THE WESTERN AUSTRALIAN REGISTER OF MULTIPLE BIRTHS: A TWIN-FAMILY STUDY OF ASTHMA

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DECLARATION

This thesis has been completed during my period of candidature for the degree of Doctor of Philosophy at The University of Western Australia. It is wholly my own composition and contains no material that has been accepted for any degree of diploma by the University or any other institution, except by way of background information and duly acknowledged in the text. It contains published works which have been co-authored. The bibliographic details of the works and where they appear in the text are set out overleaf.

Janice Hansen
December 2006

THE WESTERN AUSTRALIAN REGISTER OF MULTIPLE BIRTHS: A TWIN-FAMILY STUDY OF ASTHMA. Janice Hansen, PhD Candidate

There have been two published papers using WA twin register data.

Hansen J, de Klerk NH, Croft ML, Alessandri PT, Burton PR (2000). The Western Australian Twin Child Health (WATCH) study: work in progress. *Aus Epidemiol*; 7: 16-20.
This paper is referenced throughout the thesis and is included as Appendix 1.

Hansen J, Alessandri PT, Croft ML, Burton PR, de Klerk NH (2004). The Western Australian Register of childhood multiples: effects of questionnaire design and follow-up protocol on response rates and representativeness. *Twin Res*; 7: 149-161.
This paper appears in its entirety as Chapter 3: The WA Twin Register.

The contribution of each author was the same on both papers and has been assessed as:

Hansen	75%;
Alessandri	10%;
Croft	5%;
Burton	5%;
de Klerk	5%

Janice Hansen Nick de Klerk Matthew Knuiman

December 13th 2006

I dedicate this thesis to my dear Dad

THOMAS JOHN WHITBY

24th February 1917 – 5th March 2004

Not "How did he die?" But "How did he live?"
Not "What did he gain?" But "What did he give?"
Not "What was his station?" But "Had he a heart?"
And "How did he play his own special part?"
Not "What was his shrine?" Nor "What was his creed?"
But "Had he befriended those really in need?"
Not "What did the piece in the newspaper say?"
But "How many were sorry when he passed away?"
Was he ever ready with a word or good cheer,
To bring back a smile, to banish a tear?
These are the units to measure the worth
Of a man as a man, regardless of birth.
(Author unknown)

ABSTRACT

Background: Genetic epidemiology draws on the mechanisms of heredity and the reproductive characteristics of populations to formulate methods to investigate the role of genetic factors and their interaction with the environment in disease aetiology. Asthma and atopy are complex genetic disorders and are among the most common diseases to affect the developed world. Twin studies provide an elegant means of disentangling genetic and environmental contributions to the aetiology of conditions that have a significant impact on the health of the general population in ways that cannot be achieved by any other study design, by comparing disease frequency in monozygotic (MZ) or identical twins, who share 100% of their genes with that in dizygotic (DZ) or non-identical twins who share, on average, 50% of their genes. Twin-family studies allow the complete partitioning of phenotypic variation into components representing additive genetic, dominance, shared environment and non-shared environment.

Aims: (a) to establish a population-based Register of all multiple births born in Western Australian (WA) since 1980; (b) to describe the Western Australian Twin Child Health (WATCH) study of the contribution of genetic and environmental factors in the aetiology of childhood asthma and atopy; (c) to examine the representativeness of the WATCH study by comparing outcomes and exposures in twins and families in the study with those in: (i) twins and families not in the study, and (ii) singletons born during the same period; (d) to determine whether exposure to passive smoking increases the risk of childhood asthma and atopy in WA twin families; (e) to examine the genetic epidemiology of asthma in twins and twin families by extending existing variance components models, and to test the validity of the equal environments assumption with respect to doctor-diagnosed asthma in WATCH study families.

Methods: The WA Twin Register was established by identifying twins and other multiple births from the Maternal and Child Health Research database (MCHRDB), which was established and is maintained by the Telethon Institute for Child Health research (TICHR). Families who were not known to have experienced the death of one of their multiples, and for whom a current address could be found, were invited to participate in the WA Twin Child Health

(WATCH) study, by completing a series of mailed questionnaires covering every member of their family. A detailed family structure was collected to allow the relationships between family members to be determined. Doctor-diagnosed asthma (DDA) and zygosity were determined via questionnaire using a standard series of questions. The representativeness of the Register was assessed by using the socio-economic indexes for areas (SEIFA) codes developed by the Australian Bureau of Statistics (ABS), and comparison of maternal characteristics using the MCHRDB. Generalized estimating equations (GEEs) were used to identify risk factors for the development of asthma and atopy in twins. Twin concordance and correlation of asthma was then used to estimate heritability. Variance components models using the Bayesian inference using Gibbs sampling (BUGS) software, and based on the methodological work of Burton, Scurrah and colleagues (Burton *et al* 1999, Scurrah *et al* 2000), were extended to analyze binary phenotypes in twins and twin families, and to assess the validity of the equal environments assumption (EEA).

Results: The WATR comprises all multiple birth children born in WA between 1980 and 1997 inclusive. It consists of 5,340 twin pairs, 156 sets of triplets and 10 sets of quadruplets and quintuplets, a total of 11,189 children. Six hundred and thirty six multiple birth children from 506 families died during the perinatal period, 578 twins and 58 higher order multiples (HOMs) giving a perinatal mortality rate of 54 per 1,000 and 114 per 1,000 births respectively. The WATCH study collected data from families whose multiples were born in WA from 1980 to 1992 inclusive. Seventy nine percent of eligible families (2,395/3,041) contacted replied, and 88% of them (2,115) agreed to participate in the study. Of these, 1,827 (86%) returned completed questionnaires, yielding data on 9247 individuals. There was no difference in measures of socio-economic status (SES) between participants and non-participants. Twins had lower birth weight (2,503g vs. 3,443g, p<.001) and were born at shorter gestation (36 weeks vs. 39 weeks, p<.001) when compared with their singleton siblings. The prevalence of doctor-diagnosed asthma in twin families was higher in mothers than in fathers (18% vs. 12%, p<.001), but in children, boys had a higher rate than girls (30% vs. 24%, p<.001). There was no difference in the prevalence of asthma between twins and their siblings (28% vs. 26%, p=0.16), nor between MZ and DZ twins (28% vs. 27%, p=0.66). Multivariate GEE modelling for DDA in twins showed that the risk of DDA was increased 5.8

times (95% CI: 4.6-14.8) if both parents were asthmatic. Other risk factors for an increased odds of asthma in twins included being male (OR=1.43; 95% CI: 1.1-1.8), living in the city (1.30; 1.1-1.7), having no older siblings (1.4; 1.1-1.7), mothers experiencing a threatened miscarriage during pregnancy (1.66; 1.2-2.4), having at least one episode of otitis media during childhood (1.32; 1.03-1.7), having had tonsils removed (1.51; 1.04-2.2), and being in the bottom 10% with respect to the SEIFA indexes of disadvantage (2.20; 1.2-4.0) and economic resources (4.08, 1.6-10.4). There was no relationship between asthma in twins and exposure to environmental tobacco smoke (ETS). Compared with DZ twins, MZ twins had a significantly higher concordance (76% vs. 49%, respectively) and correlation (87% vs. 48%, respectively) for asthma, resulting in an estimate of heritability at 78%. Variance components analysis showed that the models developed produced consistent results in the simulated data sets. For twin data, variance components analysis showed that the model which fit the data the best was the one which only included additive genetic effects, and that shared environment effects were not significantly related to the odds of asthma. For twin family data, the best fitting model was the one which included additive genetic effects and either genetic dominance or shared sibling environment, and that shared family environment was not important. With respect to asthma in WA twin families, there are no reasons to conclude that the EEA is not valid.

Conclusions: The WA Twin Register is the first population-based register of childhood multiples to be established in Australia, and the WATCH study is one of only a few population-based twin-family studies in the world. Families who participated in the WATCH study were no different from non-participants with respect to social class and there was no difference in the prevalence of DDA in WATCH study twins and either their singleton siblings or the general population of WA children. Results from the GEE models replicate those found in numerous studies from many different countries. The BUGS models developed have been shown to produce consistent results with both simulated and real data sets and offer alternative methods of analyzing twin and twin-family data. By including an extra term in the partitioning of the variance to account for the environment effect of being a MZ twin, a numerical value is calculated for the difference in MZ and DZ correlation with respect to the phenotype examined, which allows the validity of the EEA to be directly assessed.

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STATEMENT OF CONTRIBUTION

In my role as co-ordinator of the WA Twin Register since its inception in 1997, I have been responsible for the questionnaire designs and I supervised all aspects of data collection to enable the WATR to be established. I carried out all the statistical analyses presented in this thesis.

LIST OF ABBREVIATIONS AND ACRONYMS

ABS	Australian Bureau of Statistics
ADHD	attention deficit hyperactivity disorder
ATR	Australian Twin Registry
BHR	bronchial hyperresponsiveness
BUGS	Bayesian inference using Gibbs sampling
CI	confidence interval
CP	cerebral palsy
DDA	doctor-diagnosed asthma
DNA	deoxyribonucleic acid
DOHWA	WA Department of Health
DZ	dizygotic/dizygous
ECRHS	European Community Respiratory Health Survey
eNO	exhaled nitric oxide
ENT	ear, nose and throat
ETS	environmental tobacco smoke
FEV1	forced expiratory volume in 1 second
FVC	forced vital capacity
GEE	generalized estimating equation
GLMM	generalized linear mixed model
HDM	house dust mite
IgE	immunoglobulin E
ISAAC	International Study of Asthma and Allergies in Childhood
IUGR	intra-uterine growth restriction
LRTI	lower respiratory tract infection
MZ	monozygotic/monozygous
NHMRC	National Health and Medical Research Council

NICU	neonatal intensive care unit
NND	neonatal death
OR	odds ratio
PND	perinatal death
RSV	respiratory syncytial virus
SB	stillbirth
SEIFA	socio-economic indexes for areas
SEM	structural equation modelling
SES	socio-economic status
SCN	special care nursery
SGA	small for gestational age
SIDS	sudden infant death syndrome
SPT	skin prick test
TICHR	Telethon Institute for Child Health Research
URTI	upper respiratory tract infection
UTI	urinary tract infection
UWA	University of Western Australia
WA	Western Australia
WATCH	Western Australian Twin Child Health
WATR	Western Australian Twin Register

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CHAPTER 1
INTRODUCTION AND OBJECTIVES

1.1 Preface

In this thesis I describe the establishment of the Western Australian Twin Register (WATR), a population-based register of WA-born multiple births, born from 1980 to 1997. I then explain how this register was used to conduct the WA Twin Child Health (WATCH) study, which collected data from twins and their families to examine the roles that genes and the environment play in the aetiology of childhood asthma and atopy, with particular reference to the effect of exposure to environmental tobacco smoke (ETS) on doctor-diagnosed asthma. The methods of analyzing correlated binary data developed by Burton, Scurrah and colleagues (Burton *et al* 1999, Scurrah *et al* 2000) are extended to model continuous and binary phenotypes in twins and twin families. Finally, I describe how these models were further extended to allow the validity of the equal environments assumption (EEA), one of the most critical assumptions of the twin method, to be tested.

1.2 Genetic Epidemiology

Genetic epidemiology draws on the mechanisms of heredity and the reproductive characteristics of populations to formulate methods to investigate the role of genetic factors and their interaction with the environment in disease aetiology. Although there have been many definitions of genetic epidemiology, the one that is most often cited is that of Morton, who defined genetic epidemiology as: “a science that deals with aetiology, distribution and control of disease in groups of relatives and with inherited causes of disease in populations” (Morton 1982). It has been described by Rao as: “an emerging field with diverse interests, one that represents an important interaction between the two parent disciplines: genetics and epidemiology”, who added that: “genetic epidemiology differs from epidemiology by its explicit consideration of genetic factors and family resemblance; it differs from population genetics by its focus on disease; it also differs from medical genetics by its emphasis on population aspects” (Rao 1984).

Research strategies for genetic epidemiology include population and family studies that provide complementary methodologies for studying the role of genetic factors in disease. Population studies concentrate on describing the distribution of genetic traits and disease in populations, identifying the risk factors associated with the frequency of genetic traits in populations and examining the role of genetic factors in disease aetiology. In family studies, the concept of familial aggregation is central to genetic epidemiology and allows three important questions to be addressed (King *et al* 1984):

1. whether a specific disease clusters in families, ascertained either by comparing disease frequency in relatives of cases with that in either relatives of controls or in the general population;
2. if it does, whether it is related to a common environment, biologically inherited susceptibility, or cultural inheritance of risk factors. This can be addressed by considering both genetic and epidemiological approaches. Epidemiological methods address clustering by, for example, assessing whether increased risk of disease in relatives remains after controlling for potential confounders. Genetic methods commonly use a multi-factorial model of inheritance and use variance components approaches to infer the degree of genetic control in traits. Because relatives share genes in a predictable fashion and may also share environments in some defined manner, the covariance or correlation between relatives can be separated into common genetic and common environmental components, and this partition of the covariance forms the basis for analysis of familial data (Elston 1981); and
3. the pattern of inheritance of the genetic susceptibilty. Inheritance can either be single-gene (following Mendelian principles), or involve more than one loci. Complex diseases are characterised by multiple genetic and environmental determinants.

To determine the amount of familial aggregation, families are usually ascertained through “probands”, that is, individuals who are “cases” or “controls” for the disease or outcome of interest in the traditional epidemiological sense. Information about the disease state in their relatives is provided by the proband. However, results are likely to be biased if recall

depends on whether the proband is a case or control. To minimise bias, it is desirable to have family members also involved in the study so that they can provide information about themselves directly. However, this means that the sample size would be substantially increased (Hopper 1992). In genetic epidemiology, some of the most efficient studies are those that involve twin pairs. They are the minimum informative set of relatives and have the advantage of sharing more genetic and environmental "information" than any other pair of relatives.

Asthma and allergies are complex genetic disorders that are ideal models for studying diseases resulting from the interaction of genetic and environmental factors. Advances in several fields, including genetic epidemiology and molecular biology, have now made it possible to explore the genetics of complex diseases such as asthma and allergies at both the population and molecular level. The first known study of the heredity of asthma was in 1916 (Cooke & Van Der Veer 1916), and early investigators emphasised the familial nature of asthma, allergy and atopic disease as providing evidence of a genetic predisposition. However, developing an understanding of asthma and allergies has been difficult in view of the multiple genetic and non-genetic factors that may ultimately determine the clinical picture.

1.3 Epidemiology of Asthma and Atopy

The epidemiological definition of asthma remains controversial (Woolcock 1987), but there are several key aspects upon which there is general agreement. Period prevalence, or active symptoms during the last 12 months, is the most generally accepted measure (Anderson 1989). Asthma may be better thought of as a syndrome than a disease as clear biological markers defining disease are unavailable (Gross 1980).

Since the 19th century, asthma has been known as "episodic dyspnoe with healthy respiration between attacks caused by spasm of the bronchi" (Los *et al* 2001). In 1995, a global initiative in asthma defined asthma as:

> "a chronic inflammatory disorder of the airway in which many cells play a role, in particular mast cells, eosinophils, and T

> lymphocytes. In susceptible individuals this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and cough particularly at night and/or in the early morning. These symptoms are usually associated with widespread but variable airflow limitation that is at least partly reversible either spontaneously or with treatment. The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli" (NIH 1995).

In epidemiological studies, two methods are commonly used to identify asthma in an individual; self-report of a diagnosis of asthma, with or without physician confirmation or self-report of certain symptoms, such as wheeze; and presence of bronchial reactivity on challenge (Banerjee *et al* 1987, Burrows *et al* 1991, Speight *et al* 1983). In the population asthma tends to be under-diagnosed, and in children there is an overlap between the diagnoses of chronic and wheezy bronchitis and asthma, especially in the under five-year-olds (Taussig *et al* 1981). Although wheeze is the predominant symptom of asthma, some individuals have other symptoms such as breathlessness or cough, and are thus not included in studies focussing on wheeze. It may be more appropriate to examine the prevalence of symptoms of wheeze, breathlessness and cough rather than the syndrome labelled as asthma (Woolcock 1987).

Atopy can be defined as a condition characterised by a persistent and heritable immunoglobulin E (IgE) response to protein allergens, and includes asthma, hay fever, eczema and some other allergies (Cookson 1994). The atopic diseases are the most common chronic conditions of childhood in Australia (ABS 2002); asthma is a major cause of morbidity in children and is the most common reason for hospital admission (ACAM 2003). Across all ages, as many as 50% of individuals in Western societies suffer from some form of atopy (ISAAC 1995). In children, the prevalence of diagnosed asthma has been estimated as being as high as 20%, and to 24% report a history of recent wheeze (Peat *et al* 1994, Robertson *et al* 1992). Forty percent of children with infrequent episodic asthma and 70% of those with frequent or persistent asthma will have symptoms in mid adult life (Martin *et al* 1980).

Several genetic and environmental risk factors for asthma have been identified. These include a positive family history, age, exposure to tobacco smoke, and exposure to aero-allergens, especially house dust mite (*Dermatophagoides pteronyssinus*), cat dander and pollens, particularly grasses. Asthma is well known to cluster in families (Sibbald *et al* 1980, Sibbald & Turner-Warwick 1979, van Arsdel & Motulsky 1959), and prevalence among siblings tends to increase as the number of parents with asthma increases (Kjellman 1977, Litonjua *et al* 1998, Moffatt & Cookson 1996).

While genetic factors are clearly important in determining risk of development of asthma, environmental factors are still likely to be the primary determinants of the expression of disease. Asthma is usually associated with atopy; asthmatics tend to be more atopic than non-asthmatics (Gergen *et al* 1988, Kalliel *et al* 1989). The epidemiological literature provides some insights into the natural history of asthma, including age at onset, frequency and intensity of relapse and remission, and some information on major asthma morbidity as measured by hospitalisation, and mortality. Asthma incidence varies with age, with children under 5 years of age having the highest rate (Broder *et al* 1974, Dodge & Burrows 1980, McWhorter *et al* 1989). It occurs more frequently in boys than girls, with the male to female ratio approaching 2 to 1 (Gergen *et al* 1998, Weiss & Speizer 1993, Wieringa *et al* 1999, Yuan *et al* 2003). By adulthood, however, this trend is reversed (CDC 2001, Mannino *et al* 2002, Thomsen *et al* 2006c) with females having a higher prevalence of asthma than males.

Allergy is recognised as playing an important role in asthma. Most asthmatics are sensitised to at least one common allergen (Burrows *et al* 1991, Sears *et al* 1993b) with exposure to house dust mites being the most important documented risk factor for airway hyperresponsiveness and asthmatic symptoms (Custovic *et al* 1996, Sears *et al* 1993a, Sporik *et al* 1992). Other important allergens include those from pets, especially cats, cockroaches, the mould *Alternaria* (Sears *et al* 1993b, Warner *et al* 1990), and airborne outdoor pollens, especially grasses (Peat *et al* 1994, Suphioglu *et al* 1992).

A number of environmental risk factors have been identified, and act as early as the "*in utero*/neonatal" period. Children with low birth weight are at risk of

having narrow airways and/or early respiratory symptoms and of developing sensitisation or wheeze in early childhood. Young mothers have an increased risk of having a child with asthma when compared with mothers over 30 (Infante-Rivard 1995), which is thought to possibly relate to lower birth weights and reduced lung function. However, this effect may be confounded by smoking as smoking rates in young adult females have not shown the same declining rates as those in young males and have not changed substantially over the last decade (DOHWA 2001). Additional factors identified during the prenatal and neonatal periods include antepartum haemorrhage, early threatened labour, malpresentation of the foetus, use of opioids during labour, caesarean section, and neonatal illness in the first week of life (Annesi & Strachan 1995). In early childhood, exposure to house dust mite, development of positive skin prick tests, and continuing exposure to the same allergens as in early infancy all increase the risk of asthma. In later childhood and adolescence, asthma is consistently related to atopy, especially to house dust mites and pets, particularly cats.

Viral infections are well recognised to be precipitants of asthma attacks. Croup and lower respiratory tract infections, such as bronchiolitis and pneumonia, have been reported to be associated with the subsequent development of asthma (Anderson *et al* 1986, Konig 1978, Pullan & Hey 1982). It has been hypothesised that bacterial infections in the first few weeks of life protect against the development of an allergic phenotype. However, there is also evidence that serious respiratory infections in early life are associated with the later development of asthma (Peat *et al* 1992, Stern *et al* 1989, Voter *et al* 1988, Weiss & Gergen 1997), suggesting that early bacterial infections may protect against the development of atopy, whereas viral infections may provoke the first expression of asthma.

In many countries, increases in asthma have been reported in all social classes, but particularly in higher-income groups (Platts-Mills & Carter 1997). It is thought that decreased exposure to infections in the first few weeks of life as a result of smaller family sizes and better hygiene has played a part in the higher prevalence of asthma in affluent populations (Peat 1996). In Europe, small family size is associated with increased rates of atopy and allergic symptoms,

with the first born children being at highest risk (Strachan 1989, von Mutius *et al* 1994). Several studies have confirmed that family size and birth order, in particular, are associated with hay fever and atopy (Strachan 1997). The influence of family income is not clear. While children from less affluent families appear to have higher prevalence of wheezing, children from more affluent families are more likely to receive a diagnosis of and treatment for asthma, reflecting differential access to health care. The higher prevalence of wheezing in low-income groups may be confounded by parental smoking.

The association of active smoking and respiratory disease is well documented (AIHW 2005, DOHWA 2001, USPHS 1984). Exposure to environmental tobacco smoke (ETS), which is the most common indoor air pollutant, is the best identified risk factor for the development of allergic disease (Bjorksten 1997), particularly in childhood, and this is independent of how "allergy" is defined (Halken *et al* 1995). Maternal smoking during pregnancy is a consistent risk factor for early childhood asthma (Lewis *et al* 1995, Oliveti *et al* 1996, Stick *et al* 1996), and despite the Quit and Passive Smoking Campaigns in Western Australia, 20% of pregnant and young mothers continue to smoke (DOHWA 2001). Maternal smoking has consistently been reported to be an important risk factor for childhood asthma morbidity and the effect is still seen even if the mother only smoked during pregnancy and quit before delivery, suggesting an *in utero* effect (Taylor & Wadsworth 1987). Children whose parents smoke have been reported to have more problems with wheezing, lower respiratory tract infection, and asthma than children of parents who do not smoke, especially in the first year of life (Burchfiel *et al* 1986, Fergusson *et al* 1981, Gortmaker *et al* 1982, Neuspiel *et al* 1989). Children who are exposed to ETS at home have a significantly earlier onset of allergy, and wheezy bronchitis is five times more common in them than in children not so exposed (Bjorksten 1997).

The effects of smoking during pregnancy, which is associated with pre-term delivery and low birth weight, and of postnatal exposure to ETS, which may compound the effects of prenatal exposure, have not been separated (Peat 1996). Studies conducted in China, where few women smoke, suggest that the effects of exposure to ETS in infancy are different from those of maternal smoking during pregnancy (Chen *et al* 1988).

1.4 Twins

Monozygous or MZ twins, also called identical, arise when one fertilised ovum splits in early pregnancy to produce two genetically identical individuals. The frequency of MZ twinning is approximately 4 per 1000 births, and is not known to be effected by heredity or maternal risk factors (MacGregor *et al* 2000). Dizygotous or DZ twins share, on average, 50% of their genetic material and arise when two separate ova are fertilised and develop as part of the same pregnancy. They are also referred to as non-identical or fraternal twins. They are no more similar, genetically, than any other pair of full biological siblings. The rate of DZ twinning increases with increasing maternal age (MacGillivray 1986), and with the increased use of assisted reproductive technology in recent decades (Tough *et al* 2000). Differences in the rate of disease or trait of interest between the two different types of twins act as a natural experiment to separate genetic from environmental factors by design rather than by analysis.

1.5 Twin Studies

Twin studies provide an elegant means of disentangling genetic and environmental contributions to the aetiology of conditions that have a significant impact on the health of the general population (Hopper 1992, Neale & Cardon 1992). This is achieved by examining the extent to which monozygous (MZ) twins are more alike than dizygous (DZ) twins with respect to the trait being studied. Twin registers are a major source for genetic epidemiological studies of a wide range of phenotypes (Boomsma 1998, Boomsma *et al* 2002a) and large twin registers are particularly useful for studying whether there is a genetic component to moderately rare diseases. Many twin registers have been established around the world, including those in Sweden (Cederlof & Lorich 1978, Medlund *et al* 1977), Denmark (Hauge *et al* 1968), Norway (Kringlen 1978), Finland (Kaprio *et al* 1978), England (Spector & MacGregor 2002), Belgium (Derom *et al* 2002), the Netherlands (Boomsma *et al* 2002b) and Italy (Salvetti *et al* 1997); and in the USA (Eisen *et al* 1987, Friedman & Lewis 1978, Hrubec & Neel 1978, Lykken *et al* 1990, Miller *et al* 1998, Redline *et al* 1989). More recently, twin registers have been formed in some Asian countries, for

example, China (Yang *et al* 2002), Japan (Hayakawa *et al* 2002), Korea (Sung *et al* 2002) and Sri Lanka (Sumathipala *et al* 2002). The Scandinavian twin registers are population-based, but elsewhere they rely on the registration of volunteers (Hopper 2002a, Jang *et al* 2002, Spector & MacGregor 2002, Spinath *et al* 2002, Strassberg *et al* 2002). Some have been established to study a specifically-defined population, for example war veterans (Eisen *et al* 1987, Hrubec & Neel 1978). Recently, the European community recognised the value of collecting large, unbiased samples of twins by funding the GenomEUtwin project, a collaboration between six major European twin registers, together with the St. Thomas' twin cohort and the Australian Twin Registry (ATR), and the multi-national population cohort, MORGAM (Peltonen 2003).

Twin registers have been used to study the genetic and environmental contributions to a wide range of health conditions, including asthma and atopy (Duffy 1995, Harris *et al* 1997, Hopper *et al* 1990, Koeppen-Schomerus *et al* 2001, Laitinen *et al* 1998, Lichtenstein & Svartengren 1997, Nieminen *et al* 1991, Rasanen *et al* 2000, 2001, Skadhauge *et al* 1999), behavioural disorders (Eley *et al* 2003, Levy & Hay 1996, Sherman *et al* 1997), psychiatric disorders (Kendler *et al* 1995, Kringlen *et al* 2001, Lyons *et al* 1998, Torgersen *et al* 2001), cancer (Boyd *et al* 2002, Lichtenstein *et al* 2000, Zhu *et al* 1999), cardio-vascular disease (Boomsma *et al* 2002b, Brinsuk *et al* 2004, Harrap *et al* 2000, Iliadou 2003, Kringlen 1978, Medlund *et al* 1977, van den Bree *et al* 1996) and arthritis (Bellamy *et al* 1992, Jawaheer *et al* 1994, Kujala *et al* 2000a, Spector & MacGregor 2004).

A study of 7,000 Swedish twin pairs found allergic symptoms concordance of 19% for MZ twins versus 4.8% for DZ twins (Edfors-Lubs 1971). The importance of genetic susceptibility to asthma was highlighted in a retrospective study of 5,864 Norwegian twins aged 18 to 25. The relative risk of asthma developing among twins whose co-twin had a history of asthma compared with those whose co-twin had not was 17.9 (95% CI: 10.3-31.0) for identical twins but only 2.3 (1.2-4.4) for DZ twins, despite the shared environment (Harris *et al* 1997). In that study, 75% of the variation in propensity to asthma was explained by genetic factors.

Australia already has a national database of twins, the National Health and Medical Research Council (NHMRC)-funded Australian Twin Registry (ATR) (Clifford & Hopper 1986). It is an invaluable research tool and has been used to study a number of conditions. However, it is volunteer-based and covers only 12.5% of Australian twins of all ages from all States and Territories (Clifford & Hopper 1986). Although this is not a problem in some areas of application, it could lead to problems of non-generalisability for many studies in the area of Public Health. This is because the expression of many conditions is strongly influenced by factors such as social class, which also modulate response to voluntary data collection. The importance of establishing twin registers at a state and national level in the US was endorsed to maintain a more complete clinical description of the perinatal and childhood morbidity occurring in twins (Kirby 1995). There is therefore great scientific value in complementing the national volunteer registry, the ATR, with a population-based register.

Twin studies therefore provide a useful means of studying the epidemiology and natural history of these conditions, and of quantifying the burden that they represent both for their families and for the health services in general. Since WATCH is a twin-family study (twins+parents+siblings), it provides one of the most powerful nuclear-family-based designs for resolving a range of different genetic and environmental contributions to phenotypic variation of asthma and allergy and, to my knowledge, no-one has used this study design to study asthma and atopy.

1.6 The classic twin design

The classic twin design has been in existence since early last century, when the use of twins in research was acknowledged in 1924 simultaneously by German dermatologist Hermann Siemanns and American psychologist Curtis Merriman (Spector 2000). They both recognised the existence of two types of twins and how analysis of differences in disease or other endpoints between them could separate genetic from environmental factors. The twin design is based on a number of fundamental assumptions that stem from the differences between MZ and DZ twins. These basic assumptions are:

(i) a greater phenotypic similarity among MZ than DZ twins must be caused by the greater genetic similarity, that is, the trait or disease under study is influenced by genetic factors; and
(ii) discordance in MZ pairs can be attributed to environmental effects only.

Fundamental to these assumptions is the notion that MZ and DZ twins experience the same degree of environmental exposure, the so-called "equal environments assumption" (EEA).

When considering the implications of the results of a twin study it is important to consider validity of the EEA, and also to determine that the twins under study are representative of the population from which they are drawn. The EEA is crucial to the validity of the classic twin method. If MZ twins are in fact more similar than DZ twins, then the EEA will not be valid, and there will be a resultant over-estimation of genetic effects. The validity of the EEA should be determined with respect to each trait under study, and not just assumed to be true. Another criticism of the twin method that needs to be considered is the ability to apply any results found from twin studies to the general population. Twins are different from singletons in a number of ways, for example, they are born with lower average birth weight and at shorter gestation than singletons (Taffel 1995). However, these differences are not necessarily significant to each disease or trait under study. It is therefore important to ascertain that the prevalence of the disease being studied is the same in twins and singletons, and that there is no association between the disease state and zygosity or sex (Kyvik 2000). The twins can then be considered to be a representative sample, and results can be generalized to the general population.

1.7 Methods of Analyzing Twin and Twin-Family Data

The basis of the analysis of twin data is the comparison of the phenotypic covariance between MZ and DZ twins, which allows the decomposition of the phenotypic variance into genetic and environmental components. Genetic effects can be divided into additive (A) and non-additive, or dominance (D) components, and the environmental effects into shared (C), and non-shared or individual components (E). All genetic effects are perfectly correlated in MZ

twins, whereas DZ twins, like other siblings, share half their additive genetic effects, and one quarter of genetic dominance. Shared environmental effects are perfectly correlated in both MZ and DZ twins. Models to analyze twin data are then parameterised in terms of an ACE or ADE model, since the C and D components of variance are confounded in data for twins reared together (Eaves *et al* 1978, Martin *et al* 1978). Variance components analysis can be extended to twin-family data, where relatives within a family are related in a genetically predictable fashion. The advantage of a twin-family study is that it allows for the complete parameterization of the ACDE model.

Various statistical methods have been developed to analyze twin and family data, and a brief description of some of them is given below.

1.7.1 Regressive models

Regressive models were developed by Bonney (Bonney 1984) to analyze family data where families were ascertained via probands. The premise for regressive models to analyze family data is to condition each subject's phenotype on those of his or her antecedents, and requires the arbitrary ordering of family members, usually by age. Twin data is analyzed by predicting a co-twin's score from a proband's score and the coefficient of relationship, set at 1.0 for MZ twins and 0.5 for DZ twins. It is particularly useful for analyzing continuous data when one twin (the proband) is selected as having a deviant score (de Fries & Fulker 1985). The original Bonney models have been extended to include binary data analyzed by regressive logistic models (Bonney 1986).

1.7.2 Structural equation modelling

The most widely used method for fitting variance components models to twin data is maximum likelihood estimation within the framework of structural equation modelling (SEM). Software packages, for example Mx (Neale 1997) and LISREL (Joreskog & Sorbom 1986) have been developed specifically for SEM. SEM uses path analysis, which was developed by Sewell Wright in 1921 (Wright 1934), to evaluate the relative importance of the various components of phenotypic variance. The structural relationships among these components of variance are represented by path diagrams. SEM involves solving a series of simultaneous equations in order to estimate the variance components (Heath *et*

al 1989b). Originally developed to analyze continuously-distributed data, extensions to fit models to binary data is achieved by using the tetrachoric correlation to fit the AC or AD models. The inclusion of covariates, interactions and non-linear components is possible, but not straightforward, and SEMs are not recommended for analyzing an unbalanced family design (Heath *et al* 1989b).

1.7.3 Markov chain Monte Carlo methods

Markov chain Monte Carlo (MCMC) methods are a class of algorithms for sampling from probability distributions based on constructing a Markov chain that has the desired distribution as its stationary distribution. The state of the chain after a large number of iterations is then used as a sample from the desired distribution. MCMC approaches are so-named because previously sample values are used to randomly generate the next sample value, thereby generating a Markov chain. The Gibbs sampler, an example of a MCMC algorithm, was developed by Geman and Geman (Geman & Geman 1984) and is particularly useful in Bayesian analysis which requires complex integration across high-dimensional functions to obtain the posterior distribution.

Paul Burton and colleagues have developed models within a Bayesian framework to analyze correlated data found in nuclear families (Burton *et al* 1998, Burton *et al* 1999, Scurrah *et al* 2000), using the BUGS (Bayesian inference using Gibbs sampling) software (Spiegelhalter *et al* 1995). The Bayesian method uses a Markov chain Monte Carlo (MCMC) approach to estimate the variance components, and the Gibbs sampler is employed to draw inferences from a Markov chain, which converges to the posterior distribution of interest. Burton and colleagues used BUGS to fit a generalized linear mixed model (GLMM) to Normally-distributed data (Burton *et al* 1998), and then extended the models to analyze binary data (Burton *et al* 1999) and censored survival time data (Scurrah *et al* 2000). Once a working model had been established, it was relatively straightforward to modify it to generalize the methods to develop other models.

MCMC methods using BUGS are becoming increasingly popular for the analysis of twin data (Do *et al* 2000, Eaves & Erkanli 2003, Eaves *et al* 2005, Kuhnert &

Do 2003, van den Berg *et al* 2006), and are known to produce consistent results compared with other methods of analysis (Do *et al* 2000, Kuhnert & Do 2003). To my knowledge, these methods have not been extended for the analysis of twin-family data.

1.8 Aims of the Study

The aims of this study were to:

(a) establish a population-based Register of all multiple births born in Western Australian (WA) since 1980;

(b) describe the Western Australian Twin Child Health (WATCH) study of the contribution of genetic and environmental factors in the aetiology of childhood asthma and atopy;

(c) examine the representativeness of the WATCH study by comparing outcomes and exposures in twins and families in the study with those in:

    (i) twins and families not in the study, and

    (ii) singletons born during the same period.

(c) determine whether exposure to passive smoking increases the risk of childhood asthma and atopy;

(d) examine the genetic epidemiology of asthma in twins and twin families by extending existing variance components models, and

(e) test the validity of the EEA with respect to doctor-diagnosed asthma in WATCH study families.

1.9 Significance

Twin studies are a powerful tool for dissecting the relative contributions of genetic and environmental factors in disease aetiology. However, genetic dominance and shared environment are confounded in twins reared together, and these components of variance cannot be modelled together. Family studies can demonstrate whether or not genetic factors are important in disease aetiology, but since both genes and the environment cluster in families, it is not possible to separate one from the other using family studies. Twin-family studies combine features of both twin studies and family studies. By studying MZ and DZ twins within a family setting, not only is it possible to differentiate

between genetic and environmental factors, but it is also possible to completely disentangle the genetic and environmental components of variance for the trait under study, that is, additive genetics, genetic dominance, shared environment and non-shared environment can all be modelled together.

A number of genetic epidemiological studies, including some based on twins, have clearly demonstrated that both genetic and environmental factors play a role in determining the asthmatic and atopic phenotypes (Duffy 1995, Postma 1995). Some environmental exposures may act directly, thereby increasing the risk of asthma in all exposed. Others may act only in those with a genetically determined sensitivity to that exposure. It is unclear into which category passive smoking falls.

Twins, and other multiples, and studies based upon them are highly relevant to both the public health and the promotion of health in the community. This study could provide knowledge of genetic factors influencing the effect of tobacco smoke on asthma that may be of direct relevance to education-based anti-smoking campaigns. The ban on smoking in public places has generally reduced exposure to ETS in the community, but ETS in the home, which is the predominant place of exposure for many young children, has not been fully addressed. Families bear considerable financial burden of having an asthmatic child, especially if the asthma is severe enough to require admission to hospital (Toelle *et al* 1995). Environmental interventions, such as the reduction of exposure to ETS, could reduce the use of medical services and asthma medications and provide a cost-effective method for improving quality of life both for asthmatics and potential asthmatics, and their families.

Two papers have been published using WA twin family data as described in this thesis:

Hansen J, de Klerk NH, Croft ML, Alessandri PT, Burton PR (2000). The Western Australian Twin Child Health (WATCH) study: work in progress. *Aus Epidemiol*; 7: 16-20.

This paper appears in Appendix 1.

Hansen J, Alessandri PT, Croft ML, Burton PR, de Klerk NH (2004). The Western Australian Register of childhood multiples: effects of questionnaire design and follow-up protocol on response rates and representativeness. *Twin Res*; 7: 149-161.

This paper appears in its entirety as Chapter 3.

1.10 Conclusions

Twin studies provide a useful means of studying the epidemiology and natural history of complex diseases such as asthma and atopy, and of quantifying the burden that they represent both for their families and for the health services in general. Because the WATCH study is a twin-family study, it provides one of the most powerful nuclear-family-based designs for resolving a range of different genetic and environmental contributions to phenotypic variation of asthma and atopy.

CHAPTER 2
TWIN REGISTERS AROUND THE WORLD

2.1 Preface

In this section, I describe various twin registers that have been established around the world. A brief history of each register, along with a description of the data collection methods used and conditions studied will be given. It will inevitably be incomplete, as this is a rapidly expanding area in health and medical research, but I have tried to include those registers that have made long and significant contributions to the field. I also describe the WA Twin Register, how it was established, and give a few descriptive statistics about its composition.

2.2 Introduction

Monozygotic or MZ twins, also called identical, arise when one fertilised ovum splits in early pregnancy to produce two genetically identical individuals. The frequency of MZ twinning is approximately 4 per 1000 births, and is not known to be effected by heredity or maternal risk factors (Spector 2000). Dizygotic or DZ twins share, on average, 50% of their genetic material and arise when two separate ova are fertilised and develop as part of the same pregnancy. They are also referred to as non-identical or fraternal twins. They are no more similar, genetically, than any other pair of full biological siblings. The rate of DZ twinning increases with increasing maternal age (MacGillivray 1986), and with the increased use of assisted reproductive technology in recent decades (Tough *et al* 2000). Differences in the rate of disease or trait of interest between the two different types of twins act as a natural experiment to separate genetic from environmental factors by design rather than by analysis.

Currently in WA, there are approximately 300 multiple births per annum (~1 in 80 pregnancies) and around one in every 40 children is a twin (Gee & O'Neill 2002). Australia already has a national database of twins (the NHMRC-funded *Australian Twin Registry*). This volunteer database is an invaluable research tool and places Australia at the forefront of genetic epidemiology research

worldwide; it has generated more than 150 publications. However, it covers only 12.5% of all twins (Clifford & Hopper 1986), and although this is not a problem in many areas of application, it can lead to problems of bias or non-generalisability for many studies in the area of Public Health. This is because the expression of many of the conditions of interest to public health are strongly influenced by factors, such as social class, which also modulate response to voluntary data collection. There is, therefore, great scientific value in complementing the national volunteer registry with a state-wide population-based register.

In 1998, Boomsma and her colleagues conducted a comprehensive review of twin registers that had been established in Europe (Boomsma 1998). She identified registers that had been set up in Belgium, Germany, The Netherlands, Scandinavia (Finland, Norway, Sweden, and Denmark), Italy and the United Kingdom. Over 350,000 pairs of twins of all ages had been identified by these registers. This process was repeated in 2002 and extended to include all of these European twin registers, as well as those that were established elsewhere in the world (Boomsma *et al* 2002a). Registers have been established in Australia, Canada, China, Japan, South Korea, Sri Lanka, and various states of the USA. They have been formed by the routine examination and collection of public records, opportunistic recruitment of twins for specific research studies, or by comprehensive media campaigns encouraging volunteers to register. Some of the many data sources that have been used to establish twin registers include birth records (Skytthe *et al* 2002, Sumathipala *et al* 2002), notification of multiple pregnancies from health professionals (Derom *et al* 2002, Glinianaia *et al* 2002b), school records (Anderson *et al* 2002), military service records (Goldberg *et al* 2002, Page 2002) and national population or health insurance registers (Sung *et al* 2002, Yang *et al* 2002). Most volunteer-based registers rely on advertising to recruit participants, and this is achieved using various media outlets, referrals by health professionals, and contact through Twins Clubs, Parishes, Schools and Hospitals (Hopper 2002a, Jang *et al* 2002, Spector & MacGregor 2002, Spinath *et al* 2002, Strassberg *et al* 2002). Recently the European community recognised the value of collecting large, unbiased samples of twins by funding the GenomEUtwin project, a collaboration of six major European twin cohorts from Scandinavia, The Netherlands and Italy. Two more

registers, namely the St Thomas' twin cohort and the Australian Twin Registry (ATR) joined the collaboration, and the multi-national population cohort, MORGAM was involved to help examine the genetics of cardiovascular diseases (Peltonen 2003). It forms a collection of more than 800,000 twins, with in excess of 30,000 DNA samples being collected.

A brief description of some twin registers follows.

2.3 Belgium

The East Flanders Prospective Twin study (EFPTS) commenced in 1964 to determine the prevalence of multiple births in well-defined geographical areas, and the number of MZ and DZ twins in each area. The fact that twins and higher-order multiples are prospectively ascertained at birth means that this register is population-based with the potential for long-term follow-up (Derom *et al* 2002). It is the only register that contains information of placentation, which allows the three types of MZ twins to be identified, that is, monochorionic-monoamniotic, monochorionic-diamniotic, dichorionic-diamnotic (Derom *et al* 2002).

All multiple pregnancies in the province East Flanders, Belgium, were included on the register where one of the children weighed more than 500g at birth, or where the gestational age was at least 22 weeks if birth weight was unknown. Between 1995 and 2001, 6,279 multiple births were recorded, comprising 6,050 twin pairs and 229 sets of higher-order multiples. The register is able to accurately collect information on zygosity and chorionicity by examination of the placental membranes, blood and DNA tests. Over 2,000 twins have enrolled in up to seven different studies, and in some of them, their families were also examined. Examples of studies undertaken include: congenital malformations (Cameron *et al* 1983), differences in mortality between the three types of MZ twins (Derom *et al* 1991, Loos *et al* 1998), the inheritance of spontaneous DZ twinning, in collaboration with the Netherlands Twin Register (NTR) (Meulemans *et al* 1996), and the effect of pre-natal environment on health effects in adult life (Loos *et al* 2001, 2002).

2.4 China

A population-based twin registry was established with the initial focus on stroke and other coronary artery diseases. Its long term aims were to create a population-based twin registry, to conduct detailed phenotyping for cardio-vascular end points, and expand the twin registry to include the twins' families, thus enabling genetic linkage and association studies to be undertaken. The researchers have already ascertained twins living in Qingdao City, Shandong Province, based on the estimated number of living twins in a 10% population sample. A number of different recruitment methods were used, including neighbourhood village committees, local health care networks, residency registers and public media. Twins were included if both lived in the same region, and those over 25 years old were asked to complete questionnaires. Response from the 10 districts in the Province so far covered ranged from 10.5% (Pingdu) to over 80% in Laoshan. Up to the end of 2001, 4,374 twins had been recruited, 26% of the estimated total number of twin pairs. The researchers aim to use data collection in this province as a model to recruit 45,000 pairs from all over China in 5 years (Yang *et al* 2002).

2.5 Denmark

The Danish twin register was established in 1954 and is the oldest national twin register in the world. It comprises data on 127 birth cohorts between 1870 and 1996. More than 66,000 twin pairs of the estimated 120,000 twin births in Denmark during the period are included on the Register. Identification for all cohorts was population-based and not disease-specific. The Danish Civil Registration System (CRS) was used to identify multiple births occurring since 1931, using the 10-digit personal identification number assigned to every person. Prior to that, parish birth records were searched by local clergy to identify twin pairs where both had survived to the age of 6 years. This was carried out in the 1950s and 1960s (Hauge *et al* 1968). Twins born during 1931-1952 were included if they appeared on the 1968 CRS, that is, if they were still alive as at 1/4/1968, and data were supplemented by conducting capture-recapture analysis of a number of cohorts of twins, including male conscripts, who had been previously studied (Skytthe *et al* 2002). At the beginning of the 1990s, the CRS was used to identify twins by selecting pairs of persons born

within three days of each other to the same mother (Kyvik *et al* 1995). The Danish Medical Birth register (MBR) records all births, including stillbirths, in Denmark since 1973, and was used to ascertain twins born between 1983 and 1996. Records from the MBR were used to complete the 1973-1982 birth cohorts to include the stillbirths. Ascertainment is reported to be complete for all liveborn pairs since 1968, and estimated to be 90% complete up to that date (Skytthe *et al* 2002). Twins are followed up annually to confirm addresses and to update marital and vital status. The register is linked to a number of other registers, including the death and cancer registers, on a regular basis, and details of pregnancy and birth has been collected for all twins born since 1973 by linkage to the birth register. Zygosity was determined for same-sex twins from the answers to three or four questions about the degree of similarity, and confirmed by DNA anaysis if this is performed by any study.

Numerous surveys have been performed, and include studies examining ageing and cognitive function (Christensen *et al* 1999, McGue & Christensen 2001), Graves' disease (Brix *et al* 2001), migraines (Gervil *et al* 1999, Ulrich *et al* 1999), epilepsy (Kjeldsen *et al* 2001), eating disorders (Kortegaard *et al* 2001), and asthma (Skadhauge *et al* 1999).

2.6 Finland

The Finnish twin register consists of over 65,600 twins from a number of cohorts, and was established in 1974. Same sex twins born before 1958 and both alive in 1975 were selected from the Central Population Registry of Finland. A total of 13,888 pairs of known zygosity were ascertained. The Register was expanded in 1996 to include 5,017 opposite-sex pairs born between 1938 and 1949. Data on 21,958 pairs of younger twins, born between 1958 and 1986, and their parents, were collected in 1986. Among the many characteristics studied by the register include schizophrenia (Cannon *et al* 1998), asthma (Laitinen *et al* 1998, Nieminen *et al* 1991), breast, prostate and colo-rectal cancers (Lichtenstein *et al* 2000), osteoarthritis (Kujala *et al* 2000a) and diabetes (Kujala *et al* 2000b).

Younger twins have taken part in 2 studies - FinnTwin12 and FinnTwin16 (Kaprio *et al* 2002). FinnTwin16 is a five-cohort study of twins born between 1975 and 1979 and commenced in 1991. Baseline data were collected on 2,733 twin pairs, at or near their 16th birthday. Pairwise response was 88%. The aim of this study was to carry out analyses of genetic and environmental contributions to consistency and change in health-related behaviours during late adolescence and early adulthood. FinnTwin12 started in 1994 using another 5 birth cohorts, 1983-1987. Baseline data were collected on 2,724 families, just before their twins turned 12 years old. Follow-up was undertaken at 14 and 17.5 years. The study aimed to examine the genetic and environmental determinants of precursors of health-related behaviours, with particular focus on use and abuse of alcohol, initially in 11- to 12-year-old twins (Kaprio *et al* 2002). The data collected has been used to examine the genetic epidemiology of a number of conditions, including alcohol consumption (Dick *et al* 2001), obesity (Pietilainen *et al* 1999), asthma and allergy (Rasanen *et al* 2000), and personality (Vierikko *et al* 2003).

2.7 Germany

The Berlin twin register contains information on 65,000 twin pairs, and has been used to study a number of different health-related outcomes, particularly obesity (Jordan *et al* 2005) and cardiovascular conditions (Brinsuk *et al* 2004). In addition, a number of studies using twins have been conducted in Germany. The German Observational Study of Adult Twins (GOSAT) is an investigation of adult personality and cognitive abilities in twins aged 18 to 70 years. It contacted twins recruited by the Bielefeld Longitudinal Study of Adult Twins (BiLSAT) who had responded to a previous mailing. A total of 168 MZ and 132 same-sex DZ pairs participated (Spinath *et al* 2002). Based in Munich, the genetic-oriented longitudinal study of differential development (GOLD) has information on 250 pairs of twins (Spinath *et al* 2002).

2.8 Italy

The Italian twin registry was created in 1997 and comprises over 9,000 twins. It was established by examining a list of about 650,000 pairs of individuals who

shared the same surname, place and date of birth and where both were alive at the end of the previous year, and was constructed using social security numbers (Stazi *et al* 2002a). An excess of 40% of "pseudo" twins were identified and excluded. Other sources used included municipal registry offices and obstetric wards. Although Italy does not have a national twin register, it is anticipated that 120,000 twin pairs will enrol in the register in the future. At present, twins are enrolled either by participating in specific research projects in response to contact from the register, or by volunteering themselves to participate in a research studies when they register. The Italian twin registry currently has two main research interests, namely ageing and autoimmune diseases. It is an integral part of the GEHA (genetics of healthy ageing) project, financed by the European Union, which examined the genetics and lifestyle of disease-free twins aged over 90 years of age (Brakefield *et al* 2005). The register has already completed studies in multiple sclerosis (Cannoni *et al* 2001), and celiac disease (Cotichini *et al* 2001); further studies are underway in Alzheimer's disease and type I diabetes. In 2001, the government financed the establishment of a national twin register. Half a million people were contacted, and 120,000 twins were expected to be registered by 2003 (Stazi *et al* 2002a). The Italian twin registry also participated in GenomEUtwin, which used volunteers to study common diseases such as coronary heart disease, stroke and migraine (Peltonen 2003).

There are two important projects using twins for research into human biology and sport science in Italy (Casini *et al* 2002). They identified twins by examining records from 25 Sports Federations for athletes sharing the same name and date of birth. The registry of Italian Twin Athletes (RITA) contains 4,719 twin pairs concordant for participating in a range of sports. The Twin Epidemiological Registry of Rome (TERRY) was initiated in the early 1990s with the aim of establishing a register of all twins living in Rome, irrespective of birth place (Casini *et al* 2002). The prime source of information was the Resident Population Register of Rome. It contains 13,228 twin pairs born between 1910 and 1994 inclusive. Preliminary results are being analyzed and include health status, lifestyle, fitness and response to stress.

2.9 Japan

The Osaka University Aged Twin Registry comprises 12,000 pairs born between in Japan 1900 and 1935 and was formed to study psycho-physiological development. Ten percent of twins were raised apart. Recruitment was via newspaper advertisements, posters in hospitals, referrals from nurses and midwives, and follow-up from previous twin research. Questionnaires were mailed to 3,000 pairs each year, totalling 12,000 pairs, of which 67% were MZ (Hayakawa *et al* 2002). Two hundred and fifty pairs have had comprehensive medical examinations. Future studies include looking at cross-cultural issues among identical twins, one of whom lives in Japan, and the other having migrated to the USA before the age of 20.

2.10 Korea

The Korean Twin registry consists of 154,783 twin pairs. Lists of twins born in Korea were collected by matching several nationwide data sources. Addresses were obtained from the Ministry of Internal Affairs, membership of Medical aid, and the Korean National Health Insurance system. Younger twins were ascertained by matching individuals with the same birthday and surname, address code and householder. The first twin list was restricted to those born since 1979. New twins have since been added, but higher order multiples were excluded. Adult twins were ascertained by way of an algorithm developed, since twins are unlikely to share the same address. Birth report data were not used (Sung *et al* 2002). The register is 95% complete for twins born after 1975 and less representative for twins born prior to 1970. For twins born prior to 1950, data collection was restricted to those twins whose co-twin was still alive in 1990. So far, research has included ventricular septal defects in young twins, and stomach and colo-rectal cancers in adult twins (Sung *et al* 2002).

The Seoul Twin Family study was established in 2001 to examine cognitive abilities and other psychological traits. It recruited twins from private and public schools in Seoul in 2001, most of who were aged between 6 and 18 years. Data were collected on the twins, their non-twin siblings, and parents, and were re-assessed every 2 years. A total of 4,615 twin pairs have been ascertained. It is planned to examine medical records of the twins during the pre-natal period to

study the effect of the intrauterine environment on individual differences, and to attempt to identify the genes involved in specific cognitive abilities by undertaking molecular genetic studies (Hur 2002).

2.11 The Netherlands

The Netherlands Twin Register was established in 1987 and currently comprises two groups of twins and twin families. Young twins and multiples are registered by their parents a few weeks after birth. About 50% of all newborn twins are currently registered. In 1990-91, adolescent and adult twins were recruited through City Councils, who supplied lists of names and addresses of twins aged between 13 and 20. Older twins have since been recruited through City Councils, and advertisements in the Register's annual newsletter. Over 60,000 twins aged from birth to over 70 years of age are currently registered, together with over 3,000 of their siblings (Boomsma *et al* 2002b).

Data from twin families, comprising twins, their siblings and parents, have been collected to study health, fertility, lifestyle, depression, addiction, personality and psychopathology, religion, SES, and education. Over 6,500 adolescent and adult twins and their families have participated since 1991 (Boomsma *et al* 2002b).

Parents of twins registered at birth received a questionnaire shortly after registration, and then again when their twins were 2, 3, 5, 7, 10 and 12 years of age. Behaviour is assessed by asking the twins' teachers to complete questionnaires when the twins are 7, 10 and 12 years old. Research is conducted into many health, behavioural and emotional problems, including asthma and atopy, epilepsy, diabetes, hearing and visual problems and metabolic diseases (Boomsma *et al* 2002b). Aspects of aggressive and oppositional behaviour (van Beijsterveldt *et al* 2005), as well as attention problems (Derks *et al* 2006), have also been studied.

2.12 Norway

The Norwegian twin register covers twins born in Norway from 1895 onwards (Bergem 2002). Apart from the births from 1960 to 1967, which are missing, the register is thought to be almost complete. Information is gathered on both same-sex and opposite sex twins, except for the birth years between 1946 and 1960, where only same-sex twins are registered. There is no central twin register in Norway. Instead, data has been collected by a number of population-based sub-registers which are based at separate institutions. Hence the Norwegian twin register consists of three parts, all of whom have been collected separately for different research purposes.

- Part I: all twins born 1895-1945. Data collection commenced in 1963 when a register covering births between 1901 and 1930 was compiled using information from the Central Bureau of Statistics (Kringlen 1968). It was later extended to include births between 1895 and 1945 (Kringlen 1978) and consists of approximately 37,000 pairs of twins, both same sex and opposite sex. Zygosity is mainly unknown.
- Part II: Overlaps with part I, but contains additional information on same sexed twins born between 1915 and 1960. It has its origin from the Institute of Medical Genetics at the University of Oslo, and is called the Norwegian Twin Panel. It is population-based, except for birth years between 1915 and 1960, when only same-sexed twins were registered.
- Part III: comprises all twins born 1967-1979. This data is derived from the Medical Birth Registry of Norway. It is maintained by the Norwegian Institute of Public Health for research into the genetic epidemiology of mental and physical health (Harris *et al* 2002).

Most research has been conducted on the older cohorts (Parts I and II), and include psychoses (Kringlen 1968), coronary heart disease (Kringlen 1978), epilepsy (Corey *et al* 1998), psychiatric illness (Kringlen *et al* 2001, Torgersen *et al* 2001) and anxiety disorders (Tambs *et al* 1995, 1997).

The younger cohort, that is Part III, has been used to study the genetic epidemiology of a number of conditions, including asthma and related phenotypes (Harris *et al* 1997), personality and mental health (Tambs *et al*

1995), epilepsy (Kjeldsen *et al* 2005) and inflammatory bowel disease (Helgeland *et al* 1992).

The Norwegian twin register was one of the key players in the newly funded GenomEUtwin project.

2.13 Singapore

Using data from the Singapore National Registry of Births and Deaths (SNRBD), twins were identified by including pairs of individuals who were born within two days of each other, and with the same mother. Every person recorded on the SNRBD has a unique national registration identity card number. A total of 5,935 twin births and 212 triplet births were recorded during the 15-year period 1986 to 2001 (Chia *et al* 2004). Of the twins, 4,724 (80%) were of the same gender. Thus there was an estimated 2,422 DZ and 3,513 MZ twins, with a DZ/MZ ratio 0.69. The Singapore twin register has been established, but as yet research using register members has not been undertaken.

2.14 Sri Lanka

The Sri Lankan twin register is the first registry of twins to be established in the developing world. It comprises several twin cohorts, recruited by different means. A volunteer register was initiated in 1996 and comprises 4,602 volunteer pairs recruited through media advertising campaigns (Sumathipala *et al* 2001). Other cohorts were established through the Sri Lankan birth register, and include a nationwide population-based cohort of twins born between 1992 and 1997 (de Silva *et al* 2001), and a prospective birth cohort of twins born since 2002. Community surveys have been carried out to look at the feasibility of establishing a population-based register including older twins. Plans are now underway to recruit a larger sample of twins to study mental disorders and osteoporosis (Sumathipala *et al* 2002).

2.15 Sweden

The Swedish Twin Registry, the largest twin registry in the world, was established in 1961, initially to examine the roles of smoking and alcohol consumption on cancer and cardio-vascular diseases (Medlund *et al* 1977). It consists of more than 127,000 twin pairs born in Sweden since 1886, of which 61,000 pairs where both are still alive. Data are routinely linked to the address, cancer and death registers. There are three separate age cohorts of twins recruited by different methods:

1. 1886-1925 births. Data collection started in 1959, when all parishes in Sweden were contacted to obtain data on twins born between 1886 and 1925. Potential twin pairs were manually followed-up to establish their vital status in 1959. In 1960-1961, all same sexed twins (10,503 pairs), who were both alive and living in Sweden, were sent questionnaires to complete at home. Demographic, medical and lifestyle information was collected. The main focus of research has been cardiovascular and respiratory disease, general health, and psychosocial conditions (Pedersen *et al* 2002).
2. 1926-1958 births. Commencing in 1970, twin pairs born between 1926 and 1967 were ascertained through national birth registrations. A cohort of 50,000 twin births was established (Medlund *et al* 1977). Same sexed pairs born between 1926 and 1958 were contacted in 1972-1973, and responses received from 36,000 individuals, including 14,000 twin pairs.
3. 1959-1990 births. Twins born between 1959 and 1968 had already been identified when the previous cohort was established, but they had not been contacted. In 1993, record linkage to the Medical Birth Registry identified twins born between 1960 and 1990. Even though this cohort has been established, only the parents of twins born in 1985 and 1986 have been contacted (Lichtenstein *et al* 2002). Hence, other than for these two birth years, no zygosity information is available.

Many research projects are on-going and include those examining behavioural problems (Eley *et al* 2003), ageing (Pedersen *et al* 1991), dementia (Pedersen *et al* 2004), allergy (Lichtenstein & Svartengren 1997), cancer (Lichtenstein *et al* 2000), gastrointestinal disease (Cameron *et al* 2002), and cardiovascular disease (Iliadou 2003).

2.16 United Kingdom

Although there is no national register, several regional registers have been established in the UK. A description of three of the main registers is given.

2.16.1 St Thomas' adult UK twin registry

This registry was established in 1992 and contains data on nearly 5,000 twin pairs from all over the UK (Spector & MacGregor 2002). It started as an adult volunteer register and participants were recruited by successive media campaigns without the mention of specific diseases. The registry contains over 9,000 twins, with an average age of 45 years. The twins are predominantly female since the initial focus of research was on osteoarthritis which is more common in females than males. Research has also been conducted into cardiovascular (Snieder *et al* 2000), dermatological (Bataille *et al* 2002)and ophthalmological conditions (Hammond *et al* 2000). Linkage and association studies have been undertaken on about 3,000 DZ twins, who have also had whole genome scans performed (Spector & MacGregor 2002). Other diseases examined include osteoporosis, osteoarthritis (Antoniades *et al* 2000, Spector & MacGregor 2004), and skin diseases (Bataille *et al* 2002).

2.16.2 The twins early development study (TEDS)

TEDS was established to study communication disorders, mild mental impairment and behaviour problems (Trouton *et al* 2002). It is one of the largest twin studies to examine language and cognitive development in children. Twins born in England and Wales during between 1994 and 1996 inclusive were identified through birth records. Parents were contacted and asked to participate in the study. Over 16,000 parents agreed and were sent the survey material to complete at around the time their twins were 18 months old. Children were assessed again at 2, 3, 4 and 7 years old (Trouton *et al* 2002). The results of collaborative work has meant that several spin-off studies have been conducted on these children, and include specific language disorders (Bishop *et al* 2003, Dale *et al* 2003), eating behaviour for children at genetic risk of obesity (Wardle *et al* 2001), and hyperactivity (Price *et al* 2001). TEDS has also collected screening data to enable conditions such as ear infections (Rovers *et al*

2002), bladder control (Butler *et al* 2001), and asthma and allergy (Koeppen-Schomerus *et al* 2001), to be studied.

2.16.3 Multiple pregnancy register

The Multiple Pregnancy Register was set up in the north of England in 1998. The register was notified of the twins as they were detected during pregnancy. The diagnosis was made by consultant obstetricians, paediatricians, ultra-sonographers and pathologists. The register covered areas in the northern region of the UK, including Tyne & Wear, Cleveland, Cumbria, Durham and Northumberland. Nine hundred and twenty-six pregnancies were recorded during 1998-1999, with chorionicity established in 83% of twin pregnancies. The aim of this registry was to examine mortality and morbidity in multiples (Glinianaia *et al* 2002b).

2.17 United States of America

Although numerous states in America have established twin registers, there is not one register that covers the whole of the USA. Examples of some of these State registers are:

2.17.1 The Mid-Atlantic twin registry

The mid-Atlantic twin registry (MATR) commenced in 1997 when the Virginia and North Carolina twin registers were merged (Anderson *et al* 2002). In 1998, it expanded to include data from South Carolina. This twin register has been drawn from all twins born in the three states between 1913 and 2000. Data were obtained from birth and school records, and the register comprises more than 51,000 twins, including 23,000 complete pairs. Most of the twins (67%) are over the age of 18 years, with a mean age of 35 years (Anderson *et al* 2002). Over the years, twins and their families have participated in a number of health-related studies, which include cardiovascular disease (van den Bree *et al* 1996), depression and anxiety (Kendler & Prescott 1999), behavioural outcomes (Eaves *et al* 1997), obesity (Maes *et al* 1997) and epilepsy (Corey *et al* 1998).

2.17.2 The Minnesota Twin Registry

Established in 1983, the Minnesota twin registry comprises 901 twin pairs born between 1904 and 1934, 4,307 twin pairs born in Minnesota between 1936 and 1955, and 391 male pairs born from 1961 to 1964 (Iacono & McGue 2002). Data were obtained from birth records, and infant deaths, up to the age of 6 months, ascertained by linkage to the State's death index. Twin pairs were included if they were both known to have survived to the age of 6 months. The major initial research focus of the Registry was to examine human differences as assessed by self-report (Iacono & McGue 2002). Since then, subsets of the registry have completed various questionnaires looking at many different outcomes. The older cohort, that is, 1904-1934 births, has been used to study the effect of ageing on cognitive function (Finkel & McGue 1994), and research on the younger cohort, born between 1961 and 1964, has focused on illegal and antisocial activities (Krueger *et al* 2001).

2.17.3 Minnesota twin family study (MTFS)

The Minnesota twin family study was established to examine substance abuse and related problems (Iacono & McGue 2002). It is a longitudinal study of same-sexed twins aged 11 and 17 years, and their families (Iacono *et al* 1999). A total of 4,223 twins pairs born between 1971 and 1990 belong to the register, with 1,485 families having completed the assessment by 2002. It is expected that about 2,000 families will have completed the assessment by the end of the study. The study has allowed a number of other health conditions to be examined, and include depression and conduct disorder (Marmorstein & Iacono 2001), eating disorders (von Ranson *et al* 2002), ADHD (Sherman *et al* 1997) and aggressive behaviours (Elkins *et al* 1997).

2.17.4 NAS-NRC twin registry

The National Academy of Sciences – National Research Council (NAS-NRC) twin registry consists of 15,924 white male twin pairs born between 1917 and 1927, both of whom served in the armed services during World War II (Page 2002). It is one of the oldest twin registers in the USA, and was initiated in 1955. Data were collected from birth records, and matched to Department of Veteran Affairs records to determine war veteran status (Jablon *et al* 1967).

Over the years a large number research programmes have been carried out, including those looking at the genetic influences on smoking behaviour (Carmelli *et al* 1992), suicide (Roy *et al* 1991), and Alzheimer's disease (Meyer & Breitner 1998).

The Current Era Twins Registry (CETR) comprises 440 twin pairs who both served in the Army in 1986 or later, and 577 pairs where only one twin agreed to participate in the registry. It has registered both male and female twins. It is planned to extend this registry to include members of other military services (Page 2002).

2.17.5 Vietnam Era twin (VET) registry

The Vietnam Era Twin (VET) registry, which is a US Department of Veterans Affairs resource, comprises 7,369 male twin pairs who both served in the military services during the Vietnam conflict of 1965-1975 (Goldberg *et al* 2002). It was initially established to look at the long-term health effects of Vietnam military service, but has since evolved into a genetic epidemiology resource to examine various mental and physical health conditions (Henderson *et al* 1990). It is one of the largest twin registers in the US.

Twins were ascertained using record linkage of records held by the US Department of Defense, and were supplemented with Veterans Affairs data. Of the 15,711 potential twin pairs identified, 7,369 were later confirmed as meeting the criteria for inclusion into the register (Eisen *et al* 1987). The first health survey using the register was carried out in 1987 (Eisen *et al* 1991). Since then, various studies have been conducted either on the whole register or on selected subsets. Some examples of the outcomes examined are: cardiovascular disease, drug abuse (Tsuang *et al* 1996), post-traumatic stress disorder (Goldberg *et al* 1990) and sleep problems (Fabsitz *et al* 1997).

2.17.6 Wisconsin twin project

Established in 1994, the Wisconsin twin panel (WTP) recruited twins within six months of their birth to study the development of childhood mood and behavioural disorders (van Hulle *et al* 2002). Twins born between 1989 and

1994 were recruited in the first instance, with other birth years being added sequentially. Overall response is between 60% and 80%, with most (80-85%) responding favourably. The WTP aims to lay the foundation for the study of twins throughout life to examine the onset of various disorders (van Hulle *et al* 2002).

2.18 GenomEUtwin

This is an initiative, funded by the European Community, which uses the pooled resources of several European twin registers to conduct genomic research. It commenced in September 2002 and is expected to run for 4 years (Peltonen 2003). The cohorts used consist of the Danish, Finnish, Italian, Dutch, Norwegian and Swedish twin registers, together with the MORGAM population cohort (Evans *et al* 2005). Two further twin registers, the St Thomas' UK adult twin Registry, with over 10,000 pairs registered, and the ATR, with over 30,000 pairs registered, have joined GenomEUtwin (Peltonen 2003). The MORGAM population cohort (Monica, Risk, Genetics, Archiving and Monograph) is a continuation of the WHO-funded MONICA collaboration which has been successfully conducting multi-centre research into cardiovascular diseases over many years (Evans *et al* 2005). MONICA involved over 20 centres throughout the world to examine the genetic and environmental predictors of chronic diseases, particularly coronary heart disease and stroke. The participating twin registers have collected data on over 600,000 twin pairs, with many thousands of DNA samples having already been collected and stored. MORGAM is a prospective case-cohort study targeting cardiovascular traits. It includes approximately 6000 individuals drawn from population-based cohorts consisting of in excess of 80,000 individuals who have donated DNA samples. The joining of MORGAM data with twin data is ideal for studying the genetics of complex, multifactorial diseases.

The GenomEUtwin project aims to develop new molecular and statistical techniques to analyze twin and other cohort data to characterise the genetic, environmental and life-style factors in a number of worldwide, major health problems, such as coronary heart disease (Evans *et al* 2003), stroke (Gaist *et al*

2003), and migraine (Mulder *et al* 2003). The analysis will be based on linkage and association studies.

2.19 Summary

Numerous twin registers have been established around the world, using a variety of methods, and covering twins of all ages. The Scandinavian Twin Registers are the oldest and largest of any in the world. There is great potential to develop population-based Twin Registers in Asia, and some countries have already assembled a large collection of data. These newer Registers offer the opportunity to study the effects that genes and the environment on traits in non-Caucasian populations.

2.20 The Australian Twin Registry (ATR)

The Australian Twin Registry (ATR), established in the late 1970s, contains information on over 30,000 twin pairs (Hopper 2002a). It is a volunteer-based register of twins of all ages across all Australian states. Volunteers are unselected for their medical or health history. The ATR does not conduct research in its own right, but is used by researchers across Australia as a sampling frame for research into a wide range of health-related conditions. Over the years, over 200 studies using ATR data have been undertaken, which have resulted in comprehensive longitudinal data on around 10,000 twin pairs, and DNA samples on more than 6,000 twins being collected (Hopper 2002a). Examples of health-issues studied using ATR data include asthma (Duffy *et al* 1990), breast cancer (Boyd *et al* 2002), bone density (Hopper *et al* 1998), rheumatoid arthritis (Bellamy *et al* 1992), dental disorders (Townsend *et al* 1998), baldness (Ellis *et al* 1998), attention deficit hyperactivity disorder (Levy *et al* 1997), cardiovascular diseases (Harrap *et al* 2000), and melanoma (Zhu *et al* 1999). However, the voluntary nature of the Register could lead to problems of non-generalisability for some studies, because the expression of many of the conditions of interest to public health research are strongly influenced by factors such as social class, which also modulate response to voluntary data collection (Heath *et al* 2001). It is well-known that female MZ twins predominate volunteer registers (Lykken *et al* 1987). There is therefore great

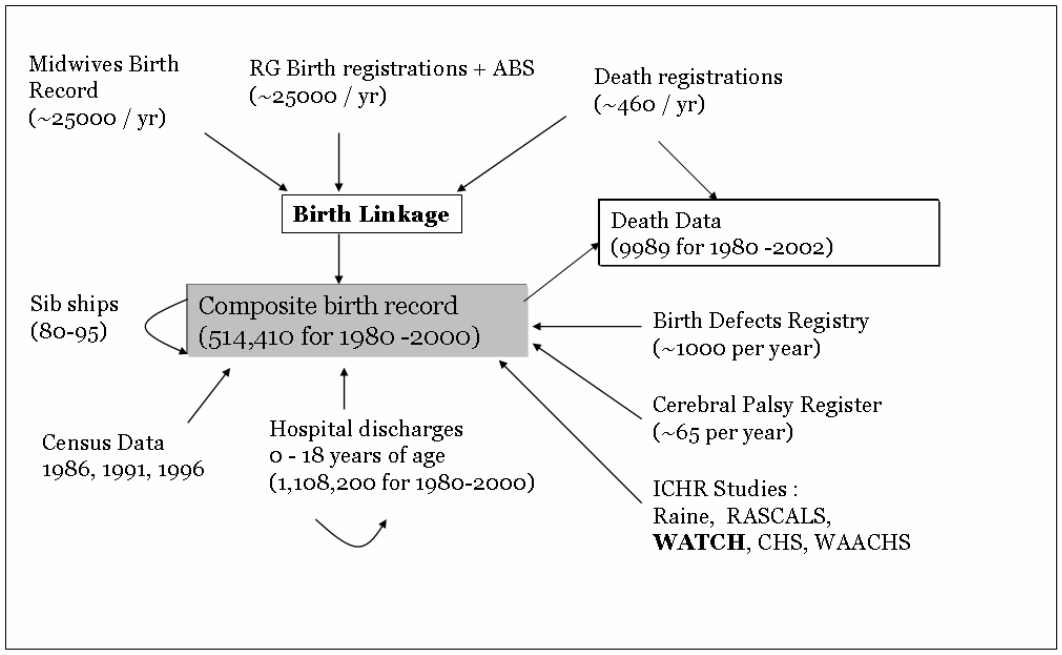
scientific merit in complementing a national volunteer register with a population-based register.

2.21 The Western Australian Twin Register (WATR)

Methods used to establish the Western Australian Twin Register (WATR) and conduct the Western Australian Twin Child Health (WATCH) study are described in more detail in the next chapter. A brief description of the WATR is given below.

The WATR is a population-based register of all multiple births to have occurred in Western Australia (WA) since 1980, and was established in 1997. Twins and other multiple births were identified entirely from the Maternal and Child Health Research database (MCHRDB), which was established and is maintained by the Telethon Institute for Child Health research (TICHR) (Stanley *et al* 1994). All births to have occurred in WA since 1980 are registered with the WA Registrar General's Office. To be registered as a viable birth, the child must weigh at least 400g, or be of 20 or more weeks gestation. These birth records are then merged with the WA Midwives' Notification system to form a composite record of every birth. Records on the MCHRDB are linked to the WA registration of deaths and hospital discharge records (Fig 2-1), and are updated when data become available.

Figure 2-1:
Structure and use of the Maternal and Child Health Research Database (MCHRDB)



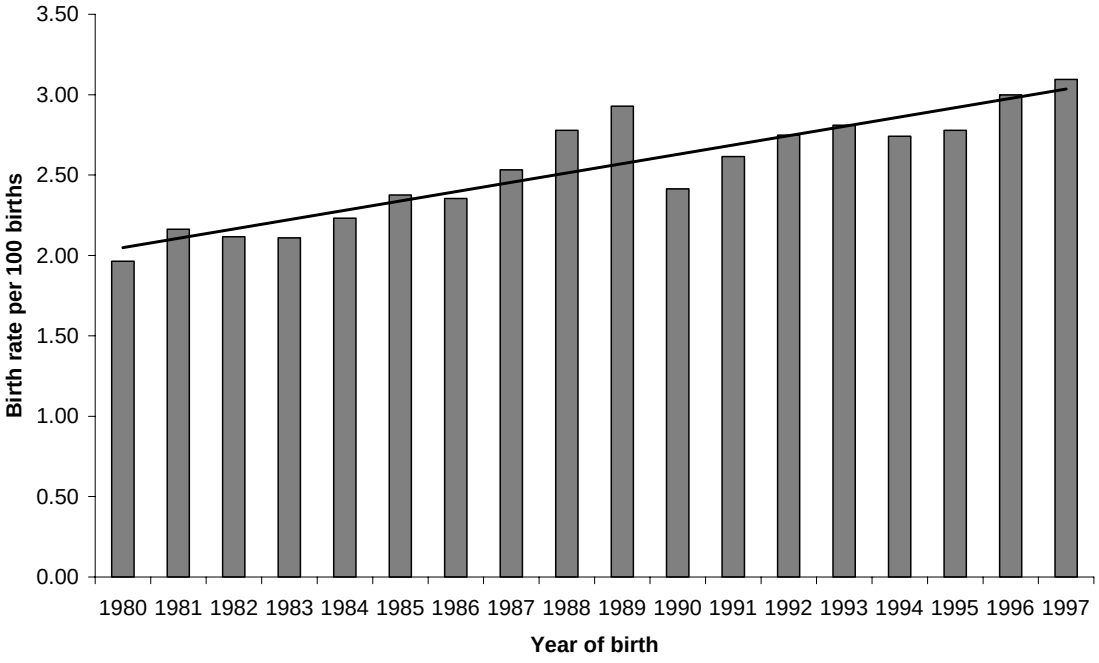
As well as pre-natal information about the mother during the index pregnancy, the MCHRDB contains data on labour and birth, some post-natal complications, all hospital admissions during childhood, and the status of the child (i.e. stillborn or live born, and whether they have died since birth). Several other registers, including the birth defects and cerebral palsy registers, have arisen from the MCHRDB.

Multiples born in WA from 1980 onwards were identified using a code for plurality, and verified using a computerised record linkage of sibships of the MCHRDB (Croft *et al* 2002). Initially, the WA Twin Register consisted of all multiple births in WA from 1980 to 1992 inclusive, using a grant from the WA Health Promotion Foundation (Healthway). The main purpose for establishing the Register was to invite families to participate in the WA Twin Child Health (WATCH) study which examined the roles that genes and the environment play in the link between childhood asthma and atopy, and exposure to environmental tobacco smoke. The Register has since been extended to include 1993-1997 births, using part of a grant from the National Health and Medical Research Council (NHMRC) for the "WATCH for Asthma" (WFA) study. This study collected detailed clinical asthma phenotype data on twins born between

1990 and 1995, and their families, to investigate and describe the familial aggregation of childhood asthma and the intermediate clinical phenotypes. No further description of this study will be given here as it is not a focus of this thesis.

Currently, the WATR houses data on all multiple birth children born in WA between 1980 and 1997 inclusive. It comprises 5,340 twin pairs, 156 sets of triplets and 10 sets of quadruplets and quintuplets, a total of 11,189 children. Just over 51% of multiple birth children were male, the same percentage being recorded for twins and higher order multiples. This percentage is consistent with that for all births in WA (Gee & O'Neill 2001). As a percentage of total births in WA, multiple births rose from just under 2% in 1980 to 3.1% in 1997 (Figure 2-2).

Figure 2-2:
WA Multiple birth rate, 1980 to 1997



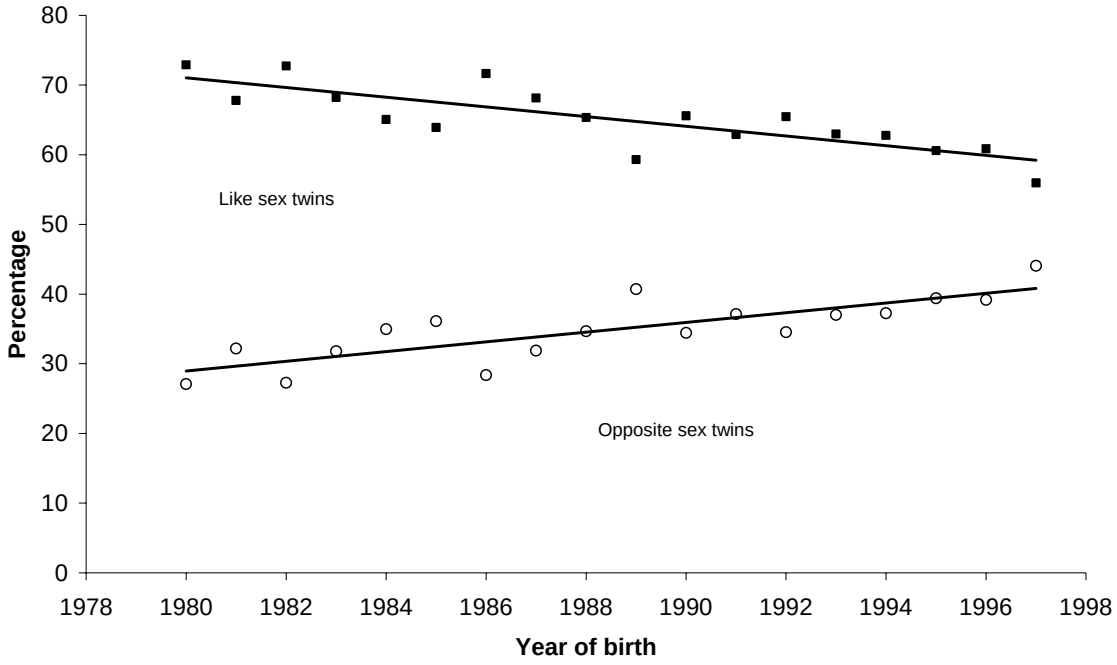
The MCHRDB records details of the child's gender, but not on zygosity of multiple births. Hence the only comparison possible is between same sexed and opposite-sexed twins (Table 2.1). Same-sex twin pairs comprised 64.5% of all twins born in WA from 1980-1997, and the percentage of same sex twins decreased steadily across the time period, from 73% in 1980 to 56% in 1997 (Figure 2-3).

Table 2.1:
Numbers and percentage of WA multiple births by gender

YEAR OF BIRTH	MM PAIRS N=1678	FF PAIRS N=1766	MF/FM PAIRS N=1896
	N (%)	N (%)	N (%)
1980	69 (34.0)	79 (38.9)	55 (27.1)
1981	81 (34.3)	79 (33.5)	76 (32.2)
1982	88 (38.1)	80 (34.6)	63 (27.3)
1983	87 (36.9)	74 (31.4)	75 (31.8)
1984	79 (32.1)	81 (32.9)	86 (35.0)
1985	68 (25.6)	102 (38.3)	96 (36.1)
1986	92 (34.3)	100 (37.3)	76 (28.4)
1987	102 (34.6)	99 (33.6)	94 (31.8)
1988	113 (34.7)	100 (30.6)	113 (34.7)
1989	100 (29.5)	101 (29.8)	138 (40.7)
1990	108 (35.1)	94 (30.5)	106 (34.4)
1991	93 (29.2)	107 (33.7)	118 (37.1)
1992	106 (32.1)	110 (33.3)	114 (34.6)
1993	97 (29.0)	114 (34.0)	124 (37.0)
1994	104 (31.2)	105 (31.5)	124 (37.2)
1995	96 (28.2)	110 (32.4)	134 (39.4)
1996	98 (27.2)	121 (33.6)	141 (39.2)
1997	97 (26.2)	110 (29.7)	163 (44.1)

M=male; F=famale

Figure 2-3:
Percentage of like and opposite sex twins in WA by year of birth



Six hundred and thirty six multiple birth children from 506 families died during the perinatal period, 578 twins and 58 higher order multiples (HOMs) (Table 2.2). The perinatal death rate was 54.1 per 1,000 births for twins, and 113.9 per 1,000 births for higher order multiples (HOMs) (Table 2.3). This compares with a perinatal death rate for all WA births of 13.1 per 1,000 in 1984 and 9.0 per 1,000 in 1998 (Gee & O'Neill 2002).

Table 2.2:
WA multiple birth children

YEAR OF BIRTH	MULTIPLE BIRTH CHILDREN	PERINATAL DEATHS	TWIN PAIRS	PERINATAL DEATHS
1980	409	30	203	29
1981	481	29	236	28
1982	474	23	231	20
1983	487	32	236	31
1984	513	26	246	23
1985	556	43	266	43
1986	564	41	268	39
1987	614	41	295	40
1988	700	48	326	45
1989	748	47	339	41
1990	628	32	308	31
1991	654	38	318	37
1992	696	39	330	36
1993	712	34	335	24
1994	697	36	333	34
1995	707	30	340	26
1996	767	36	360	26
1997	782	31	370	25
Total	**11,189**	**636**	**5,340**	**578**

The perinatal death (PND) rate was higher for male twins than female twins, and for second born twins compared with first born. However, the PND rate in male twins was lower if their co-twin was female, when compared with those whose co-twin was male (62/1000 vs. 46/1000). Twins showing the lowest PND rates were opposite sex pairs (Table 2.4).

Table 2.3:
Perinatal deaths among WA multiple births

OUTCOME	TWINS	TRIPLETS+
Total births	10,680	509
Perinatal deaths (PNDs)	578	58
PND rate/1,000 births	54.1	113.9
Stillbirths (SBs)	280	26
SB rate per 1,000 births	26.2	51.1
Live births	10,400	483
Neonatal deaths (NNDs)	298	32
NND rate per 1,000 live births	28.7	66.3

Table 2.4:
Perinatal death rates in WA twins by gender, type and birth order

	PERINATAL DEATH RATE PER 1,000 BIRTHS	SB RATE PER 1,000 BIRTHS	NND RATE PER 1,000 LIVE BIRTHS
All twins	54.1	26.2	28.6
Male twins	*58.3*	*29.1*	*30.1*
Female twins	*49.8*	*23.2*	*27.1*
Same sex pairs	57.8	28.8	29.9
MM twin pairs	*62.0*	*29.8*	*33.2*
FF twin pairs	*53.8*	*27.8*	*26.8*
Opposite sex pairs	50.1	21.6	26.4
MF pairs	*46.0*	*20.7*	*25.9*
FM pairs	*49.0*	*22.6*	*27.0*
First born twins	49.6	24.5	25.7
Male 1st born twins	53.3	27.2	26.9
Female 1st born twins	45.7	21.7	24.5
Second born twins	58.6	27.9	31.6
Male 2nd born twins	63.3	30.9	33.4
Female 2nd born twins	53.8	24.8	29.7

2.22 Summary of the WATR

The WATR consists of all multiple births to have occurred in WA from 1980 to 1997. Examination of data from the WA Department of Health showed that the WATR is complete for these years (Gee 1992, Gee & O'Neill 2001). The rate of multiple births increased over the time period, due mainly to the advent of assisted reproductive technology. Although 51% of twins were male, the percentage of same-sex male twins was less than expected (31.4% vs. 33.3%, respectively). The stillbirth, neonatal death and perinatal death rates in twins were all higher than the corresponding rates for all WA births (Gee & O'Neill 2002), and were higher in male twins than female twins.

2.23 Conclusions

Only a few truly national population-based twin registers have been formed using the systematic collection, review and validation of birth records. The most notable are those that have been established in the Scandinavian countries (Cederlof & Lorich 1978, Hauge *et al* 1968, Kaprio *et al* 1978, Kringlen 1978). Although these registers cover a wide range of birth years, data is not complete for some years. For example, the Danish twin register only collected data on same sex twins born between 1911 and 1930 (Hauge *et al* 1968), and twins born between 1960 and 1967 were not included in the Norwegian register (Kringlen 1978). Others have been established from the opportunistic collection of twin data for studying specific health outcomes, and rely on the registration of volunteers (Boomsma *et al* 2002b, Clifford & Hopper 1986, Spector & MacGregor 2002, Spinath *et al* 2002). There is no systematic collection of twin data in the USA and the UK. Registers have been formed in many States of the USA, using a variety of sources and criteria for identifying twins (Anderson *et al* 2002, Baker *et al* 2002, Krueger & Johnson 2002, Lykken *et al* 1990, van Hulle *et al* 2002). Two studies recruited twins from Armed Forces records and examined various sequalae of the involvement in either World War II (Jablon *et al* 1967) or the Vietnam War (Henderson *et al* 1990). In the UK, the main register of twins is the one based at St Thomas' Hospital in London, which had its origins in a study of osteoarthritis by recruiting adult female same-sex twin pairs via media campaigns (Spector & MacGregor 2002).

Registers that have been formed using birth records rarely collect data on twins who were not born alive (Anderson *et al* 2002, Krueger & Johnson 2002). The Danish twin register has only routinely collected data on twins who were stillborn on births since 1983 (Skytthe *et al* 2002).

The WATR is the only population-based register of twins in Australia, and one of only a few in the world to be formed from the routine collection of birth registration data. It was established in 1997 specifically to conduct the WATCH study which aimed to examine the genetic and environmental components of childhood asthma and allergy. The unique nature of the MCHRDB, which used to identify multiple births in WA, means that there are detailed data on pregnancy, birth and the neonatal period for all births, including those twins who were stillborn or who have died during the perinatal period or later in childhood. These features enable direct comparison of participants and non-participants of any study using register data to be made. In addition, periodical and systematic linkage to the hospital morbidity data allows for any health condition which requires hospitalisation to be examined in detail. As these children progress into adolescence and adulthood, it will also be possible to examine whether there are any childhood antecedents to chronic adult diseases.

CHAPTER 3
THE WA TWIN REGISTER

3.1 Preface

This chapter is presented in its entirety as the peer-reviewed paper published in the journal Twin Research, viz:

"Hansen J, Alessandri PT, Croft ML, Burton PR, de Klerk NH (2004). The Western Australian Register of childhood multiples: effects of questionnaire design and follow-up protocol on response rates and representativeness. *Twin Res*; 7: 149-161".

This paper describes the methods used to trace and contact families belonging to a population-based Register of twins and higher order multiples born in Western Australia (WA) between 1980 and 1995. Then the effects that different questionnaire designs and follow-up protocols had on response rates are assessed; and factors, if any, that influenced response are examined.

3.2 Background

Studies using twins and higher order multiple births are important for examining the relative contributions that genes and the environment make to a wide range of health-related conditions. This can be achieved either by establishing twin registers or using defined cohorts of twins to study particular outcomes. Many twin registers have now been established worldwide (Boomsma *et al* 2002a). They may collect information on twins of all ages (Skytthe *et al* 2002), restrict their data to specific age groups (Kaprio & Koskenvuo 2002, Spector & MacGregor 2002, Spinath *et al* 2002, Trouton *et al* 2002), or be established to study defined populations (Goldberg *et al* 2002, Page 2002). In Australia, the Australian Twin Registry (ATR), established in the late 1970s, contains information on over 30,000 twin pairs (Hopper 2002a). It is a volunteer-based register of twins of all ages across all Australian states, and has been used as a sampling frame for research into a wide range of health-related conditions, for example, asthma (Duffy *et al* 1990), breast cancer (Boyd *et al* 2002), bone density (Hopper *et al* 1998), rheumatoid arthritis (Bellamy *et al*

1992), dental disorders (Townsend *et al* 1998), baldness (Ellis *et al* 1998), attention deficit hyperactivity disorder (Levy *et al* 1997), cardiovascular diseases (Harrap *et al* 2000), and melanoma (Zhu *et al* 1999). However, the voluntary nature of the Register could lead to problems of non-generalisability for some studies, because the expression of many of the conditions of interest to public health research are strongly influenced by factors such as social class, which also modulate response to voluntary data collection (Heath *et al* 2001).

Researchers have accessed many data sources when establishing twin registers, including birth records (Skytthe *et al* 2002, Sumathipala *et al* 2002), notification of multiple pregnancies from health professionals (Derom *et al* 2002, Glinianaia *et al* 2002b), school records (Anderson *et al* 2002), military service records (Goldberg *et al* 2002, Page 2002) and national population or health insurance registers (Sung *et al* 2002, Yang *et al* 2002). Most volunteer-based registers rely on advertising to recruit participants, and this is achieved using various media outlets, referrals by health professionals, and contact through Twins Clubs, Parishes, Schools and Hospitals (Hopper 2002a, Jang *et al* 2002, Spector & MacGregor 2002, Spinath *et al* 2002, Strassberg *et al* 2002).

Studies in which participants are asked to complete questionnaires at home are more economic than interview studies, but usually have lower response rates (Heath *et al* 2001). Cost is an important consideration when a large number of participants need to be recruited and it is therefore very important to maximise response rates. Several strategies have been identified that appear to maximise response to questionnaire surveys (Edwards *et al* 2002). These include offering monetary incentives (Collins *et al* 2000), using coloured ink, sending letters by recorded delivery, using stamps on return envelopes instead of reply-paid (Duffy & Martin 2001), using a pre-contact alerting potential participants of receiving a questionnaire by mail, making questions “more interesting”, the study having endorsement from a University, and personal follow-up contact (Spry *et al* 1989). In recent times, researchers have examined the feasibility of using the Internet to recruit study participants, and assessing any differences in the characteristics of responders (Mavis & Brocato 1998). However, data as to which methods result in the highest response rates are inconsistent. The decision on which methods to use are usually situation-specific and are largely

dependent on the nature of the study being undertaken (Larroque *et al* 1999), and factors such as cost, timeliness and convenience of data collection and study power (Morris *et al* 2001). It is also important to be able to compare the characteristics of responders and non-responders so that any results can be generalized to the wider population. Several factors have consistently been shown to affect response. These include age, gender, educational levels, employment status and urban residence (Heath *et al* 2001, Lamers 1997), but also important seems to be the nature of the originating institution, the use of personalised letters and providing feedback to participants (Morris *et al* 2001).

A systematic review of published papers examined a number of methods thought to influence response to mailed questionnaires (Edwards *et al* 2002). However, of the 292 trials reviewed, only 32% were from medical, epidemiological or health-related journals. Of these, it was not clear how many related to contacting doctors and other health professionals, or members of the general population regarding their experiences. Age of study participants was also not known. The combination of various strategies was not considered.

3.3 Establishing the WA Twin Register

The Maternal and Child Health Research Database (MCHRDB) is maintained by the Telethon Institute for Child Health Research and comprises records of all births in WA from 1980 onwards (Stanley *et al* 1994). Data are derived from the Birth Registration records and the Midwives' Notification of Birth record, and combined to form a composite record for each birth. The MCHRDB is updated when data become available. Records are then linked to the WA registration of deaths and hospital discharge records. As well as pre-natal information about the mother during the index pregnancy, the MCHRDB contains data on labour and birth, some post-natal complications and all hospital admissions during childhood.

Multiples born in WA from 1980 onwards were identified using a code for plurality, and verified using a computerised record linkage of sibships of the MCHRDB (Croft *et al* 2002). Initially, the WA Twin Register consisted of all multiple births in WA from 1980 to 1992 inclusive, using a grant from the WA

Health Promotion Foundation (Healthway). The main purpose for establishing the Register was to invite families to participate in the WA Twin Child Health (WATCH) study which examined the roles that genes and the environment play in the link between childhood asthma and atopy, and exposure to environmental tobacco smoke. The Register has since been extended to include 1993-1995 births, using part of a grant from the Australian National Health and Medical Research Council (NHMRC) for the "WATCH for Asthma" (WFA) study. This study aimed to collect detailed clinical asthma phenotype data on twins born between 1990 and 1995, and their families, and to investigate and describe the familial aggregation of childhood asthma and atopy. We aimed for approximately 60% response to give sufficient power. The different questionnaire designs and follow-up methods adopted for the two studies allowed us to examine the effect they had on response rates and response bias.

3.4 Questionnaire design

3.4.1 1980-1992 births

Three separate questionnaires were developed; one for the multiples, another for their parents, and a third for siblings of the multiples. Each questionnaire was lengthy (over 100 questions on each), and designed to collect detailed data on asthma and allergy phenotypes in multiple birth families (the focus of the WATCH study), as well as being used as screening tools for possible future studies of health conditions other than asthma and atopy. They also contained questions about known risk factors for asthma and allergies, especially active smoking and exposure to passive smoking, as well as a number of questions on general health conditions (including attention-deficit-hyperactivity disorder (ADHD), epilepsy, physical disabilities, birth defects); occurrence of accidents; use of medications, and use of health-related services. The children's questionnaires (for both the multiples and their siblings) contained additional questions relating to each pregnancy (including the use of assisted reproduction technology, complications of pregnancy, mother's smoking during pregnancy), birth (including mode of delivery, complications and treatment immediately after birth, birth weight, gestation, maternal post-natal depression), duration of breast feeding and age at introduction of other milk products; current weight and height. We also asked a series of questions dealing with the children's

education, and parental satisfaction with their children's educational experiences. The multiples' questionnaire also contained a series of standard questions used to determine zygosity (Cohen *et al* 1975). Questionnaires for the parents included additional questions on demographics (including education and employment); family history of multiples; household rules affecting family members' exposure to passive smoking; and the reproductive history of the mother. Parents were also asked to complete a table that allowed us to determine the family structure. In total, the questionnaire for the multiples contained 131 questions each, the parents, 107 questions each, and the siblings, 118 questions each. Families estimated that it took them up to 2 hours to complete their series of questionnaires.

Some families withdrew from the study after receiving the questionnaires because they felt they were too long, and they could not spare the time to complete them. In July 1998, therefore, a shorter version of the questionnaire was developed: one questionnaire per family, containing a total of 65 questions. Questions were included if they directly related to ascertaining the prevalence of asthma and atopy, exposure to active and passive smoking, or were needed to determine the family structure. This shortened questionnaire covered all family members and took an estimated 30 minutes to complete. Families contacted at follow up were offered this shorter questionnaire as an alternative to the set of long questionnaires.

3.4.2 1993-1995 births

One questionnaire was developed to cover all members of the family and was considerably shorter than that used for the 1980-1992 cohort. As it was used as a screening tool for the WFA study, the questions mainly related to the development of asthma and allergies, along with known risk factors (birth weight, gestation, passive and active smoking, duration of breast-feeding, introduction of other milk, etc.). Details of the family structure were also collected. The questionnaire contained a total of 39 questions and took an estimated 15 minutes to complete.

3.5 Tracing and follow up procedures

3.5.1 All multiple births

Permission to use WA Department of Health data was given by the Confidentiality of Health Information Committee (CHIC). The study complied with the national Privacy Principles, and was approved by the relevant Institutional Ethics Committee. It was a requirement of CHIC that families who had experienced the death of one or more of their multiples were not to be contacted. A separate study looking at grief and loss in multiple birth families has been conducted (Swanson *et al* 2002). Identifiers of eligible mothers (full name, including previous surnames, date of birth, and address at the time of the index birth) from the MCHRDB were linked to the WA Electoral Roll to determine current addresses.

3.5.2 1980-1992 births

We began the tracing of and mailing to families in 1997. Each mother was sent a letter and information sheet explaining the WATCH study, and asked to indicate her willingness to participate in the study by returning an "Expression of Interest" form in a pre-paid envelope. On this form, she was given the option of providing the name and address of the multiples' biological father if he no longer lived with them. Fathers so identified were then contacted separately. After one month, if there was no response from the mother to this first letter, the White Pages were searched, and if a telephone number was found, the families were contacted by telephone. If not, a follow-up letter was sent to the same address. One further attempt to contact any family who had not replied was made after another month, either by telephone or letter, after which time any family still not responding was considered a non- participant.

Families who agreed to participate in the study were then sent their questionnaires. If they had not returned them after 2 months, they were contacted by telephone (the preferred method) or by letter, and then at monthly intervals until either the questionnaires were returned, the family decided to withdraw from the study, or it became impractical to continue follow up. Extra copies of the questionnaires were sent to those families who had misplaced the originals. It was decided to discontinue follow-up on families who did not return

questionnaires despite receiving at least 3 sets of questionnaires, and after several attempts to contact them had failed.

Because more families could be contacted by telephone in the evenings than during the day, follow-up telephone calls were usually made between 6 pm and 8 pm Monday to Thursday inclusive. In addition, annual newsletters were sent to all families who had agreed to participate, and, in an attempt to elicit their response, to those families who had failed to respond.

3.5.3 1993-1995 births

Tracing commenced in 2000 and is continuing. Families whose index birth record could be successfully linked to the Electoral Roll were contacted. They were mailed an introductory letter and information leaflet explaining the study, together with a short questionnaire to complete at home and return in a pre-paid envelope. The mother was also asked to complete and return a Registration Form, confirming her informed consent to take part in the study. As before, she was also given the option of providing the contact details of the multiples' biological father if he no longer resided with them. Any father so identified was contacted separately. After 1 month, the names of non-responders were searched for on the White Pages. Families were contacted either by telephone or sent another questionnaire to complete. One more attempt to contact non-responders was made, after which time the family was considered a non-participant. Those contacted by telephone were given the option to complete the questionnaire by phone, which took approximately 15 minutes, return the questionnaire by mail, or receive another copy. Families who requested that another questionnaire be sent to them, were further contacted on a monthly basis until the questionnaire had been returned, or the family had decided to withdraw from the study.

3.6 Results

A total of 9,640 multiple birth children, born in WA between 1980 and 1995 inclusive, were identified, representing 2.5% of all births during that time. They comprised 4,610 sets of twins, 138 sets of triplets, quadruplets and quintuplets (Table 3.1). Twenty-five families had two sets of multiples during the time

period. The percentage of higher order multiples (triplets plus) was about 3% of all multiple births, ranging from a low in 1980, and peaking at 6.1% in 1989.

Table 3.1:
Number and Plurality of Multiple Births by Birth Year

YEAR OF BIRTH	TOTAL	SETS OF TWINS	SETS OF TRIPLETS+*
1980	204	203	<5*
1981	239	236	<5*
1982	235	231	<5*
1983	241	236	5
1984	253	246	7
1985	275	266	8
1986	277	268	9
1987	303	295	8
1988	342	326	16
1989	361	339	22
1990	312	308	<5*
1991	324	318	6
1992	342	330	12
1993	349	335	14
1994	343	333	10
1995	349	340	9
Total	4748	4610	138

* exact numbers not given to protect the privacy of families

Of the 9,640 multiple birth children, 650 (7%) of them were known to have died. Five hundred and sixty nine were either stillborn or died within the first four weeks of life, giving a perinatal death rate of 59.0 per 1,000 births (Table 3.2), which ranged from a low of 42.3 per 1,000 births in 1995, to 77.3 per 1,000 births in 1985. Higher order multiples (triplets, quadruplets and quintuplets) had a higher rate of both total childhood deaths and perinatal deaths when compared with twins (10.5% vs. 6.6% ($p=0.002$) for all deaths, and 10.0% vs. 5.7% ($p<0.001$) for perinatal deaths, respectively).

Table 3.2:
Deaths among multiple birth children

PLURALITY	CHILDREN	TOTAL DEATHS	DEATH RATE/1,000 BIRTHS	PERINATAL DEATHS (PND)	PND RATE/1,000 BIRTHS
Twins	9220	606	65.7	527	57.2
Triplets+	420	44	104.8	42	100.0
Total	9640	650	67.4	569	59.0

The average age of mothers at the time of the multiples' birth was 28.6 years (range 15-44 years). Mothers who were under 20 years of age at the time of the multiples' birth were more likely to have experienced the loss of one of their multiples compared with other mothers (13.6% vs. 6.4%, $p<0.0001$) for all childhood deaths; 10.8% vs. 5.7% ($p<0.001$) for perinatal deaths) (Table 3.3).

Table 3.3:
Deaths of multiple birth children by mother's age at birth of multiples

MOTHER'S AGE (YRS)	TOTAL BIRTHS	DEATHS	PERCENTAGE	PERINATAL DEATHS	PERCENTAGE
Under 20	434	59	13.6	47	10.8
20-29	5476	364	6.6	331	6.0
30-39	3633	219	6.0	186	5.1
40+	97	8	8.2	5	5.2
Total	9640	650	6.7	569	5.9

Over 90% of families were traced and invited to join the WATCH study. Overall, completed questionnaires have been received from 57% of families. A greater proportion of families whose multiples were born between 1993 and 1995 returned completed questionnaires compared with those born between 1980 and 1992 (62% vs.55%, $p<0.001$). This is despite a smaller proportion of eligible families of the younger cohort having been contacted (92% vs. 87%, $p<0.0001$) (Figure 3-1).

Figure 3-1:
The WA Twin Register, 1980-1992

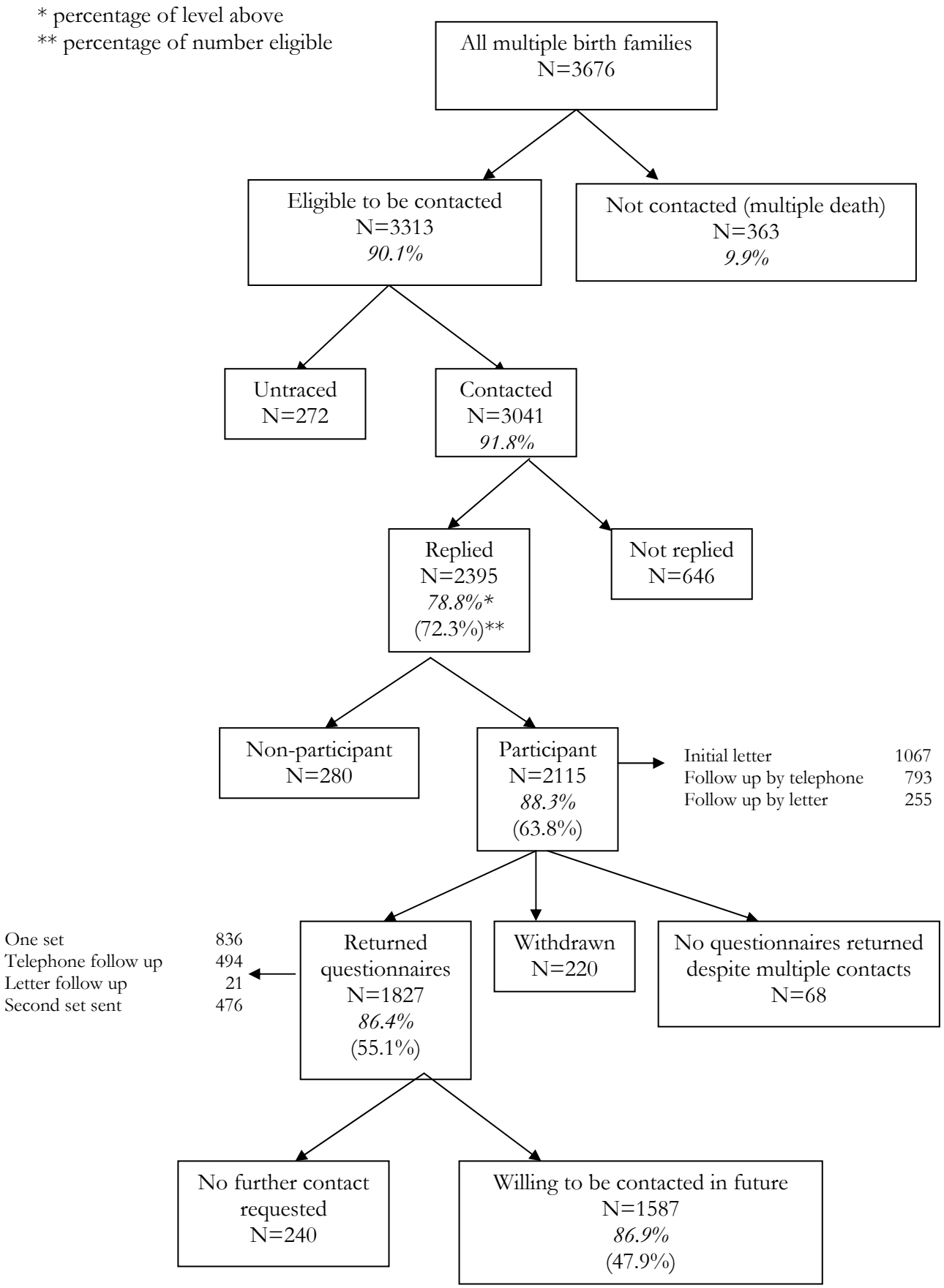
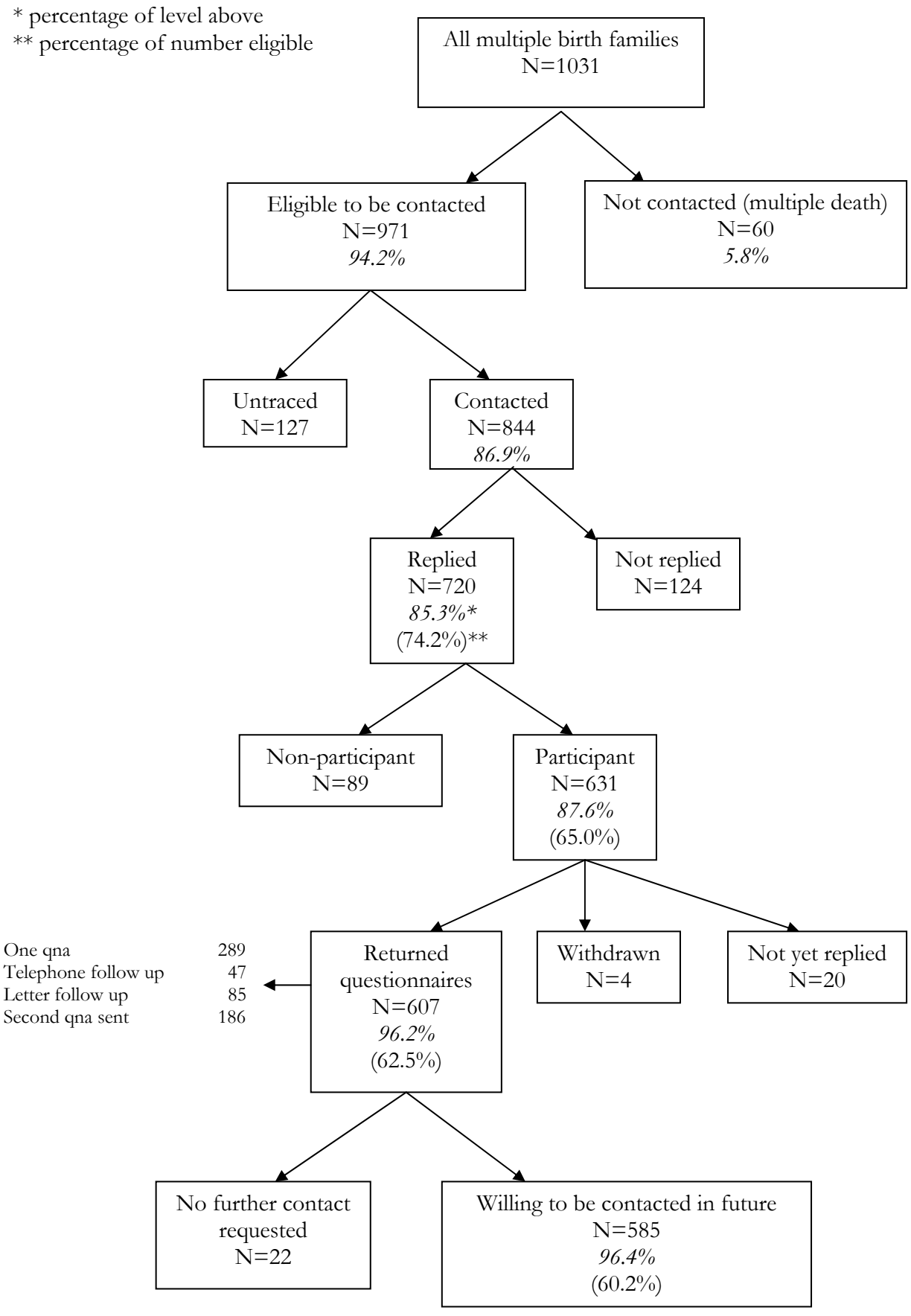


Figure 3-2:
The WA Twin Register, 1993-1995



Of the 3,041 eligible families of multiples born between 1980 and 1992 to be contacted, only 1,186 (39%) of them responded to the initial letter without further prompting and 90% of them agreed to participate (Figure 3-1). Of the remaining families, 875 were contacted by telephone and the remaining 980, by letter, with 91% and 26% respectively, participating (Table 3.4). Telephone calls made during the daytime were not answered, or resulted in us leaving messages inviting families to return our call. As only a small number of families did so, we felt that daytime calls were not the most efficient use of our limited staff and funds, and we made the calls during the evening wherever possible. Six hundred and forty six families (21%) did not reply after 4 attempts at contact. This resulted in an effective participation rate of 70% of the 3,041 families who were contacted (Table 3.4). Questionnaires were sent to the 2,115 families who agreed to participate, and 86% of them returned completed questionnaires, representing 55% of all eligible families (Table 3.5). Only 40% (836) of families returned their questionnaires without further prompting.

Table 3.4:
Number of families responding to the introductory letter – 1980-1992 births

	PARTICIPANT	NON PARTICIPANT	NO RESPONSE	TOTAL
Initial letter only	1067	119		1186
Follow-up by phone	793	82		875
Follow-up by letter	255	79	646	980
Total	2115	280	646	3041

Table 3.5:
Number of families returning completed questionnaires – 1980-1992 births

	COMPLETED	WITHDRAWN	NO RESPONSE	TOTAL
One set only	836	18		854
One set + phone call	494	103		597
One set + f/up letter	21	2		23
Two sets – long, long	298	41		339
Two sets – long, short	163	38		201
Two sets – short, short	15	7		22
Multiple contact			79	79

Total	1827	209	79	2115

A total of four hundred and ninety one families (27%) responded to the initial letter and returned their questionnaires with no follow up required at either stage. Six hundred and forty one families (30%) were sent more than one set of questionnaires. In total, 79 families failed to return their questionnaires after several contacts from us, and having been sent questionnaires on three separate occasions (Table 3.5). It was decided to discontinue follow-up on these families. Eighty seven percent of families who returned completed questionnaires indicated they were willing to be contacted about other studies in the future (Figure 3-1).

In the 1993-1995 cohort of multiples, only 335 (35%) of the 971 eligible families contacted responded to the initial letter without follow up (Figure 3-2), with 310 (93%) of them agreeing to participate (Table 3.6). Most of the remaining families were sent another questionnaire by mail, and 65 families completed their questionnaires at the time of the telephone follow-up (Table 3.6). Ninety-eight families (12%) had not replied after 3 attempted contacts. A further 25 families have not yet responded and follow-up is continuing. Completed questionnaires have been received from 607 (96%) of the 631 families who agreed to be part of the study (Table 3.7), and 585 (96%) stated that they were willing to be contacted in the future (Figure 3-2).

Table 3.6:
Number of families responding to an initial letter – 1993-1995 births

	PARTICIPANT	NON PARTICIPANT	WITHDRAWN	NO RESPONSE	TOTAL
Letter only	310	25		6	341
Phone follow-up	59	6			65
Letter follow-up	182	41		19	242
2 follow-up contacts	80	17	1	98	196
Total	631	89	1	123	844

Table 3.7:
Number of families returning completed questionnaires – 1993-1995 births

	COMPLETED	WITHDRAWN	NO RESPONSE	TOTAL
One questionnaire only	289			289
Phone follow-up	47	1		48
Two questionnaires	271	2	21	294
Total	607	3	21	631

The two stage contact process used for the 1980-92 cohort of families inherently meant that this group had a longer time between initial contact from us and our receipt of completed questionnaires. However, families of multiples born between 1980 and 1992 took more than three times longer to return questionnaires than families of multiples born between 1993 and 1995 (217 days vs. 60 days) (Table .3.8). Nearly half of all families whose multiples were born between 1993 and 1995 took less than four weeks to return completed questionnaires from the time they received the initial letter. In contrast, more than half of the 1980-92 multiple-birth families took in excess of six months to return their questionnaires.

Table .3.8:
Length of time between initial contact and return of completed questionnaires

TIME TO REPLY	1980-1992 BIRTHS	1993-1995 BIRTHS
< 2weeks	39 (2%)	155 (26%)
2-4 weeks	136 (8%)	129 (21%)
4-13 weeks	374 (21%)	190 (31%)
13-26 weeks	306 (17%)	85 (14%)
Over 26 weeks	944 (52%)	47 (8%)
Average	217 days (31 weeks)	60 days (9 weeks)

When considering the length of time between our sending questionnaires to families and receiving them back, families of multiples of the older cohort took slightly longer to return completed questionnaires than families of multiples born between 1993 and 1995 (78 days compared with 60 days) (Table 3.9). However, the percentage of the older cohort who still took longer than six months was significantly greater when compared with the younger cohort (11% vs. 8%, p=0.03).

Table 3.9:
Length of time between sending and receiving questionnaires

TIME TO REPLY	1980-1992 BIRTHS	1993-1995 BIRTHS
< 2weeks	376 (21%)	155 (26%)
2-4 weeks	323 (18%)	129 (21%)
4-13 weeks	604 (33%)	190 (31%)
13-26 weeks	327 (18%)	85 (14%)
Over 26 weeks	199 (11%)	47 (8%)
Average	78 days (11 weeks)	60 days (9 weeks)

In total, 2,286 families (57%) had changed their address between the time of their multiples' birth and our contact with them. Families of the younger multiples were less likely to have changed their address (50% vs. 59%, p<0.0001). Mothers who were under 20 at the time of the multiples' birth were less likely to reply to the introductory letter, and less likely to agree to participate than older mothers (45.2% vs. 79.1%, p<0.001; 41.7% vs. 70.4%, p<0.001). They were also less likely to return completed questionnaires (29.8% vs. 61.0%, p<0.001) (Table 3.10).

Table 3.10:
Response rates by mother's age at birth of multiples

	UNDER 20	20-29	30-39	40+
Contacted	84	1660	1244	49
Replied	38 (45%)	1278 (77%)	1036 (83%)	41 (84%)
Agreed to participate	35 (42%)	1128 (68%)	915 (74%)	36 (74%)
Returned questionnaires	25 (71%)	990(81%)	781 (85%)	31 (86%)
Overall response	30%	59%	63%	63%

Compared with families living in rural areas of WA, families who lived in the Perth metropolitan area were more likely to respond to the initial letter (79.9% vs. 75.5%, p=0.007), but no more likely to participate in the study (69.6% vs. 69.0%, p=0.76). Families who lived outside of Perth were more likely to return completed questionnaires (89.9% vs. 84.6%, p=0.002) than those who lived in Perth. There was no difference in the overall response rates between metropolitan and rural families (58.9% vs. 62.0%, p=0.11) (Table 3.11).

Table 3.11:
Response rates by place of residence

	METRO RESIDENCE	RURAL RESIDENCE
Contacted	2102	885
Replied	1680 (80%)	668 (76%)
Agreed to participate	1463 (70%)	611 (61%)
Questionnaires returned	1238 (85%)	549 (90%)
Overall response rate	59%	62%

3.7 Discussion

It has been shown that it is feasible to establish a population-based Register of multiple births in WA, using probabilistic record linkage techniques (Croft *et al* 2002). Here, we have demonstrated that it is possible to use this Register to contact families for a questionnaire survey, achieving acceptable response rates, and that respondents appear to be representative of all WA multiple birth families.

The MCHRDB, established and maintained by the Telethon Institute for Child Health Research, is a very powerful resource, and consists of data on all children born in WA since 1980 (Stanley *et al* 1994). Its unique nature has enabled us to compare participants and non-participants and to examine the representativeness of the Register. We have shown that there is no difference between respondents and non-respondents with respect to maternal age at first birth, paternal age at first birth, and racial origin (Hansen *et al* 2000). Our overall response rate of 57% is lower than that usually considered acceptable for such studies (Howell *et al* 2003), but was adequate in terms of study power.

Tracing people, especially women, can be difficult when the population is mobile and name changes not infrequent. Our attempts to trace and contact families commenced in 1997 when the multiples were aged between 5 and 17 years. The names and date of birth of the mother were used to link to the WA Electoral Roll and subsequently to search the White Pages. However, both sources of data have limitations. To be registered on an Electoral Roll, people must either be an Australian citizen, or be British subjects enrolled before 25th

January 1984. The WA Electoral Roll covers approximately 86% of the Western Australian population[1]. It contains information on full name, date of birth and current address. At the time the 1980-92 birth records were linked, the year of birth was rounded to the closest year ending in either "0" or "5". The White Pages have limited use for tracing females, as many women do not have telephone numbers registered in their own name. We also used the Commonwealth Electoral Roll to search for women resident in States other than WA. This is available for public perusal at State Electoral Roll Offices, but as it has no date of birth listed, is of limited use. The Commonwealth Electoral Rolls and White Pages are most useful for tracing residents who have unusual surnames. We believe that most of the mothers that we have been unable to trace have moved away from WA. However, we still managed to find a current address for over 90% of families, a large number of whom have changed their residential address since the birth of their multiples.

When assessing factors influencing response, this study has shown that:

- there was a lower response to the series of three questionnaires per family, than to the shorter questionnaire which covered all family members.
- response was increased if families only had to reply to one mailing, that is, they received their questionnaires along with information leaflets and consent forms, rather than having to respond twice, firstly by returning an expression of interest form and then, returning their completed questionnaires.
- telephone was the preferred mode of contacting non-responders.
- telephoning families at home was most productive when it was conducted during the early evening hours.
- giving potential responders the option of completing their questionnaires by telephone was well received by families. This option appeared only to be feasible for the shorter version of our questionnaires.

What is still unclear, however, is which of these factors had the most impact, and whether they operated singly or jointly. We used a combination of strategies, and were unable to examine the effect of any of them in isolation from the others.

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[1] Calculated using the total number registered on the WA Electoral roll, divided by the WA population (excluding visitors) aged 18 years or older at the last census (2001).

The length of the series of questionnaires sent to the families of the 1980-92 birth cohort did affect the response rate. Families were happy to agree to participate in the study, but some decided to withdraw after receiving the questionnaires, and realising the amount of time needed to complete them. The shorter questionnaire was readily accepted and resulted in a lower percentage of families subsequently deciding to withdraw. Other studies have found that shorter questionnaires result in higher response rates (Bean & Roszkowski 1995, Edwards *et al* 2002), although there seems to be no agreement as to the optimal questionnaire length. We decided to persevere with the longer version of the questionnaires as a first resort, as we felt that the data obtained would be very useful in identifying families for future studies into other health outcomes.

As with some other studies (Mueller *et al* 1986, Parker & Dewey 2000), we found that the most efficient method of follow up contact was by telephone. Many families appreciated the personal contact, and commented that they were pleased that researchers were taking an interest in "ordinary families like ours". However, the telephone calls needed to be made in the early evening hours when most families could be contacted.

The use of incentives to increase response is a contentious one. Many surveys use incentives to motivate volunteers (Collins *et al* 2000, Spry *et al* 1989), although others who offer incentives target specific groups such as physicians (Tambor *et al* 1993, Ward *et al* 1998). One study of twins offered monetary rewards to registrants (Jang *et al* 2002). However, researchers should take into account the likely impact on the total cost per completed response, and whether the respondents are representative of the population being sampled. It could perhaps even be argued that a University-based study offering a monetary incentive would be seen by potential participants as unethical, or a waste of taxpayer's money, or both. This could actually deter people from participating. Hence, we did not consider using monetary rewards or other incentives. This study originated from the Telethon Institute for Child Health Research, a non-Government Research Institute affiliated with the University of WA, and was funded solely by nationally-competitive grants.

One of the most important aspects to consider when undertaking a large questionnaire study is the characteristics of the study staff. We found that some important requirements for staff carrying out follow up include: having a special telephone manner, the ability to be able to devote 2-3 hours during the evening to contact families; empathy/sympathy with the needs and concerns of multiple birth families, and being a willing listener. Of course, they must strictly adhere to the study follow up protocols, and pay particular attention to detail when using the Electoral Rolls and White Pages to trace mothers. The nature of telephone contact should be friendly, especially if non-responders are to be contacted regularly. Many families require time to decide whether or not to participate in the study. Multiple birth families have many demands made on their time, but we have found that many mothers have appreciated the opportunity to be able to discuss some of their concerns with a member of our staff.

This study has also shown that families of multiples born in WA between 1993 and 1995 are more likely to join the WA Twin Register and more likely to return completed questionnaires, than families of WA multiples born between 1980 and 1992. Several factors could account for this. For the younger cohort, questionnaires were mailed with an introductory letter and information package, which meant that families only had to respond to one letter. Also, questionnaires were significantly shorter, and therefore took much less time to complete. At the time the families were contacted, mothers were younger and their multiples were aged between 7 and 8; details of the pregnancy and birth were more recent and therefore more likely to be remembered. Several other studies have also found that the age of respondents effects response, (Larroque *et al* 1999) as do the number of mailings (Sauerland & Neugebauer 2002).

The reasons why families living outside the Perth metropolitan area were more likely to respond than their non-metropolitan counterparts are unclear. Multiple birth children are more likely to be born prematurely, and at a lower birth weight, than singleton children, and are therefore of special interest to researchers looking at the long-term sequelae of adverse birth outcomes. They are often invited to participate in studies that require them to attend clinics in Perth. But limited research funds do not allow for any reimbursement of costs

incurred, and so families living at great distances from Perth, often over a thousand kilometres away, are usually unable to participate. It is possible that metropolitan families could feel that they already take part in enough research, and this could explain why, in this case, their participation rate is lower than their country counterparts. Facilities and support services for multiple birth families are often lacking in rural areas; until recently, the Australian Multiple Birth Association (WA branch) (AMBAWA) only held regular meetings for parents of multiples in metropolitan regions. Many families who lived in country areas expressed their gratitude at being contacted and invited to participate, and were grateful for someone to talk to.

To get a definitive answer as to which method, or combination of methods results in the maximum response rate, more randomised studies examining all the various methods are needed. However, what is clear is that a combination of methods using questionnaire length, number and type of follow-up contacts, characteristics of likely participants, and characteristics of staff should be taken into account when planning a questionnaire survey.

3.8 Summary

Twin registers have been established worldwide to study the roles of genes and the environment in health and behaviour. While questionnaire surveys are thought to be the most cost-effective way of collecting large amounts of data, low response rates can result in response bias. Many different strategies have been proposed to maximise response rates.

A register of all multiple births occurring in Western Australia (WA) from 1980 onwards has been established using probabilistic record linkage techniques. Families who had not experienced the death of one or more of their multiples were invited to participate in the Western Australian Twin Child Health (WATCH) study, which studied the genetic and environmental determinants of childhood asthma and atopy. Several questionnaire designs and follow-up methods were assessed.

Over 90% of families have been contacted, and 63% of them have returned questionnaires, giving an overall response rate of 57%. Shorter questionnaires were more likely to be completed than the longer versions (62% vs. 55%, $p<0.001$). Telephoning non-responders was the most effective method of follow up, with a 91% participation rate compared with 26% of those contacted by mail ($p<0.001$). Mothers aged under 20 years at the time their multiples were born, were less likely to respond than older mothers (45% vs. 79%, $p<0.001$), and families living in rural areas of WA were more likely to respond than those living in the Perth metropolitan area (80% vs. 75%, $p=0.007$).

We have shown that it was feasible to use a population-based Register of multiple births to contact families for a questionnaire study. Questionnaire length, mode of follow-up, the number of responses required, and age of participants, all appeared to affect response.

CHAPTER 4
THE WATCH STUDY POPULATION

4.1 Preface

This chapter describes the characteristics of the WATCH study families whose twins were born between 1980 and 1992 inclusive, and who returned completed questionnaires. Data were collected from these families to look specifically at the genetic epidemiology of asthma and atopy, which is discussed in subsequent chapters. Triplets and higher order multiples were not included, as analyses examined the differences in outcomes by zygosity. Also, data from the 1993-1995 multiple birth cohort were not included; the aims for this part of the study were quite different, different questionnaires were used, and data collected by the two sets of questionnaires did not completely correspond with each other.

4.1 Review of the literature

4.1.1 Introduction

Twin pregnancies differ from singleton pregnancies in several respects. Twin mothers, especially of DZ twins, tend to be older and of higher parity (Taffel 1995). A multiple pregnancy confers additional risk to the mother with risk of mortality during pregnancy and delivery being significantly higher than for a singleton pregnancy (Conde-Agudelo *et al* 2000, Senat & Ancel 1998). Higher rates of hypertension during pregnancy and post-partum haemorrhage are thought to contribute to this excess in risk. There is also a significant association between multiple gestation and anaemia and urinary tract infection during pregnancy (Conde-Agudelo *et al* 2000). Twins are more likely than singletons to be delivered by caesarean section (Scher *et al* 2002), and there is a higher rate of pre-eclampsia during a multiple pregnancy than during a singleton pregnancy (Cooperstock *et al* 2000). Duration of gestation is shorter, twins are of lower birth weight, and their perinatal mortality is higher than that of singletons (Taffel 1995). Over 40% of twins are born preterm (before 37 weeks) and around 50% weigh less than 2500g at birth (Taffel 1995). The incidence of sudden infant death syndrome (SIDS) in twins is about twice that in singletons (Malloy & Freeman 1999, Platt & Pharoah 2003). Twins have a higher risk of

cerebral palsy (CP) (Petterson *et al* 1993) and congenital malformations (Ben-Ami *et al* 2005, Hansen *et al* 2002, Li *et al* 2003, Mastroiacovo *et al* 1999) than singletons. During childhood, multiples are more likely than singletons to display behaviours associated with attention-deficit hyperactivity disorder (ADHD) (Hay 2005), and as a result, are at increased risk of learning difficulties (Hack *et al* 2002), and are more prone to accidents and injuries than singletons (Lalloo *et al* 2003). Multiple births also have an effect on maternal emotional well-being long after delivery, with mothers more likely to suffer from depression (Thorpe *et al* 1991) than mothers after a singleton pregnancy.

4.1.2 Changes in the rate of multiple births over time

During the 1980s and 1990s, there was an increase in the rate of multiple births across the developed world (Fellman & Eriksson 2003, Levene *et al* 1992). A 40% increase in twin births was observed in the USA, rising from 1.8% of births in 1971 to 2.7% in 1997 (Martin & Park 1999, Taffel 1992, Ventura *et al* 1998), with similar increases being reported from the United Kingdom (Botting *et al* 1987, Levene *et al* 1992), Japan (Imaizumi & Nonaka 1997) and Israel (Mordel *et al* 1992). A European multi-centre study reported that, as a percentage of all births, the rate of multiple births increased from 1.9% in 1980 to 2.4% in 1990 (Topp *et al* 2004). In Western Australia (WA), the proportion of twins in WA rose from 1.9% in 1980 to 2.9% in 1989 (Gee 1992) and 3.0% in 1999 (Gee & O'Neill 2001). These increases are thought to be mainly due to higher maternal age at first pregnancy and the increased use of assisted reproductive technology (Bergh *et al* 1999, Tough *et al* 2000, Tough *et al* 2002).

4.1.3 Birth weight and gestation

It is well known that, on average, twins have lower birth weight and shorter gestation than singletons, with twins weighing as much as 1 kg less at birth, and being delivered on average three weeks earlier than singletons (Alexander *et al* 1998). Although twins comprise a small percentage of all births, they contribute a high percentage of low birth weight and premature infants (Alexander *et al* 1998, Day *et al* 1997, Donoghue 1996). In 2003 in Australia, multiple births represented 23% of all preterm births, and 22% of all level 3 neonatal intensive care unit (NICU) admissions (Laws & Sullivan 2005). In the USA, multiple births accounted for less than 3% of births, but 20% of all low birth weight

infants and 13% of all preterm deliveries (Alexander *et al* 1998). Furthermore, more than 50% of twins were preterm and low birth weight (Alexander *et al* 1998, Vintzileos *et al* 2003). Prior to 28 weeks' gestation, there appears to be little difference in the mean birth weight of twins and singletons, but thereafter, the average birth weight of twins is less than that of singletons for every week of gestation, with the difference being as much as 300g by 38 weeks gestation (Alexander *et al* 1998). However, it is not clear at what stage of pregnancy the point of divergence in birth weight occurs. Some studies found no difference in birth weights below 30 weeks (Alexander *et al* 1998), whereas others found significant differences at 30 weeks (Kiely 1990, Luke 1993, 1996). Several studies showed that the growth patterns of twins and singletons were similar to 32 weeks' gestation (Glinianaia *et al* 2000, Skjaerven *et al* 2000). It is clear, however, that in the last trimester of pregnancy, twins become more growth retarded compared with singletons (Bleker *et al* 1988, Liu & Blair 2002), and the birth weight of twins for comparable lengths of gestation are less than that of singletons (Leroy *et al* 1982, Liu & Blair 2002, Naeye *et al* 1978, Rydhstroem 1992). Definitions of low birth weight and prematurity used in most studies were based on <2500g and <37 weeks respectively. They were derived from singleton births, and represent optimal birth weight and gestation, with respect to perinatal mortality.

4.1.4 Perinatal mortality and morbidity

Perinatal mortality is defined as a stillbirth or neonatal death, that is death within 28 days of birth. Although the rate of twinning is only between 2% and 3% of all births, twins account for a much higher proportion of fetal and neonatal deaths (Boggees & Chisholm 1997, de Veciana *et al* 1995, Gardner *et al* 1995, Grothe & Ruttgers 1985, Osbourne & Patel 1985). In Western Australia, multiple births accounted for 12% of all perinatal deaths in the period 1999-2001 (Gee & Green 2005). In the USA, multiple births account for 14% of all neonatal deaths (Alexander *et al* 1998). Other studies report that twins are between 3 and 11 times more likely to die perinatally or during infancy than singletons (Bajoria & Kingdom 1997, Imaizumi 1994, Kiely 1990, Luke & Keith 1992, McCarthy *et al* 1981, Nylander 1979, Powers *et al* 1995, Scher *et al* 2002). In WA, rates of stillbirth and neonatal death in multiple births were 20 per 1000 births and 15 per 1000 live births, respectively, both considerably higher than

the corresponding rates in singletons (6.8 per 1000 births and 2.5 per 1000 live births, respectively) (Gee & Green 2005). These results are consistent with those found in other States of Australia (Laws & Sullivan 2005) as well as elsewhere in the world (Fakeye 1986, Ghai & Vidyasagar 1988, Imaizumi 2001, Kouam *et al* 1988, Liapis *et al* 1997, Luke & Keith 1992, Naeye *et al* 1978). There is considerable evidence that the majority of the increased morbidity and mortality associated with twin pregnancy is as a result of the higher likelihood of being born prematurely and of low birth weight (Boggees & Chisholm 1997, El-Jallad *et al* 1998, Fakeye 1986, Kilpatrick *et al* 1996, Kouam *et al* 1988, Liapis *et al* 1997, Luke 1996, Nielson *et al* 1997, Nylander 1979).

Caesarean delivery has been shown to be associated with a lower stillbirth rate, and twins are more likely than singletons to be delivered by this mode (Scher *et al* 2002). However, a recent study found no significant difference in perinatal mortality and neonatal morbidity between planned caesarean section and planned vaginal delivery of twins (Haest *et al* 2005).

Fetal death and neonatal mortality rates in multiple births could potentially be reduced by the attainment of optimal gestational age and birth weight. Perinatal mortality rate for twins was lower than for singletons in earlier gestational age or lower birth weight ranges (Kato & Matsuda 2006), suggesting that the optimal gestational age for twins was earlier than for singletons and optimal birth weight was lower. Optimal birth weight and gestational age for twins, defined as those ranges for which the perinatal mortality rate was lowest, has been estimated to be between 2500g and 3000g, and 36-39 weeks respectively (Alexander *et al* 1998, Ananth *et al* 1998, Cheung *et al* 2000, Kato & Matsuda 2006, Kiely 1998, Luke 1996, Min *et al* 2000, Minakami & Sato 1998). However, even at the most favourable birth weight, twins still had approximately twice the risk of early mortality than in singleton infants (Scher *et al* 2002).

Neonatal outcomes in the first-born twin were significantly better than in the second-born twin (Arnold *et al* 1987, Ghai & Vidyasagar 1988, Haest *et al* 2005, Wells *et al* 1991). The risk of death of a twin was greater if the co-twin died *in utero* or shortly after birth (Scher *et al* 2002).

4.1.5 Cerebral palsy

Infants from multiple births are known to be at higher risk of cerebral palsy (CP) compared with singleton infants (Bejar *et al* 1990, Grether *et al* 1993, Nelson & Ellenberg 1995, Petterson *et al* 1993, Pharoah & Cooke 1996, Pharoah *et al* 2002, Williams *et al* 1996, Zosmer *et al* 1994). Estimates of the increased risk of CP in twins ranged from four-fold in a multicentre study in Europe (Topp *et al* 2004), to 8 to 12-fold in the USA and Western Australia (Grether *et al* 1993, Petterson *et al* 1993). Many other studies report similar results (Goodman & Alberman 1996, Grether *et al* 1993, Petterson *et al* 1993, Pharoah & Cooke 1996, Scher *et al* 2002, Williams *et al* 1996, Williams & O'Brien 1998, Yokoyama *et al* 1995). The higher rate of CP in multiple births is closely related to low gestational age and birth weight, two of the most important risk factors for CP (Goodman & Stevenson 1989, Grether *et al* 1993, Petterson *et al* 1993, Pharoah & Cooke 1996, Williams *et al* 1996, Yokoyama *et al* 1995). In twins, CP rates vary less with birth weight and gestational age than the rates for singletons (Bonellie *et al* 2005). Comparison of birth weight specific prevalence rates in twins and singletons shows that the greatest disparity in rates occurs among those of normal birth weight, that is, those weighing ≥2500g (Pharoah & Cooke 1996).

It is not clear whether the risk of CP in twins is affected by birth order. One study showed that twin CP infants are more likely to be second-born than first-born (Topp *et al* 2004), while another showed no difference in the risk of CP between first- and second-born twins (Bonellie *et al* 2005). However, there is strong evidence that the risk of CP is affected by the outcome of the co-twin, with the death of a co-twin being a powerful predictor of CP in the surviving twin (Bergh *et al* 1999, Bonellie *et al* 2005, Scher *et al* 2002). Studies from the USA and Western Australia found that intrauterine death of a co-twin is associated with a 13-15-fold higher risk for CP compared with twins where both are born alive (Grether *et al* 1993, Petterson *et al* 1993). Where both twins survived infancy, same sex were at greater risk of CP than unsame sex twins (Glinianaia *et al* 2002a, Grether *et al* 1993, Pharoah 2001, Pharoah & Adi 2000, Pharoah *et al* 2002).

Increased perinatal mortality and infant in twins means that mortality is a competing risk to the diagnosis of CP. Twins are more likely to die as infants (Kleinman *et al* 1991, McCarthy *et al* 1981, Nelson & Ellenberg 1995, Powers *et al* 1995), precluding a diagnosis of CP which generally cannot be made with any certainty until months or years after birth.

4.1.6 Congenital malformations

Many studies have reported an increased risk of congenital malformations in twins when compared with singletons (Bower *et al* 1995, Doyle *et al* 1990, Hay & Wehrung 1970, Kallen *et al* 1994, Layde *et al* 1980, Little & Bryan 1896, Mastroiacovo *et al* 1999, Myrianthopoulos 1974, 1975), in particular, malformations of the central nervous, digestive and cardio-vascular systems (Doyle *et al* 1990, Hay & Wehrung 1970, Kallen *et al* 1994, Layde *et al* 1980). More recently, children conceived with the assistance of reproductive technology, particularly intracytoplasmic sperm injection (ICSI), were found to be at higher risk of birth defects than children conceived naturally (Ben-Ami *et al* 2005, Hansen *et al* 2002), which has serious implications for multiple births.

4.1.7 Zygosity

In a large, population-based study of twins, it is often not feasible or economically viable to determine zygosity by way of blood or DNA tests. Hence, it is important to be able to use an alternative method to determine zygosity that produces consistent and reliable estimates of true zygosity. One such method was developed by Cohen and colleagues (Cohen *et al* 1973, 1975), who used discriminant analysis of a series of questions answered by parents about their perception of their twins' similarity at the time of interest. A crude estimate of zygosity over a population can be calculated using the Weinberg method, which assumes that the ratio of male:female births is 1:1. It is assumed that the four sex combinations of DZ twins (FF, MM, MF, FM) are equally likely (p=0.25), giving the equal numbers of like- and unlike-sexed DZ twins. The expected number of MZ twins is then the total number of pairs minus the estimated number of DZ pairs (Parisi 1995). Hence, in a population of twins, it would be expected to have equal number of MZ, same sex DZ and opposite-sex DZ twins.

4.1.8 Other issues

The importance of prematurity is not restricted to the perinatal period, where twins are at increased risk of perinatal mortality and morbidity, such as CP. It has been shown that later in life, problems including cardio-respiratory failure and chronic lung disease can occur particularly in those born extremely pre-term (<32 weeks) (Henderson-Smart 1995). Gestational age at birth is a powerful determinant of child development and is associated with motor function, language, and mental development (Akerman & Thomassen 1992, Stauffer et al. 1988). Studies have shown that children that are born small-for-gestational age (SGA) experience more mental health morbidity than children who attain ideal birth weight for gestational age (Rooney *et al* 2003, Zubrick *et al* 2000). There is also evidence that twins experience a higher risk of ADHD compared with singletons (Hay 2005), which in turn is associated with increased impulsivity, hyperactivity and inattention. The higher rates of these behaviours are associated with speech and language delay (Mogford-Bevan 1999), reading problems and poorer academic achievement (Hack *et al* 2002, Henderson-Smart 1995), which are all more common in twins than singletons. These conditions are particularly significant for male twins (Hay *et al* 1986, Hay *et al* 1984, Levy & Hay 1996). Twins are also known to experience more accidents and injuries during childhood, a direct result of poor co-ordination and impulsive behaviour stemming from ADHD (Bijur *et al* 1988, Lalloo *et al* 2003).

The arrival of twins has an impact on the whole family and has been shown to be associated with a decline in physical health (Gjerdingen & Center 2003), and an increase in the risk of mental health problems, particularly depression, in both parents (Gjerdingen & Center 2003, Hay 1994). Data from the US and Japan (Bryan 1992) suggest that there may be up to a 10-fold increased risk of child abuse in twins or in older siblings of twins. It has also been reported that the older siblings of multiples are more likely to exhibit more behavioural problems than those of single-born children (Hay *et al* 1998). The extra cost to the family of having multiple-birth children can also place extra pressure on the family and cause increased tension between parents (Davies 1995).

4.1.9 Summary

The entire family is influenced by the arrival of twins. Whilst approximately 1 child in 40 is a twin, about 1 person in 15 is directly affected by the birth of twins through being a parent or sibling or twin. Parents are affected by their increased likelihood of experiencing emotional problems, especially depression, and siblings of twins are at increased risk of displaying behavioural problems when compared with siblings of singletons.

Establishing this population-based twin register at a state level will enable a more complete clinical picture of the perinatal and childhood morbidity and mortality occurring in twins to be described. In addition, this work is complemented by a thorough study of the impact of health problems in twins on their families and co-twins. The need for this is emphasised by observations such as that by Thorpe *et al*., that maternal depression may be present even five years after the loss of one twin (Thorpe *et al* 1991). A comprehensive study using WA Twin Register data has described the impact of the loss of a twin on the whole family, and is thought to be one of a few studies to examine the impact of loss on co-twins (Swanson *et al* 2002).

4.2 Aims

The aims of this section are to:

(1) describe the WATCH study population;
(2) describe differences between twins and their siblings with respect to health outcomes and behaviours, health services utilisation, and educational needs;
(3) describe differences between twins and their siblings in risk factors associated with poorer outcomes in childhood health and behaviour;
(4) examine the distributions of birthweight and gestation for twins and their singleton siblings; and
(5) report on the representativeness of the sample collected.

4.3 Characteristics of WATCH Study Population

The WA Twin Register comprises all families known to have had multiple birth children born in WA during the period 1980 to 1997 inclusive. One of the conditions stipulated by the Confidentiality of Health Information Committee (CHIC) in allowing WA Health Department data to be used in establishing the Register was that no family known to have experienced the death of one or more of their multiples were to be contacted. These families were therefore deemed to be ineligible for the WATCH study. A separate study examining the impact of the loss of a multiple birth child has been conducted using these families (Swanson *et al* 2002).

All eligible families of multiple birth children born in WA from 1980 to 1992 inclusive, who could be traced to a current address, were contacted and invited to participate in the Western Australian Twin Child Health (WATCH) study (as described in Chapter 3). A comprehensive set of questionnaires, covering all family members, was sent to all families who agreed to participate. A full set of these questionnaires is contained in Appendices 2 to 4. Some families opted to complete one shorter questionnaire covering all family members (Appendix 5). However, due to the nature of the data collected by these shorter questionnaires, the results presented in this chapter are limited to data obtained from the full set of questionnaires completed by families of twins only.

One thousand seven hundred and fifty eight twin families returned completed questionnaires, yielding data on 9247 individuals. These families comprised 1779 sets of twins, 2138 full siblings, 182 step- and half-siblings, 1749 mothers, 1510 fathers and 89 step-parents. Twenty-one families had two sets of twins. The average family size was 5.3 persons. Mother's age at the time of their multiples' birth ranged from 14 to 44 years, with an average age of 29 years. Mothers of MZ twins were, on average, younger than mothers of DZ twins when they gave birth (27.9 vs. 29.3 years, $p<.001$). Fathers were on average 35 years old, ranging from 19 to 64 years, when their twins were born. Nearly all families had English as their first language (99%), and most parents were in their first marriage (77% for mothers and 84% of fathers).

4.4 Descriptive statistics of parents

4.4.1 Occupation and Education

Nearly a third of mothers reported that they worked at home, and nearly half of all parents claimed to be employed in either a professional or semi-professional occupation (Table 4.1). Mother's and father's occupation were positively correlated (ρ=0.28, p<.0001).

Table 4.1:
Current or previous occupation of parents of twins

	MOTHERS (N=1405)	FATHERS (N=1249)
Work at home/unemployed	32.5%	2.1%
Professional (eg lawyer, engineer, teacher)	23.0%	28.2%
Semi-professional (eg salesperson, clerical)	24.6%	17.0%
Skilled trades (eg plumber, electrician, mechanic)	2.0%	26.6%
Unskilled trades (eg labourer)	5.1%	8.7%
Other	12.8%	17.4%

One in five parents reported that they had obtained tertiary qualifications, with less than one percent having no high school education (Table 4.2). There was a significant correlation between the parents' educational level (ρ=0.40, p<.0001).

Table 4.2:
Highest level of education attained by parents of WA twins

	MOTHERS (N=1405)	FATHERS (N=1249)
Primary school	0.3%	0.6%
High school	60.1%	44.7%
Apprenticeship or trade qualifications	8.6%	28.2%
Tertiary qualifications	18.0%	21.3%
Other	13.0%	5.2%

4.4.2 Family structure

On average, parents of twins had 3 siblings each, with a range of 0 to 15. The twins' grandmothers had an average of 3.7 siblings (range 0-17), and their grandfathers, 3.6 siblings (range 0-17). Sixty-five parents (2.4%) reported that they were a member of a twin pair (Table 4.3), and 89 parents (3.6%) had

multiples among their siblings. In addition, 168 parents (6.7%) reported that they had cousins who are twins.

Table 4.3:
Twinning in the parents of WA twins

	MOTHERS (N=1405)	FATHERS (N=1249)
Has an identical twin	10	8
Has a fraternal twin of the same sex	16	12
Has a fraternal twin of the opposite sex	11	8

4.4.3 Mother's reproductive history

Although the total number of pregnancies reported by mothers ranged from 1 to 11 (with a median of 2.0), the majority of mothers (95%) had only had the one multiple pregnancy. Five percent of mothers had had a stillborn child, and three percent had given birth to a child who had subsequently died.

4.4.4 Health conditions and operations in parents

Mothers were more likely than fathers to report having had a diagnosis of arthritis or rheumatism (6.6% vs. 4.6%, p=0.034) and migraine or severe headaches (21.4% vs. 8.8%, p<0.001), but there was no difference in the percentage of parents diagnosed with any of the other health conditions examined. Mothers were also more likely to have had their tonsils and adenoids removed than fathers, and very few parents had had grommets inserted (Table 4.4).

Table 4.4:
Operations on the ear, nose and throat in parents of WA twins

	MOTHERS (N=1405)	FATHERS (N=1249)	P VALUE
Tonsils removed	35.7%	28.8%	<.001
Adenoids removed	13.7%	9.5%	<.001
Grommets inserted	1.2%	0.9%	.406

4.4.5 Accidents experienced by parents

One hundred and eight mothers and 133 fathers reported that they had had at least one accident in the last 12 months. This represents 7.7% of mothers and 10.5% of fathers (p-value for difference<0.001). Most accidents only required consultation with a health professional for either treatment or advice, but fathers more likely to be admitted to hospital than mothers (Table 4.5). Fathers were also more likely than mothers to have had an accident serious enough to require bed rest, and only a small percentage reported that their accident had not affected the level of their daily activity (Table 4.6).

Table 4.5:
Treatment required for accidents

	MOTHERS (N=108)	FATHERS (N=133)	P VALUE
Admission to hospital	7.4%	22.6%	<.001
Treatment at hospital emergency department	22.2%	30.8%	.13
Treatment by health professional	44.4%	49.6%	.42
Advice from a health professional	13.9%	18.8%	.31
Minor treatment at home	21.3%	15.8%	.27

Table 4.6:
Action required after accidents

	MOTHERS (N=108)	FATHERS (N=133)	P VALUE
Staying in bed	12.0%	24.1%	.017
Some time off study or work	33.3%	40.6%	.25
Reduced activity only	50.9%	42.9%	.21
No change in amount or level of daily activity	2.8%	8.3%	.070

The most common types of injury reported were broken or fractured bones, and sprains or strains, which together accounted for over half of all injuries (Table 4.7).

Table 4.7:
Type of injury experienced by parents of WA twins

	MOTHERS (N=108)	FATHERS (N=133)	P VALUE
Head injury with loss of consciousness	3.7%	4.5%	.76
Broken bones or fractures	20.4%	18.8%	.76
Dislocations	2.8%	6.0%	.23
Sprains or strains	39.8%	31.6%	.18
Broken teeth or teeth knocked out	0.9%	0.8%	.88
Internal injuries	2.8%	2.3%	.80
External wound needing stitches	7.4%	21.8%	.002
External bruising	18.5%	12.8%	.22
Burns and scalds	4.6%	2.3%	.31

About 20% of the accidents experienced by parents occurred while they were playing sport. Fathers were more likely to be injured at work, while mothers in their own home (Table 4.8).

Table 4.8:
Places where accidents occurred

	MOTHERS (N=108)	FATHERS (N=133)	P VALUE
Inside own home	15.7%	7.5%	.04
Inside someone else's home	3.7%	0.8%	.11
In own garden	8.3%	15.0%	.11
In someone else's garden	2.8%	4.5%	.48
At work	14.8%	39.1%	.001
Playing sport	22.2%	18.0%	.42
In a road traffic crash as the driver of a vehicle	4.6%	3.8%	.74
Bike riding	1.8%	2.3%	.83

4.4.6 Parental use of health-related services

Mothers, but not fathers, have consulted with a physiotherapist more often since the birth of their twins than they did before they were born. Both parents reported that they had used the services of mental health experts more often after their twins were born than before they were born (Table 4.9).

Table 4.9:
Percentage of parents who used health-related services before and after the birth of their twins

SERVICE USED	BEFORE %	AFTER %	P-VALUE
Mothers (n=1405)			
Physiotherapist	19.9	25.9	<.0001
Psychologist	3.1	6.6	<.0001
Psychiatrist	2.0	3.6	.0019
Fathers (n=1249)			
Physiotherapist	23.5	22.4	.34
Psychologist	1.2	3.8	<.0001
Psychiatrist	1.0	2.4	.0014

4.4.7 Parental Smoking

The majority of parents (58%) reported that they had smoked at some time during their lifetime, with a greater percentage of fathers than mothers having ever smoked (62% vs. 54%, respectively, $p<0.001$) (Table 4.10). Smoking was strongly related to the SES of the family, with fewer mothers and fathers in the top 10% of SEIFA indices for disadvantage, advantage/disadvantage and education and occupation (see section 4.7 for description of these indices), than in the other 90% of these indices indicating that they had ever smoked. Parental smoking status was not related to the SEIFA index of economic resources. Most households reported that they had rules about not allowing smoking inside their homes (74.5%) or having a smoke-free car (83.5%). Around 20% of mothers reported that they had smoked during pregnancy, with the rate being higher during the pregnancy of their singleton children than that of their twins (Table 4.11). Of the mothers who said they gave up smoking while they were pregnant, over three-quarters of them started smoking again once the twins were born.

Table 4.10:
Smoking status of parents of WA twins

	MOTHERS (N=1405)	FATHERS (N=1249)
Never smoked	45.8%	38.3%
Ex smoker	34.4%	37.6%
Current smoker	19.8%	24.1%

4.5 Descriptive statistics of the twins and their siblings

4.5.1 Maternal behaviours before and during pregnancy

Twins were more likely than their siblings to have been conceived after their parents had sought medical assistance before pregnancy (p=0.001). Mothers were more likely to have experience symptoms of post-natal depression after the birth of the twins than after the birth of their singleton children (Table 4.11).

Table 4.11:
Percentage of mothers reporting various behaviours before and during pregnancy

VARIABLE	TWINS (N=2848)	SIBLINGS (N=1196)	P VALUE
Assisted reproduction			
Became pregnant without really trying	44.5	38.4	.001
Sought medical assistance before pregnancy	18.7	9.2	.001
Periconceptional multivitamin use			
In the month before pregnancy	15.3	12.8	.038
In the first three months of pregnancy	55.4	54.0	.42
Depression			
Mother more depressed after delivery	22.7	14.7	.001
Smoking			
Mother smoked during pregnancy	18.8	21.7	.001

4.5.2 Complications of pregnancy

Mothers reported no complications in two-thirds of their singleton pregnancies, but in under half of twin pregnancies (Table 4.12). They were more likely to require bed rest during a twin pregnancy than a singleton pregnancy, and were more likely to experience a threatened miscarriage, toxaemia, high blood pressure, or premature rupture of membranes for their twins compared with their singleton children (Table 4.12).

Table 4.12:
Pregnancy complications of the twins and other children

VARIABLE	TWINS (N=2848)	SIBLINGS (N=1196)	P VALUE
Bleeding serious enough to require bed rest	9.1%	5.8%	.001
Threatened miscarriage under 20 weeks	8.4%	4.9%	.001
Urinary tract infection	4.4%	3.1%	.061
Toxaemia	9.4%	4.1%	.001
High blood pressure	19.9%	10.3%	.001
Gestational diabetes	1.6%	1.4%	.65
Placenta praevia	2.2%	1.3%	.077
Premature rupture of membranes	6.7%	1.8%	.001
No complications	43.6%	67.1%	.001

4.5.3 Mode of delivery

Twins were less likely to be delivered by a spontaneous vaginal delivery than their singleton siblings (Table 4.13). Over one third of twins were delivered by caesarean section, compared with 13% of singletons.

Table 4.13:
Mode of delivery of twins and their siblings

VARIABLE	TWINS (N=2848)	SIBLINGS (N=1196)	P VALUE
Normal	35.9%	61.9%	.001
Breech	13.7%	2.0%	.001
Forceps	14.4%	15.2%	.50
Vacuum extraction	7.0%	9.2%	.016
Booked caesarean section	17.4%	6.3%	.001
Emergency caesarean section	16.5%	6.8%	.001

4.5.4 Birth weight and gestation

Twins weighed less at birth, and had shorter gestation when compared with their singleton siblings. Nearly half of all twins weighed under 2,500g at birth, compared with only 5% of singletons (Figure 4-1). Birth weight for twins ranged from 720g to 4,423g, with a mean of 2,503g. This mean was significantly less than that for their siblings (3,443g, $p<0.001$), whose birth weight ranged from 800g to 5,103 (Figure 4-2). On average, first-born twins weighed more than second-born twins ($p=0.037$). For all children, boys were heavier than girls at birth (2,817g vs. 2,690 g, $p<0.001$), and this difference was evident for both twins (2,562g vs. 2,435g respectively, $p<0.001$) and their siblings (3,514g vs. 3,390g respectively, $p<0.001$). Children whose mothers smoked during pregnancy were born at significantly lower average birth weight than children of non-smoking mothers: 2,426g vs. 2,522g, $p=.0089$, for twins, and 3,317g vs. 3,483g, $p=.0002$, for their siblings.

Figure 4-1:
Cumulative distribution of birth weight for twins and their singleton siblings

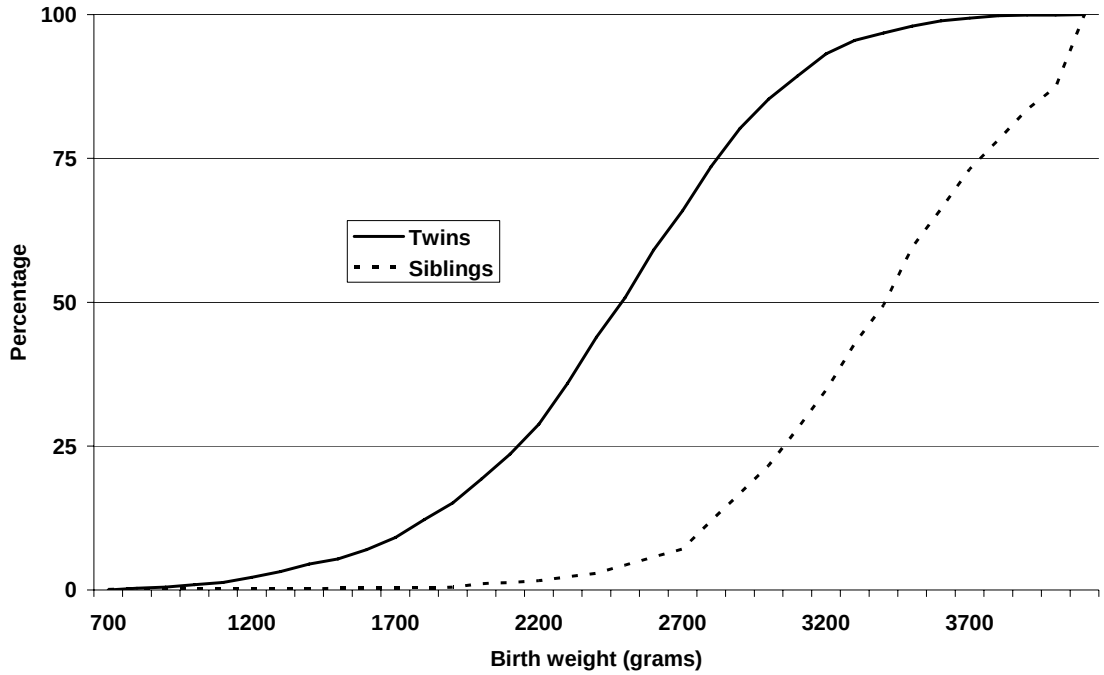
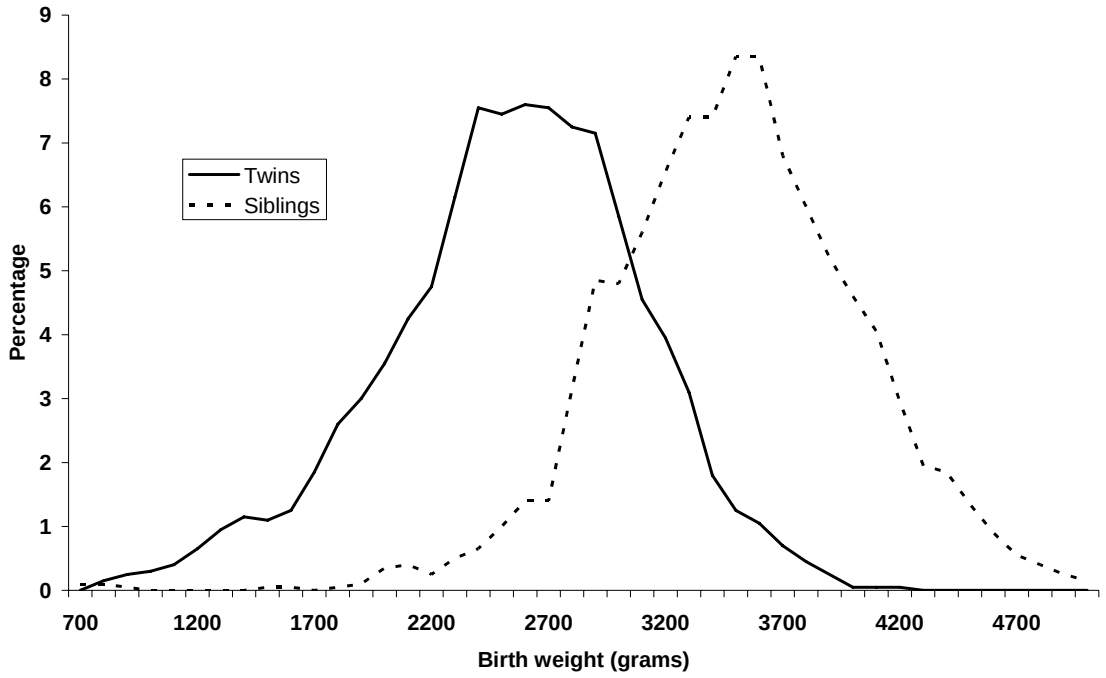
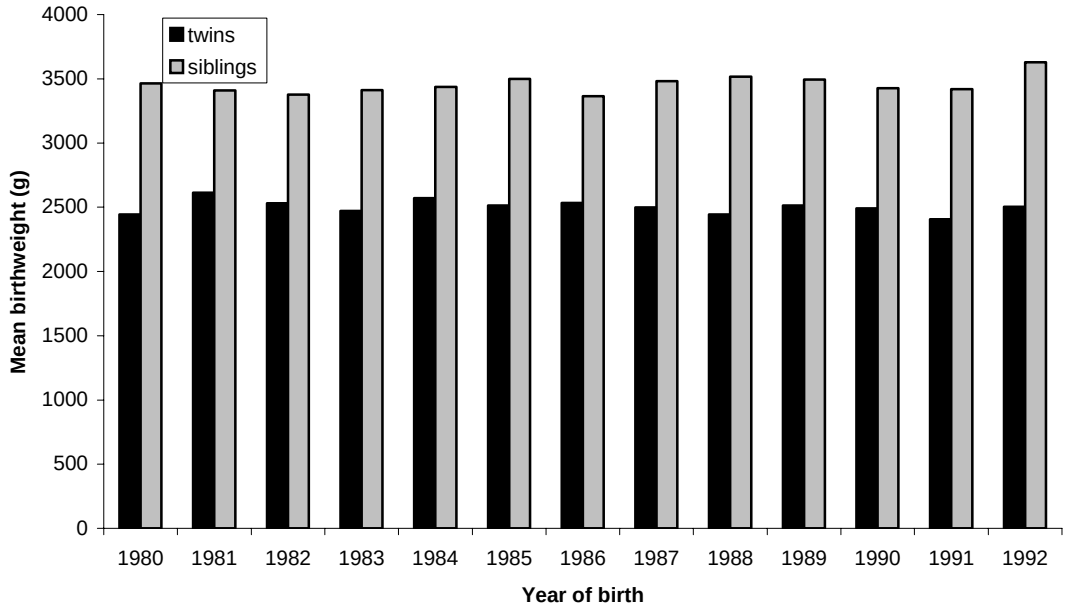


Figure 4-2:
Distribution of birth weight for twins and their singleton siblings



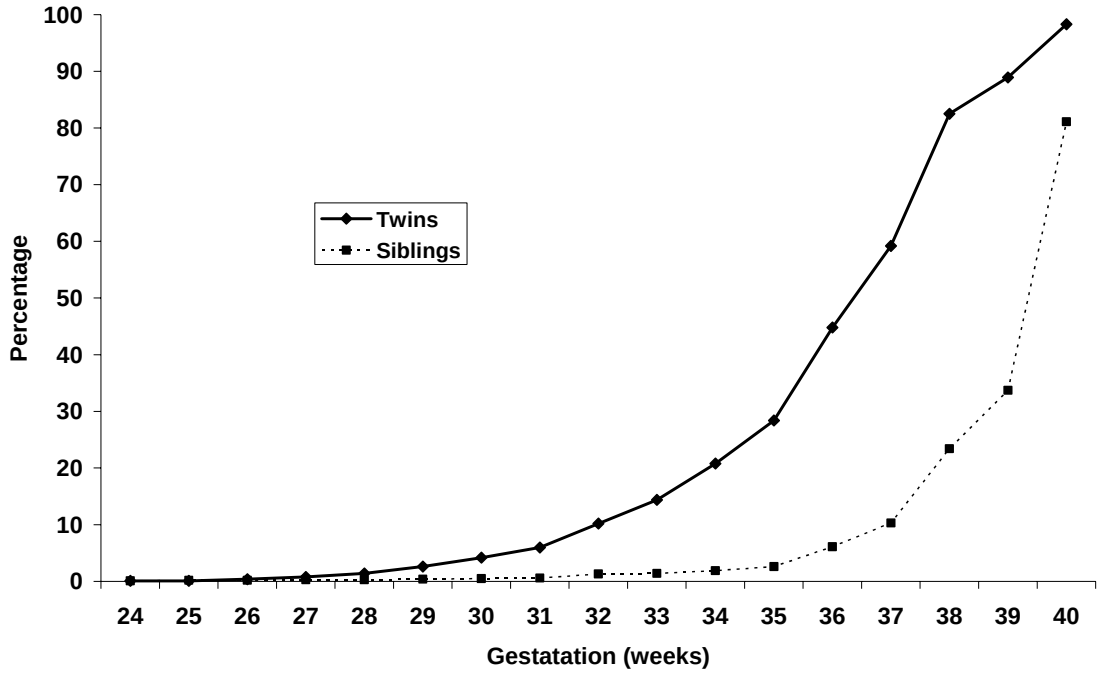
Mean birth weight did not vary across years of birth for both twins and their siblings (Figure 4-3).

Figure 4-3:
Mean birth weight by year of birth, for twins and their singleton siblings



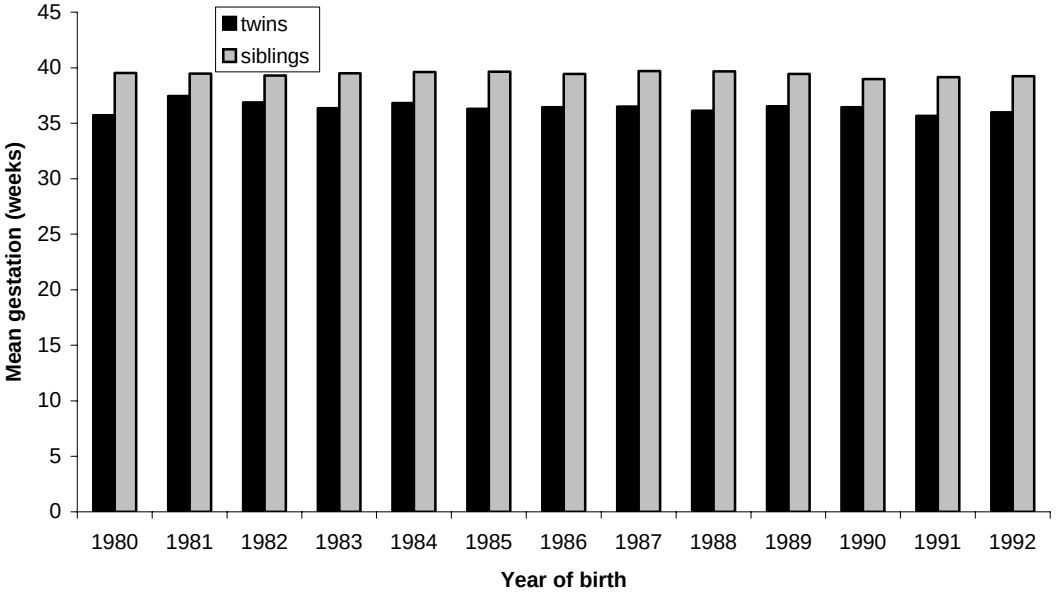
Gestation for twins ranged from 24 to 42 weeks, with a mean gestation of 36.4 weeks. This was significantly shorter than for singletons, whose mean gestation was 39.4 weeks (Figure 4-4). A greater percentage of twins than singletons were born prematurely, that is, under 37 weeks of gestation (59.3% vs. 9.9%, p<.0001). Length of gestation was not affected by the smoking status of mothers during pregnancy, for both twins and their singletons (36.1 weeks vs. 36.2 weeks for twins, 39.3 weeks vs. 39.3 weeks for siblings, respectively).

Figure 4-4:
Cumulative distribution of gestation for twins and their singleton siblings



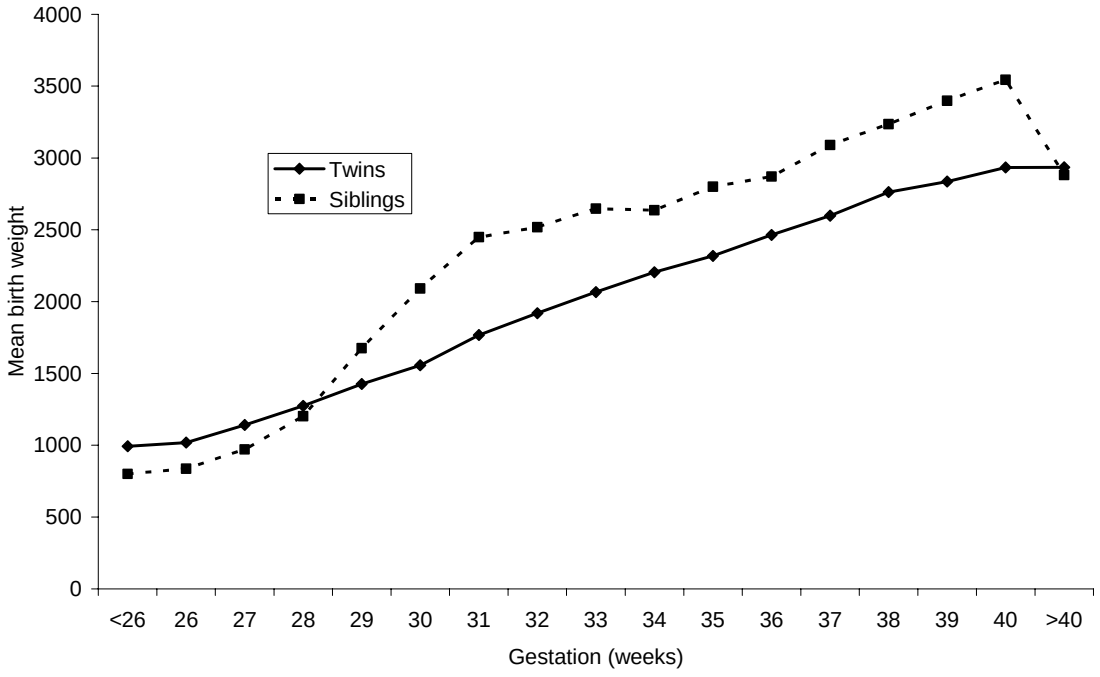
Mean gestation for twins and their singleton siblings did not change over the time period examined (Figure 4-5).

Figure 4-5:
Mean gestation by year of birth, for twins and their singleton siblings



For each completed week of gestation between 28 and 40, twins had a lower mean birth weight than their singleton siblings (Figure 4-6).

Figure 4-6:
Mean birth weight by gestation for twins and their siblings



However, there was no difference in birth weight at each week gestation, between same-sex twins and opposite sex twins, for both girls (Figure 4-7) and boys (Figure 4-8).

Figure 4-7:
Mean birth weight by gestation for girls in WA twin families

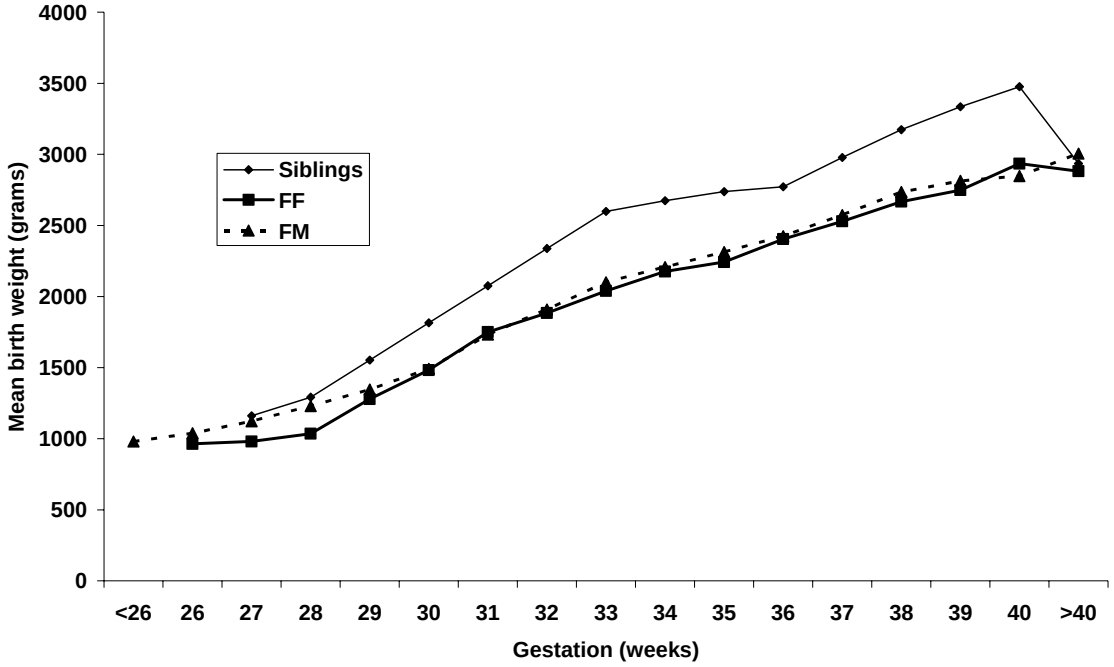
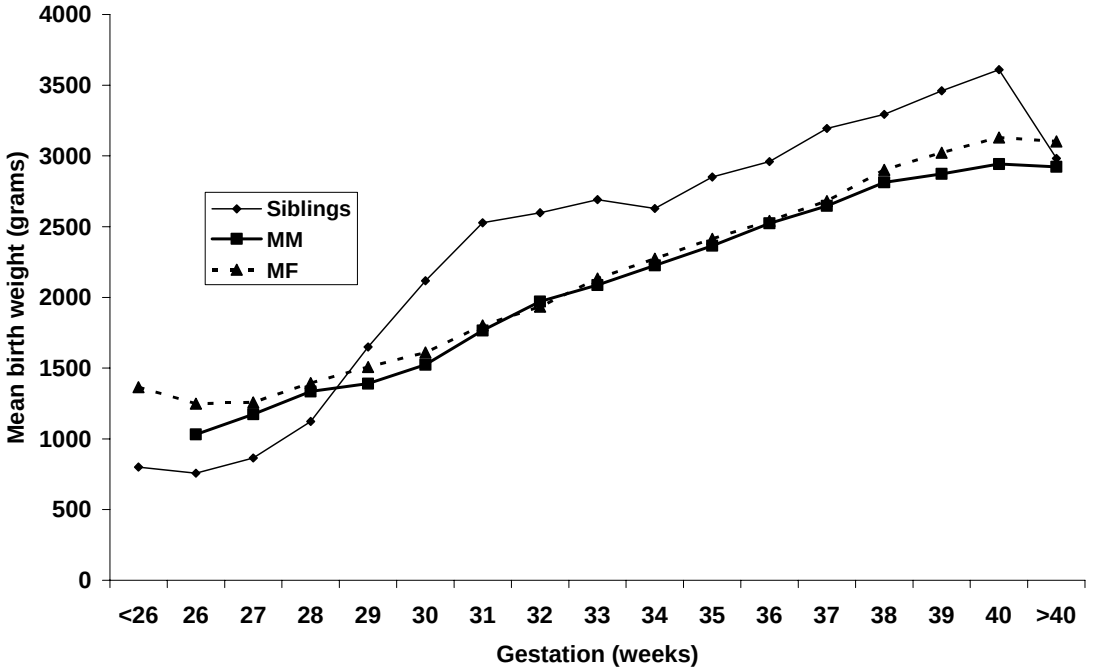


Figure 4-8:
Mean birth weight by gestation for boys in WA twin families



4.5.4.1 Risk factors for low birth weight and gestation

Twins were likely to be born weighing under 2500g if their mother was under 25 years old and if the mother smoked during pregnancy (p=0.014 and p=0.0037, respectively) (Table 4.14).

Table 4.14:
Percentage of twins and singleton siblings under 2,500g at birth for each potential risk factor

RISK FACTOR	TWINS		SIBLINGS	
	% low birth weight	*p value for difference*	*% low birth weight*	*p value for difference*
Mother's age at time of birth				
Under 25	53.0	.014	4.3	.38
25 and over	45.4		3.2	
Mother's marital status				
In first marriage	47.0	.34	3.1	.13
Other	44.0		5.3	
Mother's occupation				
Professional	47.1	.56	3.0	.44
Other	45.8		3.8	
Mother's education				
Tertiary	46.7	.93	3.5	.91
Other	46.4		3.4	
Mother had ever smoked				
Yes	53.7	<.001	4.4	.21
No	43.7		3.0	
Mother smoked during pregnancy				
Yes	53.2	.0037	5.3	.047
No	45.0		2.7	
SEIFA index for advantage/disadvantage				
Bottom 10%	54.0	.18	3.0	.90
Other	46.2		3.4	
SEIFA index for disadvantage				
Bottom 10%	53.8	.18	8.1	.11
Other	46.2		3.3	
SEIFA index for economic resources				
Bottom 10%	44.4	.86	6.2	.53
Other	46.5		3.4	
SEIFA index for education				
Bottom 10%	50.0	.49	5.4	.50
Other	46.3		3.4	

None of the risk factors examined was associated with premature birth in either twins or singletons (Table 4.15).

Table 4.15
Percentage of twins and singleton siblings born under 37 weeks gestation, for each potential risk factor

RISK FACTOR	TWINS		SIBLINGS	
	% born prematurely	*p value for difference*	*% born prematurely*	*p value for difference*
Mother's age at time of birth				
Under 25	46.2	.57	7.7	.16
25 and over	44.4		5.3	
Mother's marital status				
In first marriage	44.7	.98	5.6	.44
Other	44.6		7.1	
Mother's occupation				
Professional	46.1	.28	5.1	.31
Other	43.7		6.5	
Mother's education				
Tertiary	46.0	.59	4.3	.31
Other	44.5		6.1	
Mother had ever smoked				
Yes	45.9	.49	5.3	.59
No	44.2		6.1	
Mother smoked during pregnancy				
Yes	41.8	.21	4.4	.33
No	45.3		6.0	
SEIFA index for advantage/disadvantage				
Bottom 10%	52.6	.16	3.3	.56
Other	44.4		5.9	
SEIFA index for disadvantage				
Bottom 10%	52.5	.15	2.9	.47
Other	44.4		5.9	
SEIFA index for economic resources				
Bottom 10%	33.3	.33	7.1	.83
Other	44.8		5.8	
SEIFA index for education				
Bottom 10%	45.7	.85	0	
Other	44.7		6.0	

4.5.5 Time spent in Intensive Care or Special Care nurseries after birth.

Nearly 50% of twins spent time in special care nursery, which was a significantly higher percentage than siblings who did so (p<.0001). A significantly higher percentage of twins than their siblings were also admitted to neonatal intensive care after birth (Table 4.16).

Table 4.16:
Percentage of children who spent time in intensive care or special care nurseries after birth

VARIABLE	TWINS (N=2848)	SIBLINGS (N=1196)	P VALUE
Neonatal intensive care (NICU)	38.1	5.4	<.001
Special care nursery (SCN)	48.5	9.0	<.001

4.5.6 Conditions and treatments needed immediately after birth.

Parents reported that a greater percentage twins than their siblings experienced respiratory distress after birth (p=0.001), and that they were more likely to require treatment from a respirator or ventilator, or feeding through a naso-gastric tube than their singleton siblings (Table 4.17). However, parents reported that the majority of their children required no treatment or had any adverse health outcomes immediately after birth.

Table 4.17:
Percentage of children with various conditions and treatments, after birth

VARIABLE	TWINS (N=2848)	SIBLINGS (N=1196)	P VALUE
Conditions			
Respiratory distress	17.7	4.1	.001
Convulsions	.21	.17	.78
None	68.3	89.8	.001
Treatments			
Respirator/ventilator	16.2	2.3	.001
Naso-gastric tube	31.2	3.3	.001
None	52.7	90.0	.001

4.5.7 Breast feeding

The majority of twins were breast fed for less than 6 months, while most of their siblings were breast fed for at least 6 months (Table 4.18). As a consequence, a greater percentage of twins than singletons first received other milk products before the age of 4 months (71% vs. 44%, p=.001).

Table 4.18:
Breast feeding patterns for twins and their siblings

LENGTH OF BREAST FEEDING	TWINS (N=2848)	SIBLINGS (N=1196)
Was not breast fed	16.5	6.5
Less than 3 months	24.3	13.8
At least 3 months but less than 6 months	19.2	15.3
At least 6 months	40.0	64.4

4.5.8 Health conditions diagnosed by a doctor

Mothers reported that 47% of their twins, and 56% of their singleton children had experienced no significant health conditions. However, twins were more likely to have been diagnosed with febrile convulsions and migraine headaches, and more likely to have been diagnosed with developmental delay or lag than their siblings (Table 4.19). No cases of Fragile X syndrome were reported among the children, and none of the siblings had cystic fibrosis or cerebral palsy.

Table 4.19:
Doctor diagnosed health conditions in children

CHILDHOOD CONDITION	TWINS (N=2848)	SIBLINGS (N=1196)	P VALUE
Heart problems	3.4	2.4	.091
Febrile convulsions	2.8	1.8	.044
Epilepsy	0.9	0.6	.34
Kidney Problems	0.3	0.6	.15
Arthritis or rheumatism	0.1	0.3	.11
Cancer	0.1	0.1	.89
Blood disorder	0.7	1.0	.39
Migraine or severe headaches	5.2	3.6	.028
At least one episode of otitis media	65.1	60.3	.003
Down Syndrome	0.1	0.3	.14
Spina Bifida	0.04	0.08	.53
Intellectual disability	0.6	0.4	.56
Developmental delay or lag	3.1	1.4	.002
Birth defects	2.7	2.3	.47

4.5.9 Attention deficit disorder variables

A series of questions was included in the questionnaires aimed at assessing the prevalence of some of the known behaviours associated with Attention Deficit hyperactivity disorder (ADHD). These were questions 40 to 45 inclusive on the twins' questionnaire (Appendix 2) and questions 27 to 32 inclusive on the siblings' questionnaire (Appendix 4). All but one of these symptoms was more common in twins than in their siblings (Table 4.20). Symptoms were combined to give some indication of the likely prevalence of ADHD (hyperactive type) and ADHD (inattentive type) (Professor David Hay, personal communication). A

positive response, defined as answering "pretty much/often" or "very much/very often", to at least 2 of: fidgeting or squirming in seat, difficulty waiting their turn, or interrupting or intruding on others; indicated that the children were likely to have the hyperactive type of ADHD. Children were assessed as likely to have the inattentive type of ADHD if there was a positive response to at least 2 of: difficulty keeping attention, losing necessary items, and difficulty organising tasks and activities. Both types of ADHD were more prevalent in twins than in their siblings. However, there was no difference between twins and their siblings in the percentage medicated for ADHD, either ever, or currently (Table 4.20).

Table 4.20:
Maternal-reported symptoms of ADHD in their children

VARIABLE	TWINS (N=2848)	SIBLINGS (N=1196)	P VALUE
Has difficulty keeping attention	10.7	7.7	.004
Loses things necessary for tasks	11.2	7.1	.001
Has difficulty organising tasks	8.4	6.9	.12
Fidgets, or squirms in seat	13.8	8.8	.001
Has difficulty waiting their turn	12.1	7.6	.001
Interrupts or intrudes on others	17.7	10.7	.001
ADHD (hyperactive)	11.9	6.9	.001
ADHD (inattentive)	8.5	6.3	.016
Medication for ADHD	3.3	3.3	.94
Still on medication for ADHD	2.3	2.3	.91

4.5.10 Conditions and operations relating to eyes, ears, nose and throat.

There was no difference between twins and their siblings in the reported prevalence of operations on the ear, nose and throat (Table 4.21). Most children were reported to have normal vision and hearing (86% of twins and 99% of siblings).

Table 4.21:
Operations on the ear, nose and throat

VARIABLE	TWINS (N=2848)	SIBLINGS (N=1196)	P VALUE
Tonsils removed	7.7	7.8	.92
Adenoids removed	8.4	7.5	.36
Grommets inserted	8.2	8.2	.98

4.5.11 Accidents

Seven hundred and fifty six children, or nearly 19% of all children, had experienced at least one accident in 12 months prior to the questionnaires being completed, with a higher percentage of twins than siblings recording accidents (19.9% vs. 15.9%, p=0.003). Children displaying symptoms of ADHD (hyperactive type) had a higher prevalence of accidents in the last year compared with children with no such symptoms (25% vs. 18%, p=.003). Most accidents were not serious in nature, with under 10% of them requiring admission to hospital (Table 4.22), and the majority (57.5%) resulting in either a reduction in or no change to daily activity (Table 4.23).

Table 4.22:
Treatment required for accidents experienced by twins and siblings

	TWINS (N=2848)	SIBLINGS (N=1196)	P VALUE
Admission to hospital	10.4	8.4	.42
Treatment at hospital emergency department	36.8	41.0	.29
Treatment by health professional	37.3	35.3	.62
Advice from a health professional	14.5	14.2	.92
Minor treatment at home	22.3	23.2	.80

Table 4.23:
Action required after accidents to twins and siblings

	TWINS (N=2848)	SIBLINGS (N=1196)	P VALUE
Staying in bed	12.9	10.5	.39
Some time off school	34.4	37.9	.39
Reduced activity only	47.7	45.8	.65
No change in amount or level of daily activity	10.8	9.0	.47

The most common types of injury were breaks or fractures, and sprains or strains, which accounted for nearly 50% of all injuries in children (Table 4.24).

Table 4.24:
Type of injury experienced by twins and their siblings

	TWINS (N=2848)	SIBLINGS (N=1196)	P VALUE
Head injury with loss of consciousness	5.1	6.8	.37
Broken bones or fractures	22.4	21.0	.69
Dislocations	2.8	4.2	.35
Sprains or strains	22.3	34.2	.003
Broken teeth or teeth knocked out	2.1	3.2	.42
Internal injuries	1.9	2.6	.57
External wound needing stitches	20.5	14.7	.08
External bruising	18.9	19.0	.99
Burns and scalds	2.3	3.7	.30

The most common places for accidents to occur were while the children were at school (23%), or while they were playing sport (26%) (Table 4.25).

Table 4.25:
Places where accidents occurred in children

	TWINS (N=2848)	SIBLINGS (N=1196)	P VALUE
Inside own home	14.7	13.2	.61
Inside someone else's home	4.8	2.6	.20
In own garden	14.8	13.2	.57
In someone else's garden	6.9	5.3	.43
At school	24.4	17.4	.05
Playing sport	24.2	32.6	.02
In a park or playground	8.3	6.3	.38
Bike riding	11.8	7.4	.08

Overall, boys were more accident-prone than their sisters (21.8% vs. 15.7%, $p<0.001$). Boys were more likely to be injured while bike-riding than girls (12.9% vs. 7.8%, $p=0.024$), but less likely to sustain an internal injury (1.2% vs. 3.4%, $p=0.032$).

4.5.12 Education

Twins experienced more difficulties with their education than their singleton siblings (Table 4.26). Mothers believed that a greater percentage of their twin children than their singleton children needed further remedial instruction at school, and that a greater percentage of their twin children needed extra help in all the areas that were examined (Table 4.26). However, a greater percentage of siblings than twins (16.0% vs. 11.4%, p=0.001) had attended classes for gifted and talented students.

Table 4.26:
Educational needs of children in twin families

VARIABLE	TWINS (N=2848)	SIBLINGS (N=1196)	P VALUE
Education			
General remedial class	5.0	2.3	.001
Remedial English	5.0	5.0	.97
Remedial reading	9.9	5.5	.001
Remedial maths	4.5	3.7	.22
No remedial work	69.4	70.2	.61
Extra work needed			
Oral language	5.8	2.4	.001
Written language	11.3	7.1	.001
Reading	10.8	5.6	.001
Number	8.2	5.6	.004
Motor skills	3.5	1.8	.003
None	75.8	84.7	.001

4.5.13 Parental satisfaction with their children's development and behaviour

Overall, parents reported that they were satisfied, or very satisfied, with all aspects of their children's development (Table 4.27), although they were less satisfied with the education of their twins than that of the twins' siblings.

Table 4.27:
Parent's reported satisfaction with aspects of their children's development

VARIABLE	TWINS (N=2848)	SIBLINGS (N=1196)	P VALUE
Education	89.0	91.5	.021
Getting along with siblings	75.0	69.9	.001
Getting along with other children	96.1	96.4	.64
Behaviour	93.6	93.8	.79
Co-ordination	96.6	97.5	.11

4.5.14 Use of health-related services

When compared with their siblings, twins had ever used many health-related services (Table 4.28), and had done so more often (Table 4.29). However, there was no difference in the rate of consulting mental health professionals between twins and their siblings.

Table 4.28:
Health services ever used by twins and their siblings

VARIABLE	TWINS (N=2848)	SIBLINGS (N=1196)	P VALUE
Paediatrician	62.0	40.3	.001
Hospital emergency department	53.2	49.4	.028
Hospital out patient clinic	35.5	31.7	.021
Admitted to hospital for at least 1 night	45.0	43.6	.40
Physiotherapist	14.2	11.2	.011
Speech pathologist	22.9	13.5	.001
Occupational therapist	7.5	4.4	.001
School psychologist	14.6	12.4	.065
Child mental health clinic	2.2	1.9	.56
Other Psychologist	4.4	4.3	.90
Other Psychiatrist	1.5	1.2	.59
Disability services commission	14.9	14.4	.66
Family and children's services	2.4	2.0	.42

Table 4.29:
Health services used 5 or more times by twins and their siblings

VARIABLE	TWINS (N=2848)	SIBLINGS (N=1196)	P VALUE
Paediatrician	11.2	8.2	.004
Hospital emergency department	4.3	4.2	.82
Hospital out patient clinic	7.0	4.8	.012
Admitted to hospital for at least 1 night	2.6	2.3	.59
Physiotherapist	5.2	4.8	.60
Speech Pathologist	12.6	6.8	.001
Occupational therapist	3.9	2.1	.003
School psychologist	1.9	1.0	.035
Child mental health clinic	0.8	0.6	.52
Other Psychologist	0.7	0.3	.14
Other Psychiatrist	0.2	0	NA
Disability services commission	0.7	0.4	.35
Family and children's services	0.6	0.8	.57

4.6 Descriptive statistics of the twins

4.6.1 Birth weight and gestation by zygosity

On average, DZ twins weighed more than MZ twins (2554g vs. 2407g, p<0.001), with males weighing more than females for both MZ and DZ twins. Twins of the same sex, irrespective of zygosity, weighed on average 80g less than twins of opposite sex (p=0.002). Gestation for DZ twins was, on average, half a week longer than for MZ twins (p=0.001), but there was no difference in gestation between same sex twins and opposite sex twins (36.3 weeks vs. 36.5 weeks, p=0.24) (Table 4.30).

Table 4.30:
Birthweight and gestation for twins by zygosity

ZYGOSITY	MEAN BIRTH WEIGHT (G)	MEAN GESTATION (WKS)
All MZ twins	2407.3	36.1
MZ female twins	*2346.0*	*36.1*
MZ male twins	*2462.2*	*36.2*
All DZ twins	2554.4	36.6
DZ female twins	*2481.3*	*36.5*
DZ male twins	*2629.2*	*36.8*
DZ opposite sex twins	*2555.5*	*36.5*
All same sex twins	2475.6	36.3
All opposite sex twins	2555.5	36.5

4.6.2 Zygosity

In the WATCH study, it was not feasible for all twins to have their zygosity determined from DNA analysis, as the cost for each pair was between $100 and $200. Hence, it was therefore necessary to determine zygosity by another means, and that any alternative method should provide a reliable and valid surrogate for the blood test. In this study, zygosity was assessed using a series of questions included on the twins' questionnaire (Appendix 2), and based on a method of determining zygosity developed by Cohen and colleagues (Cohen *et al* 1973, 1975) who used twins of known zygosity to test the validity of two alternative methods of assessing zygosity. For same-sex twins, parents were asked to report on their belief of the twins' zygosity (question 7) and then answer the next 12 questions (questions 8 to 19 inclusive) rating their

perception of the twins' similarity at the time they completed the questionnaire. The first six questions (numbers 8 to 13) concerned the degree to which parents rated their children's similarity for physical characteristics: height, weight, facial appearance, hair colour, eye colour and complexion, and five questions (numbers 14 to 18) concerned general identity and confusion: "Do they look as alike as two peas in a pod?" "Does their mother ever mistake one for the other?" "Does their father ever mistake one for the other?" "Are they sometimes mistaken for each other by other relatives?" and "Is it hard for strangers to tell them apart?" Question 19, "Do they have very similar personalities?" was not used in this analysis. Each of the six questions 8 and 13 were coded: 0=not at all similar, 1= somewhat similar, and 2=exactly similar. Questions 15 and 16 were combined to form the question: "Does either parent ever mistake one for the other?" This new question, together with questions 14, 17 and 18 were then coded: 0=no and 2=yes.

The two methods used by Cohen and colleagues were based on intuitive scores, which gave equal weighting to the 10 questions, and the discriminant score, using the weighted scores from a discriminant analysis of the above 10 questions (Cohen *et al* 1975). The intuitive score was computed by adding the responses to the 10 questions, and gave a range of scores of 0 to 20. Twins scoring 7 or less were designated DZ, those scoring 12 and over, MZ, while those in the middle range (that is, 8 to 11) could not have their zygosity determined precisely, and were therefore considered to have unknown zygosity (Cohen *et al* 1973). The discriminant score was calculated using the weightings developed by Cohen (Cohen *et al* 1973) and ranged from -0.29 to 44.47. All twin pairs whose discriminat score was greater than 26.71 were assigned to the MZ group, while pairs scoring below this figure, the DZ group. The two scores were very highly correlated (ρ=.971, $p<.001$), and there was 98% agreement between zygosity determined by DNA analysis and results from these two alternative methods (Cohen *et al* 1973).

Based on parental perception, nearly 10% of WATCH study parents were unsure of their twins' zygosity (Table 4.31).

Table 4.31:
Parental perception of zygosity in WA twins born 1980-1992

ZYGOSITY	N=1,769 PAIRS	PERCENTAGE
MZ	454	25.7
MZ female	215	
MZ male	239	
DZ	1163	65.8
DZ female	323	
DZ male	308	
DZ male-female	532	
Unknown	152	8.5
Unknown female	76	
Unknown male	76	

The mean total intuitive score for MZ twins in the WATCH study was 16.6, DZ twins, 10.9 and twins whose zygosity was uncertain, 15.6. All questions showed higher mean scores for MZ twins than for DZ twins (Table 4.32), with nearly all parents reporting that their MZ twins had similar eye colour and were mistaken for each other by relatives and strangers.

Table 4.32:
Intuitive scores for similarity of physical characteristics and identity/confusion as assessed by parents, by perceived zygosity

SIMILARITY OF PHYSICAL CHARACTERISTICS	MZ (N=403)		DZ (N=571)		UNSURE (N=136)	
	Mean	SD	Mean	SD	Mean	SD
Height	1.39	.56	.81	.63	1.29	.55
Weight	1.26	.57	.72	.65	1.26	.56
Facial appearance	1.44	.51	.52	.58	1.27	.46
Hair colour	1.92	.30	.91	.78	1.90	.33
Eye colour	1.95	.23	1.09	.83	1.89	.34
Complexion	1.90	.33	.92	.73	1.88	.36
Identity/confusion						
Like two peas in a pod	1.46	.89	.19	.59	1.00	1.00
Mistaken for each other by parents	1.39	.92	.33	.74	1.29	.96
Mistaken for each other by relatives	1.89	.46	.67	.94	1.82	.57
Mistaken for each other by strangers	1.92	.39	.70	.95	1.85	.52

Twins that were assessed as MZ and DZ by parents are perceived quite differently. Over 90% of MZ twins were rated "exactly similar" for both hair and eye colour, while for DZ twins, 26% are rated exactly similar for hair colour, and 39% for eye colour (Table 4.33). Nearly all MZ twins were mistaken for each other by other relatives and strangers, while only a small percentage of DZ twins were mistaken for each other by other relatives (6%) and strangers (4%).

Table 4.33:
Frequencies of each question, by zygosity

SIMILARITY OF PHYSICAL CHARACTERISTICS	DZ			MZ		
	0	**1**	**2**	**0**	**1**	**2**
Height	31.3	56.6	12.1	3.7	53.1	43.2
Weight	38.6	50.6	10.8	6.2	61.1	32.7
Facial appearance	52.0	43.9	4.1	0.5	54.8	44.7
Hair colour	34.8	38.9	26.3	0.8	6.7	92.5
Eye colour	30.4	30.4	39.2	0.2	4.5	95.3
Complexion	31.2	45.6	23.2	1.0	7.7	91.3
Identity/confusion	**No**		**Yes**	**No**		**Yes**
Like two peas in a pod	90.3		9.7	25.2		74.8
Mistaken for each other by parents	83.5		16.5	30.3		69.7
Mistaken for each other by relatives	66.6		33.4	5.7		94.3
Mistaken for each other by strangers	65.0		35.0	3.7		96.3

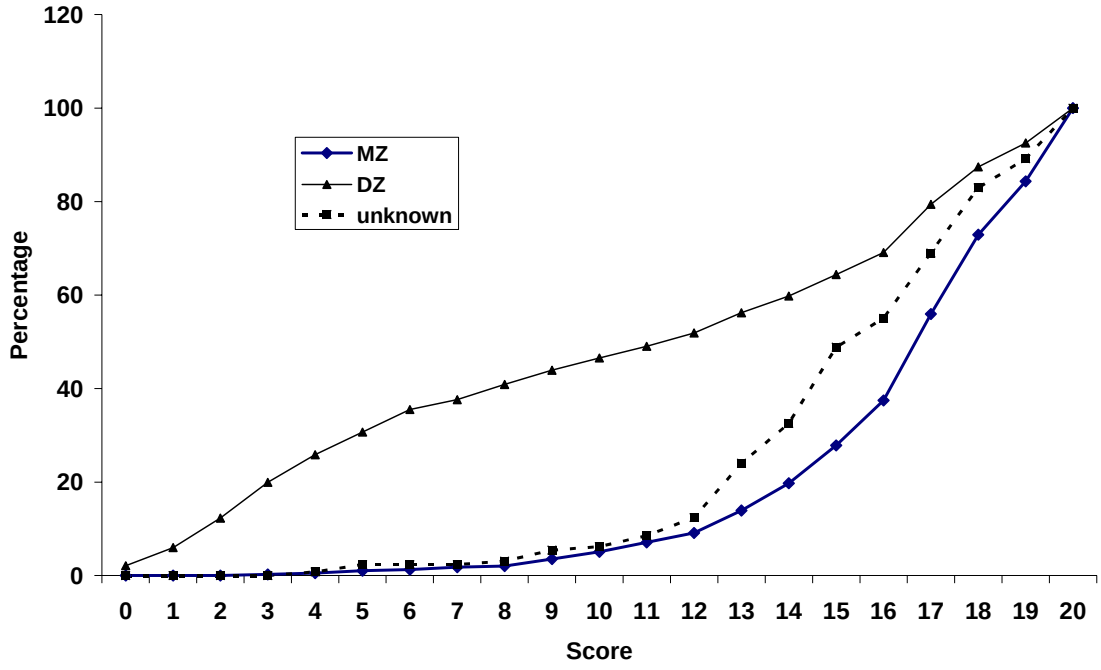
For twins whose parents were unsure of their zygosity, there was a statistically significant difference in the distribution of intuitive scores between them and DZ twins in all variables, while the only significant differences between them and MZ twins were in similarity of facial appearance, and being "alike as two peas in a pod" (Table 4.34).

Table 4.34:
Comparison of twins with unknown zygosity with MZ and DZ twins

SIMILARITY IN PHYSICAL CHARACTERISTICS:	UNKNOWN VS MZ	UNKNOWN VS DZ
Height	.16	<.0001
Weight	.96	<.0001
Facial appearance	.003	<.0001
Hair colour	.71	<.0001
Eye colour	.07	<.0001
Complexion	.34	<.0001
Identity/confusion		
Alike as two peas in a pod	<.0001	<.0001
Mistaken for each other by parents	.28	<.0001
Mistaken for each other by relatives	.31	<.0001
Mistaken for each other by strangers	.27	<.0001

Examination of the cumulative distribution of intuitive scores for twins of unknown zygosity showed a closer resemblance to the distribution of scores for MZ twins than for DZ twins (Figure 4-9).

Figure 4-9:
Cumulative distribution of total intuitive scores by perceived zygosity



Based on the intuitive scores for the 132 twin pairs of unknown zygosity, 120 of them, or 91% would have been classified as MZ using Cohen's method (Cohen *et*

al 1973). However, since no definite conclusion could be drawn on the zygosity of individual pairs, this group of twins was excluded from the analysis undertaken in the subsequent chapters.

4.7 Representativeness of WATCH study families

A measure of the socio-economic status of WATCH families is corroborated by the SEIFA codes (Socio-Economic Indexes For Areas) developed by the Australian Bureau of Statistics (ABS), which allows the ranking of regions/areas to assess the welfare of Australian communities (ABS 2000). They provide a method of determining the level of social and economic well being in that region. Four indexes that have been developed are:

- Index of advantage/disadvantage
- Index of disadvantage
- Index of economic resources
- Index of education and occupation

These indexes show where the affluent (as opposed to just high income earners) live; where disadvantaged (as opposed to the unemployed) live; and where the highly skilled and educated (as opposed to the tertiary educated people) live.

The SEIFA indexes of WATCH families were compared with those of the WA population according to the postcode of residents. Values for each index for the population at various percentiles are given in Table 4.35.

Table 4.35:
Cut-off scores for SEIFA index codes

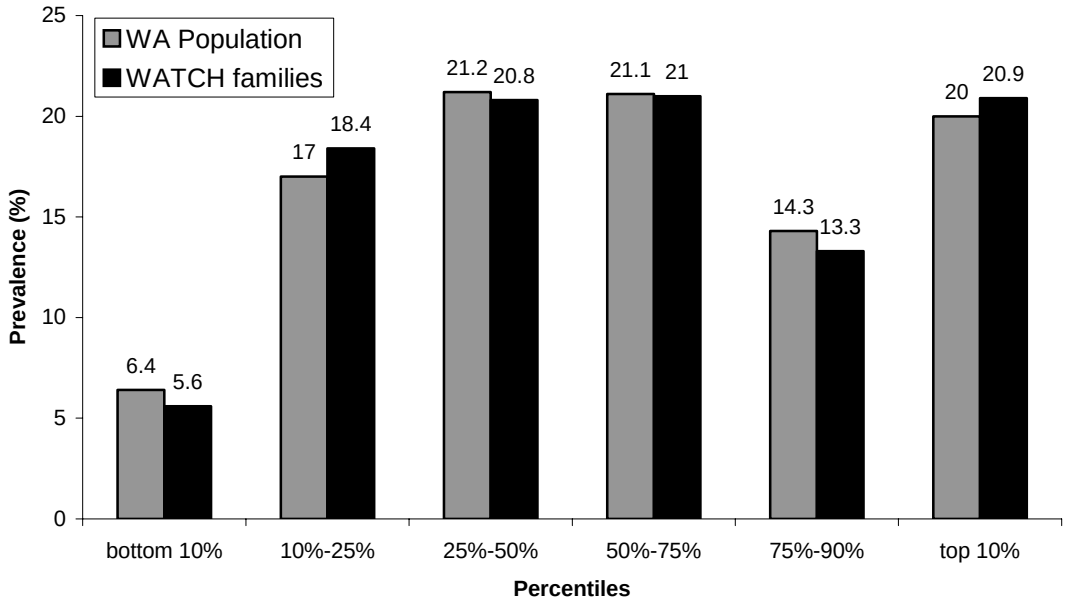
PERCENTILE	DISADVANTAGE	ADVANTAGE/ DISASDVANTAGE	ECONOMIC RESOURCES	EDUCATION & OCCUPATION
Bottom 10%	918.2	909.1	882.5	898.8
25%	959.8	932.1	912.3	930.3
50%	989.8	957.4	952.2	956.3
75%	1023.4	1008.9	1009.8	994.0
90%	1062.5	1077.2	1083.0	1086.5
Top 10%	1151.7	1207.3	1205.9	1222.7
WA Average	1003.6	1006.8	1006.7	998.5

4.7.1 Index of disadvantage

This index represents a continuum of advantage to disadvantage available for both urban and rural areas. Low values indicate areas of disadvantage, and high values indicate areas of advantage. It takes into account variables such as the proportion of families with high incomes, people with a tertiary education, and employees in skilled occupations.

The mean SEIFA index of disadvantage for WATCH families was 1008.9, which was significantly higher than that for the general population of WA (1003.6, $p<0.001$). WATCH study families were less likely to be in the bottom 10% when compared with the WA population ($p=0.04$), but equally likely to be in the top 10% ($p=0.14$) (Figure 4-10).

Figure 4-10:
SEIFA index of disadvantage, comparing WATCH study families with the WA population

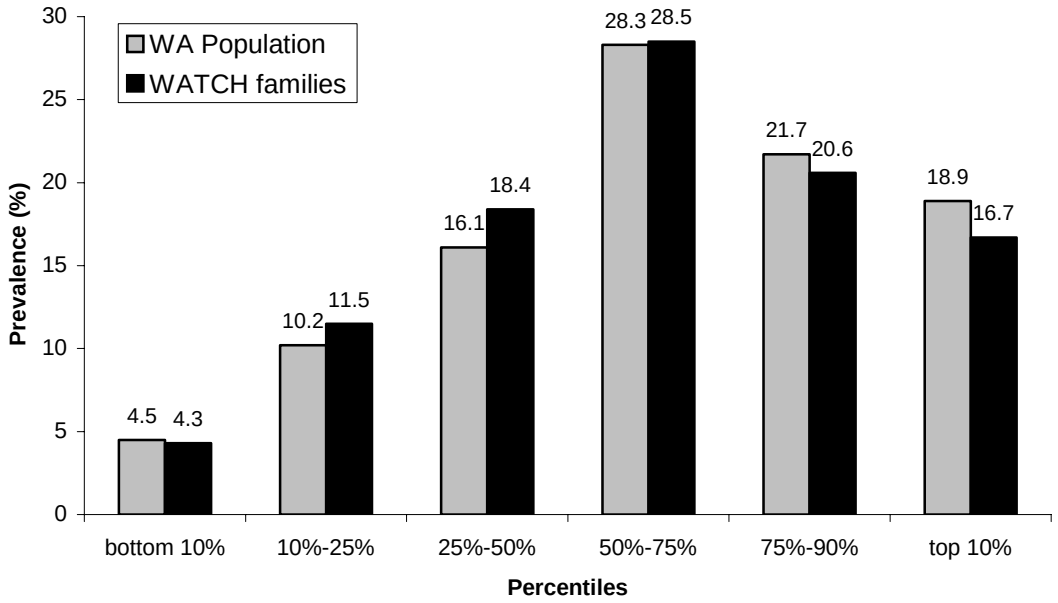


4.7.2 Index of advantage/disadvantage

This index is derived from attributes such as income, educational attainment, unemployment, and dwellings without motor vehicles. In particular it focuses on low-income earners, relatively lower educational attainment and high unemployment.

The WATCH study population had a mean index for advantage/disadvantage of 1005.6, which was not significantly different from the WA population average of 1006.8 (p=0.20). They were more likely to be in the top 10%, but equally likely to be in the bottom 10%, when compared with the WA population (p=0.0002 and p=0.584, respectively) (Figure 4-11).

Figure 4-11:
SEIFA index of advantage/disadvantage, comparing WATCH study families with the WA population



4.7.3 Index of Economic Resources

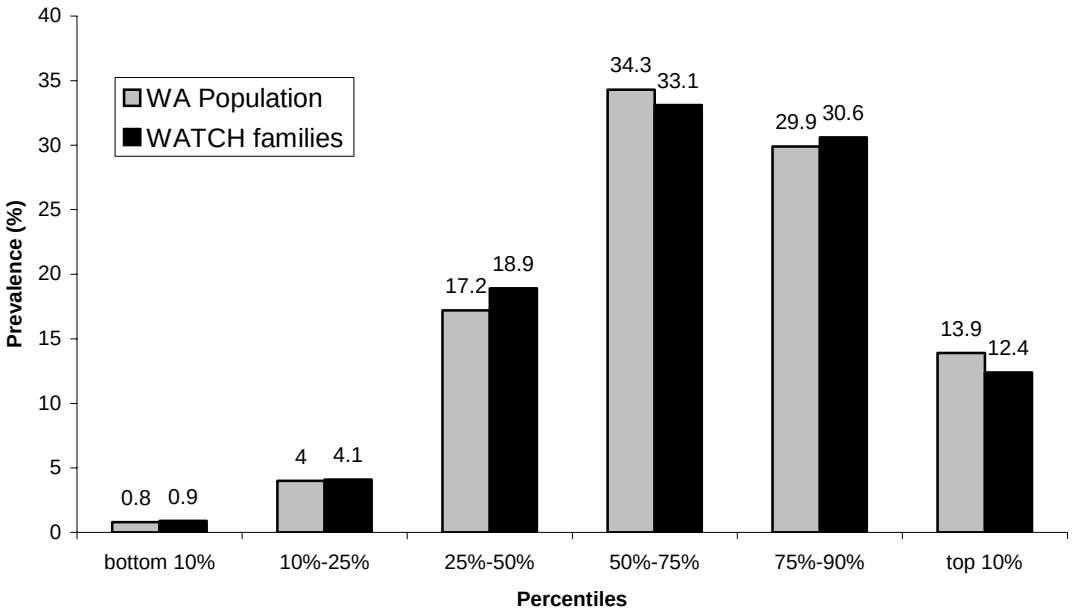
Variables in this index include those relating to the income, expenditure and assets of families, such as family income, rent paid, mortgage repayments, and dwelling size.

The Census variables which are summarised by this index reflect the income and expenditure of families, such as income and rent and home ownership. Additionally, variables which reflect non-income assets, such as dwelling size and number of cars, are also included. The income variables are specified by family structure, since this affects disposable income. A higher score on this index indicates that the area has a higher proportion of families on high income, a lower proportion of low income families, more households purchasing or owning dwellings and living in large houses. A low score indicates the area has a

relatively large proportion of households on low incomes and living in small dwellings (ABS 2000).

There was no difference in the mean index of economic resources between WATCH study families and the WA population (1006.5 vs. 1006.7 respectively, p=0.43). However, WATCH study families were more likely to be in the top 10%, but equally likely to be in the bottom 10%, when compared with the WA population (p=0.005 and p=0.147, respectively) (Figure 4-12).

Figure 4-12:
SEIFA index of economic resources, comparing WATCH study families with the WA population



4.7.4 Index of education and occupation

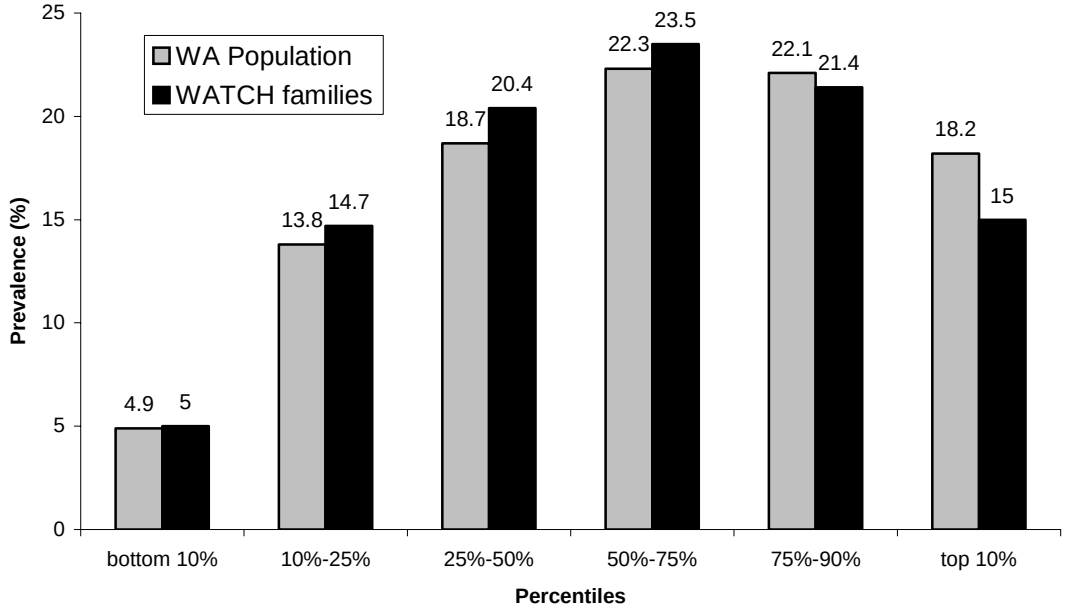
This index includes variables relating to the educational and occupational characteristics of communities, such as the proportion of people with a higher qualification or those employed in a skilled occupation.

The education variables in this index show either the level of qualification achieved or whether further education is being undertaken. The occupation variables classify the workforce into the Australian Standard Classification of Occupation (ASCO) major groups and the unemployed. This index does not include any income variables. An area with a high score on this index would have a high concentration of persons with higher education or undergoing

further education, with people being employed in the higher skilled occupations, rather than being labourers or unemployed. A low score indicates an area with concentrations or either persons with low educational attainment or unskilled or unemployed people (ABS 2000).

WATCH study families had a significantly lower mean index of education and occupation when compared with the WA population (994.8 vs. 998.5 respectively, p=0.006). WATCH study families were less likely to be in the top 10%, but equally likely to be in the bottom 10%, when compared with the WA population (p<0.0001 and p=0.767, respectively) (Figure 4-13).

Figure 4-13:
SEIFA Index of Education and Occupation, comparing WATCH study families with the WA population



4.8 Summary of results

The WATR is a complete population-based register of multiple births born in WA from 1980-1997, when compared with data from the WA Department of health (Gee 1992, Gee & O'Neill 2001). The WATCH study is a unique collection of twin-family data, and an important resource for genetic epidemiological research. It has accumulated data not only on the twins, but also on their parents and siblings. The collection of detailed family structure allowed the precise relationship between every individual within each family to be

characterised, and makes it possible to conduct the analysis of the genetic epidemiology of asthma with confidence (see Chapter 6). By developing a comprehensive set of questionnaires, data on a wide variety of health and behaviour outcomes have been collected. These data can be used as a sampling frame for future research into a wide range of conditions that impact on the health and well-being of WA families.

There was no difference in demographic characteristics of parents when comparing study participants with non-participants (Hansen *et al* 2000). WATCH study parents were no different from the WA population with respect to SEIFA indices of advantage/disadvantage and economic resources, suggesting that there was no difference in the both occupational and educational characteristics of families, and income and dwelling size. There was concern that the nature and length of questionnaires sent to families would discourage parents who had attained a lower level of education from participating. This did not prove to be the case, as, on average, WATCH parents had a lower mean index of education and occupation when compared with the WA population. 1996 Australian census figures reveal that 23% of the working population in WA aged 15 years or over had a tertiary qualification (ABS 2000). This compared with 21% of WATCH study parents who were employed at the time they completed the questionnaires. It seems reasonable, therefore, to conclude that WATCH study families were not significantly different from the general population of WA.

Results from the WATCH study confirm results from many previous studies that, on average, twins are born earlier and at lower birth weight than singleton children. In addition, first-born twins were heavier at birth than their co-twin, and MZ twins had a lower mean birth weight than DZ twins. The stillbirth, neonatal and perinatal death rates were higher in twins than in the general population of WA. A low rate of CP in the twin population was probably due to the fact that families who had experienced the death of one of their twins were not contacted, and it is known the surviving co-twin is at increased risk of CP.

Nine percent of parents were unsure of the zygosity of their twins. Results from a discriminant analysis developed by Cohen and colleagues (Cohen *et al* 1973,

1975) suggests that most of these twins are likely to be MZ. Although most people may have trouble distinguishing MZ twins from each other, mothers can almost always tell them apart, and would therefore be unlikely to assess them as being “alike at two peas in a pod”, and this confusion may lead them to be unsure of the zygosity. Quite often in the past, if there were two placentae, mothers would be told that their twins were non-identical when it is possible for MZ twins to have two placentae. Again, this information, together with perceived likeness in appearance, could put doubt in parents’ minds making them unsure of the zygosity.

Several important differences between twins and their siblings were found. Twins were more likely to have been delivered by caesarean section, and have been admitted to the NICU immediately after birth than their siblings. During pregnancy, mothers reported more complications with their twins than with their singleton children. Singleton children were breast-fed for a longer time on average than twins, and as a result, had other milk products introduced later. Twins were more likely than their singleton siblings to display behaviours characteristic of ADHD, but were no more likely to have been medicated for the condition, or consulted a mental health professional. This difference in behavioural patterns may explain why twins were also more likely to have experienced at least one accident in the last year than their siblings. The fact that twins needed and received more remedial instruction at school could have led to parents being less satisfied with their twins’ education and behaviour than their singleton children. Twins visiting a paediatrician more than their siblings was probably the result of their prematurity.

The majority of parents reported that they had ever smoked, and about one-fifth of them still smoked. Twenty percent of mothers reported that they had smoked during pregnancy. However, the majority of families had household rules covering no smoking being allowed inside the family home and car.

It was impossible to determine the ethnic background of parents of twins, as participants seemed not to understand the question about the national group with which they identified themselves (question 7 on parents’ questionnaire, Appendix 3). Answers to this question included labour party, anglo-saxon,

Anglican and Roman Catholic, but the majority left this question blank. It was only possible to ascertain that the majority of parents were born in Australia and had English as their main language at home.

The WATCH study has collected comprehensive demographic, health and behaviour data from WA twins born between 1980 and 1992, as well as from their parents and siblings. To my knowledge, this is the first such data set assembled in Australia, and will prove to be a unique and valuable resource for future research in Australia.

CHAPTER 5
EPIDEMIOLOGY OF ASTHMA AND ATOPY IN WA TWINS AND TWIN FAMILIES

5.1 Preface

Numerous genetic and epidemiological risk factors for asthma and atopy have been identified. This section reviews the literature relating to epidemiological risk factors. Genetic risk factors will be discussed in chapter 6. Using questionnaire data from the WATCH study, I will describe factors characterising asthma in twins and twin families, including health, behavioural and educational outcomes. Results from both the univariate and multivariate analyses to identify variables that have significant effects on the risk of asthma and atopy are given.

5.2 Review of the Literature

5.2.1 Introduction

Asthma is one of the most common diseases of childhood, and one of the leading causes of hospital admission across all ages, while as many as 50% of individuals in Western societies suffer from some sort of atopy (Cookson 1994, Hopper *et al* 1995). It has been identified as one of the ten leading causes of disease burden in WA (DOHWA 2004). Worldwide, it is estimated that around 300 million people currently have asthma (GINA 2006). As the proportion of the world's population that becomes urbanised increases, there is likely to be a marked increase in the number of asthmatics over the next few years. There could be as many as an extra 100 million people diagnosed with asthma by the year 2025 (GINA 2006). In many areas of the world, especially in developing countries, people with asthma do not have access to basic medical care and hence to asthma medication (GINA 2006). Asthma accounts for about 15 million or around 1%, of all disability-adjusted life years (DALYs) lost every year. This is a similar number as those lost for diabetes, cirrhosis of the liver, or schizophrenia (GINA 2006). Asthma accounts for about 1 in 250 deaths

worldwide, many of them being preventable due to suboptimal long-term medical care or delay in seeking medical attention during the final attack.

There are many barriers to reducing the burden of asthma, and one of the most obvious is the low public health priority it tends to be assigned. This is mainly due to the importance of other diseases such as cancer and cardio-vascular diseases, and other respiratory diseases such as tuberculosis and pneumonia. The lack of reliable data on asthma morbidity and mortality limits the chances of improving this priority. There are also important environmental and societal barriers to reducing asthma, which include indoor and outdoor air pollution, with tobacco smoking, both active and passive, forming a substantial component of such exposure, and factors relating to poverty, poor education and poor infrastructure, such as access to health care facilities.

Both genes and the environment play a role in the aetiology of asthma and atopy (Los et al. 2001). The continued rise in prevalence and incidence of these conditions over the last few decades is unlikely to be due to changes in genetic composition, and therefore it is important to identify those environmental risk factors that are amenable to change, so that the burden of asthma and atopy on individuals, families and society can be reduced.

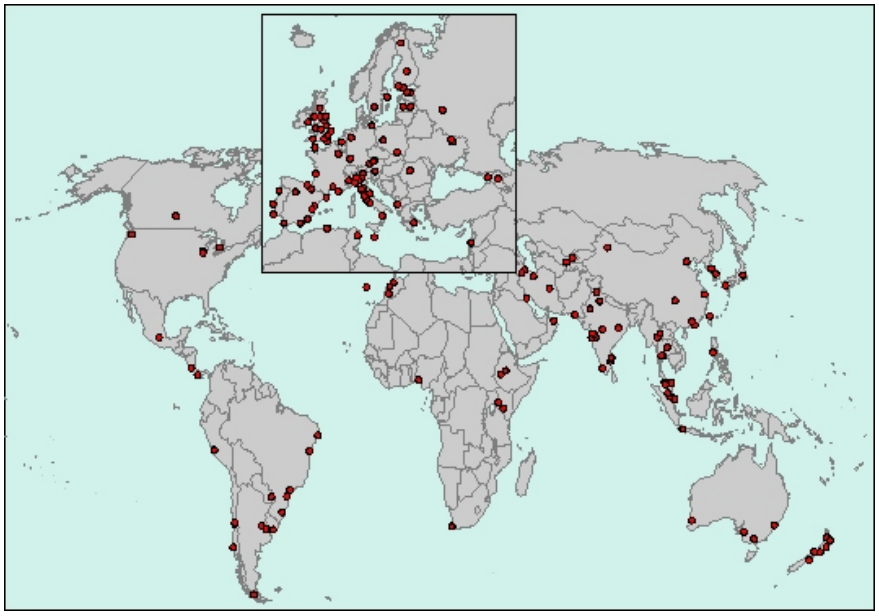
5.2.2 International comparisons

In Australia, recent surveys show that 14-16% of children and 10-12% of adults have a diagnosis of asthma, and a higher percentage (20-30%) report that they had wheezed in the last year (AIHW 2005). These rates were high by international standards, and had increased during the 1980s and 1990s (ECRHS 1997, ISAAC 1995). In WA, asthma has been identified as one of the ten leading causes of disease burden (DOHWA 2004).

Two of the major international collaborative studies to be conducted since the 1980s have compared the prevalence and incidence of asthma and atopy in countries around the world. The International Study of Asthma and Allergies in Childhood (ISAAC) aimed to describe the prevalence and severity of asthma of asthma, rhinitis, and eczema in children living in different centres and to make comparisons with and between countries (ISAAC 1995). It studied children aged

13-14 years in 155 centres across 56 countries, and children aged 6-7 years old in 91 centres from 38 countries (http://isaac.auckland.ac.nz) (Figure 5-1).

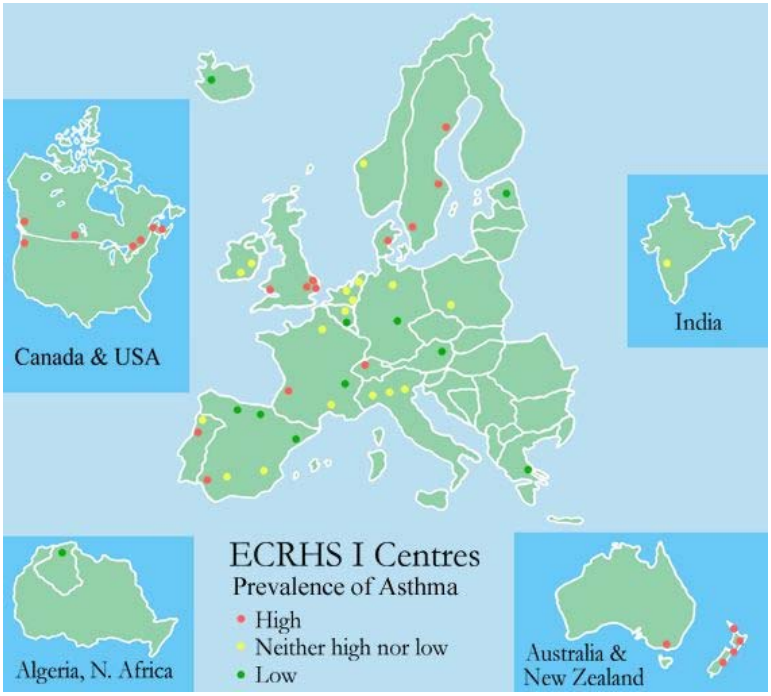
Figure 5-1:
Centres involved in Phase I of ISAAC



(from: http://isaac.auckland.ac.nz/PhaseOne/Centres/CentFrame.html)

The European Community Respiratory Health Survey (ECRHS) studied adults to determine the variation in the prevalence of asthma, asthma-like symptoms and bronchial reactivity in Europe, as well as some non-European centres, including Melbourne, Australia. Fifty-six centres from 35 countries completed at least part of the first survey in the 1990s. This study also aimed to estimate the variation in exposure to known or suspected risk factors for asthma, to measure their association with asthma, and to further assess the extent to which they may explain variations in prevalence across Europe (www.ecrhs.org/home.htm) (Figure 5-2).

Figure 5-2:
Centres involved in ERCHS I



(From www.ecrhs.org/home.htm)

Both studies reached similar conclusions, that is:

1. asthma prevalence is increasing worldwide;
2. asthma is more common in Western countries and less common in developing countries;
3. asthma is more prevalent in English-speaking countries;
4. asthma prevalence is increasing in developing countries as they become more Westernised or communities become more urbanised; and
5. the prevalence of other allergic disorders may also be increasing worldwide.

In the ISAAC study, the prevalence of self-reported wheeze in the last 12 months among 13-14 year olds ranged from 1.9% to 35.3% (Asher *et al* 1995). The corresponding figure for 6-7 year olds was 1.6% to 27.2%. The highest rates were found in English-speaking countries, with lower rates recorded in centres from Eastern Europe and developing countries (Asher & Weiland 1998, Burney *et al* 1996).

A strong correlation between the ISAAC and ECRHS prevalence data was found when the 17 countries who participated in both studies were examined (Pearce *et al* 2000) (Table 5.1).

Table 5.1:
Summary of prevalence of asthma in adults and children from the ECRHS and ISAAC surveys

COUNTRY	ADULTS	CHILDREN 6-7 YRS	CHILDREN 13-14 YRS
Australia	11.9	27.1	28.2
New Zealand	11.3	26.5	24.4
England	8.4	22.9	20.7
USA	7.1		16.5
Canada		14.7	16.5
Sweden	6.8	8.0	10.4
France	5.5	9.3	12.6
Germany	4.1	3.6	5.7
Italy	4.5	8.6	9.9
Spain	6.3	6.2	10.5
Estonia	2.0	1.4	3.0
Latvia		1.6	4.3
Poland		2.5	2.4
India	3.5	3.7	4.5
Japan		18.2	18.9
Hong Kong		7.7	11.2

Among the countries included in ISAAC, Australia had the third highest 12-month prevalence of asthma symptoms, the 7th highest 12-month prevalence of allergic rhino-conjunctivitis and the 12th highest 12-month prevalence of atopic eczema syndrome (ISAAC Steering Committee 1998). Among the Australian ISAAC centres, the one in Perth reported a higher prevalence of asthma in 13-14-year-olds than both Melbourne and Sydney (30.2%, 26.6% and 24.8%, respectively), and slightly lower than Adelaide (30.4%), whilst in the 6-7-year age group, the highest prevalence was reported in Melbourne (28.6%), with Perth (28.4%) being higher than both Adelaide (27.5%) and Sydney (24.4%).

Both the ISAAC and ECRHS surveys have confirmed that asthma prevalence has increased over the last 3 decades (Beasley *et al* 2000, ISAAC Steering

Committee 1998). This is evident in both Westernised and developing countries (Table 5.2).

Table 5.2:
Changes in prevalence of asthma and asthma-symptoms in children and young adults

COUNTRY	PERIOD	1ST STUDY (%)	2ND STUDY (%)
Australia	1982-1992	5.6	10.5
England	1966-1990	3.9	6.1
New Zealand	1975-1989	7.9	13.3
USA	1981-1988	3.1	4.3
Finland	1961-1986	0.1	1.8
France	1968-1982	3.3	5.4
Sweden	1971-1981	1.9	2.8
Israel	1986-1990	7.6	9.6
Japan	1982-1992	7.1	13.5
Papua New Guinea	1973-1984	0.0	0.6
Taiwan	1974-1985	1.3	5.1

Although there were substantial increases seen in the prevalence of asthma during the 1980s and early 1990s, there is now some evidence that the rate has plateaued. Researchers in the USA (Akinbami & Shoendorf 2002), Switzerland (Braun-Fahrlander *et al* 2004), and Italy (Ronchetti *et al* 2001) all observed no further increase in the prevalence of asthma during the latter half of the 1990s when compared with the 1980s and early 1990s. In Melbourne school children, there was a significant decrease in the prevalence of wheeze between 1993 and 2000, although the rates of eczema and allergic rhinitis continued to rise (Robertson *et al* 2003).

5.2.3 Economic burden of asthma

Asthma represents a considerable cost to the economy, both to the health-care system and to the community in general. The *National Asthma Campaign* estimated that the total cost of asthma in Australia was in the $585 to $720 million range each year (AIHW 2005). In Western Australia, it was estimated that hospital admissions due to asthma cost $6 million per year, and asthma

accounted for 3% of all GP visits (DOHWA 2004). The costs of asthma in the UK and the USA were also considerable. Weiss and colleagues (Weiss *et al* 1992b) estimated that the cost of asthma in the USA was $US6.2 billion in 1990, with 59% of the cost being contributed by the health care system. In the UK, asthma was estimated to cost the society around £843 million per year (at 1988 prices), with £344 million per year being the total health-care costs (Sculpher & Price 2003). In Switzerland asthma costs amounted to CHF 1,200 million per year and were considered a major health care cost factor (Szucs *et al* 1999).

Both direct and indirect costs contribute to the overall cost of asthma. Direct costs are those related to the cost to the health-care system and include the costs of medical consultations and pharmaceuticals, ambulance and hospital admissions, as well as various indirect and allied health treatment costs. Indirect costs result from absenteeism from work and/or school, lost productivity at work and travel time to attend treatment. For children, there are the additional costs borne by the family to provide care for the sick child, either by parents being absent from work, or employing a special carer. These indirect costs account for about half of the total cost of asthma.

Medical specialists estimate that only about 6% of adult asthmatics have severe or very severe disease, meaning that a high proportion of the cost of asthma relates to a minority of adult cases (National Asthma Campaign of Australia 2006). However, very mild asthmatics, who account for about 80% of cases, contribute considerably to the overall economic burden of the disease, as they still require emergency, out-patient and hospital care during acute exacerbations of their symptoms.

Much of the cost of asthma could be reduced or avoided by improved disease control in the individual. Poor asthma control is characterised by inappropriate management plans or poor compliance with medication regimes (Debley *et al* 2004). From a community perspective, optimal individual disease control will ultimately lead to reduced cost of the disease. However, this net benefit to society may only be achieved through an increase in some costs to the individual, such as pharmaceuticals.

5.2.4 Quality of life and disability

Many studies have shown that asthmatics can experience a reduced quality of life. In a major Australian survey, people with asthma rated their health lower than people without asthma, with females reporting a lower perceived health status than males (Pearce *et al* 2000). Adolescents with asthma also reported having a lower perceived well-being when compared with well teenagers (Forrest *et al* 1997). In children, asthmatics report lower perceived well-being, limitations in physical activity and more emotional symptoms than children without chronic disease (Bussing *et al* 1995, Creer *et al* 1992, Ortega *et al* 2002). Children with asthma have also been found to have more learning disabilities than those without asthma (Fowler *et al* 1992). A greater proportion of asthmatics reported having days away from work or study (11.4% in the last 2 weeks) compared with 7.9% of non-asthmatics. In the 2 weeks preceding the 2001 National Health Survey, 2.6% of all asthmatics reported taking time off work or study, and 3.2% reported having days of reduced activity that they attributed to asthma (ABS 2002). There was a higher prevalence of depression reported among people with asthma when compared with people without asthma (Goldney *et al* 2003).

Asthma accounts for about 15 million or around 1%, of all disability-adjusted life years (DALYs) lost every year (GINA 2006). This is a similar number as those lost for diabetes, cirrhosis of the liver, or schizophrenia. In the US during 1994-5, 1.4% of children experienced some degree of disability due to asthma. Disabling asthma resulted in an annual average of 20 restricted activity days, including 10 days lost from school – almost twice the level of illness burden as experienced by children with disabilities due to other types of chronic conditions (Newacheck & Halfon 2000).

5.2.5 Definition of asthma

The epidemiological definition of asthma remains controversial (Woolcock 1987), and as yet there is no single test or gold standard. But there are several key aspects upon which there is general agreement. Period prevalence, or active symptoms during the last 12 months, is the most generally accepted measure (Anderson 1989). Asthma may be better thought of as a syndrome than a

disease as clear biological markers defining disease are unavailable (Gross 1980). Since the 19th century, asthma has been known as "episodic dyspnoe with healthy respiration between attacks caused by spasm of the bronchi" (Los et al. 2001). In 1995, a global initiative in asthma defined asthma as:

> "a chronic inflammatory disorder of the airway in which many cells play a role, in particular mast cells, eosinophils, and T lymphocytes. In susceptible individuals this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and cough particularly at night and/or in the early morning. These symptoms are usually associated with widespread but variable airflow limitation that is at least partly reversible either spontaneously or with treatment. The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli" (NIH 1995).

In epidemiological studies, two methods are commonly used to identify asthma in an individual; self-report of a diagnosis of asthma, with or without physician confirmation or self-report of certain symptoms, such as wheeze; and presence of bronchial reactivity on challenge (Banerjee *et al* 1987, Burrows *et al* 1991, Speight *et al* 1983). In the general population, asthma tends to be under-diagnosed, and in children there is an overlap between the diagnoses of chronic and wheezy bronchitis and asthma, especially in the under 5 year olds (Taussig *et al* 1981). Although wheeze is the predominant symptom of asthma, some individuals have other symptoms such as breathlessness or cough, and are thus not included in studies focussing on wheeze. It may be more appropriate to examine the prevalence of symptoms of wheeze, breathlessness and cough rather than the syndrome labelled as asthma (Woolcock 1987). Many researchers advocate the use of bronchial-hyperresponsiveness (BHR) to make a diagnosis of asthma. In the clinical setting, where asthmatics predominate the case mix of subjects, sensitivity to BHR has been found in over 90% of subjects (Boushey *et al* 1980). However, population studies show poor sensitivity to BHR in detecting asthma. This may be because the general population included many mild or borderline asthmatics, as well as non-asthmatics who have atopy, a family history of asthma, or other respiratory disorders, which have all be shown to correlate with BHR (Britton & Tattersfield 1986). A large population-

based epidemiological study of asthma using BHR to diagnose the condition is probably not feasible due to the prohibitive costs involved.

Given the difficulty of defining asthma, even in a clinical setting, one aim of an epidemiological study is to use epidemiological methods to compare the prevalence and incidence of asthma within and between populations using some standardised method. To date, an existing physician diagnosis or the presence of a given number or type of symptoms (e.g., cough wheeze, etc.) as reported on questionnaires has been the primary means of identifying asthma in epidemiological studies. Self-report of information on questionnaires and medical record data have been shown to be highly correlated, with agreement between physician-diagnosis of asthma and clinical records being as high as 98.5% (McGill *et al* 1998). High sensitivity (75%) and specificity (81%) were found in a sub-set of children surveyed at school by questionnaire and then later receiving a clinical diagnosis of asthma (Wolf *et al* 1999). Questionnaires have been used by researchers in a number of highly regarded community-wide epidemiological studies (Samet 1987, Woolcock 1987), as well as by ECRHS and ISAAC, whose aims were to compare asthma prevalence between and within countries (ECRHS 1997, ISAAC Steering Committee 1998).

5.2.6 Atopy and its relationship with asthma

In the past, atopy was considered to be any allergic condition - hay fever, asthma or atopic dermatitis/eczema (Host & Halken 2000, Johansson & Bennich 1967). However, since the discovery of immunoglobulin E (IgE), atopy can be considered to be a condition that is characterised by the persistent and heritable production of specific IgE in response to common environmental allergens (Jarvis & Burney 1998, Morton 1982). The atopic diseases are now the most common chronic conditions of childhood in Australia (ABS 2002), and across all ages, as many as 50% of individuals in Western societies suffer from some form of atopy (Cookson 1994, Hopper *et al* 1995). Up to 90% of asthmatic children have been reported to be atopic, with a higher frequency of family history of atopy (Sears *et al* 1993b). However, it has also been shown that up to 40% of non-asthmatics are atopic, as defined by positive skin prick test and/or serum specific IgE to at least one common allergen (Pearce *et al* 1999).

Asthma is usually associated with atopy; asthmatics tend to be more atopic than non-asthmatics (Gergen *et al* 1988, Kalliel *et al* 1989). Allergy is recognised as playing an important role in asthma. Most asthmatics are sensitised to at least one common allergen (Burrows *et al* 1989, Sears *et al* 1993a) with exposure to house dust mites (HDMs) being the most important documented risk factor for airway hyperresponsiveness and asthmatic symptoms (Custovic *et al* 1996, Sears *et al* 1993a, Sporik *et al* 1992). Other important allergens include those from pets, especially cats, cockroaches, the mould *Alternaria* (Sears *et al* 1993b, Warner *et al* 1990), and airborne outdoor pollens, especially grasses (Peat *et al* 1993, Suphioglu *et al* 1992).

While most studies have shown no association between the prevalence of atopy and asthma, some studies have (Charpin *et al* 1988, Wieringa *et al* 1997). In Australia, little or no association between the prevalence atopy and asthma was shown (Peat *et al* 1995), and in Hong Kong, Malaysia and China, who have similar prevalence rates for atopy (58%, 64% and 49%, respectively), different rates of asthma were reported (7%, 3% and 2%, respectively) (Pearce *et al* 1999).

As a result of the ISAAC phase II cross-sectional study in school children aged 5-7 years, two distinct asthma phenotypes have been identified: “infectious asthma” and “atopic asthma”. Infectious asthma is characterised by the induction or triggering of asthma through repeated early infections, with environmental allergens not playing a major role (Host & Halken 2000). It is thought to have a more favourable prognosis than atopic asthma (Stein *et al* 1999, von Mutius *et al* 1999). Asthma and wheezing in pre-school children is mainly associated with infections, whereas in school-aged children, it is usually associated with atopy (Silverman 1993, Stein *et al* 1997).

The allergens associated with atopic asthma depend on many factors: age, climatic, seasonal and social factors, and housing conditions (Pearce *et al* 1999). House dust mite (HDM) allergy shows the strongest association with asthma in temperate and humid areas (Sporik *et al* 1992), whereas allergy to the fungus *Alternaria* has been shown to be important in arid regions of the USA (Platts-Mills & Carter 1997).

5.2.7 Risk factors

5.2.7.1 Introduction

The epidemiological literature provides some insights into the natural history of asthma, including age at onset, frequency and intensity of relapse and remission, and some information on major asthma morbidity as measured by hospitalisation, and mortality. A number of risk factors of asthma and atopy have been identified and can be classified as genetic or environmental (AIHW 2005). Constitutional or genetic risk factors are those which are thought to predispose individuals to the development of asthma and include a positive family history, genetic mutation, age, sex and the presence of co-existing atopy. They define at-risk individuals, but as they cannot be modified by intervention, surveillance of them is of limited value to the potential reduction of the disease. They are important, however, in determining which individuals are at risk, so that intervention with modifiable factors will be effective in reducing the prevalence of disease.

While genetic factors are clearly important in determining risk of development of asthma, environmental factors are still likely to be the primary determinants of the expression of disease. Exposure to environmental risk factors occurs throughout the life-span and includes the *in utero* period, early childhood, later childhood and adulthood. All are potentially amenable to modification, with the hope of reducing the prevalence of the disease.

Some risk factors, such as diet and lifestyle, may act by affecting the risk of acquiring asthma, changing the course of a pre-existing disease or triggering symptoms. Some examples of triggers are exercise, viral infections, irritants, such as air pollution, specific allergens, for example, house dust mites (HDM), moulds and fungi, and certain food preservatives. Although these factors have been extensively studied and reviewed, the evidence on the effects of exposure remains controversial. There is conflicting evidence on the relationship between asthma and exposure to pets and other allergen sources, breast feeding, other aspects of diet and feeding, overweight and obesity, and exposure to childhood infections.

The adverse effects of smoking, both active and passive, are well-known. Asthmatics who smoke have more asthma exacerbations, poorer asthma control and less beneficial response to medication, than asthmatics who do not smoke (Chalmers *et al* 2002, Pedersen *et al* 1996). For children, exposure to environmental tobacco smoke (ETS) has been shown to be associated with both the development of asthma and exacerbation of symptoms (ABS 2002, Martinez *et al* 1992). Evidence from international studies confirms that parental smoking is related to more severe asthma in their children (Strachan & Cook 1998). It has also been shown that asthmatic children exposed to ETS have increased morbidity and asthma symptoms (Murray & Morrison 1988), more frequent attacks (Chilmonczyk *et al* 1993), more severe asthma (Murray & Morrison 1988, Strachan & Cook 1998), and increased health care utilisation (Evans *et al* 1987).

Risk factors for childhood asthma can also be divided into medical and socio-economic factors. Among the most common medical risk factors identified are allergy (Lundback 1988), eczema (Wickens *et al* 1999a), antibiotic use (Wickens *et al* 1999b), food allergies (Wright *et al* 2001), respiratory syncytial virus (RSV) infection (Martinez *et al* 1995), breast feeding (Horwood *et al* 1985, Oddy *et al* 1999), gender (Lundback 1988), and parental asthma (Horwood *et al* 1985). Among the socio-economic factors, the one most consistently identified has been indoor smoke exposure (Stein *et al* 1999). Conflicting results have been found when evaluating any relationship with family income and parental education (Horwood *et al* 1985, Wickens *et al* 1999b), and day-care attendance (Kramer *et al* 1999, Nystad *et al* 1999).

5.2.7.2 Age and gender

Asthma incidence varies with age, with children under 5 years of age having the highest rate (Broder *et al* 1974, Dodge & Burrows 1980, McWhorter *et al* 1989). It occurs more frequently in boys than girls, with the male to female ratio approaching 2 to 1 (Gergen *et al* 1988, Weiss & Speizer 1993, Wieringa *et al* 1999, Yuan *et al* 2003). However, in adulthood this trend is reversed (CDC 2001, Mannino *et al* 2002, Thomsen *et al* 2006c) with females having a higher prevalence of asthma than males. The timing of this reversal is unclear, with some studies suggesting that it happens during adolescence (Gergen *et al* 1988,

Manfreda *et al* 1993, Norrman *et al* 1994, Venn *et al* 1998), whereas others highlight the 30s as being when the gender reversal occurs (de Marco *et al* 2002, Laor *et al* 1993).

Follow-up studies of asthma show that a large percentage of children, in some cases up to 79%, will out grow their asthma before adulthood (Grol *et al* 1996). However, in a later prospective study, Grol et al. (Grol *et al* 1999) found that only 25% of asthmatic children were asthma-free by the age of 42 years. Another study found that 40% of children with infrequent episodic asthma, and 70% of those with frequent or persistent asthma still had symptoms in mid adult life (Martin *et al* 1980). The variations in the recovery rate could be due to a number of methodological issues, such as differences in study design, diagnostic criteria, age at inclusion, and definition of recovery from asthma.

5.2.7.3 *In utero* and neonatal exposures

Several risk factors for asthma that occur in the pre- or perinatal period have been identified. These include premature birth, low birth weight, and maternal smoking during pregnancy (Gilliland *et al* 2003, Infante-Rivard 1995, Kelly *et al* 1995, Steffensen *et al* 2000, von Mutius *et al* 1993). Information about *in utero* influences on lung development is limited, and studies describing early airway function have concentrated on describing placental function, intra-uterine growth retardation (IUGR), sex and ethnicity, as well as environmental factors such as smoking, maternal nutrition, and respiratory infections in the neonate (Vrijlandt & Gerritsen 2004). Adverse effects on lung development can result from either mother-related factors, such as hypoxia, intoxication, pre-eclampsia leading to premature birth, or child-related factors, such as congenital abnormalities relating to renal function and diaphragmatic hernia (Greenough *et al* 2004). Both wheezing and respiratory infections have been linked to IUGR (Brooke *et al* 1995, Vik *et al* 1996), and a significant relationship between gestation-adjusted birth weight and lung function has been shown in children who were small for gestational age (SGA) when born (Rona *et al* 1993).

Maternal infections during pregnancy have been shown to be related to the development of asthma in the child (Calvani *et al* 2004, Hughes *et al* 1999, Xu *et al* 1999), while pregnancy complications, such as ante-partum haemorrhage

(Strachan *et al* 1996), and threatened abortion (Calvani *et al* 2004, Nafstad *et al* 2003, Stazi *et al* 2002b) have been associated with both wheezing and asthma. Other uterus-related complications of pregnancy, such as infections of the genital tract, ectopic pregnancy, haemorrhage, anaemia, insufficient cervix, placental dysfunction, rhesus immunisation, and preterm contractions, were associated with asthma in 4 year old Norwegian children (Nafstad *et al* 2000, Nafstad *et al* 2003). Additional factors identified during the prenatal and neonatal periods as possibly having a relationship with atopic diseases include malpresentation of the foetus, use of opioids during labour, caesarean section, and neonatal illness in the first week of life (Annesi & Strachan 1995).

Low birth weight has been associated with asthma in some studies (Braback & Hedberg 1998, Seidman *et al* 1991, Shaheen *et al* 1999, Steffensen *et al* 2000) but not others (Fergusson *et al* 1997, Lewis *et al* 1995). Premature infants who require mechanical ventilation after birth have also been shown to be at increased risk of wheezing illnesses (Frischer *et al* 1993, von Mutius *et al* 1993). Children with low birth weight are at risk of having narrow airways and/or early respiratory symptoms and of developing sensitisation or wheeze in early childhood. Young mothers have an increased risk of having a child with asthma when compared with mothers over 30 (Infante-Rivard 1995), which is thought to possibly relate to lower birth weights and reduced lung function. However, this effect may be confounded by smoking as smoking rates in young adult females have not shown the same declining rates as those in young males and have not changed substantially over the last few decades (DOHWA 2001). Associations between perinatal factors and other manifestations of atopy have shown opposite trends to those with asthma, with greater gestational age, and high maternal age both leading to increased risk of hay fever (Braback & Hedberg 1998, Strachan *et al* 1996), and eczema (Olesen *et al* 1997).

5.2.7.4 Breast feeding

The relationship between breast feeding and risk of atopy was first shown in 1936, when it was reported that the incidence of infantile eczema was higher in both partially breast fed and non breast fed babies when compared with fully breast fed babies (Grulee & Sanford 1936). Since then, the evidence linking breast feeding with the aetiology of asthma and atopy has been inconsistent. Studies have reported a protective effect of breast feeding on asthma (Dell & To 2001, Infante-Rivard 1993, Marini *et al* 1996, Miller 2001, Oddy *et al* 1999, Saarinen & Kajosaari 1995, Tariq *et al* 1998) and recurrent wheezing (Rusconi *et al* 1999, Schwartz *et al* 1990, Wright *et al* 1995), an increased risk of asthma related to breast feeding (Astarita *et al* 1988, Kaplan & Mascie-Taylor 1985, Sears *et al* 2002, Takemura *et al* 2001, Wright *et al* 2001), as well as showing no association (Burr *et al* 1993, Gruskay 1982, Wilson *et al* 1998). Differences in these relationships may be explained by the definition of breast feeding. It has been defined as any versus none (McKeever *et al* 2001, Schwartz *et al* 1990, Wright *et al* 1995), by duration (Dell & To 2001, Rusconi *et al* 1999, Sears *et al* 2002), or as exclusive versus non-exclusive breast feeding (Oddy *et al* 1999, Takemura *et al* 2001, Wright *et al* 2001). In another study, Wilson and colleagues reported an increased risk of wheezing in those infants who had solid food introduced before the age of 4 months (Wilson *et al* 1998).

Breast feeding seems to protect young children (up to age 6 years) from asthma (Dell & To 2001, Miller 2001, Oddy *et al* 1999), while studies of older children (Takemura *et al* 2001) and young adults (Sears *et al* 2002) reported that breast feeding was likely to increase the risk of asthma. Areas of high asthma prevalence have found breast feeding to have both a protective effect (Oddy *et al* 1999) and to be associated with an increased risk of asthma (Sears *et al* 2002). However, Wright et al. (1999) reported a protective effect of breast feeding only for children of non-asthmatic mothers (Wright *et al* 1999), and the Canadian Task Force on the Periodic Health Examination concluded that breast feeding may reduce the incidence of atopy in children with a family history of atopy, but not in the general population (Wang 1994).

The increase in asthma has paralleled the increase in obesity (Magarey *et al* 2001, WHO 1998), which is a leading cause of morbidity from a variety of

diseases in later life (Beilin 1999, Berenson *et al* 1998). But the relationship between obesity, breast feeding and childhood asthma is still unclear. A number of studies have shown a positive association between obesity and asthma (Genuso *et al* 1998, Luder *et al* 1998, von Kries *et al* 2001, von Mutius *et al* 2001), but not between obesity and atopy (von Mutius *et al* 2001). Breast feeding has been shown to be protective against both obesity (Butte 2001, Gillman *et al* 2001, Hediger *et al* 2001, von Kries *et al* 1999) and childhood asthma (Oddy *et al* 1999, Saarinen & Kajosaari 1995). In a large prospective study, no association between breast feeding and being overweight was found, but a higher BMI was a risk factor for asthma (Oddy *et al* 2004).

5.2.7.5 Socio-economic status

Lower rates of asthma and atopy in developing countries than in the developed nations have led to suggestions that socio-economic status plays an important role. It has been shown that the prevalence of asthma is not consistently related to SES, but that severe asthma may be more frequent in poorer communities (Mielck *et al* 1996).

Studies in Britain, Singapore and southern China/Hong Kong found higher parental-reported rates of childhood asthma in subjects of high SES (Goh *et al* 1996, Kaplan & Mascie-Taylor 1985, Lai *et al* 1996, Peckham & Butler 1978), whereas studies in the USA tend to find the reverse (Litonjua *et al* 1999, Perskey *et al* 1998). But mostly no association has been found (Mitchell *et al* 1989, Peat *et al* 1989, SIDRIA 1997, Strachan *et al* 1994). Studies in poorer countries, particularly Africa, have examined urban/rural differences rather than traditional Western markers of SES (Addo Yobo *et al* 1997, Keeley *et al* 1991). The ISAAC study found that countries in the lowest quartile of GNP had the lowest positive responses to questions about symptoms of asthma, rhinitis and eczema (Stewart *et al* 2001).

Poverty is thought to be a risk factor for asthma mortality and hospital admission (Carr *et al* 1992, Claudio *et al* 1999, de Palo *et al* 1994, Ehrlich & Bourne 1994, Goh *et al* 1996, Higgins & Britton 1995, Jones & Bentham 1997, Mielck *et al* 1996, Strachan *et al* 1994, Watson *et al* 1996, Wissow *et al* 1988). However, poor management of asthma in patients from a poor background is a

consistent theme in the literature, and may be explained by poor access to health care services (Jones & Bentham 1997), patients' characteristics (Morgan & Olding 1995), and patients preferring to attend hospital emergency departments rather than a general practitioner (Watson *et al* 1996).

Regional variations have been shown in Australia (Peat *et al* 1994, Robertson *et al* 1992, Robertson *et al* 1991), Britain (Duran-Tauleria & Rona 1999, Jones & Bentham 1997), the USA (Aligne *et al* 2000), Norway (Lindbaek *et al* 2003), Italy (SIDRIA 1997), and New Zealand (Mitchell *et al* 1989). And even within cities, there are differences in the prevalence of asthma and atopy by racial group. In the USA, asthma is more prevalent in black children than in white children (Crain *et al* 1994, Cunningham *et al* 1996b, Weiss & Wagener 1990), but residents of impoverished areas of inner-cities are predominantly black, and asthma is worse in inner cities than elsewhere (Grant *et al* 1999, Kattan *et al* 1997, Weiss *et al* 1992a). In Australia, asthma is higher in the Indigenous population than the non-Indigenous population (AIHW 2005). All children living in urban areas are at increased risk of asthma compared with children living in rural areas, and the higher prevalences are not due to race or low income *per se*, but possibly to other factors characteristics of urban dwelling. These include: passive smoking, substandard housing, increased time spent indoors, poor diet, decreased access to health care etc (Aligne *et al* 2000, Evans 1992, Platts-Mills & Carter 1997). The high asthma mortality among inner city children in the USA has been related to cockroach allergen (Rosenstreich *et al* 1997), but in Britain, the burden of cockroach allergen in children was much lower (Luczynska *et al* 1995).

It is unlikely that there is a genetic basis for the link between race and asthma, as black populations in Africa have low asthma prevalence (Mohamed *et al* 1995, Platts-Mills & Carter 1997) and immigrants from countries with low asthma prevalence (e.g. India or Malaysia) who now live in high asthma-prevalence countries such as England or Australia tend to develop asthma at similar rates similar to those in the countries to which they have migrated (Leung 1996).

Living in a farm environment has shown to have a protective effect on allergic diseases (Johansson *et al* 2001), rhinitis (Braun-Fahrlander *et al* 1999, Kilpelainen *et al* 2000, Riedler *et al* 2001, von Ehrenstein *et al* 2000) and on reported asthma and wheeze (Kilpelainen *et al* 2000, Riedler *et al* 2001, von Ehrenstein *et al* 2000), and on doctor-diagnosed current asthma (Kilpelainen *et al* 2002). However, children who lived on a farm were more likely to belong to larger families (Kilpelainen *et al* 2002), and more likely to have had a cat or dog as a pet in childhood. Pet exposure has been associated with a decreased risk of atopy and hay fever (Hesselmar *et al* 1999, Nafstad *et al* 2001, Roost *et al* 1999, Svanes *et al* 1999). However, the relationship between farm environment and risk of atopy remained after adjusting for pet ownership.

5.2.8 The "Hygiene Hypothesis"

It has now been well established that the rate of asthma and atopy increased dramatically during the 1980s and 1990s, and in many Western countries, this occurred at the same time as improvements in hygiene and health standards, with the reductions in family size, and with increased uptake of vaccination against common childhood conditions such as measles, mumps, rubella and varicella.

In 1989, Strachan reported an inverse relationship between family size and the risk of hay fever. This led to the development of the so-called "hygiene hypothesis", that exposure to infection in early life protects against the development of allergy (Strachan 1989). This hypothesis claims that growing up in a more hygienic environment with decreased exposure to infections, could lead to an altered immune response resulting in an increase in the risk of the atopic diseases. At birth, the immune system is skewed towards the characteristics of allergic individuals, but during infancy and early childhood this profile is usually shifted towards the non-allergic phenotype, perhaps through exposure to infections and other environmental stimuli (Holt *et al* 1997, Martinez & Holt 1999, Prescott *et al* 1999). It is thought that a delayed or absence of exposure to infections in childhood may cause the immune system to retain the early allergic profile present at birth, thus increasing the risk of allergy.

The results from Strachan's 1989 UK study have since been replicated in German (von Mutius *et al* 1994) and Italian (Forastiere *et al* 1997) children. Three other studies have provided indirect support for the "hygiene hypothesis": children who suffered from measles in Guinea-Bissau, West Africa, were less likely than those who did not contract measles, to develop skin sensitivity to mites, an indication of atopy (Shaheen *et al* 1996); Japanese children who were tuberculin-positive were less likely than tuberculin-negative children to have symptoms of asthma and allergy (Shirakawa *et al* 1997); and Italian military recruits were less likely to have asthma, allergic rhinitis or atopy if they were serologically positive to hepatitis A (Matricardi *et al* 2000). The hygiene hypothesis is also supported by findings from studies showing that populations with a high incidence of respiratory infections report lower rates of allergic disease (Flynn 1994, von Mutius *et al* 1994).

Larger family size, the number of older siblings and child-care attendance have all been used as indirect markers of infectious burden, as they are associated with increased exposure to infections (Holberg *et al* 1993, Infante-Rivard *et al* 2001, Marbury *et al* 1997, Osterholm 1994). The strongest support for the hygiene hypothesis comes from studies of hay fever and eczema (Bodner *et al* 1998, Pekkanen *et al* 1999, Ponsonby *et al* 1998, Wickens *et al* 1999b), and the evidence for asthma is less clear (Bodner *et al* 1998, Lewis *et al* 1996, Pekkanen *et al* 1999, Ponsonby *et al* 1998, Rona *et al* 1999, Wickens *et al* 1999b). In one review article (Karmaus & Botezan 2002), all 17 studies reviewed showed an inverse relation between hay fever and the number of siblings; 9 of the 11 studies reported showed the same relationship for eczema, but for asthma and wheezing, only two-thirds of 31 studies reported an inverse association (Karmaus & Botezan 2002). The number of siblings was found to be related to the risk of asthma in some studies (Ponsonby *et al* 1998, Rona *et al* 1999, Rusconi *et al* 1999, Sunyer *et al* 1997, Westergaard *et al* 2005, Wickens *et al* 1999b) but not in others (Nystad *et al* 1999). Another study found that the risk of asthma decreased with increasing number of siblings, but only for cases diagnosed at 3-4 years of age (Infante-Rivard *et al* 2001). For persistent cases, the protective effect was no more for two or more siblings compared with one sibling. In studies where an inverse relation between birth order and asthma was found, the number of older siblings, and not younger siblings, was

important (Lewis *et al* 1995, Rona *et al* 1999, Sunyer *et al* 1997, Westergaard *et al* 2005). Other studies have found no relation between the risk of asthma with either the number of older siblings or being first born (Bodner *et al* 1998, Crane *et al* 1994, Ponsonby *et al* 1998, Rasanen *et al* 1997, Rona *et al* 1999, Sears *et al* 1996, Wickens *et al* 1999b).

The evidence on the effect of attending day care on the subsequent development of asthma, hay fever and eczema is also unclear. One study found that day care attendance was protective against the development of allergic disease (Kramer *et al* 1999), while others showed no association (Backman *et al* 1984, Ponsonby *et al* 1998, Strachan 1997). Attendance at day care was found to be a risk factor for asthma diagnosed under the age of 5 (Marbury *et al* 1997, Nafstad *et al* 1999), but was inversely related to asthma in children aged between 5 and 14 years (Kramer *et al* 1999). The age at which the child first attended day care seems to be important on the subsequent development of disease. Day care attendance in the first year of life was inversely related to eczema (Celedon *et al* 2003). The relationship is further confounded by social class, as day care attendance is lowest in children from low-income families, who have the highest asthma morbidity (Wissow *et al* 1988). It seems that day-care attendance before the age of 2 may protect against transient wheeze, but increase the risk of persistent cases (Infante-Rivard *et al* 2001, Rusconi *et al* 1999). Transient cases of asthma are thought to be less severe, or are misdiagnosed as asthma at an early age because of a concurrent infectious wheezy episode. Therefore, it is reasonable to conclude that, in these children, day-care attendance would increase the risk, whereas early exposure to infection is protective among “true” (and persistent) cases.

If the “hygiene hypothesis” was true, it could be expected that multiple birth children should have a decreased incidence of allergic disease because they are likely to have increased exposure to infections from being in close contact with their co-twin. However, studies have shown that being part of a multiple birth reduces the risk of hay fever in children (Braback & Hedberg 1998, Timonen *et al* 1995), adolescents (Varjonen *et al* 1992) and adults (Duffy *et al* 1990, Nieminen *et al* 1991), and there is no evidence to suggest that the rate of asthma or hay fever is different in twins and singletons (Rasanen *et al* 1997).

Both respiratory and non-respiratory infections have been associated with the risk of asthma and atopy. Respiratory infections are one of the most common diseases in humans. They are important causes of disease and disability in high-income families (Chanock & Parrot 1965) and some of the most common causes of death, especially among children, in low-income countries (Denny & Loda 1986). Lower respiratory tract infections (LRTIs) account for high morbidity, large numbers of hospital admissions and considerable health care costs. A study in the Netherlands found higher frequencies of upper respiratory tract infections (URTIs) in children with asthma than in controls (Koopman *et al* 2001). Recurrent URTIs in asthmatic children are more likely to be caused by viral infections than by bacterial infections, with evidence to suggest that acute otitis media, generally considered to be bacterial in nature, is more likely to be caused by a virus (Heikkinen *et al* 1999, Johnston *et al* 1995, Openshaw & Lemanske 1998). Viral respiratory tract infection of the upper airways explained 85% of asthma exacerbations in 9-11 year old children; however, no control group was available in this study (Johnston *et al* 1995). It has been shown that influenza vaccination reduces acute respiratory diseases by 55% in pre-school children with asthma during an influenza epidemic (Smits *et al* 2002).

It has been suggested that exposure to measles and other common non-respiratory childhood viral infections may protect against the development of allergy, but the results have been inconsistent. Some studies report an inverse association (Shaheen *et al* 1996, Wickens *et al* 1999b), some an association varying with age at exposure and type of outcome (Bodner *et al* 2000, Bodner *et al* 1998), some no association (Farooqi & Hopkin 1998, Illi *et al* 2001, Lewis & Britton 1998, Matricardi *et al* 2000), and others a positive association (Paunio *et al* 2000). Others investigated the possible association between atopy and varicella, mumps or rubella infection, and found either no association or an increased risk of atopy (Bodner *et al* 2000, Bodner *et al* 1998, Farooqi & Hopkin 1998, Mummers *et al* 2004, Paunio *et al* 2000). Other studies have reported on the effect of other infections on the risk of asthma and atopy. One study found an inverse association between *Mycobacterium tuberculosis* and asthma (Mommers *et al* 2004), another found that *Mycoplasma* and *Chlamydia* species were frequent causes of asthma exacerbations (Isaacs & Joshi 2002), while

another reported on children who had repeated ear infections were more likely to be diagnosed with asthma (Eldeirawi & Persky 2004).

The effects of infection described in epidemiological studies seem to be stronger for atopy than for asthma. An asthmatic's susceptibility to react to viral infections with lower respiratory tract symptoms could suggest a positive causal relationship, but the reverse causal association could also be true. The timing of exposure may be important in determining whether the effects are harmful or beneficial. Data suggests that fever episodes in early life may affect the natural history of asthma by preventing the development of atopy (Calvani *et al* 2002). Exposure in the first few months of life, before asthma becomes evident, may be protective, whereas exposure later in life may exacerbate symptoms of the disease once it is established. Results show that having had pneumonia or tonsillitis to be associated with an increased risk of asthma (Infante-Rivard *et al* 2001), which may argue against the protective effect of viral infections. However, both diseases are likely to have a bacterial aetiology, and therefore act differently from viral infections (Infante-Rivard *et al* 2001).

The inconsistent results for asthma may be due to the complex aetiology of asthma and wheezing disorders. While wheezing early in life is thought to be provoked by respiratory infections, which are increased by the number of older siblings, whereas later wheezing may be due to allergic sensitisation, which is decreased by the presence of older siblings (Martinez *et al* 1995). Therefore it is important to consider the age of the children being studied when examining any relationship between asthma and infection.

There is a lack of data relating age of exposure to infections to risk of atopy. However, in one study, a higher risk of atopy was associated with measles in the first year of life, but not after the first year of life, when compared with no measles before age 7, but there was no association between atopy and mumps, rubella or varicella in the first year of life (Bager *et al* 2002). Questions remain concerning the interplay among host characteristics, the timing and nature of microbial and viral exposures with respect to the development of asthma (Christiansen 2000).

Total microbial burden, rather than exposure to a single infection, is thought to be of greater importance in the regulation of the immune system in early life (Martinez 2001). This is supported by a studies reporting significantly increased risk of atopy (Bager *et al* 2002, Bodner *et al* 1998) and asthma (Illi *et al* 2001) with increasing numbers of infections in the first 2 or 3 years of life.

5.2.9 Relationship with smoking

The adverse effects of both active and passive smoking are well known. The association of active smoking and respiratory disease is well documented (AIHW 2005, DOHWA 2001, USPHS 1984). Smokers who have asthma have more asthmatic symptoms, worse asthma control (Siroux *et al* 2000), more airway inflammation (Chalmers *et al* 2002), and a less beneficial response to asthma treatment (Chalmers *et al* 2002, Pedersen *et al* 1996) compared with non-smoking asthmatics. Overall, in Australia, the prevalence of smokers in people with asthma was higher than that in people without asthma (26% vs. 24.1%) (AIHW 2005).

Exposure to environmental tobacco smoke (ETS) is an important public health issue and has been shown to have deleterious effects on the health of both adults and children (AIHW 2005, DOHWA 2001, Tager 1988). It is the most common source of indoor air pollution and the best identified risk factor for the development of allergic disease (Bjorksten 1997), particularly in childhood. The effects of ETS on children's respiratory health have been extensively studied (CEPA 1997, USDHHS 1986, USEPA 1992), and they have concluded that ETS adversely effects the growth and development of lungs, with most studies demonstrating reduced levels of pulmonary function in exposed children. There is also evidence that exposure *in utero* leads to reduced lung function at birth which may persist into adulthood (Cunningham *et al* 1996a, Lodrup *et al* 1997, Tager *et al* 1995).

In Australia, the 2001 National Health survey found that around 40% of children with asthma lived in the same household as one or more smokers, and that boys with asthma were more likely to live with regular smokers than boys without asthma (41.9% vs. 36.7%, respectively) (ABS 2002). Despite the Quit and Passive Smoking campaigns, around 23% of pregnant and breast feeding

mothers continue to smoke (DOHWA 2001). This resulted in around 58,000 Australian babies being born in 2001 to mothers who smoked during their pregnancy. Although 20-30% of smoking mothers ceased while they were pregnant, there is evidence to suggest that the majority of them recommenced the habit once their babies were born (Lumley *et al* 2006). It has been estimated that 15 million children, more than 25% of the population in the USA, are exposed to ETS and are at risk of adverse health effects resulting from this exposure (CDC 1997).

There is substantial evidence that parental smoking increases the risk of asthma and respiratory symptoms in school children, and is associated with more severe disease among those with established asthma (Butland *et al* 1997, Cook & Strachan 1999, Lam *et al* 1998, Maier *et al* 1997, Ronmark *et al* 1998, Saraclar *et al* 1998). Exposure to ETS is known to increase the risk of wheezing in young children (Martinez *et al* 1992) and that this association is most consistent at high levels of exposure (NHMRC 1997). These findings are supported by evidence from international studies (Strachan & Cook 1998). It has also been reported that children exposed to ETS are more likely to attend hospital (Evans *et al* 1987), and that the prevention of indoor smoking results in a reduction of hospital admissions in children with asthma (Gurken *et al* 2000). Maternal smoking has consistently shown a stronger association with respiratory health of children than paternal smoking (Agabiti *et al* 1999).

Children whose parents smoke have been reported to have more problems with wheezing, lower respiratory tract infection, and asthma than children of parents who do not smoke, especially in the first year of life (Burchfiel *et al* 1986, Fergusson *et al* 1981, Gortmaker *et al* 1982, Neuspiel *et al* 1989). Children who are exposed to ETS at home have a significantly earlier onset of allergy, and wheezy bronchitis is five times more common in them than in children not so exposed (Bjorksten 1997).

Maternal smoking during pregnancy is a consistent risk factor for early childhood asthma (Lewis *et al* 1995, Oliveti *et al* 1996, Stick *et al* 1996). Maternal smoking has consistently been reported to be an important risk factor for childhood asthma morbidity and the effect is still seen even if the mother

only smoked during pregnancy and quit before delivery, suggesting an *in utero* effect (Taylor & Wadsworth 1987). The effects of smoking during pregnancy, which is associated with pre-term delivery and low birth weight, and of postnatal exposure to ETS, which may compound the effects of prenatal exposure, are highly correlated, and need to be considered separately. Studies conducted in China, where few women smoke, suggest that the effects of exposure to ETS in infancy are different from those of maternal smoking during pregnancy (Chen *et al* 1988). Studies in Boston, USA and Perth, were able to exclude the effects of ETS by examined the lung function of babies whose mothers smoked during pregnancy, soon after birth (Hanrahan *et al* 1992, Stick *et al* 1996). They found that the deficits in lung function at birth persisted into childhood and adolescence.

There is growing evidence that *in utero* exposure can lead to persistent deficits in lung function in the neonatal period (Hanrahan *et al* 1992, Stick *et al* 1996), but that the relative contributions of *in utero* smoke exposure and exposure to ETS in early childhood on the persistence of lung deficits are not clear. Some studies found that *in utero* exposure had no effect, or the effects of ETS were independent of *in utero* exposure (Dijkstra *et al* 1990, Sherrill 1992), another suggested an independent effect of *in utero* exposure and maternal smoking (Cunningham *et al* 1995), while yet another reported that the effect of maternal smoking during pregnancy was larger than for current smoking, and did not change after adjustment for the mother's current smoking (Cunningham *et al* 1996a).

The influence of parental smoking on their children's health is not confined to the resultant passive smoke to which they are exposed. It has been shown that parental smoking doubles the risk of smoking uptake by their children (Jarvis 1998). While the effects of exposure to ETS on health are small in comparison with those of active smoking, ETS exposure for children is involuntary, especially during pregnancy. The major source of ETS exposure remains the home, as there have been successful campaigns and legislation in developed to reduce the levels of smoking in public places. In many developing countries male smoking rates remain high, even though few women smoke. The challenge will be to promote household changes in smoking patterns if education

campaigns are targeted at women, because of the balance of power within families.

5.2.10 Summary of the literature

Asthma and atopy are among the most significant health conditions to affect the developed world, and prevalence and incidence of these conditions continues to rise in the developing world as countries increasingly adopt the western lifestyle. They account for a substantial proportion of the health budget in many countries, and the burden of cost to families of asthmatics is considerable. The incidence and prevalence of asthma in Australia is amongst the highest in the world, with between 14 and 16% of children, and 10-12% of adults experiencing this condition (National Health Priority Action Council 2006). Although genetic risk factors for asthma are not amenable to change, there is potential to reduce the impact of environmental risk factors, especially exposure to active or passive smoking, in the development of these conditions. Exposure to ETS in the home is still the major environment risk factor that children experience. Legislation has been passed to limit the amount of smoking in public places, but no such restrictions apply to smoking in the home.

5.2.11 Significance

A number of studies, including some based on twins, have clearly demonstrated that both genetic and environmental factors play a role in determining the asthmatic and atopic phenotypes (Duffy 1995, Postma 1995). Some environmental exposures may act directly, thereby increasing the risk of asthma in all exposed. Others may act only in those with a genetically determined sensitivity to that exposure. It is unclear into which category passive smoking falls. The answer to this question would have major implications for those attempting to understand the physiological mechanism underlying asthma/atopy and it is such an understanding which is most likely to lead to a primary prevention strategy for asthma. Furthermore, knowledge of genetic factors influencing the effect of tobacco smoke on asthma could be of direct relevance to education-based anti-smoking campaigns.

5.3 Aims

The aims of this section are to:

(1) describe the characteristics of twins with asthma;

(2) describe the prevalence of wheezing, asthma, hay fever and eczema in WA multiple birth families;

(3) determine the risk factors for wheezing, asthma, hay fever and eczema in twins; and

(4) determine the risk factors for asthma in twin families.

5.4 Methods

5.4.1 End points

Multiple end points were considered. They were:

Wheezing disorders: ever wheezing; current wheezing, as defined as wheezing in the last 12 months.

Asthma: reported doctor diagnosis of asthma (DDA); current asthma, as defined by those who had ever received a doctor diagnosis of asthma and had also wheezed in the last 12 months.

Hay fever: hay fever ever and hay fever in the last 12 months, and

Eczema: eczema ever and eczema in the last 12 months.

Atopy: A composite variable was created to reflect the total burden from atopy. In this context, atopy was defined as doctor-diagnosed asthma, hay fever or eczema. Both atopy ever and current atopy were considered.

Analyses of current wheezing, asthma, hay fever, eczema and atopy were limited to those who had ever wheezed, been diagnosed with asthma, hay fever, eczema and atopy, respectively.

5.4.2 Variables

A large number of variables were considered as potential risk factors for the end point of interest. These variables are listed below

- Demographics: age, gender, season of birth, place of residence, zygosity, birth weight, gestation, body mass index

- Mother's behaviours before and during pregnancy: use of assisted reproduction, maternal smoking during pregnancy.
- Complications of pregnancy: bleeding serious enough to require bed rest, threatened miscarriage under 20 weeks, urinary tract infection (UTI), toxaemia of pregnancy, gestational diabetes, placenta praevia, premature rupture of membranes.
- Mode of delivery: caesarean section (booked or elective), versus vaginal delivery.
- Maternal depression after the birth.
- Admission to the neonatal intensive care unit (NICU) or special care nursery (SCN) after birth.
- Conditions and treatments immediately after birth: respiratory distress, treatment with respirator or ventilator, feeding through naso-gastric tube.
- Infant feeding: length of breast feeding, age at which other milk products were first introduced.
- Health conditions diagnosed by a doctor: febrile convulsions, migraine or severe headaches, otitis media (ear infection).
- Childhood behaviour: attention-deficit disorder, both hyperactive and inattentive types.
- Ear, nose and throat surgery: tonsils or adenoids removed, grommets inserted.
- Family variables: having older siblings, mother's and father's phenotype, parental smoking, exposure to ETS in the home and car, parental occupation, education and marital status; age of parents at time of twins' birth.
- Socio-economic status: as defined as being in the top or bottom 10% with respect to the SEIFA indices of disadvantage, advantage/disadvantage, economic resources, and education and occupation, as described in section 4.7.

5.4.3 Birth weight and gestation

It is well known that, on average, twins have a lower birth weight than singletons (Taffel 1995). Literature suggests that low birth weight, that is under 2,500g, confers an increased risk of the subsequent diagnosis of asthma.

However, this figure was arrived at when considering singleton-born children. In this cohort, the majority of twins were under this weight when born, and so this definition may not be a true representative of low birth weight in twins. In the WATCH cohort, the percentage of singletons that could be categorised as low birth weight was 4.6%. The equivalent birth weight for twins was 1,700g. As I could find no conclusive figure in the literature that defined low birth weight in twins, I decided to categorise birth weight as quartiles with respect to this cohort, low birth weight being defined as the lowest quartile, referred to as Q1 in the analysis. Similarly, the low gestational age definition of 37 weeks was also based on data from singletons. My analysis defined low gestation in twins to be 32 weeks and under, as the percentage of twins who fell into this category corresponded to that in the singletons in the WATCH study population who were under 37 weeks gestation.

5.4.4 Environmental tobacco smoke (ETS) exposure

Details of smoking were collected for every individual in the family. To examine the effect of exposure to environmental tobacco smoke on asthma and atopy end points in twins, I excluded those twins who had smoked. This ensured that any potential effect of active smoking was eliminated. For all family members, their smoking status was adjusted to represent individual smoking status at the time the twins and their siblings were born. For example, if a parent reported that he/she had smoked but quit before the birth of the twins, they were deemed to be non-smokers at the time of the birth. This was especially important when considering current and ex-smoking. Two separate passive smoking variables were created, one for each of “ever” smoking and “current” smoking. They were based on the total number of smokers in each household, and coded as:

0 = no smokers in household
1 = one smoker in the household
2 = 2 or more smokers in the household

When analyzing twin-family data, an extra category was added to the passive smoking variable, indicating whether the individual (except for twins) was an “active” smoker, either at the time of the birth of their children (for parents) or the twins (for siblings) or currently.

5.4.5 Creating other variables to be used as covariates

A variable to indicate that the family lived in a rural area was created from the post code of current residence. Postcodes in each Australian state commence with a unique number; for WA this is "6". For the Perth metropolitan area, the postcode range was 6000-6199, together with a special postcode starting with "69" to denote a private mail box at a Post Office in Perth. Postcodes outside these numbers were coded as "1" for the rural variable, and Perth residence coded "0".

An overweight variable was created and defined as being in the top 15% of BMI for each age and sex group of children, and for each sex for adults. It was used in preference to BMI as the definitions for being overweight and obese used for adults, that is BMI>25 and BMI>30 respectively, was considered inappropriate for children (WHO 2002).

5.4.6 Modelling age

The age variable was fitted to each model to assess the significance of its relationship with each end point. It was decided to force age into each model, as it has been reported as a risk factor for asthma and allergy in numerous studies. To assess the best transformation for age for each end point, it was modelled using fractional polynomials, according to the formula below:

$$\log (OR) = a + b_1 \times age^p + b_2 \times age^p \times \log_n(age)$$

where a is the constant, and b_1 and b_2 are the regression coefficients in the regression equation, and p is the power of the polynomial, ranging from –2 to +2 (Royston & Altman 1994). This technique allows a flexible curve to be fitted to the data as a combination of 2 fractional polynomial response curves that express the change in odds ratios (ORs), usually in 2 functions of the "risk" variable. The usual convention of a power of 0 indicating the log transformation was adopted. For all end points, there was little difference between all the models, but that model which resulted in the smallest deviance was chosen.

5.4.7 Statistical Analysis

Descriptive statistics of asthma and atopy in twin families were derived from families where data on both parents were available (1327 families). Significance

was assessed at the 5% level. Analysis of asthma and atopy in twins used only twin pairs where the zygosity was known, and where neither twin had ever smoked.

For each phenotype, univariate and multivariate analyses using logistic regression were carried out and the final model was determined by following the method proposed by Hosmer & Lemeshow (Hosmer & Lemeshow 2000). Both univariate and multivariate analyses used generalized estimating equations (GEEs) using PROC GENMOD in SAS. GEEs were used in preference to the standard logistic regression analysis to take account of the non-independence of members of each twin pair. In both the univariate and multivariate analyses, age (defined as the best fitting fractional polynomial specific to the end-point) was included in each model. In addition, exposure to ETS was also included in the multivariate analyses, since its effect on the development of asthma and atopy was one of the priniciple aims of the analyses. For the univariate analysis, each covariate was fitted individually, after adjusting for age, and those found to be significant at the 25% level were then fitted together in a multivariate analysis, after adjusting for age and exposure to ETS. The final model was achieved by removing the least significant variables singly, until only those variables significant at the 5% level remained. Then all variables previously eliminated were re-fitted, and those found to be significant at the 5% level were re-added to the final model.

Sections for each end-point analyzed give a summary table of only the variables that were significant at the 5% level in the univariate and multivariate analyses. A complete list of all the variables considered separately in the univariate analyses can be found in Appendix 6. P-values are given correct to two significant figures.

5.5 Wheezing Disorders

5.5.1 Wheezing ever

5.5.1.1 Twin-family prevalences

The overall prevalence of ever wheezing in WA twin families was 25%, with children experiencing a higher rate than their parents (28% vs. 21%, p<0.001). Among the parents, mothers had a higher prevalence of wheezing than fathers (p<.001), while among the children, boys had a higher prevalence than girls (p<.001). There was no significant difference in the prevalence of wheezing between twins and their siblings (p=.063), or between MZ and DZ twins (p=.077) (Table 5.3).

Table 5.3:
Wheezing in WA multiple birth families

	NUMBER	PREVALENCE
Parents	2654	20.9
Mothers	1405	23.8
Fathers	1249	17.5
Children	4044	27.9
Females	2054	23.1
Males	1990	33.0
Twins	2848	28.8
MZ	700	31.5
DZ	1860	27.9
Siblings	1196	25.8

5.5.1.2 Wheezing in twins.

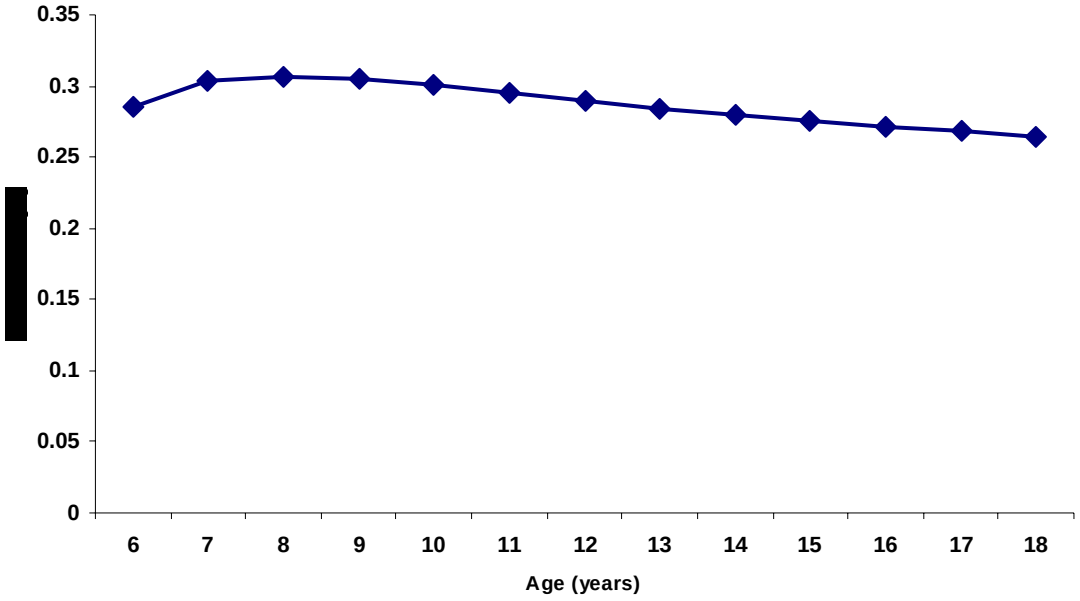
In the unadjusted univariate analysis, there was no effect of age on the prevalence of wheezing in twins (p=.27), but boys had a significantly higher rate than girls (OR=1.64, p<.001). There was no effect of exposure to ETS (p=.85). The fractional polynomial with p=-2 provided the best fit for age, giving the relationship as:

$$\log (OR) = -1.2439 - 86.5263 \times age^{-2} + 54.8434 \times age^{-2} \times \log_n(age)$$

(deviance=2593.12 on 2162 df).

Prevalence showed a small, non-significant reduction after the age of 8 years (Figure 5-3).

Figure 5-3:
Prevalence of ever wheezing by age in WA twins aged 6 to 18 years



5.5.1.3 GEE models

5.5.1.3.1 Univariate analysis

Boys had a 64% increased risk of ever wheezing compared with girls, while wheezing was more common in twins living in urban areas than in rural areas. Wheezing was not related to any of the prenatal or pregnancy variables examined. Twins who spent some time in the neonatal intensive care unit (NICU) or in special care nursery (SCN) immediately after birth had a higher prevalence of wheezing than those who did not. These results were supported by the higher risk of wheezing in those twins who had received treatment or had respiratory distress after birth. Twins with low birth weight experienced a higher prevalence of wheezing than those whose birth weight was in the highest quartile, but wheezing in twins was not related to gestation. Any breast feeding resulted in a small, non significant decrease in the risk of wheezing, when compared with no breast feeding. However, the risk was not related to the early introduction of other milk products. Wheezing was associated with twins having had at least one episode of otitis media (ear infection) and having had their tonsils removed. No other childhood conditions were significant. Parental wheezing was significantly associated with wheezing in twins, with mothers having a slightly higher effect than fathers. Wheezing in twins was not associated with the number of smokers in the household, nor with any of the

other smoking variables examined. Only twins whose families were in the bottom 10% with respect to the SEIFA index of disadvantage had a significantly higher rate of wheezing (Table 5.4).

5.5.1.3.2 Multivariate analysis

Positive family history of wheezing, male gender, having had a tonsillectomy and being fed through a naso-gastric tube immediately after birth were all risk factors for wheezing in twins. After adjusting for age and family history, exposure to ETS had no significant effect on wheezing in twins. After adjusting for age and exposure to ETS, the risk of wheezing was increased nine-fold in twins where both parents who had wheezed compared with twins where neither parent had wheezed (OR=9.27; 5.5-15.9), mothers conferring a higher risk than fathers (Table 5.4).

Table 5.4:
Significant variables in the final multivariate model and univariate analysis for wheezing in WATCH study twins, after adjusting for age

	UNIVARIATE MODEL		MULTIVARIATE MODEL	
Variable	**OR 95% CI**	**p-value**	**OR 95% CI**	**p-value**
Individual variables				
Male gender	1.64 (1.4-2.0)	<.0001	1.67 (1.3-2.1)	<.0001
Family variables				
Lives in a rural area	0.77 (.6-1.0)	.043		
Birth variables				
Was in NICU	1.35 (1.1-1.7)	.0071		
Was in SCN	1.40 (1.1-1.7)	.0026		
Had respiratory distress after birth	1.41 (1.1-1.8)	.0058		
Needed respirator after birth	1.42 (1.1-1.8)	0.0091		
Fed through naso gastric tube	1.59 (1.3-2.0)	0.0001	1.61 (1.2-2.1)	0.0002
Birth weight – Q1 vs. Q4	1.56 (1.2-2.1)	.0030		
Childhood conditions				
At least one episode of otitis media	1.32 (1.0-1.7)	0.023		
ENT operations				
Had tonsils removed	1.50 (1.0-2.2)	0.039	1.70 (1.1-2.6)	0.014
Parental phenotypes				
Mother wheezed	3.30 (2.6-4.3)	<.0001	3.12 (2.4-4.1)	<.0001
Father wheezed	2.79 (2.1-3.7)	<.0001	2.64 (2.0-3.5)	<.0001
Socio-economic status				
In bottom 10% of index of disadvantage	1.76 (1.0-3.1)	.048		
Smokers in household				
None	1		1	
One	1.24 (.9-1.6)	.12	1.33 (1.0-1.8)	.054
Two or more	0.92 (.7-1.6)	.61	0.92 (.7-1.3)	.60

5.5.2 Current wheeze

In this section, current wheeze is defined as wheezing in the last 12 months, among those twins who had ever wheezed.

5.5.2.1 Twin-family prevalences

Among twins who had ever wheezed, 54% of them had wheezed in the last 12 months, with children reporting a lower rate than their parents (51% vs. 59%, p=0.006). There was no difference in the rate among mothers and fathers (p=.57), or among the boys and girls (p=.52). Twins experienced a lower rate than their siblings (p=.046), but there was no difference in the rate of current wheezing among MZ and DZ twins (p=.091) (Table 5.5).

Table 5.5:
Reported current wheeze in WA twin families

	NUMBER	PREVALENCE
Parents	526	58.6
Mothers	323	57.6
Fathers	203	60.1
Children	1060	51.2
Females	439	52.4
Males	621	50.4
Twins	772	49.4
MZ	204	45.1
DZ	489	52.2
Siblings	288	56.2

5.5.2.2 Current wheezing in twins

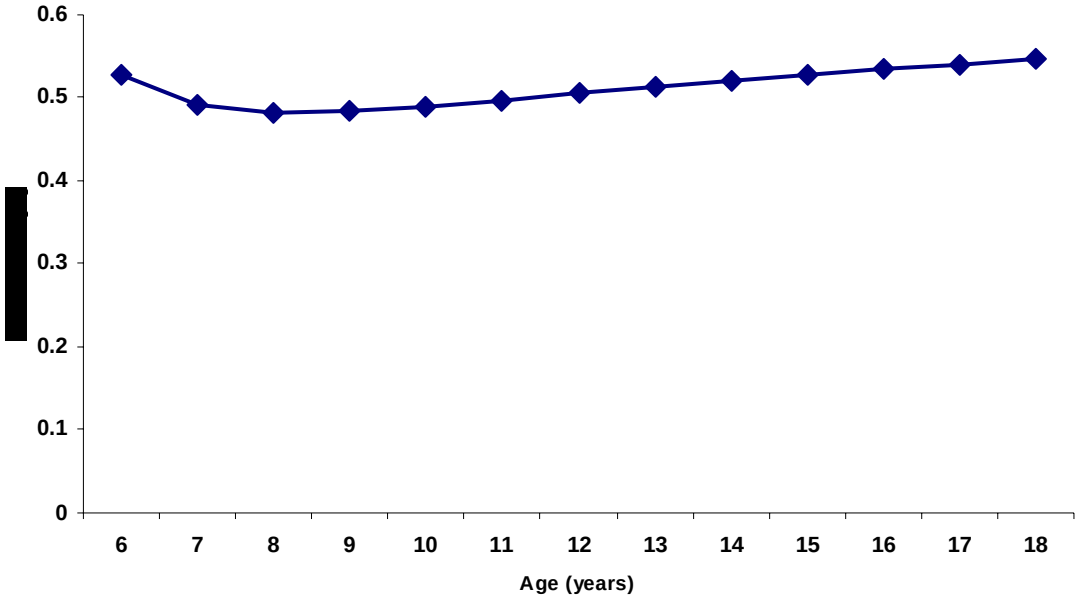
Among twins who had ever wheezed, there was no difference in the prevalence of current wheezing in boys and girls (p=.56), or with age (p=.67). There was also no effect of passive smoking exposure on wheezing in the last 12 months (p=.32). The polynomial for age with the smallest deviance was the one with p=-2, giving the equation

$$\log (OR) = 0.4879 + 125.0556 \times age^{-2} - 77.3517 \times age^{-2} \times \log_n(age)$$

(deviance=829.27 on 596 df)

Prevalence of current wheeze was highest in 6 year olds (52.8%), and exceeded 50% for most ages (Figure 5-4).

Figure 5-4:
Prevalence of current wheeze by age in twins aged 6 to 18 years



5.5.2.3 GEE Models

5.5.2.3.1 Univariate analysis

Wheezing in the last 12 months was not associated with any individual or family characteristic, nor any prenatal or pregnancy variables. Low birth weight twins had an increased risk of wheezing in the last 12 months, but this risk was not significant. Both length of breast feeding and age at which other milk was introduced were significantly associated with the prevalence of persistent wheeze, with those who were breast fed for at least 6 months having about half the risk as those who were not breast fed. There was no association between current wheezing and any of the childhood conditions examined. Twins who wheezed in the last 12 months were more likely to have parents who wheezed, but no other parental variables affected the prevalence. Current wheeze in twins was not associated with either exposure to passive smoke or socio-economic status (Table 5.6).

5.6.3.3.2 Multivariate analysis

Risk factors for current wheezing in twins were having a positive family history, being born during the summer months and the early introduction of other milk products. After adjusting for age and family history, twins who had one or more

current smoker living in the same house had an increased, but non-significant, risk of wheezing in the last 12 months. After adjusting for age and exposure to ETS, parental wheezing in the last 12 months resulted in a significantly increased risk of current wheezing in twins (OR=2.94; 1.3, 6.8) (Table 5.6).

Table 5.6:
Significant variables in the final multivariate model and univariate analysis for current wheeze in WATCH study twins, after adjusting for age

	UNIVARIATE ANALYSIS		MULTIVARIATE MODEL	
Variable	**OR 95% CI**	**p-value**	**OR 95% CI**	**p-value**
Twin variables				
Born during summer months			1.72 (1.1-2.7)	.022
Infant feeding				
Other milk introduced before 4 months	1.83 (1.2-2.8)	.0038	1.72 (1.1-2.5)	.0101
Parental phenotypes				
Mother ever wheezed	1.56 (1.1-2.3)	.023	1.68 (1.1-2.5)	.010
Father ever wheezed	1.63 (1.1-2.4)	.018	1.75 (1.2-2.7)	.0091
Smokers in household				
None	1		1	
One	1.31 (.8-2.1)	.26	1.47 (.9-2.4)	.13
Two or more	1.20 (.6-2.2)	.56	1.22 (.6-2.3)	.54

5.6 Asthma

5.6.1 Doctor-diagnosed asthma

5.6.1.1 Twin-family prevalences

The prevalence of asthma was higher in children than in their parents (27.0% vs. 14.8%, p<0.001). Mothers had a higher rate than fathers (17.5% vs. 11.7%, p<0.001), but in children, girls had a lower rate than boys (24.2% vs. 29.9%, p<0.001). In children aged 6-12 years, the prevalence of asthma was higher in boys than girls (33.6% vs. 24.0%, p<.0001), but there was no difference in the prevalence in children aged 13-18 years (25.2% vs. 25.3%, p=.81). There was no difference in asthma prevalence between twins and their siblings (27.6% vs. 25.5%, p=0.16), and between MZ and DZ twins (28.3% vs. 27.4%, p=0.66) (Table 5.7).

Table 5.7:
Doctor-diagnosed asthma in WA twin families

	NUMBER	PREVALENCE
Parents	2654	14.8
Mothers	1405	17.5
Fathers	1249	11.7
Children	4044	27.0
Females	2054	24.2
Males	1990	29.9
Twins	2848	27.6
MZ	700	28.3
DZ	1860	27.4
Siblings	1196	25.5

5.6.1.2 Asthma in twins

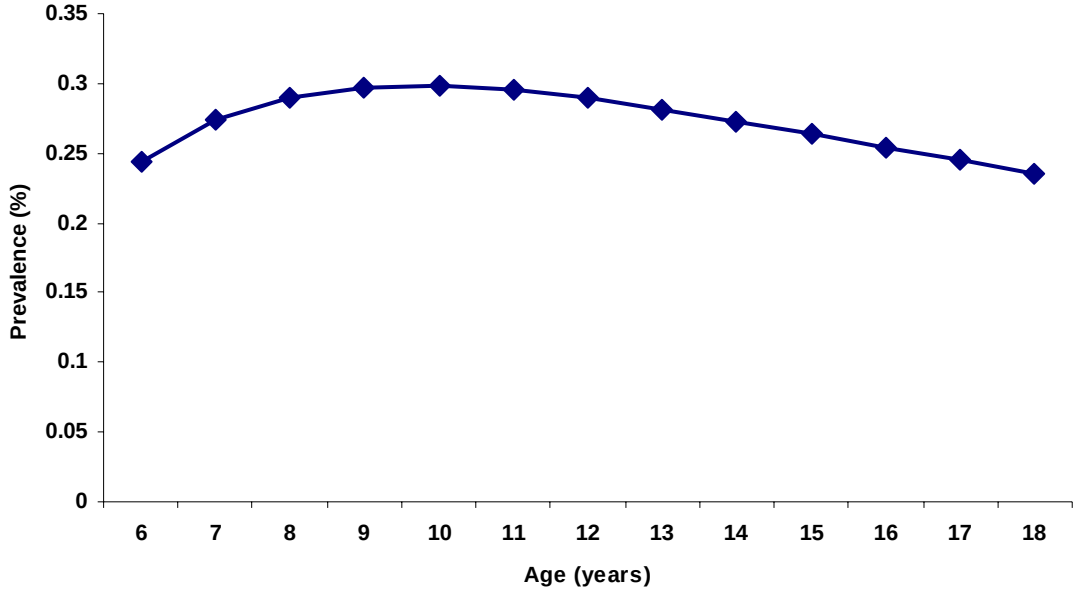
Asthma was more prevalent in boys than girls (OR=1.37, p=.0041) but there was the rate did not vary with age (p=.45). Exposure to ETS had no effect on asthma prevalence (p=.40). Age best fit the data when modelled with a polynomial of order –0.5, giving the equation

$$\log (OR) = -9.1235 - 3.4789 \times age^{-1/2} + 12.8657 \times age^{-1/2} \times \log_n(age)$$

(deviance=2616.59 on 2225 df)

Prevalence of asthma peaked at 10 years of age, with a steady decline thereafter (Figure 5-5).

Figure 5-5:
Prevalence of doctor-diagnosed asthma by age in twins aged 6 to 18 years



5.6.1.3 GEE models

5.6.1.3.1 Univariate analysis

The rate of asthma was higher in boys than girls, and in twins with older siblings than those without older siblings. Twins who lived in the metropolitan area experienced a higher rate of asthma compared with those living in rural areas. Complications of pregnancy, involving either bleeding or threatened miscarriage, were associated with an increased risk of asthma, as were delivery by caesarean section, and developing conditions requiring treatment immediately after birth. Being born at or before the 32nd week of pregnancy increased the risk of asthma in twins. Birth weight was a risk factor for asthma in twins, with low birth weight conferring an increased risk over higher birth weight. There was no association with either length of breast feeding or age at which other milk was introduced. Asthma in twins was associated with migraines, ear infections, tonsillectomy and adenoidectomy, and showed a strong association with asthma in both parents. Having at least one smoker in the household resulted in an 11% increase in risk of asthma in twins, which was not significant. Those twins whose families were among the lowest 10% with

respect to the index of disadvantage, had more than a two-fold risk of asthma compared with twins in the other 90% (Table 5.8).

5.6.1.3.2 Multivariate analysis

Having asthmatic parents, being male, having no older siblings and living in urban areas were all strongly associated with an increased risk of asthma in twins. There was also an association with being fed through a naso-gastric tube immediately after birth, and having tonsils removed. Twins whose families were in the bottom 10% of 2 SEIFA codes (index of disadvantage, and index of economic resources) were more likely to develop asthma than those in the remaining 90% of these codes. After adjusting for age and exposure to ETS, twins with both asthmatic parents had a eightfold increased risk compared with twins whose parents were not asthmatic (OR=8.27; 4.6-13.6). The risk was greater for an asthmatic mother compared with an asthmatic father. After adjusting for age and family history, living in the same household as one or more smokers increased the risk of asthma by 11% when compared with to living with no smokers, but this increase was not significant (Table 5.8).

Table 5.8:
Significant variables in the final multivariate model and univariate analysis for the risk of asthma in WATCH study twins, after adjusting for age

	UNIVARIATE ANALYSIS		MULTIVARIATE MODEL	
Variable	**OR 95% CI**	**p-value**	**OR 95% CI**	**p-value**
Individual variables				
Male gender	1.36 (1.1-1.7)	.0041	1.43 (1.1-1.8)	.0020
Twin variables				
Has older siblings	0.68 (.6-.9)	.0006	0.71 (.6-.9)	.0045
Family variables				
Lives in rural area	0.77 (.6-1.0)	.038	0.77 (.6-1.0)	.046
Complications of pregnancy				
Bleeding serious enough for bed rest	1.48 (1.0-2.1)	.028		
Threatened miscarriage under 20 weeks	1.65 (1.1-2.4)	.0074	1.66 (1.2-2.4)	.0070
Birth variables				
Gestation over 32 weeks	0.86 (.8-1.0)	.0088		
Delivered by caesarean section	1.33 (1.1-1.7)	.016		
Birth weight – Q1 vs. Q4	1.64 (1.21-2.2)	.0011		
Spent time in NICU	0.75 (.6-.9)	.0068		
Spent time in SCN	0.76 (.6-.9)	.0095		
Had respiratory distress after birth	1.44 (1.1-1.8)	.0030		
Needed respirator after birth	1.41 (1.1-1.8)	.0088		
Fed through naso-gastric tube after birth	1.49 (1.2-1.9)	.0005		
Childhood conditions				
Migraines	1.75 (1.1-2.7)	.010		
Had at least one episode of otitis media	1.37 (1.1-1.7)	.0058	1.32 (1.03-1.7)	.026
ENT operations				
Had tonsils removed	1.66 (1.2-2.4)	.0046	1.51 (1.04-2.2)	.030
Had adenoids removed	1.64 (1.2-2.3)	.0055		
Parental phenotypes				
Mother asthmatic	3.10 (2.4-4.0)	<.0001	3.12 (2.4-4.1)	<.0001
Father asthmatic	2.54 (1.9-3.4)	<.0001	2.65 (1.9-3.6)	<.0001

Table 5.8 (continued)

	UNIVARIATE ANALYSIS		MULTIVARIATE MODEL	
Variable	**OR 95% CI**	**p-value**	**OR 95% CI**	**p-value**
Socio-economic status				
Bottom 10% of index of disadvantage	2.26 (1.3-3.8)	.0027	2.20 (1.2-4.0)	.0082
Bottom 10% of index of economic resources			4.08 (1.6-10.4)	.0031
Smokers in household				
None	1		1	
One	1.12 (.8-1.5)	.44	1.18 (.9-1.6)	.26
Two or more	1.11 (.8-1.5)	.47	1.06 (.8-1.4)	.70

5.6.2 Current asthma

5.6.2.1 Twin-family prevalences

Persistent asthma, as defined by asthma in the last 12 months among those who had received a doctor-diagnosis of asthma, occurred in 79% of asthmatics in twin families. This rate was higher in parents than their children (p=.003), but no different between twins and their siblings (p=.45), mothers and fathers (p=.94), or MZ and DZ twins (p=.59). Boys had a higher rate than girls (p<.001) (Table 5.9).

Table 5.9:
Current asthma in WA twin families

	NUMBER	PREVALENCE
Parents	392	84.4
Mothers	246	84.6
Fathers	479	84.2
Children	1092	77.5
Females	497	72.0
Males	595	82.0
Twins	787	76.9
MZ	198	79.2
DZ	510	76.1
Siblings	305	79.0

5.6.2.2 Current asthma in twins

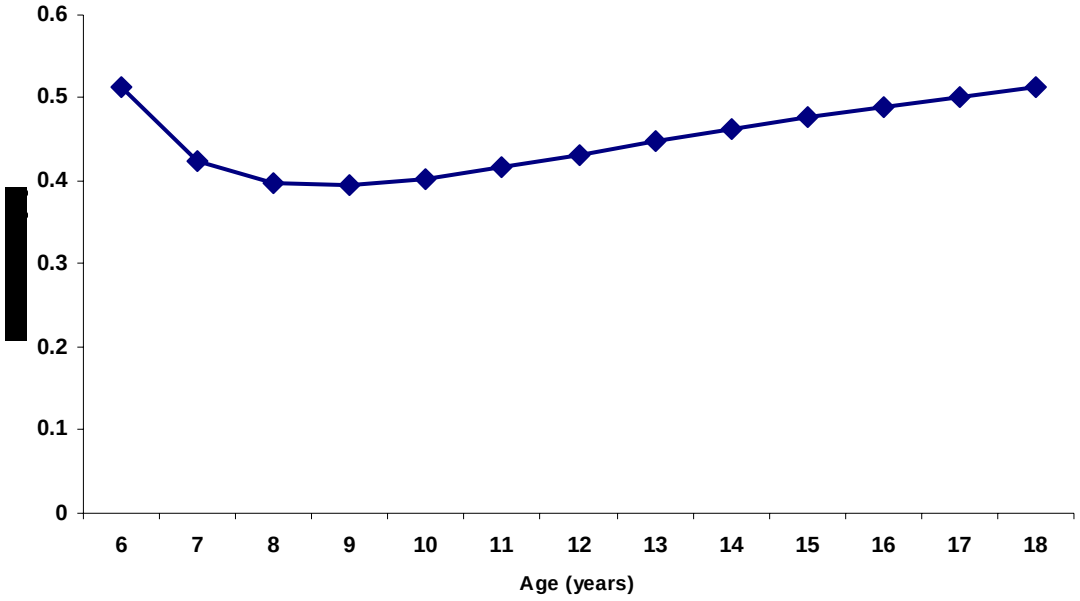
In asthmatic twins, there was no difference in the prevalence of current asthma with age (p=.54) or gender (p=.71). The prevalence of asthma in the last 12 months was independent of passive smoking exposure (p=.75). The best fitting polynomial for age was the one with p=-2, giving the equation:

$$\log (OR) = 0.6832 + 275.8939 \times age^{-2} - 166.5781 \times age^{-2} \times \log_n(age)$$

(deviance=794.53 on 580 df).

Prevalence ranged from a low of 39.4% at age 9 years, to a high of 51.4 at age 6 years (Figure 5-6).

Figure 5-6:
Prevalence of current asthma by age in twins aged 6 to 18 years



5.6.2.3 GEE models

5.6.2.3.1 Univariate analysis

No individual, twin or family characteristics were related to current asthma in twins. It was associated with threatened miscarriage and respiratory distress after birth, but not with birth weight. Although there was no association with breast feeding, those twins who had other milk introduced before the age of 4 months had an 80% increased risk of asthma in the last 12 months, when compared with those who had other milk introduced at an older age. Asthma in the last 12 months in twins was independent of any of the childhood conditions or operations examined. Having asthmatic or highly educated parents increased the risk of current asthma in twins, but there was no association with either exposure to passive smoking or socio-economic status (Table 5.10).

5.6.2.3.2 Multivariate analysis

Exposure to ETS was not associated with current asthma after adjustment for age and family history. After controlling for age and exposure to ETS, history of asthma in both the mother and father increased the risk of current asthma in twins (OR=3.12; 1.3-7.8). Twins who had older siblings had an increased risk of asthma in the last 12 months, compared with twins who did not. Risk was reduced in twins whose mother experienced a threatened miscarriage at under

20 weeks gestation. Conversely, respiratory distress after birth, early introduction of other milk, and having a mother with a tertiary qualification all increased the risk of current asthma in twins (Table 5.10).

Table 5.10:
Significant variables in the final multivariate model and univariate analysis for current asthma in WATCH study twins, after adjusting for age

	UNIVARIATE ANALYSIS		MULTIVARIATE MODEL	
Variable	**OR 95% CI**	**p-value**	**OR 95% CI**	**p-value**
Twin variables				
Has older siblings			1.52 (1.0-2.3)	.048
Pregnancy complications				
Threatened miscarriage under 20 weeks	0.49 (.3-.9)	.020	0.49 (.2-.9)	.032
Birth variables				
Had respiratory distress after birth	1.99 (1.3-3.1)	.0015	2.06 (1.3-3.2)	.0016
Infant feeding				
Other milk introduced before 4 months	1.79 (1.2-2.7)	.0057	1.67 (1.1-2.5)	.016
Parental phenotypes				
Mother asthmatic	1.60 (1.1-2.4)	.027	1.66 (1.1-2.6)	.022
Father asthmatic	1.95 (1.2-3.1)	.0053	1.88 (1.2-3.0)	.0088
Parental variables				
Mother has tertiary qualifications	2.06 (1.2-3.4)	.0043	1.88 (1.1-3.2)	.022
Father as tertiary qualifications	1.78 (1.1-2.8)	.017		
Smokers in household				
None	1		1	
One	1.02 (.6-1.6)	.92	1.24 (.8-2.1)	.40
Two or more	0.85 (.5-1.5)	.57	0.87 (.5-1.6)	.65

5.7 Atopic diseases

5.7.1 Hay fever

5.7.1.1 Twin-family prevalences

The overall rate of hay fever in WA twin families was 36%, which was higher in mothers than fathers (p<.001). Among the children, there was no difference in the rate between twin and their siblings (p=.46), or between boys and girls (p=.12). However, MZ twins had a significantly higher rate of hay fever than DZ twins (p=.025) (Table 5.11).

Table 5.11:
Hay fever in WA twin families

	NUMBER	PREVALENCE
Parents	2654	46.6
Mothers	1405	53.2
Fathers	1249	39.2
Children	4017	29.0
Females	2044	27.9
Males	1973	30.1
Twins	2848	29.3
MZ	700	33.1
DZ	1860	28.6
Siblings	1169	28.1

5.7.1.2 Hay fever in twins

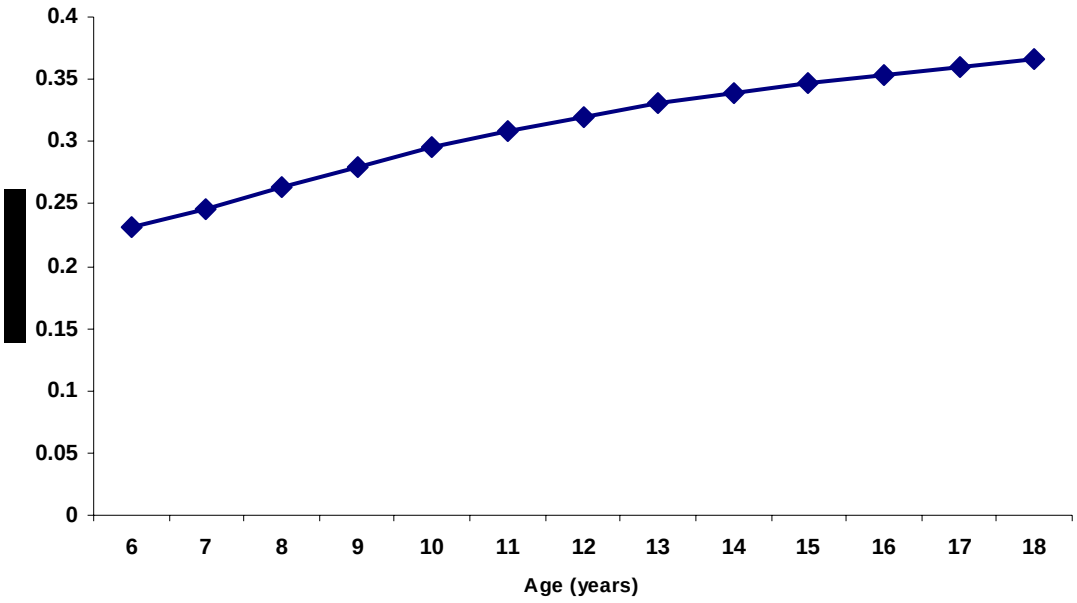
The prevalence of hay fever in twins increased 5.4% per year with increasing age (p=.0005), and boys had a marginally significantly higher rate than girls (p=.052). There was no effect of exposure to ETS on hay fever (p=.41). The equation for the best fitting polynomial for age was:

$$\log (OR) = -0.2721 + 59.3693 \times age^{-2} - 51.8065 \times age^{-2} \times \log_n (age)$$

(deviance=2712.04 on 2225 df)

Prevalence increased steadily from a low of 23.1% at age 6 to 36.6% at age 18 (Figure 5-7).

Figure 5-7:
Prevalence of hay fever by age in twins aged 6 to 18 years



5.7.1.3 GEE models

5.7.3.3.2 Univariate analysis

Boys had a higher rate of hay fever than girls, as did twins with no older siblings and those living in urban areas compared with no older siblings and city living respectively. Hay fever was related to delivery by caesarean section, and spending time in either NICU or SCN immediately after birth. Twins in the lowest quartile with respect to birth weight had an increased risk of hay fever compared with those in the highest quartile, but there was no relationship with length of gestation. There was no association between hay fever and the infant feeding practices that were examined. Hay fever was associated with migraine headaches, ear infections and all of the ear, nose and throat procedures examined. Parents' education and occupation were associated with an increased risk of hay fever in twins, as was a history of hay fever in both the mother and father. There was no relationship between any passive smoking variables and hay fever in twins. Twins whose families were in the top 10% of both the index of advantage/disadvantage, and the index of education and employment, had a higher risk of hay fever than twins who were not (Table 5.12).

5.7.3.3.3 Multivariate analysis

After adjusting for age and family history of hay fever, twins who had at least one smoker in the same household had a non-significant reduced risk of hay fever when compared with twins who had no smokers living in the same household. After controlling for age and exposure to ETS, twins with both parents with hay fever had six times the risk of hay fever than twins whose parents did not have hay fever (OR=6.15; 3.8-9.6). Hay fever in twins was also associated with living in urban areas and having older siblings. An increased risk of hay fever was found in children who suffered from migraines, have had at least one episode of otitis media, and have had their adenoids removed (Table 5.12).

Table 5.12:
Significant variables in the final multivariate model and univariate analysis for hay fever in WATCH study twins, after adjusting age

	UNIVARIATE ANALYSIS		MULTIVARIATE MODEL	
Variable	**OR 95% CI**	**p-value**	**OR 95% CI**	**p-value**
Individual variables				
Male gender	1.24 (1.0-1.5)	.031		
Twin variables				
Has older siblings	0.64 (.5-.8)	.0001	0.67 (.5-.8)	.0005
Family Variables				
Lives in rural area	0.58 (.4-.7)	<.0001	0.62 (.5-.8)	.0001
Complications of pregnancy				
Toxaemia	1.60 (1.1-2.3)	.011		
Birth variables				
Delivered by caesarean section	1.39 (1.1-1.7)	.0071		
Was admitted to NICU	1.29 (1.0-1.6)	.014		
Was admitted to SCN	1.23 (1.0-1.5)	.050		
Fed through naso-gastric tube after birth	1.34 (1.1-1.7)	.0099		
Birth weight – Q1 vs. Q4	1.37 (1.0-1.8)	.034		
Conditions diagnosed by a doctor				
Suffers from migraines	1.76 (1.2-2.6)	.0051	1.67 (1.1-2.5)	.017
At least one episode of otitis media	1.49 (1.2-1.9)	.0004	1.29 (1.02-1.6)	.034
ENT operations				
Had tonsils removed	1.57 (1.1-2.2)	.0075		
Had adenoids removed	1.80 (1.3-2.5)	.0005	1.80 (1.2-2.6)	.0020
Had grommets inserted	1.57 (1.1-2.2)	.0080		
Parental phenotypes				
Mother has hay fever	2.69 (2.1-3.4)	<.0001	2.46 (1.9-3.1)	<.0001
Father has hay fever	2.70 (2.2-3.4)	<.0001	2.50 (2.0-3.1)	<.0001
Parental variables				
Mother has tertiary qualifications	1.38 (1.1-1.8)	.016		
Father in a professional occupation	1.36 (1.1-1.7)	.0061		
Father has tertiary qualifications	1.44 (1.1-1.9)	.0067		

Table 5.12 continued

	UNIVARIATE ANALYSIS		MULTIVARIATE MODEL	
Variable	**OR 95% CI**	**p-value**	**OR 95% CI**	**p-value**
Socio-economic status				
In top 10% of index of advantage/disadvantage	1.38 (1.1-1.8)	.017		
In top 10% of index of education & employment	1.43 (1.1-1.9)	.012		
Smokers in household				
None	1		1	
One	0.77 (.6-1.0)	.055	0.82 (.6-1.1)	.16
Two or more	0.94 (.7-1.2)	.67	1.06 (.8-1.4)	.70

5.7.2 Current hay fever

5.7.2.1 Twin-family prevalences

Hay fever symptoms persisted in 81% of those WA twin family members who had ever had hay fever. Although there was no difference in the rate between parents and their children (p=.21), but mothers had a significantly higher rate than fathers (p=.019). No difference was seen between boys and girls (p=.30), twins and their siblings (p=.35), nor between MZ and DZ twins (p=.44) (Table 5.13).

Table 5.13:
Current hay fever in WA twin families

	NUMBER	PREVALENCE
Parents	1204	79.9
Mothers	731	82.1
Fathers	473	76.5
Children	1109	82.0
Females	547	83.2
Males	562	80.8
Twins	795	82.6
MZ	221	85.1
DZ	505	82.8
Siblings	314	80.2

5.7.2.2 Current hay fever in twins

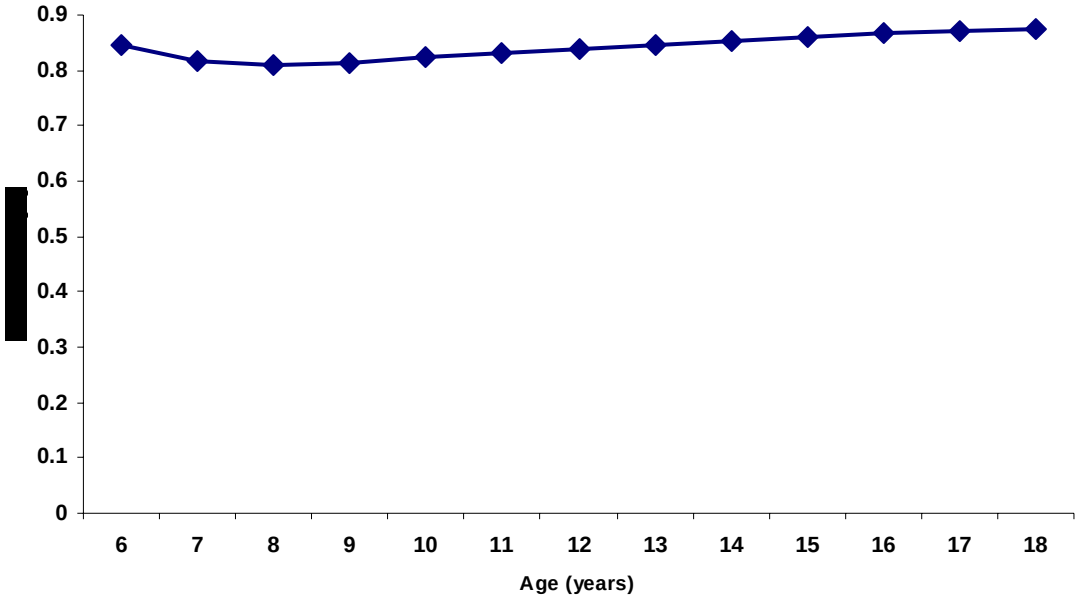
Neither gender nor age was associated with the prevalence of current hay fever among twins who had ever had hay fever (p=0.16, p=0.36, respectively). The rate was also not significantly related to passive smoking (p=.27). The best fitting polynomial model for age was the one with p=-2, giving the model equation:

$$\log (OR) = 2.4691 + 204.2986 \times age^{-2} - 129.3681 \times age^{-2} \times \log_n(age)$$

(deviance=567.26 on 641 df).

Over 80% of children who had reportedly had hay fever still had symptoms in the last 12 months, with the highest rate being recorded in 18 year olds (Figure 5-8).

Figure 5-8:
Prevalence of current hay fever by age in twins aged 6 to 18 years



5.7.2.3 GEE models

5.7.3.3.2 Univariate analysis

The only variable that was significantly associated with hay fever in the last 12 months was having been admitted to NICU immediately after birth (Table 5.14).

5.7.3.3.3 Multivariate analysis

Hay fever in the last year was not associated with family history of hay fever. Increased risk of hay fever in the last 12 months was found in twins who had been delivered by caesarean section, and whose father was in a professional occupation. However, the risk was reduced by more than 50% in twins whose families imposed no smoking rules in the home, in the bottom 10% of SEIFA code for disadvantage, and top 10% for advantage/disadvantage. Having one or more smoker in the household decreased with risk of current hay fever, but this was not significant (Table 5.14).

Table 5.14:
Significant variables in the final multivariate model and univariate analysis for current hay fever in WATCH study twins, after adjusting for age

	UNIVARIATE ANALYSIS		MULTIVARIATE MODEL	
Variable	**OR 95% CI**	**p-value**	**OR 95% CI**	**p-value**
Birth variables				
Delivered by caesarean section			1.86 (1.1-3.2)	.030
Was admitted to NICU	0.77 (.6-.95)	.014		
Parental variables				
Father in professional occupation			1.91 (1.1-3.5)	.037
Smoking variables				
No smoking rules in the home			0.49 (.3-.9)	.022
Socio-economic status				
Bottom 10% of index for disadvantage			0.30 (.1-.9)	.030
Top 10% for index of advantage/disadvantage			0.38 (.2-.7)	.0035
Smokers in household				
None	1		1	
One	1.23 (.5-2.8)	.62	1.22 (.6-2.5)	.54
Two or more	1.36 (.6-2.9)	.41	1.89 (.9-5.0)	.09

5.7.3 Eczema

5.7.3.1 Twin-family prevalences

The overall rate of eczema in twin families was 18%, which was higher in children than their parents (p<.001). Mothers had a higher rate than fathers (p<.001), but there was no difference between girls and boys (p=.084). MZ twins had a higher rate than DZ twins (p=.021), but overall, there was no difference in the rate of eczema in twins and their siblings (p=.18) (Table 5.15).

Table 5.15:
Eczema in WA twin families

	NUMBER	PREVALENCE
Parents	2654	13.2
Mothers	1405	16.9
Fathers	1249	9.0
Children	4012	21.1
Females	2040	22.2
Males	1972	20.0
Twins	2848	21.7
MZ	700	25.1
DZ	1860	20.9
Siblings	1164	19.8

5.7.3.2 Eczema in twins

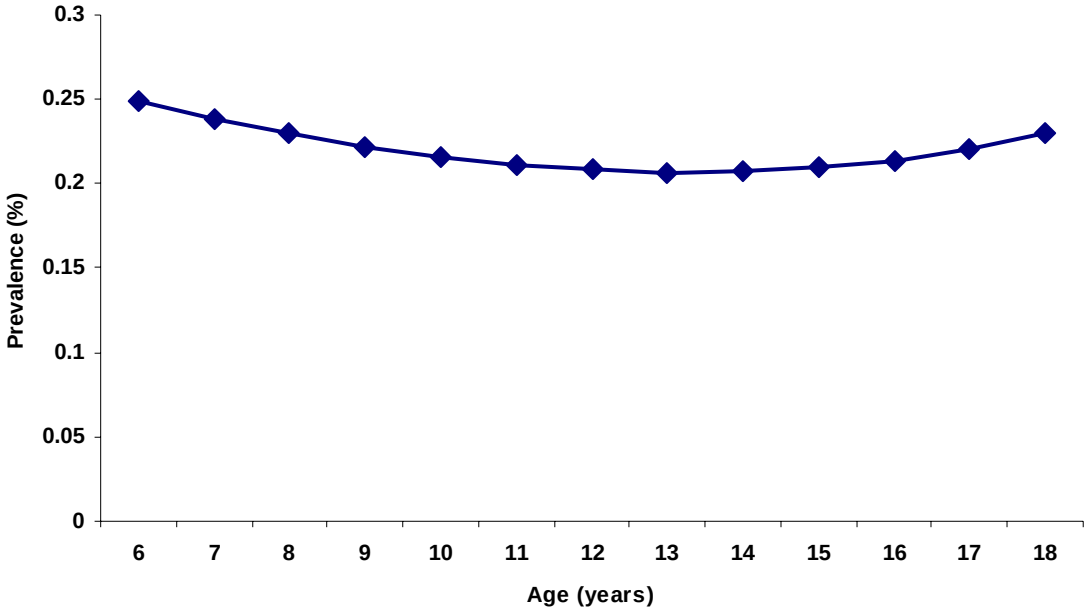
The prevalence of eczema was not related to gender (p=.88), age (p=.28) or exposure to passive smoke (p=.62). Squared terms for age best fit the data, with the model equation being:

$$\log (OR) = -0.8410 - 0.0176 \times age^2 + 0.0057 \times age^2 \times \log_n(age)$$

(deviance=2347.71 on 2225 df).

Twins aged 13 years had the lowest rate of eczema (20.7%), while twins aged 6 recorded the highest rate (24.8%) (Figure 5-9).

Figure 5-9:
Prevalence of eczema by age and sex in twins aged 6 to 18 years



5.7.3.3 GEE models

5.7.3.3.2 Univariate analysis

Eczema in twins was not related to any of the individual, twin or family characteristics examined, nor with birth weight and gestation. The premature rupture of membranes was associated lower risk of eczema in twins, but all other birth-related variables were not significant. The only childhood condition related to eczema in twins was migraine headaches. Parental eczema was a strong predictor of eczema in twins, as was the father's education. There was also no effect of exposure to ETS or socio-economic status on the risk of eczema (Table 5.16).

5.7.3.3.3 Multivariate analysis

After adjustment for age and exposure to ETS, an increased risk of eczema in twins was seen in twins whose parents also had eczema, compared with twins whose parents did not have eczema (OR=5.66; 3.0-10.6). There was no association between eczema and passive smoking. Other risk factors for eczema in twins included living in urban areas, premature rupture of membranes and father's education (Table 5.16).

Table 5.16:
Significant variables in the final multivariate model and univariate analysis for eczema in WATCH study twins, after adjusting for age

	UNIVARIATE ANALYSIS		MULTIVARIATE MODEL	
Variable	**OR 95% CI**	**p-value**	**OR 95% CI**	**p-value**
Family Variables				
Lives in rural area			0.76 (.6-1.0)	.049
Complications of pregnancy				
Premature rupture of membranes	0.57 (.3-1.0)	.047	0.50 (.3-.9)	.022
Childhood conditions				
Migraines	1.62 (1.0-2.5)	.036		
Parental phenotypes				
Mother has eczema	2.12 (1.6-2.8)	<.0001	2.10 (1.6-2.8)	<.0001
Father has eczema	2.67 (1.9-3.8)	<.0001	2.51 (1.8-3.6)	<.0001
Parental variables				
Father has tertiary qualifications	0.64 (.5-.9)	.0038	0.57 (.4-.8)	.0006
Smokers in household				
None	1		1	
One	1.10 (.8-1.5)	.51	1.00 (.8-1.4)	.97
Two or more	1.07 (.8-1.4)	.66	1.01 (.8-1.4)	.93

5.7.4 Current eczema

5.7.4.1 Twin-family prevalences

There was no difference in the rate of persistent eczema between parents and children (p=.062), twins and their siblings (p=.32), mothers and fathers (p=.86), and MZ and DZ twins (p=.095). However, among children, the rate was significantly higher in girls than boys (p=.009). The overall rate of current eczema in twin families was 51% of those who had ever had eczema (Table 5.17).

Table 5.17:
Current eczema in WA twin families

	NUMBER	PREVALENCE
Parents	342	55.3
Mothers	233	54.9
Fathers	109	56.0
Children	838	49.3
Females	451	53.4
Males	387	44.4
Twins	610	50.3
MZ	173	54.9
DZ	385	47.3
Siblings	228	46.5

5.7.4.2 Current eczema in twins

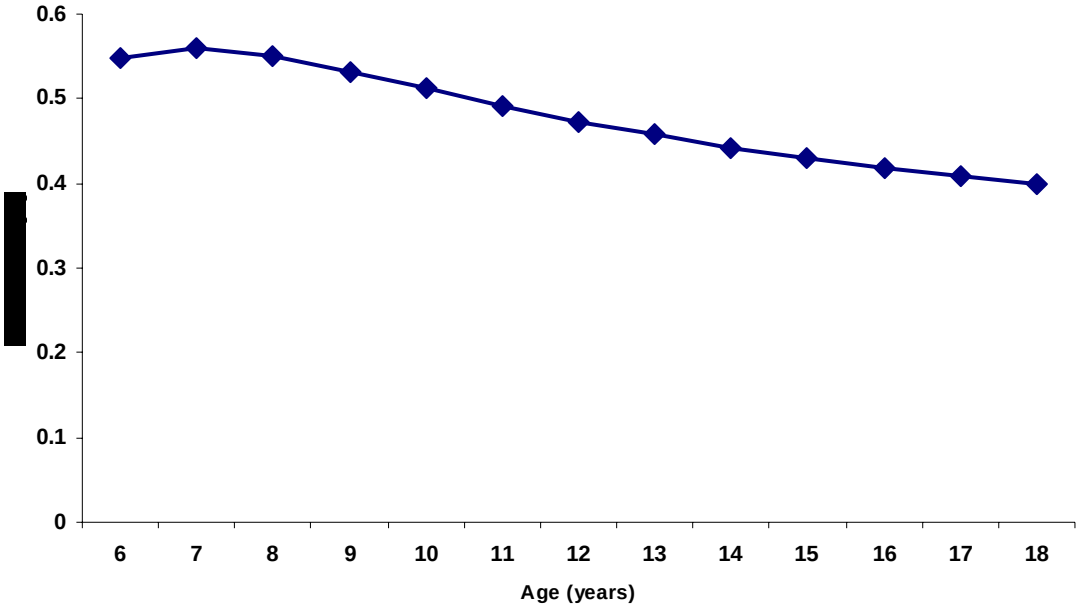
Age, gender and passive smoking all had no significant effect on the prevalence of current eczema in twins (p=.051, p=.071 and p=.72, respectively). The best fitting polynomial for age was when p=-2, giving the model equation:

$$\log (OR) = -0.8991 - 154.0526 \times age^{-2} + 107.9704 \times age^{-2} \times \log_n(age)$$

(deviance=664.49 on 481 df).

The prevalence of eczema in the last 12 months showed a decline from 56.1% at age 7 to 39.9% at age 18 (Figure 5-10).

Figure 5-10:
Prevalence of current eczema by age in twins aged 6 to 18 years



5.7.4.3 GEE models

5.7.4.3.1 Univariate analysis

Girls had a higher rate of current eczema than boys, as did those twins born during the autumn months (September, October or November). Current eczema was associated with the mother having a urinary tract infection during pregnancy. Having other milk introduced before 4 months of age increased the risk of current eczema in twins, but the risk was independent of breast feeding, birth weight and gestation. The only childhood condition that was associated with the risk of current eczema in twins was having migraine headaches. Young paternal age decreased the risk of current eczema in twins, but no other parental characteristics were significant. The rate of current eczema was independent of exposure to ETS and socio-economic status (Table 5.18).

5.7.4.3.2 Multivariate analysis

Girls were at higher risk of current eczema compared with boys (OR=1.52; 1.02-2.5). The only other factors that were significantly related to the risk of current eczema were if the twins were born during autumn (March, April or May), and the mother experiencing a urinary tract infection (UTI) during pregnancy (Table 5.18).

Table 5.18:
Significant variables in the final multivariate model and univariate analysis for current eczema in WATCH study twins, after adjusting for age

	UNIVARIATE ANALYSIS		MULTIVARIATE MODEL	
Variable	**OR 95% CI**	**p-value**	**OR 95% CI**	**p-value**
Individual variables				
Male gender	0.67 (.4-1.0)	.044	0.66 (.4-.98)	.040
Season of birth				
Born during autumn months	0.45 (.2-.8)	.0071	0.56 (.4-.9)	.017
Complications of pregnancy				
UTI	2.85 (1.1-7.5)	.034	2.81 (1.04-7.6)	.041
Infant feeding				
Other milk introduced before 4 months	0.63 (.4-1.0)	.032		
Childhood conditions				
Migraines	0.46 (.2-1.0)	.049		
Parental variables				
Under 25 when twins born	0.46 (.2-1.0)	.042		
Smokers in household				
None	1		1	
One	0.80 (.5-1.3)	.36	0.80 (.5-1.3)	.36
Two or more	1.06 (.5-2.1)	.88	0.99 (.5-1.3)	.98

5.7.5 Atopy

In this section, atopy is defined as being diagnosed with at least one of asthma, hay fever and eczema.

5.7.5.1 Twin-family prevalences

Atopy occurred in 52% of twin family members. This rate was higher in parents than their children (p=.003), but there was no difference in the rate between twins and their siblings (p=.060). Mothers reported more atopy than fathers (p<.001), and the rate was higher in MZ twins compared with DZ twins (p=.008). Among the children, there was no significant difference between boys and girls (p=.099) (Table 5.9).

Table 5.19:
Atopy in WA twin families

	NUMBER	PREVALENCE
Parents	2654	54.6
Mothers	1405	61.6
Fathers	1249	46.6
Children	4044	50.7
Females	2054	49.4
Males	1990	52.0
Twins	2848	51.6
MZ	726	56.1
DZ	1858	50.2
Siblings	1196	48.4

5.7.5.2 Atopy in twins

Overall, 56% of atopic twins had asthma, 53% had hay fever and 39% had eczema. Nearly 10% of twins had all of asthma, hay fever and eczema (Table 5.20).

Table 5.20:
Percentage of twins with asthma, hay fever and/or eczema

PHENOTYPES	N (%)
Asthma+hay fever+eczema	152 (9.5)
Asthma+hay fever	236 (14.8)
Asthma+eczema	119 (7.5)
Hay fever+eczema	116 (7.3)
Asthma only	389 (24.4)
Hay fever only	342 (21.5)
Eczema only	239 (15.0)

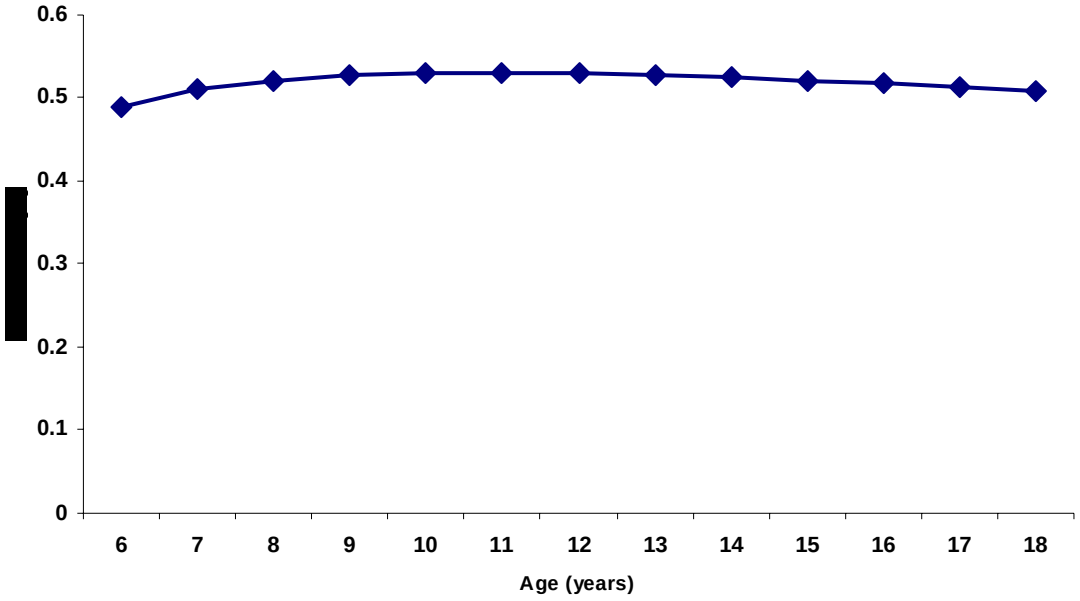
The rate of atopy in twins was independent of age (p=.82) and passive smoking exposure (p=.50), but boys had a 25% increased risk compared with girls (p=.013). The best fitting polynomial for age was when p=-0.5, which give the model equation:

$$\log (OR) = -3.0452 - 1.9473 \times age^{-1/2} + 5.1940 \times age^{-1/2} \times \log_n(age)$$

(deviance=3084.0 on 2225 df).

The prevalence of atopy showed little change over the age range of 6 to 18 years (Figure 5-11).

Figure 5-11:
Prevalence of atopy by age in twins aged 6 to 18 years



5.7.5.3 GEE models

5.7.6.3.1 Univariate analysis

Atopy was decreased in girls, twins with older siblings, and twins living in rural areas, when compared with boys, being first born in the family, and urban residence, respectively. Twins whose mothers experienced either a UTI or toxaemia during their pregnancy were more likely to be atopic. Being admitted to NICU or SCN was associated with a higher prevalence of atopy compared with not being admitted to either. Low birth weight, defined as being in the bottom quartile, was associated with a higher prevalence of atopy when compared with being in the highest quartile. There was no effect of gestation, breast feeding or age at which other milk products were introduced on atopy in twins. Atopic twins were more likely to suffer from migraines, have had at least one ear infection, and had their tonsils and adenoids removed, compared with non-atopic twins. Parental atopy was a strong risk factor for atopy in twins. Exposure to ETS had no effect on the risk of atopy in twins. Only those twins in the lowest 10% of the SEIFA index for disadvantage showed an increased risk of atopy (Table 5.21).

5.7.6.3.2 Multivariate analysis

After adjusting for age and exposure to ETS, having atopic parents significantly increased the risk of atopy in twins (OR=5.90; 3.8-9.0). After adjusting for age and all other variables, boys were at higher risk of atopy compared with girls, and those who had older siblings or who lived rural areas had a lower risk. Other factors increasing the risk were toxaemia of pregnancy, ear infections and tonsillectomy (Table 5.21).

Table 5.21:
Significant variables in the final multivariate model and univariate analysis for atopy in WATCH study twins, after adjusting for age

	UNIVARIATE ANALYSIS		MULTIVARIATE MODEL	
Variable	**OR 95% CI**	**p-value**	**OR 95% CI**	**p-value**
Individual variables				
Male gender	1.26 (1.1-1.5)	.012	1.27 (1.04-1.5)	.018
Twin variables				
Has older siblings	0.67 (.6-.8)	.0001	0.72 (.6-.9)	.0018
Family variables				
Lives in rural area	0.69 (.6-.8)	.0006	0.71 (.6-.9)	.0023
Complications of pregnancy				
UTI	1.82 (1.1-3.1)	.026		
Toxaemia	1.79 (1.2-2.6)	.0020	1.57 (1.1-2.3)	.018
Was admitted to NICU	1.26 (1.04-1.51)	.020		
Was admitted to SCN	1.21 (1.0-3.1)	.048		
Fed through naso-gastric tube after birth	1.37 (1.1-1.7)	.0031		
Birth weight: Q1 vs. Q4	1.37 (1.1-1.8)	.020		
Childhood conditions				
Migraines	1.59 (1.1-2.4)	.025		
Had at least one episode of otitis media	1.42 (1.2-1.7)	.0007	1.25 (1.01-1.6)	.045
ENT operations				
Had tonsils removed	1.64 (1.2-2.3)	.0029	1.56 (1.1-2.2)	.017
Had adenoids removed	1.58 (1.1-2.2)	.0058		
Parental phenotypes				
Mother atopic	2.61 (2.1-3.2)	<.0001	2.41 (1.9-3.0)	<.0001
Father atopic	2.26 (1.8-2.8)	<.0001	2.13 (1.7-2.6)	<.0001
Socio-economic status				
In bottom 10% for index of disadvantage	1.78 (1.0-3.1)	.044	1.90 (1.1-3.2)	.019
Smokers in household				
None	1		1	
One	0.96 (.8-1.2)	.72	0.98 (.8-1.3)	.89
Two or more	1.09 (.8-1.4)	.49	1.20 (.9-1.6)	.19

5.7.6 Current atopy

In this section current atopy is defined as doctor-diagnosed asthma, or hay fever, or eczema in the last 12 months, among those who had ever reported being atopic.

5.7.6.1 Twin-family prevalences

The overall rate of atopy in the last 12 months among atopic twins and their families was 79%. The rate was higher in parents than their children (p=.014) and mothers than fathers (p=.016). However there was no difference in the rate of current atopy between twins and their siblings (p=.54), boys and girls (p=.096) or MZ and DZ twins (p=.071) (Table 5.22).

Table 5.22:
Current atopy in WA twin families

	NUMBER	PREVALENCE
Parents	1448	80.9
Mothers	866	82.9
Fathers	582	77.8
Children	2050	77.4
Females	1015	75.9
Males	1035	78.9
Twins	1471	77.8
MZ	407	81.1
DZ	933	76.6
Siblings	579	76.5

5.7.6.2 Current atopy in twins

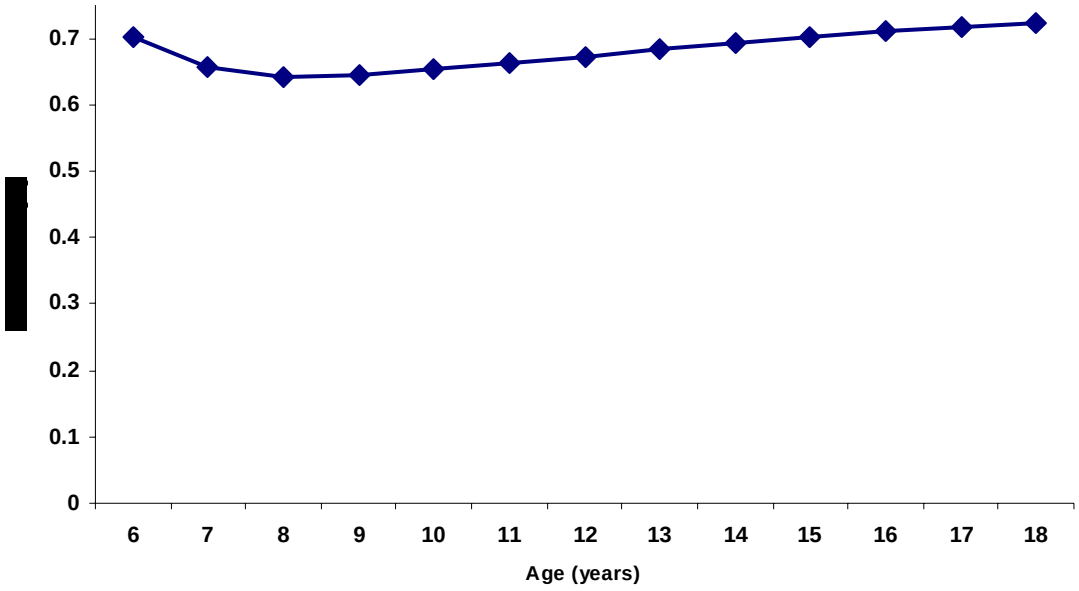
Atopy in the last 12 months was not significantly associated with age (p=.27), gender (p=.47), or exposure to ETS (p=.13). Age was modelled in all further analysis by a polynomial with p=-2, which gave rise to the equation:

$$\log (OR) = 1.4028 + 182.7432 \times age^{-2} - 112.9562 \times age^{-2} \times \log_n(age)$$

(deviance=1454.5 on 1154 df).

The prevalence of atopy in the last 12 months was least at age 8 and rose slightly each year after than (Figure 5-12).

Figure 5-12:
Prevalence of current atopy in twins by age



5.7.6.3 GEE models

5.7.6.3.1 Univariate analysis

The only individual, twin or family characteristic found to be associated with current atopy was place of residence. Twins who were delivered by caesarean section had an increased risk of atopy in the last 12 months when compared with twins delivered vaginally. All other pre-natal, birth or perinatal factors did not affect the risk. Twins in the lowest quartile of birth weight had a 60% increased risk of current atopy when compared with those in the highest quartile. There was no significant effect of gestation on current atopy in twins. The age at which other milk products were introduced, but not the length of breast feeding, was associated with the prevalence of atopy in the last year. None of the childhood conditions examined affected the prevalence of current atopy. Family history of atopy was strongly associated with current atopy in twins. However, having a young father decreased the risk. Neither exposure to ETS nor socio-economic status was related to atopy in the last 12 months (Table 5.23).

5.7.6.3.2 Multivariate analysis

There was no association between passive smoke exposure and current atopy in twins. After adjusting for age and exposure to ETS, twins with two atopic parents were twice as likely to have experienced symptoms of atopy in the last

year than those without such family history (OR=2.25; 1.1-3.8). Living in a rural area, the early introduction of other milk products, and young paternal age all reduced the risk of current atopy in twins (Table 5.23).

Table 5.23:
Significant variables in the final multivariate model and univariate analysis for current atopy in WATCH study twins, after adjusting for age

	UNIVARIATE ANALYSIS		MULTIVARIATE MODEL	
Variable	**OR 95% CI**	**p-value**	**OR 95% CI**	**p-value**
Family Variables				
Lives in rural area	0.70 (.5-.96)	.027	0.71 (.5-.99)	.038
Birth variables				
Was delivered by caesarean section	1.37 (1.0-1.9)	.046		
Birth weight – Q1 vs. Q4	1.61 (1.1-2.4)	.015		
Infant feeding				
Other milk introduced before 4 months	0.62 (.4-.8)	.0016	0.60 (.4-.8)	.0013
Parental phenotypes				
Mother atopic	1.55 (1.2-2.1)	.0046	1.44 (1.1-2.0)	.025
Father atopic	1.45 (1.1-1.9)	.011	1.40 (1.04-1.9)	.029
Parental variables				
Father has tertiary qualifications	1.54 (1.1-2.2)	.023		
Father under 25 when twins born	0.47 (.3-.8)	.0020	0.49 (.3-.8)	.0059
Smokers in household				
None	1		1	
One	0.86 (.6-1.2)	.42	0.99 (.7-1.4)	.94
Two or more	0.74 (.5-1.2)	.18	0.90 (.6-1.4)	.66

5.8 Examination of the three components of the atopy variable

A positive family history was a consistent risk factor for all of the end points which comprise atopy. Boys were at increased risk of asthma, but there was no difference in the risk of hay fever and eczema between boys and girls. Twins living in rural areas had a reduced risk of asthma, hay fever and eczema when compared with those living in the city. Having older siblings did not affect the risk of eczema in twins, but reduced the risk of both asthma and hay fever, compared with twins who do not have older siblings. The risk of asthma and hay fever, but not eczema, was increased in those twins who had experienced at least one episode of otits media compared with those who had never had otitis media. Having had the tonsils removed affected the risk of asthma, and removal of the adenoids increased the risk of hay fever (Table 5.24).

Table 5.24:
Comparison of risk factors for atopy and its component diseases in WA twins, after adjusting for age and exposure to ETS

	ATOPY	ASTHMA	HAY FEVER	ECZEMA
Variable	**OR 95% CI**	**OR 95% CI**	**OR 95% CI**	**OR 95% CI**
Individual variables				
Male gender	1.27 (1.04-1.5)	1.43 (1.1-1.8)		
Twin variables				
Has older siblings	0.72 (.6-.9)	0.71 (.6-.9)	0.67 (.5-.8)	
Family variables				
Lives in rural area	0.71 (.6-.9)	0.77 (.6-1.0)	0.62 (.5-.8)	0.76 (.6-1.0)
Complications of pregnancy				
Threatened miscarriage under 20 weeks		1.66 (1.2-2.4)		
Premature rupture of membranes				0.50 (.3-.9)
Toxaemia	1.57 (1.1-2.3)			
Childhood conditions				
Migraine headaches			1.67 (1.02-1.6)	
Had at least one episode of otitis media	1.25 (1.01-1.6)	1.32 (1.03-1.7)	1.29 (1.02-1.6)	
ENT operations				
Had tonsils removed	1.56 (1.1-2.2)	1.51 (1.04-2.2)		
Had adenoids removed			1.80 (1.2-2.6)	
Parental phenotypes				
Mother	2.41 (1.9-3.0)	3.12 (2.4-4.1)	2.46 (1.9-3.1)	2.10 (1.6-2.8)
Father	2.13 (1.7-2.6)	2.65 (1.9-3.6)	2.50 (2.0-3.1)	2.51 (1.8-3.6)
Parental variables				
Father has tertiary qualifications				0.57 (.4-.8)
Socio-economic status				
In bottom 10% for index of disadvantage	1.90 (1.1-3.2)	2.20 (1.2-4.0)		
In bottom 10% for index of economic resources		4.08 (1.6-10.4)		

5.9 Characteristics of chronic asthmatic twins

A number of questions were asked to determine characteristics of those twins who had received a diagnosis of asthma from a doctor. Of these twins, 53% of them reported symptoms in the past 12 months. More reported that their asthma was worse in winter than at any other time of the year (48%), which could be explained by symptoms worsening during episodes of colds and influenza, which was reported by 68%. Nearly half of twins (45%) reported that no particular activity triggered their asthma, but 33% and 36% reported their asthma worsened while playing sport or running, respectively. Eleven percent felt that their asthma was severe enough to affect their usual daily activities. Apart from colds and the 'flu, the most common triggers of an asthma attack were cold weather (39%), changes in temperature (26%), exposure to cigarette smoke (16%), exposure to dust (19%) and exposure to cats (11%). Only nine percent of twins reported that they could recall no specific triggers.

The most common medication that asthmatics had ever taken was relievers (Ventolin, salbutamol etc.), which was reported by nearly 50% of asthmatic twins. However, nearly a quarter of them reported using inhaled steroids. Fifteen percent of twins who had been diagnosed with asthma under the age of 15 took medication every day, or nearly every day; once again the most common medication being Ventolin (46%) and inhaled steroids (59%). The use of inhaled steroids every day could be viewed as a measure of asthma severity, and this was taken by a majority of children whose parents reported that they took medication for their asthma every or on most days. Another 21% of children took Intal, which is used in the same way as inhaled steroids.

5.10 Effect of having asthma on behaviour and education

Asthmatic twins were more likely than non-asthmatic twins to display behaviours characteristic of ADHD (hyperactive type), have had at least one accident in the last year, been absent from school for at least one week in the last year and required remedial education at school (Table 5.25). Although not significant, they also displayed poorer outcomes in other educational variables examined.

Table 5.25:
Percentage of asthmatic and non-asthmatic twins with certain characteristics of behaviour and eduation outcomes

OUTCOME	ASTHMATIC TWINS	NON-ASTHMATIC TWINS	P-VALUE
ADHD – hyperactive	13.9	11.2	.05
ADHD – inattentive	9.5	8.1	.22
Parental satisfaction with behaviour	6.5	6.4	.91
Had at least one accident in last year	22.7	18.8	.018
Absent from school for more than 1 week in the last year	31.5	19.7	<.001
Attended general remedial class	7.1	4.2	.0016
Remedial English class	4.7	5.1	.67
Remedial reading class	11.2	9.4	.15
Remedial maths class	5.1	4.3	.38
No remedial class	67.3	70.1	.23
Needs work in oral language	6.6	5.4	.23
Written language	12.7	10.7	.13
Reading	12.2	10.3	.14
Number	8.1	8.2	.92
Motor skills	4.6	3.1	.048
No extra work needed	75.2	76.1	.64
Parents satisfied with education	11.4	10.8	.68

5.11 Childhood asthma in the WA population

The prevalence of asthma of six year old children in the Raine study was 23% (Joseph-Bowen *et al* 2004). The prevalence of current asthma in the same cohort was 18% (Joseph-Bowen *et al* 2004). In the ISAAC study, the prevalence of asthma in 6-7-year-olds was 28.4% and in 13-14 year-olds, 30.2% (Asher *et al* 1998). This compares with 28% in WATCH study twins.

5.12 Asthma in WATCH study families

The prevalence of asthma in children was higher in boys than in girls, but in their parents, mothers had a higher rate than fathers. To allow for this in the model, a variable "child" was created, coded 1 if the individual was a child and 0 if they were a parent; and the interaction of this variable and sex was included in the model. Age best fit the data when modelled with a polynomial of order 1, giving the equation

$$\log (OR) = 0.7404 + 0.0225 \times age - 0.0022 \times age \times \log_n(age)$$

(deviance=5806.64 on 5214 df)

After adjusting for age, there was no effect of smoking status on the risk of asthma in WA twin families. The multivariate model of DDA in twin families showed that, for children, boys had a higher risk than girls (OR=1.27; 0.7-2.2), and, in parents, mothers had a higher risk than fathers (OR=1.43; 1.1-2.0) (Table 5.26). As was found in the twin analysis, living in rural areas reduced the risk of asthma in twin families, but having the tonsils removed and being in the lowest 10% of both the index for disadvantage/advantage and economic resources increased the risk. Tables giving results of the univariate analysis for all the covariates considered can be found in Appendix 7.

Table 5.26
Significant variables in univariate and multivariate analysis of DDA in WATCH twin families, after adjusting for age

	UNIVARIATE ANALYSIS		MULTIVARIATE MODEL	
Variable	**OR 95% CI**	**p-value**	**OR 95% CI**	**p-value**
Individual variables				
Child	1.18 (.7-2.0)	.53	1.20 (.7-2.0)	.51
Male gender	0.67 (.5-.8)	.0011	0.70 (.5-.9)	.0047
Child*sex interaction	1.88 (1.4-2.5)	<.001	1.81 (1.4-2.4)	<.001
Family variables				
Lives in rural area	0.80 (.7-1.0)	.028	0.80 (.6-1.0)	.025
Conditions diagnosed by a doctor				
Migraines	1.34 (1.1-1.7)	.017		
Had at least one episode of otitis media	1.22 (1.0-1.5)	.031		
ENT operations				
Had tonsils removed	1.61 (1.3-2.0	<.001	1.58 (1.3-1.9)	<.001
Had adenoids removed	1.64 (1.3-2.0)	<.001		
Socio-economic status				
Bottom 10% of index of disadvantage	1.75 (1.2-2.6)	.009	1.72 (1.1-2.6)	.0094
Bottom 10% of index of economic resources	2.12 (1.0-4.6)	.046	2.33 (1.1-4.9)	.025
Smoking status				
No exposure	1		1	
One smoker in house	1.07 (.9-1.4)	.55	1.05 (.8-1.3)	.67
Two + smokers in house	1.04 (.8-1.3)	.74	1.02 (.8-1.3)	.86
Active smoker	0.96 (.8-1.2)	.76	1.02 (.8-1.3)	.90

5.13 Summary of results

Data collected by the WATCH study has allowed the epidemiology of numerous end points associated with asthma and atopy to be examined. A wide range of known and potential risk factors have been analyzed, and include those thought to act during the pre-natal and immediate post-natal periods, as well as during pregnancy and later in childhood. The collection of family data has also allowed

the effects of parental characteristics and those common to the family to also be analyzed.

The most important risk factor identified for the development of asthma and atopy in WA twins was a positive family history. No significant association with exposure to ETS was found with any of the end points examined. The risk of asthma was not associated with length of breast feeding or with the age at which other milk products were introducted. Twins living in the Perth metropolitan area were at increased risk of asthma and hay fever compared with those living in rural areas. Only asthma showed any association with a measure of SES as determined by the four SEIFA codes.

As the questionnaires did not include questions on respiratory infections during childhood, child care attendance or vaccination history, having older siblings was used as a proxy for respiratory infections during childhood in order to test the validity of the hygiene hypothesis. Indoor air pollution is an important risk factor to the development of asthma and atopy, but likely sources of this type of pollution, namely, exposures to furry pets, type of indoor heating in the home, and floor coverings, could not be directly assessed.

Some studies have suggested that mothers who experience asthma during pregnancy are more likely to have asthmatic children than those who do not. Although materal asthma was included as a covariate in the analysis of asthma in twins, whether or not they had an episode of asthma during pregnancy could not be determined.

CHAPTER 6
GENETIC EPIDEMIOLOGY OF ASTHMA IN TWIN FAMILIES

6.1 Preface

This chapter firstly describes the basic principles of genetic epidemiology and how they apply to asthma. Data from the WATCH study are then used to estimate the relative contributions of genetic and environmental factors to the aetiology of doctor-diagnosed asthma (DDA) in twins and their families. New methods are developed to model and analyze continuous and binary phenotypes in twin-family data. These methods are an extension of the methodological work of Burton, Scurrah and colleagues (Burton *et al* 1999, Scurrah *et al* 2000) who developed methods of analyzing family data using BUGS (**B**ayesian inference **U**sing **G**ibbs **S**ampling)(Spiegelhalter *et al* 1995) and later, WinBUGS software (Spiegelhalter *et al* 2000). Models are developed and then validated using simulated data to ensure they give unbiased and accurate estimates. Results obtained by these new methods are then compared with those from the logistic models described in the previous chapter. Finally, the validity of the EEA with respect to doctor-diagnosed asthma in WA twin families is tested, by estimating the extra covariance arising due to being a MZ twin.

6.2 Genetic epidemiology

The discipline of genetic epidemiology is relatively young and was thought to have originated in the 1960s as the fusion of traditional epidemiology and classical/molecular genetics. Pioneers in the field include Newton Morton, Douglas Falconer, Robert Elston, Elizabeth Thompson and Neil Risch. The journal *Genetic Epidemiology* was first published in 1985, and the International Genetic Epidemiology Society formed some eight years later. Traditionally, genetics and epidemiology differed in their objectives; epidemiology focussed on the environmental causes of disease, such as smoking and exposure to asbestos and cancer (de Klerk *et al* 1996, Hansen 1995), whereas genetics has been interested in discovering the genes responsible for causing diseases such as breast cancer (Garrett *et al* 1995) and Wilms' tumour (Zhao *et al* 1997).

However, both disciplines share the same mission, that is, an understanding of the aetiology of human disease, whether genetic or environmental. Genetic epidemiology means different things to different people, but the most widely accepted definition is that of Newton Morton, viz.

> "genetic epidemiology is a science that deals with aetiology, distribution and control of disease in groups of relatives and with inherited causes of disease in populations" (Morton 1982).

Rao pointed out several differences between genetic epidemiology and its parent disciplines: "genetic epidemiology differs from epidemiology by its explicit consideration of genetic factors and family resemblance; it differs from population genetics by its focus on disease; it also differs from medical genetics by its emphasis on population aspects" (Rao 1984).

Historically, epidemiological studies used population-based case-control or cohort studies to examine the role of specific environmental exposures, whereas genetic studies involve families and/or extended pedigrees to investigate familial aggregation of traits, segregation of major disease genes, and locations of disease genes on the human genome by the use of linkage analysis. By integrating both objectives, genetic epidemiology aims to assess the *associations* of environmental factors with disease status, to quantify the *aggregation* of cases within families, to characterize putative disease genes via *segregation* analysis, and finally to localize disease genes via *linkage* analysis with genetic markers.

Most of the earlier successes in genetic epidemiology research were made with monogenic diseases such as cystic fibrosis, which follow the simple Mendelian pattern of inheritance, but today much of its focus is the study of complex diseases, such as cardiovascular disease, cancer, diabetes mellitus and asthma. These diseases are polygenic in nature and do not follow Mendelian inheritance.

Research strategies used in genetic epidemiology include population and family studies, which provide complementary methodologies for studying the role of genetic factors in disease. Population studies concentrate on describing the distribution of genetic traits and disease in populations, identifying the risk

factors associated with the frequency of genetic traits in populations and examining the role of genetic factors in disease aetiology. In family studies, the concept of familial aggregation is central to genetic epidemiology and allows three important questions to be addressed (King *et al* 1984):

1. whether a specific disease clusters in families, ascertained either by comparing disease frequency in relatives of cases with that in either relatives of controls or in the general population.
2. if it does, whether it is related to a common environment, biologically inherited susceptibility, or cultural inheritance of risk factors, and
3. the pattern of inheritance of the genetic susceptibilty. Inheritance can either be single-gene (following Mendelian principles), or involve more than one loci. Complex diseases are characterised by multiple genetic and environmental determinants.

The aggregating of the disease or trait of interest is the first step in a genetic epidemiology study. This phenomenon can be examined by determining whether the relatives of an affected individual have an increased risk of the disease or trait than either the relatives of an unaffected individual, or the general population. This clustering can either be due to genetics or environment, as both are known to aggregate within families. Hence, familial aggregation of a disease is a necessary but not sufficient condition to infer the importance of a genetic contribution. Not taking into account the effects of environment leads to an over-estimation of any genetic effect. Epidemiological methods address clustering by, for example, assessing whether increased risk of disease in relatives remains after controlling for potential confounders. Genetic methods commonly use a multi-factorial model of inheritance and use variance components approaches to infer the degree of genetic control in traits. Because relatives share genes in a predictable fashion and may also share environments in some defined manner, the covariance or correlation between relatives can be separated into common genetic and common environmental components, and this partition of the covariance forms the basis for analysis of familial data (Elston 1981).

Once a genetic basis for the disease has been established, the next stage is to determine the mode of inheritance of that disease, that is, is the disease affected

by only one gene (monogenic), such as cystic fibrosis, or is it a complex or polygenic disease where several genes play a part in its aetiology? This is achieved by using segregation analysis of data from extended pedigrees or multi-generational families. The gene(s) that make up the genetic component of disease aetiology can then be identified by linkage and association studies. Linkage is used to determine the location of the gene(s) by selecting affected relative pairs, usually siblings, and assessing whether they are also concordant for a known genetic marker on the same chromosomal region as the gene. It is not within the scope of this thesis to perform segregation, linkage or association studies, and no further descriptions will be given.

A number of different study designs have been used to address the issue of familial aggregation, and include family studies and twin studies. In family-based study design, families are ascertained through probands, and disease occurrence in their first degree relatives is examined. Probands can be considered as either "cases" or "controls" in the traditional epidemiological sense, and information about disease in their relatives can be obtained either from the probands or by recruiting the relatives themselves into the study. However, results are likely to be biased if recall depends on whether the proband is a case or control (Hopper 1992). By including the relatives themselves in the study, the increase in sample size may mean that the cost of conducting such a study would be prohibitive to some researchers. In genetic epidemiology, some of the most efficient studies are those that involve twin pairs (Eaves *et al* 1978, Neale & Cardon 1992). They are the minimum informative set of relatives and have the advantage of sharing more genetic and environmental "information" than any other pair of relatives.

6.3 The classic twin design

The classic twin design has been in existence since early last century, when the use of twins in research was acknowledged in 1924 simultaneously by German dermatologist Hermann Siemanns and American psychologist Curtis Merriman (Spector 2000). Although Francis Galton had earlier viewed twins as being useful for studying "nature vs. nurture" (Rende *et al* 1990), it is not clear that he recognised that there were two types of twins. The same could be said of

Thorndike and Fisher, who both believed that all twins were of the same kind (Parisi 1995, Rende *et al* 1990).

There are three basic assumptions which underpin the classic twin study; firstly, there are two types of twins (MZ and DZ) and researchers can reliably distinguish between them; secondly, the results from twin studies are generalizable to the population from which the twins are drawn; and thirdly, the environments of MZ and DZ twins are equal. This third assumption is commonly referred to as the "equal environments assumption" (EEA). If the twin method is valid, it can be concluded that a greater phenotypic similarity among MZ than among DZ twins must be caused by the greater genetic similarity, and that any discordance in MZ pairs can be attributed to environmental factors only (Kyvik 2000).

The twin method has been the subject of much criticism, although it is becoming more popular again after reaching a low point in the 1960s following Don Jackson's critique relating to five published studies on schizophrenia, where he pointed out the female twins were consistently more concordant than male twins, and that DZ twins were more concordant than ordinary siblings (Joseph 2001). Perceived problems with the method have included the lack of a consistent definition of the trait under study, inadequate or biased methods of zygosity determination, and the use of non-representative sample populations. But the main criticism focuses around the validity of the EEA (Joseph 2000), and has led to two different definitions of EEA being formed. The "traditional EEA definition" was the only definition in use until the 1960s. It simply states that the environmental conditions experienced by MZ and DZ twins are roughly the same, and that a greater phenotypic similarity of the MZs must therefore be due to their greater genetic similarity. However, many twin researchers acknowledged that this assumption was not necessarily true (Kendler *et al* 1994, Morris-Yates *et al* 1990, Rose 1991, Scarr & Carter-Saltzman 1979). MZs are treated more similarly than DZ twins by their parents and by the social environment, spend more time together, and are more likely to be dressed identically during childhood (Kendler *et al* 1986). Sandra Scarr, a behaviour geneticist, noted that, "the evidence of greater environmental similarity for MZ than DZ twins is overwhelming" (Scarr & Carter-Saltzman 1979). This then led

researchers to consider the concept of a "trait-relevant EEA" (Carey & Di Lalla 1994). One of the first examples to use this definition was in the field of schizophrenia research, where it was defined as: "the assumption of equal environments .... in respects which can be shown to be of etiological significance to schizophrenia" (Gottesman & Shields 1972). Some 20 years later, Kendler and colleagues described the EEA as: "that MZ and DZ twins are equally correlated for their exposure to environmental influences that are of etiological relevance to the trait under study" (Kendler *et al* 1993).

The literature on the validity or otherwise of the EEA has been dominated by studies in the fields of psychology, psychiatry, behaviour, intelligence and personality, and researchers in these fields have employed various methods to test its validity (Kendler *et al* 1993). Some have endeavoured to adjust for environment in their models by including such terms as the amount of time twins spend together (Cronk *et al* 2002), or by stratifying twin correlations by indices of the amount of shared environment (Eaves *et al* 2003). Attempts have been made to examine the notion that resemblance in twins may be influenced by the similarity in which they are treated by their social environment, which is a result of their degree of physical resemblance. In other words, are twins more similar because they are treated more similarly, or does the fact that MZ twins are more physically similar cause them to be treated more similarly? Three studies examining intelligence and personality (Matheny *et al* 1976, Plomin *et al* 1976) and schizophrenia (Kendler 1983), however, found no evidence that resemblance in twin behaviour was influenced by physical similarity. By directly observing twins in a social situation, one study found that parental behaviour that was in response to twin behaviour was responsible for the excess resemblance observed in the parental treatment of MZ twins compared with DZ twins (Lytton 1977).

It is well-known, that compared with same-sex DZ twins, MZ twins are more likely to share the same bedroom, share play mates and be dressed alike during childhood (Kendler *et al* 1986, Kendler *et al* 1992c), and are in more frequent social contact as adults (Rose *et al* 1990). The majority of studies in the field of psychiatry have been unable to find any connection between the degree of environmental similarity and twin similarity for anxiety and depression

(Kendler *et al* 1992a, b, c, Morris-Yates *et al* 1990) and alcoholism (Heath *et al* 1989a). In contrast, two studies found that frequency of contact as adults was related to twin similarity of self-reported psychiatric symptoms (Clifford *et al* 1984) and for alcohol intake and personality (Kaprio *et al* 1990). However, a correlation between frequency of adult contact and phenotypic similarity in twin pairs need not be a causal one, and degree of phenotypic similarity could influence frequency of contact. For example, while twins in frequent contact may develop similar drinking patterns, twins with more similar drinking patterns may tend to see each other more frequently.

The impact on twin resemblance of "real" versus "perceived" zygosity has been examined in a number of studies, as it is possible that twins and parents of twins may be misinformed about the twins' true zygosity. Studies examining intelligence (Matheny 1979), hyperactivity (Goodman & Stevenson 1989) and psychiatric illness (Kendler *et al* 1993) have, however, found little evidence that perceived zygosity influenced twin similarity.

If the EEA is not true, and MZ twins experience a more similar environment than DZ twins, then the effect of genetic factors to the aetiology of the disease or trait of interest would be over-estimated, as any excess in MZ correlation compared with DZ correlation is often interpreted as being due to genetic factors. It is important that the validity of the EEA be determined with respect to each trait or disease under study (Burton *et al* 2005, Hopper 2000). Researchers should be able to test the validity of the EEA rather than just assume it is true. If violations of the EEA are detected, results need not be automatically rejected. With so many statistical methods now available, researchers should be able to adjust for these violations in their analysis when determining the role of genetic and other familial environmental factors. As far as I am aware, analysis of twin data which includes specific terms claiming to measure the environment of MZ and DZ twins in studies of health issues other than behaviour has not been described in the literature.

Another important criticism of the twin method is the ability to apply any results from twin studies to the general population. Twins are known to differ from singletons in several important aspects; they share the same uterine

environmental exposure, such as maternal smoking and drinking, and maternal infections during pregnancy, at the same time, which is not the case for two single-born siblings. They are usually raised in the same family as children, and are the same age. Although twins are born lighter and at earlier gestation than singletons (O'Brien & Hay 1987, Petterson *et al* 1993, Phillips 1993), these factors are not necessarily important to every disease or trait under study. It is important to ascertain that the prevalence of the disease or trait being studied is the same in twins and singletons, and that there is no association between the disease state and zygosity or sex (Kyvik 2000). Confirming these results means that any results found in a twin study can then be generalized to the population from which they are drawn.

6.4 Measures used in genetic epidemiology

6.4.1 Familial aggregation

In family-based study designs, where study families are often ascertained through probands, familial aggregation for a disease is measured by the relative recurrence risk (RRR), λ_R, where R denotes the type of relationship, for example, S=siblings, O=offspring, DZ=dizygotic twin, etc. The value of λ_R is calculated by dividing the risks of relatives of type R of being affected by the population prevalence of the disease. Examination of λ_R for various classes of relatives can then potentially suggest that the disease may have a genetic component.

6.4.2 Heritability

The basis of any analysis of twin data is a comparison of the phenotypic covariance in monozygotic (MZ) and dizygotic (DZ) twins, which allows the decomposition of the phenotypic variance into genetic and environmental components.

A measure of genetic risk attributable to a binary trait, that is, the individual has or does not have the disease of interest, is calculated by the concordance rate. This rate can be either pairwise or probandwise (MacGregor 2000). Pairwise concordance is defined as the probability that a twin is affected, given that at

least one member of the twin pair is affected, and is estimated by the ratio of concordant (C) to the sum of concordant (C) and discordant (D) pairs. Probandwise concordance (Cc), the preferred measure (McGue 1992), is the probability that a twin is affected, given that the co-twin is affected. For a sample that has identified all affected twins, it is given by the formula:

$$Cc = 2C / (2C + D)$$

The heritability (h^2) of a trait is defined as the proportion of phenotypic variance attributable to genetic effects. Narrow-sense heritability is the variance of additive genetic effects divided by the total variance, whereas broad-sense heritability is defined as the total genetic variance (additive + non-additive) divided by the total phenotypic variance. It is formally defined for quantitative traits (Hopper 2002b). For binary traits, a hypothetical construct known as "liability" is used, and heritability is calculated by applying a version of variance components modelling to the liability. Liability is defined as an underlying, unobservable, Normally-distributed trait that is assumed to determine the probability that an individual develops the disease of interest (Falconer 1965, Hopper 1993, 2002b, Khoury *et al* 1993). The correlation of liability between relatives is termed the tetrachoric correlation. Unfortunately, with a binary phenotype, the heritability of the liability does not have a clear meaning and is prone to confused interpretation (Burton & Tobin 2003, Hoover 2000, Lichtenstein *et al* 2000, Spector 2000).

The intraclass correlation coefficients of MZ and DZ twins (ρ_{MZ} and ρ_{DZ} respectively) can be used to calculate the heritability of a trait and to estimate the size of the environmental components of variance. In this context, heritability, h^2, is defined as twice the difference of the intra-class correlation coefficients of MZ and DZ twins (Jensen 1967), that is:

$$h^2 = 2(\rho_{MZ} - \rho_{DZ})$$

The proportion of variance attributable to common environment, c^2, can be estimated by:

$$c^2 = 2\rho_{DZ} - \rho_{MZ}$$

Environmental effects on a trait can be estimated by subtraction, namely:

$$e^2 = 1 - (h^2 + c^2) = 1 - \rho_{MZ}$$

Twin studies have been used to assess the importance of a genetic component in the aetiology of many diseases, including celiac disease (Greco *et al* 2002), Alzheimer's disease (Raiha *et al* 1996), schizophrenia (Sullivan *et al* 2003), and cancer (Risch 2001). They have also been used to estimate heritability, which has typically ranged from 50-90%, indicating that genetic effects play an important role in disease susceptibility (Table 6.1).

Table 6.1:
Estimates of heritability of common complex diseases from twin studies

PHENOTYPE	HERITABILITY (%)
Asthma	60
Bone mineral density	60-80
Cervical and lumbar disc degeneration	60-80
Insulin-dependent diabetes mellitus	70
Osteoarthritis	50-70
Rheumatoid arthritis	60
Ulcerative colitis	50

6.4.3 Partitioning of phenotypic variance

Much of the analysis of twin data centres around variance component analysis. Phenotypic variation between MZ and DZ twins can be divided into effects due to genes and effects due to environment. Further, the genetic effects can be divided into additive (A) and non-additive (D), also called dominance, and the environmental effects into that shared by twins (C) and non-shared or individual-specific (E). Additive genetic effects are the effects of genes taken singly and over multiple loci, whereas genetic dominance represents genetic interaction, within loci. Additive genetic effects and genetic dominance are perfectly correlated in MZ twins, whereas DZ twins, like ordinary siblings, share only half their additive genetic effects, and one quarter of genetic dominance. Shared environmental effects are perfectly correlated in both MZ and DZ twins.

On the assumption of random mating in the population, the expected phenotypic correlations for MZ and DZ twins are respectively:

$$\begin{aligned}\rho_{MZ} &= h^2 + c^2 + d^2 \\ &= \sigma^2_A + \sigma^2_C + \sigma^2_D; \text{ and} \\ \rho_{DZ} &= \tfrac{1}{2}h^2 + c^2 + \tfrac{1}{4}d^2 \\ &= \tfrac{1}{2}\sigma^2_A + \sigma^2_C + \tfrac{1}{4}\sigma^2_D\end{aligned}$$

Since estimates of σ^2_C and σ^2_D are confounded in data for twins reared together (Eaves *et al* 1978, Martin *et al* 1978), the MZ and DZ phenotypic correlations may be parameterised as either an ACE or ADE model.

The relevant variance components model can be derived by considering the relative size of MZ and DZ correlations. If the MZ correlation is twice the DZ correlation, additive genetic effects play a role in the phenotypic variance, and an AE model is plausible. Alternatively, an ACE model is suggested if the MZ correlation is less than twice the DZ correlation, and MZ correlation is also greater than the DZ correlation. If the MZ correlation is greater than twice the DZ correlation, then genetic dominance is likely, with the ADE model being the most likely. Genetic factors are not important if the two correlations are equal. In summary:

$\rho_{MZ}=2\rho_{DZ}$	=> additive genetics	=> AE model
$\rho_{MZ}<2\ \rho_{DZ}$ and $\rho_{MZ} > \rho_{DZ}$		=> ACE model
$\rho_{MZ}=\rho_{DZ}$	=> genetics not important	=> CE model
$\rho_{MZ}>2\ \rho_{DZ}$	=> genetic dominance	=> ADE model

6.5 Methods of analysis

The development of statistical methods to study familial disease has progressed along two different lines. Epidemiologists use the logistic regression model to assess the role of genes in disease aetiology, whereas geneticists favour multi-factorial methods to clarify the mode of transmission of major disease by studying segregation within study families, and through their association and linkage to known genetic markers.

Variance components analysis can be undertaken with conventional statistical models such as maximum likelihood (Hopper & Mathews 1982) and generalized least squares (Neale & Cardon 1992), or MCMC-based approaches (Burton *et al* 1999). Genetic epidemiologists use various approaches to aide the specification of such models, including path analysis, which was invented by Sewall Wright nearly a century ago (Wright 1921), and the fitting is achieved by a number of programmes such as LISREL (Joreskog & Sorbom 1986) and Mx (Hopper 1993, Lange *et al* 1988, Neale & Cardon 1992, Rasbash *et al* 1999, Spiegelhalter *et al* 2000, Zeger & Liang 1992) to continuously-distributed data. Equivalent approaches can also be used for binary phenotypes (Burton *et al* 1999, Falconer 1965, Neale & Cardon 1992), and for survival time analysis (Gauderman & Thomas 1994, Scurrah *et al* 2000). MCMC methods (Smith & Roberts 1993) are playing an increasing role in genetic epidemiology. Gibbs sampling has been used in fitting variance components models for Normally distributed traits in complex pedigrees (Guo & Thompson 1991), and for combined segregation and linkage analysis (Guo & Thompson 1992, Sobel & Lange 1993).

Alternatively, twin models can be formulated as linear mixed effects models in standard statistical packages such as SAS, SPlus, SPSS and STATA. These models can be extended to generalized linear mixed models when analyzing discrete data. These methods are theoretically plausible and practically feasible for continuous univariate and multivariate normal data, but are often impractical when the models become more complex as their solution involves solving high-dimensional integrals.

Short descriptions of some of the various methods of analysis employed in genetic epidemiology are given below.

6.5.1 Regressive methods

Simple genetic and environmental models can be tested using a regression approach. Regressive models were developed by Bonney (Bonney 1984) to analyze family data, where the families were ascertained via probands. These methods condition each subject's phenotype on those of his or her antecedents, and generate a Markov structure reflecting the serial dependency of family

members. This method requires the arbitrary ordering of family members, usually by age. The original models were derived for continuous variables, but later extended to include binary data via regressive logistic models (Bonney 1986). For genetic analysis of family data, Bonney (Bonney 1984) proposed four classes of regressive models as alternatives to the mixed model of complex segregation analysis. They are that:

1. the adjusted residuals of children are independent after adjusting only for parental effects;
2. the first few older sibs, as well as the parents, are needed to account for dependencies in the younger sibs;
3. in a large sibship, sibs close together are more highly correlated than sibs further apart; and
4. sib-sib correlations are assumed to be equal for all pairs of sibs within a sibship, but the common sib-sib correlation is not necessarily due to common parentage alone.

Another widely used method was that developed by de Fries and Fulker (de Fries & Fulker 1985), designed particularly for the analysis of proband-ascertained samples, and is particularly useful when the "proband" has been selected as recording a "deviant" score measured on a continuous variable. This method describes analysis in which a co-twin's score is predicted from the proband's score and the degree of relationship (1.0 for MZ twins and 0.5 for DZ twins). These methods can be extended to include other family members such as siblings and parents by the inclusion of additional coefficients as dummy variables to model shared environmental influences that vary as a function of the degree of relationship (de Fries & Fulker 1985).

One of the major limitations of regressive models is the interpretation of the parameters estimating familial dependence, and its inability to handle multiple, possibly interacting, dependent variables, or multiple family relationships (Bonney 1984, 1986, 1987, de Fries & Fulker 1985).

6.5.2 Path analysis and structural equation modelling

The most widely used method for fitting variance components models to twin data is maximum likelihood (ML) estimation within the framework of structural equation modelling (SEM). Several software packages have been developed for SEM, including Mx (Neale 1997) and LISREL (Joreskog & Sorbom 1986). They were originally designed to analyze the genetic associations of continuously distributed data. The underlying method of analysis employed by SEM is path analysis, developed by Sewall Wright in 1921. The purpose of path analysis is the evaluation of the relative importance of the various causes of variation influencing the trait of interest (Li 1975, Wright 1978, Wright 1968, Wright 1921). In a path model, the inter-relationships among variances are described through structural linear regression equations of standardized variables. The structural relationships are often presented by path diagrams, and correlations among variables follow a defined set of rules (Li 1975). In genetic epidemiology, the causal structure of a path model can be used to express familial correlations as a function of the path coefficients (Rao 1975, Rao et al. 1994). These software packages make it possible to fit such models to summary covariance or correlation matrices by maximum likelihood or other methods, and they provide a chi-squared test of the goodness of fit of a model, and give estimates of the model parameters and their standard errors.

When analyzing binary data, after ascertaining that it is reasonable to fit a genetic model, the next stage in SEM methods is to use tetrachoric correlations to fit AE, CE, DE, ACE, or ADE models. The inclusion of covariates is possible but not straightforward, and is achieved by modelling a series of contingency tables. For example, if the analysis needs to be adjusted for sex, six separate contingency tables are modelled to account for the sex structure in MZ and DZ twins. If it is then required to also adjust for age, it needs to be dichotomised, and used in combination with the sex variable to break the contingency tables up further to reflect the age/sex distribution of the twins. But the critical decision is at which point along the distribution age should be dichotomized, and this could involve extensive analysis. The problem becomes increasing more complex as more covariates are considered. There is also a resultant loss of information associated with dichotomizing continuous variables. The incorporation of interactions, non-linear components, and spatial and temporal

effects is not easily achieved in a SEM framework (Kuhnert & Do 2003). SEMs are inappropriate for the analysis of twin-family data where the family structure is variable and these data are better handled by purpose-designed programmes (Heath *et al* 1989b).

6.5.3 Markov chain Monte Carlo (MCMC) methods

Markov chain Monte Carlo (MCMC) methods are a class of algorithms for sampling from probability distributions based on constructing a Markov chain that has the desired distribution as its stationary distribution. The state of the chain after a large number of iterations is then used as a sample from the desired distribution. MCMC methods are widely used in the field of Bayesian statistics to overcome the computationally complex integrations involved in obtaining the posterior distribution. MCMC approaches are so-named because previously sample values are used to randomly generate the next sample value, thereby generating a Markov chain. The Gibbs sampler is an example of a MCMC algorithm, and was developed by Geman and Geman (Geman & Geman 1984). Gibbs sampling is an algorithm to generate a sequence of samples from the joint probability distribution of two or more random variables, in order to approximate the joint distribution. This is achieved by generating an instance from the distribution of each variable in turn, conditional on the current values of the other variables. It is particularly applicable when the joint distribution is unknown, but the conditional distribution of each variable is known.

In BUGS, the Gibbs sampler starts with estimated or "guessed" values for all unknown parameters, say, x, y and z, designated x_0, y_0 and z_0. A new value for 'x', designated x_1, is then sampled from its full conditional distribution

$$f_x(x|y_0, z_0, \text{data}).$$

Next, y_1 is drawn from $f_y(y|x_1, z_0, \text{data})$, and then z_1 is sampled from $f_z(z|x_1, y_1, \text{data})$. This completes the first iteration. This sampling continues for a large number of iterations, say k, until the equilibrium distribution of the process will be such that x_k, y_k and z_k are sampled as if from the full joint posterior distribution (Smith & Roberts 1993). These first k iterations are referred to as the "burn in" and allow the MCMC chain to achieve stationarity. They are not

included in any parameter estimates. Formally, $\{x_{k+1}, x_{k+2}, x_{k+3}, \ldots, x_{k+n}\}$ represents a correlated marginal sample for 'x' from the full joint probability distribution. Appropriate summaries of the marginal samples, empirical means, medians and other quantiles, modes, and standard deviations allow inferences to be drawn about the parameters of interest.

The sequential values generated for a given parameter at each iteration are called a "chain". If necessary, the correlation in the marginal samples can be addressed analytically, or by using a suitably large "thinning interval", for example, by restricting the analyzed sample to $\{x_k, x_{k+10}, x_{k+20} \ldots.\}$. In BUGS, once the basic structure of the model has been specified, the full conditional sampling distributions are created automatically. By taking repeated samples from the posterior distribution, Gibbs sampling avoids the need for extensive numerical integrations across the joint distribution of random effects (Breslow & Clayton 1993, Zeger & Karim 1991).

Burton and colleagues developed models in BUGS to analyze Normally-distributed phenotypes in nuclear families (Burton *et al* 1998). Only minor modifications to the BUGS code for the working Normal model were required to generalize the methods to a binary model (Burton *et al* 1999), and censored survival data (Scurrah *et al* 2000).

MCMC methods using the BUGS software are becoming increasing popular for the analysis of twin data (Do *et al* 2000, Eaves & Erkanli 2003, Eaves *et al* 2005, Eaves *et al* 2004, Kuhnert & Do 2003, van den Berg *et al* 2006), and are known to produce comparable results to other methods of analysis (Kuhnert & Do 2003). To my knowledge, these methods have not been extended for the analysis of twin-family data. This chapter describes such an extension of these models.

6.6 Genetic epidemiology of asthma

Asthma and atopy are complex genetic disorders that are strongly familial and do not follow the Mendelian pattern of inheritance which characterise single-gene disorders such as cystic fibrosis. The complex genetic nature of asthma

results from polygenic inheritance, that is, a number of genes are thought to be involved in its pathogenesis, genetic heterogeneity (different combinations of genes act in different families), and pleiotropy, that is, the same gene or set of genes influence multiple traits, such as asthma and eczema. They are ideal models for studying diseases resulting from the interaction of genetic and environmental factors. Advances in several fields, including genetic epidemiology and molecular biology, have now made it possible to explore the genetics of complex diseases at both the population and molecular level.

It was not until the early 1900s that several family studies showed evidence for a substantial familial aggregation in asthma (Los *et al* 2001). Although these studies were important to assess the familial component of asthma, they could not estimate heritabilities to analyze patterns of inheritance. From the 1980s, segregation analysis has further developed the understanding of the genetics of asthma. Most of these analyses have been carried out by using questionnaires (Chen *et al* 1998, ECRHS 1997, Holberg *et al* 1996, Jenkins *et al* 1997, Lawrence *et al* 1994). They have all concluded that there is not a single gene that gives rise to asthma, although they have come to varying conclusions regarding the pattern of inheritance (Bleecker *et al* 1997, Marsh & Meyers 1992, Sandford *et al* 1996). But it is without doubt that a combination of multiple genes and environment are responsible for the development of asthma, independent of how it was measured. Similar patterns have been found when intermediate asthma phenotypes, such as serum IgE, BHR, SPT and lung function as measured in a clinical setting were studied (Gray *et al* 2000, Holberg *et al* 1999, Palmer *et al* 2000).

Family studies have established that asthma and atopy aggregate within families (Aberg 1993, Bouzigon *et al* 2004, Burrows *et al* 1995, Jenkins *et al* 1997, Litonjua *et al* 1998). They found an increased risk of atopy when one or both parents were affected (Aberg 1993), and the odds of asthma in a child increased from 3 when one parent was affected to 6 when both were (Litonjua *et al* 1998). Maternal asthma appears to be more influential than paternal asthma (ECRHS 1997, Holberg *et al* 1996, Johnson *et al* 1996, Litonjua *et al* 1998), although one study suggested the opposite (Dold *et al* 1992). Since both genes and environmental influences aggregate within families it is not possible, using

traditional family studies, to distinguish between the genetic and environmental contributions to the disease.

6.7 The role of twin studies

Twin studies were among the first to demonstrate the importance of genetic factors in the aetiology of asthma and allergy (Edfors-Lubs 1971). The advantage they have over traditional family studies is that they permit the analysis of environmental risk factors independent of genetic factors, without necessarily knowing the genes involved (Los *et al* 2001, Rasanen *et al* 2001, Rasanen *et al* 1998, Strachan *et al* 2001).

One of the earliest studies of twins and asthma was conducted in Sweden. This study of 7,000 twin pairs found a concordance of allergic symptoms of 19% for MZ twins versus 5% of DZ twins (Edfors-Lubs 1971). There have been many replications of this finding, for example Harris *et al* 1997, Koeppen-Schomerus *et al* 2001. The large population-based Scandinavian Twin Registers have been responsible for many subsequent twin studies into asthma and atopy, including those from Finland (Rasanen *et al* 2001, Rasanen *et al* 1997), Norway (Nystad *et al* 2005) and Denmark (Skadhauge *et al* 1999, Thomsen *et al* 2006a, Thomsen *et al* 2006b, Thomsen *et al* 2006c). Elsewhere, asthma in twins has been studied in the UK (Koeppen-Schomerus *et al* 2001, Strachan *et al* 2001), and in Australia (Clarke *et al* 2000, Duffy 1995, Duffy *et al* 1990, Ferreira *et al* 2006, Hopper *et al* 1990). Studies have generally found that the risk of asthma or atopy in a co-twin of an affected MZ twin is increased compared with the co-twin of an affected DZ twin, relative to the general population (Duffy *et al* 1990, Edfors-Lubs 1971, Larsen *et al* 1986, Skadhauge *et al* 1999).

Studies have used a variety of methods to define the asthma phenotype, ranging from self- or parent-reported via questionnaire (Duffy 1995, Edfors-Lubs 1971, Ferreira *et al* 2006, Koeppen-Schomerus *et al* 2001, Lichtenstein & Svartengren 1997, Miller *et al* 2005, Nystad *et al* 2005, Rasanen 2000, Skadhauge *et al* 1999, Strachan *et al* 2001, Thomsen *et al* 2006c), a combination of questionnaire data and clinical examination (Clarke *et al* 2000, Ferreira *et al* 2006, Strachan *et al* 2001, Thomsen *et al* 2006a), and linkage to data sets containing information of

prescribed asthma medication use (Huovinen *et al* 2001, Nieminen *et al* 1991), hospital admissions and death certificates (Nieminen *et al* 1991). Most studies used a series of standard questions concerning twin physical similarity and confusion to assess zygosity (Duffy 1995, Koeppen-Schomerus *et al* 2001, Skadhauge *et al* 1999, Strachan *et al* 2001), with some confirming zygosity so determined with results from blood and DNA tests (Strachan *et al* 2001). One study (Clarke *et al* 2000) used a hierarchical method to assign zygosity, ranging from results of a DNA test, down to the zygosity as recorded on the ATR, based on the parents' response to the zygosity question at the time of registration.

All large population-based twin studies have consistently shown higher correlations (Duffy *et al* 1990, Hopper *et al* 1990, Koeppen-Schomerus *et al* 2001, Lichtenstein & Svartengren 1997) and concordances (Edfors-Lubs 1971, Harris *et al* 1997, Koeppen-Schomerus *et al* 2001) for asthma in MZ twins than DZ twins (Table 6.2), and that these results held for twins of all ages.

Table 6.2:
Tetrachoric correlation and probandwise concordance of asthma between MZ and DZ twins

STUDY	PAIRS STUDIED	AGE RANGE (YEARS)	TETRACHORIC CORRELATION	PROBANDWISE CONCORDANCE
Sweden 1971	6996	46-85		MZ-19% DZ-4.8%
Australia 1990	3808	18-65+	MZM-0.48* DZM-0.09 MZF-0.33 DZF-0.12	MZ-20% DZ-7%
Australia 1990	3808	18-88	MZ-0.65 DZ-0.24	
Sweden 1997	1480	7-9	MZM-0.79 MZF-0.64 DZM-0.25 DZF-0.27	
Norway 1997	2932	18-25	MZ-0.75 DZ-0.21	MZ-0.45 DZ-0.12
Denmark 1999	34076 individuals including 15558 pairs	12-41 (12-26, 27-41)	MZM-0.76, 0.81 MZF-0.71, 0.65 DZM-0.36, 0.37 DZF-0.47, 0.15 DZO-0.25, 0.05	MZM-0.49 MZF-0.40 DZM-0.09 DZF-0.09 DZO-0.11

*MZM=MZ male pairs, MZF=MZ female pairs, DZM=DZ male pairs, DZF = DZ female pairs, DZO=DZ oppostive sex pairs.

Studies of twins of all ages from a number of countries have estimated heritability of asthma to be between 36% and 79%, with most studies reporting heritability to be above 60% (Table 6.3).

Table 6.3:
Summary of estimates of heritability from twin studies

STUDY	PAIRS STUDIED	AGE RANGE (YEARS)	ESTIMATE OF HERITABILITY
Sweden, 1971	MZ-2434* DZ-4302	46-85	0.65
Finland, 1991	MZ-4307 DZ-9581	18-70+	0.36
Australia, 1990	MZM-567, MZF-1232 DZM-352, DZF-751 DZO-906	18-88	0.60-0.75
Norway, 1997	MZM-412, MZF-527 DZM-384, DZF-442 DZO-794	18-25	0.75
Sweden, 1997	MZM-211, MZF-210 DZM-223, DZF-246 DZO-387	7-9	0.70-0.76
Denmark, 1999	MZM-1677, MZF-2013 DZM-1677, DZF-2228 DZO-3651	12-41	0.73
The Netherlands	MZM-397, MZF-536 DZM-349, DZF-416 DZO-661	12-24	0.72

*MZM=MZ male pairs, MZF=MZ female pairs, DZM=DZ male pairs, DZF = DZ female pairs, DZO=DZ oppostive sex pairs.

Only a few twin studies have exclusively focussed on asthma in childhood (Clarke *et al* 2000, Koeppen-Schomerus *et al* 2001) or adolescence (Laitinen *et al* 1998). While the Finnish study (Laitinen *et al* 1998) was of twins and their parents, none have studied twins, plus parents plus siblings.

Biometrical analysis of twin data consistently show that shared environment does not explain the familial patterns seen for asthma, and that the genetic effects are mainly additive (Table 6.4).

Table 6.4:
Summary of genetic models for asthma

STUDY	GENETIC MODEL	COMMENTS
Australia, 1990	Males: ADE Females: AE	Self-reported asthma No evidence for shared environment
Denmark, 1999	AE	Self-reported asthma No evidence of shared environment
Finland, 1998	AE	No evidence of shared environment
Norway, 1997	ADE and AE	Self-reported asthma
Sweden, 1997	AE	Parent-reported asthma No evidence of shared environment

In summary, twin studies have confirmed results from family studies that there is a strong genetic component to asthma. MZ twin show higher concordance, correlation and heritability to asthma than DZ twins, and biometrical models confirm that shared environment is unlikely to play a part in the aetiology of the disease.

6.8 Results from the WATCH study

6.8.1 Family correlations

All end points as described in the previous chapter were examined and results for doctor-diagnosed asthma (DDA) are reported here. There was little correlation in DDA between parents, and the mother-child relationship was stronger than that between the father and his children (Table 6.5). These relationships were true irrespective of the gender of the child.

Table 6.5
Probandwise concordance rate and tetrachoric correlation doctor-diagnosed asthma in WA twin families

RELATIONSHIP	C/D*	CORRELATION±SE
Mother-father	61/606	.08±.05
Mother-child	240/803	.38±.03
Father-child	137/710	.31±.04
MZ twins	76/48	.87±.03
DZ twins	125/260	.48±.05

*c=concordant pairs; d=discordant pairs

6.8.2 Twin concordance and correlation

Twin similarity was assessed using the proband-wise concordance rate (McGue 1992) and tetrachoric correlations, which is defined as the within-pair correlation of a dichotomous trait assuming underlying Normal distribution (Neale & Cardon 1992).

In WATCH study twins, $\rho_{MZ} > \rho_{DZ}$, and $\rho_{MZ} < 2\rho_{DZ}$, suggesting that both genetic and environmental influences are important in the aetiology of asthma in this population (Table 6.6).

Table 6.6
Probandwise concordance and tetrachoric correlation for doctor-diagnosed asthma in WA twins by zygosity

ZYGOSITY	C/D*	PROBANDWISE CONCORDANCE±SE	TETRACHORIC CORRELATION±SE
All MZ	76/48	.76±.03	.87±.03
MZ (F)	28/24	.70±.03	.84±.06
MZ (M)	47/24	.80±.04	.89±.04
All DZ	125/260	.49±.03	.48±.05
DZ (F)	41/49	.63±.04	.75±.06
DZ (M)	32/68	.48±.03	.47±.10
DZ (O)	52/143	.42±.03	.29±.08

*c=concordant pairs; d=discordant pairs

Using these estimates of concordancy, heritability was calculated using the formulae shown in section 6.4.2, and was estimated to be 78%, with unshared environment 9%. The remaining 13% was due to shared environment.

6.9 Variance components models for twins using BUGS

Twin data were simulated in SPlus, with the proportion of MZ and DZ twins comparable to the WATCH data. The basic model was a generalized linear mixed model (GLMM) with a binomial error and a logistic link as described in Burton *et al.* 1999 (Burton *et al* 1999), and was fitted in WinBUGS 1.3 (Spiegelhalter *et al* 2000). The model included a grand mean (mu), fixed effects for binary and continuous covariates (bvar and qvar, with coefficients bvbeta and qvbeta respectively), and separate estimates of the MZ and DZ variance (sigma2mz and sigma2dz, respectively). Two different models were fit, the AC and the AD models, as the variance components due to shared environment (C) and genetic dominance (D) are confounded in data from twins reared together (Eaves *et al* 1978, Martin *et al* 1978). Random effects associated with variance components for additive polygenic effects (sigma2a), genetic dominance (sigma2d) and common environment (sigma2c) are then estimated by using the solving the simultaneous equations for the variance shared by MZ and DZ twins, viz:

AC model:	**AD model:**
$\text{var(MZ)} = \sigma^2_A + \sigma^2_C$	$\text{var(MZ)} = \sigma^2_A + \sigma^2_D$
$\text{var(DZ)} = \frac{1}{2}\sigma^2_A + \sigma^2_C$	$\text{var(DZ)} = \frac{1}{2}\sigma^2_A + \frac{1}{4}\sigma^2_D$

giving:

$\sigma^2_A = 2\{\text{var(MZ)} - \text{var(DZ)}\}$	$\sigma^2_A = 4\ \text{var(DZ)} - \text{var(MZ)}$
$\sigma^2_C = 2\ \text{var(DZ)} - \text{var(MZ)}$	$\sigma^2_D = 2\ \text{var(MZ)} - 4\ \text{var(DZ)}$

The BUGS programme code for the AD model is given in Appendix 8. As described in (Scurrah *et al* 2000), in order to correctly specify the Normal component of the residual variation, it is necessary to use either cycling (Burton *et al* 1999), or the cut() function in WinBUGS (Spiegelhalter *et al* 2000). The analysis in this particular instance uses the cut() function. By breaking appropriate links in the graphical model, the cut() function allows the random effects reflecting the Normal component of residual variation to be generated from a distribution with an appropriate covariance (as defined by sigma2a and sigma2d) but prevents the random effects that are so generated from feeding back information when new values of the variance components are to be sampled. Non-informative priors were used, burn-in was 1000 iterations and the thinning interval was 10. A total of 10,000 iterations in 3 cycles completed the analysis.

6.10 Simulation of BUGS models for analysis of twin data with a binary outcome

The BUGS model gave unbiased and consistent results for the simulated data, for both the AC (Table 6.7) and AD (Table 6.8) models.

Table 6.7:
Results of simulations of twin data – AC model

SIMULATION NUMBER	1	2	3	4
True value of covariates	***Estimate*** (95% CI)	***Estimate*** (95% CI)	***Estimate*** (95% CI)	***Estimate*** (95% CI)
mu=-0.5	***-0.57*** (-.6, .5)	***-0.57*** (-.6, .5)	***-0.57*** (-.6, .5)	***-0.57*** (-.6, .5)
bvbeta=0.5	***0.56*** (.4, .7)	***0.55*** (.4, .6)	***0.55*** (.5, .7)	***0.55*** (.5, .6)
qvbeta=0.3	***0.31*** (.15 .4)	***0.31*** (.2, .5)	***0.31*** (.2, .5)	***0.31*** (.2, .5)
sigma2a=2	***3.49*** (2.2, 5.0)	***3.44*** (2.1, 5.0)	***3.46*** (2.1, 5.0)	***3.43*** (2.1, 4.9)
sigma2c=1	***0.70*** (-.1, 1.5)	***0.72*** (-.2, 1.6)	***0.71*** (-.2, 1.5)	***0.72*** (-.2, 1.6)
mu=0.5	***0.47*** (.4 .5)	***0.47*** (.4, 5)	***0.47*** (.4, .5)	***0.47*** (.4, .5)
bvbeta=0.7	***0.64*** (.5, .7)	***0.64*** (.5, .8)	***0.64*** (.5, .8)	***0.64*** (.5, .8)
qvbeta=0.5	***0.47*** (.3, .6)	***0.47*** (.3, .6)	***0.47*** (.3, .6)	***0.47*** (.3, .6)
sigma2a=3	***3.12*** (1.7, 4.7)	***3.13*** (1.7, 4.8)	***3.18*** (1.7, 4.8)	***3.17*** (1.7, 4.8)
sigma2c=2	***1.76*** (.8, 2.7)	***1.76*** (.8, 2.7)	***1.74*** (.8, 2.7)	***1.75*** (.8, 2.7)
mu=1.0	***1.061*** (.9, 1.1)	***1.062*** (1.0, 1.2)	***1.061*** (.98, 1.2)	***1.061*** (.98, 1.2)
bvbeta=0.3	***0.28*** (.2, .4)	***0.2842*** (.2, .4)	***0.28*** (.2, .4)	***0.28*** (.2, .4)
qvbeta=1.0	***0.93*** (.8, 1.1)	***0.93*** (.8, 1.1)	***0.93*** (.8, 1.1)	***0.93*** (.8, 1.1)
sigma2a=4	***5.14*** (3.4, 7.2)	***5.13*** (3.4, 7.1)	***5.11*** (3.4, 7.0)	***5.12*** (3.3, 7.2)
sigma2c=0.5	***0.48*** (-.7, 1.6)	***0.50*** (-.6, 1.6)	***0.495*** (-.6, 1.5)	***0.50*** (-.7, 1.6)
mu=1.0	***1.062*** (1.0, 1.1)	***1.064*** (.99, 1.1)	***1.065*** (.99, 1.1)	***1.06*** (.99, 1.1)
bvbeta=1.5	***1.62*** (1.4, 1.8)	***1.62*** (1.5, 1.8)	***1.62*** (1.5, 1.8)	***1.62*** (1.5, 1.8)
qvbeta=2.0	***2.06*** (1.9, 2.2)	***2.06*** (1.9, 2.3)	***2.06*** (1.9, 2.2)	***2.07*** (1.9, 2.3)
sigma2a=1	***1.23*** (-.01, 2.6)	***1.27*** (.01, 2.6)	***1.27*** (.03, 2.7)	***1.28*** (.06, 2.6)
sigma2c=2	***2.36*** (1.4, 3.2)	***2.32*** (1.4, 3.2)	***2.33*** (1.5, 3.2)	***2.31*** (1.5, 3.2)

Table 6.8:
Results of simulations of twin data – AD model

SIMULATION NUMBER	1	2	3	4
True value of covariates	***Estimate*** (95% CI)	***Estimate*** (95% CI)	***Estimate*** (95% CI)	***Estimate*** (95% CI)
mu=-0.5	***-0.50*** (-.6, -.4)	***-0.50*** (-.6, -.4)	***-0.50*** (-.6, -.4)	***-0.50*** (-.6, -.4)
bvbeta=0.5	***0.44*** (.3, .5)	***0.44*** (.3, .5)	***0.44*** (.3, .5)	***0.44*** (.3, .5)
qvbeta=0.3	***0.11*** (-.05, .3)	***0.11*** (-.05, .3)	***0.11*** (-.05, .3)	***0.10*** (-.05, .3)
sigma2a=3.0	***2.68*** (1.4, 4.0)	***2.67*** (1.4, 4.0)	***2.67*** (1.4, 4.0)	***2.69*** (1.3, 4.0)
sigma2d=2.0	***1.27*** (-.4, 3.0)	***1.23*** (-.4, 2.9)	***1.23*** (-.4, 3.0)	***1.29*** (-.4, 3.1)
mu=0.5	***0.48*** (.4, .6)	***0.48*** (.4, .6)	***0.48*** (.4, .6)	***0.48*** (.4, .6)
bvbeta=0.7	***0.74*** (.6, .9)	***0.74*** (.6, .9)	***0.74*** (.6, .9)	***0.74*** (.6, .9)
qvbeta=0.5	***0.64*** (.5, .8)	***0.64*** (.5, .8)	***0.64*** (.5, .8)	***0.64*** (.5, .8)
sigma2a=3.0	***1.95*** (.01, 3.9)	***1.91*** (-.1, 3.9)	***1.83*** (-.1, 3.7)	***1. 80*** (-.14, 3.7)
sigma2d=4.0	***4.98*** (2.4, 7.8)	***5.05*** (2.4, 7.8)	***5.10*** (2.5, 8.0)	***5.14*** (2.6, 7.9)
mu=1.0	***1.06*** (.98, 1.2)	***1.06*** (.98, 1.2)	***1.06*** (.98, 1.2)	***1.06*** (.98, 1.2)
bvbeta=0.3	***0.33*** (.2, .4)	***0.33*** (.2, .4)	***0.33*** (.2, .4)	***0.33*** (.2, .4)
qvbeta=1.0	***1.08*** (.9, 1.3)	***1.08*** (.9, 1.3)	***1.08*** (.9, 1.3)	***1.08*** (.9, 1.3)
sigma2a=4.0	***3.29*** (1.5, 5.1)	***3.26*** (1.5, 5.0)	***3.27*** (1.5, 5.1)	***3.31*** (1.5, 5.1)
sigma2d=1.0	***2.30*** (.04, 4.8)	***2.29*** (.02, 4.8)	***2.29*** (.04, 4.7)	***2.23*** (-.02, 4.7)
mu=1.0	***0.98*** (.9, 1.1)	***0.98*** (.9, 1.0)	***0.98*** (.9, 1.1)	***0.98*** (.9, 1.1)
bvbeta=1.5	***1.58*** (1.4, 1.7)	***1.58*** (1.5, 1.7)	***1.58*** (1.4, 1.7)	***1.58*** (1.5, 1.7)
qvbeta=2.0	***1.97*** (1.8, 2.2)	***1.97*** (1.8, 2.2)	***1.97*** (1.8, 2.2)	***1.97*** (1.8, 2.2)
sigma2a=1.0	***1.62*** (.4, 2.8)	***1.56*** (.4, 2.8)	***1.62*** (.4, 2.8)	***1.55*** (.3, 2.8)
sigma2d=2.0	***1.51*** (.001, 3.2)	***1.55*** (-.05, 3.2)	***1.50*** (.03, 3.2)	***1.50*** (.02, 3.2)

6.11 Comparison with GEE models

When the BUGS models were applied to the models obtained from logistic regression, only the additive genetic effects were significant (Table 6.9). The estimates for the covariates were consistent with those obtained via logistic

regression, in that the BUGS estimates fell within the CIs for the corresponding logistic regression estimates, with the exception of parental phenotypes which appeared to be over-estimated.

Table 6.9:
Comparison of GEE and BUGS models for doctor-diagnosed asthma in twins, after controlling for age

VARIABLE	GEE MODEL	BUGS AC MODEL	BUGS AD MODEL
	OR (95% CI)	*OR (95% CI)*	*OR (95% CI)*
Individual variables			
Male gender	1.43 (1.1, 1.8)	1.57 (1.2, 2.1)	1.57 (1.2, 2.2)
Twin Variables			
Has older siblings	0.71 (.6, .9)	0.58 (.4, .8)	0.58 (.4, .8)
Family variable			
Lives in rural area	0.77 (.6, 1.0)	0.65 (.4, .9)	0.65 (.4, .9)
Complications of pregnancy			
Threatened miscarriage under 20 weeks	1.66 (1.2, 2.4)	2.06 (1.1, 3.8)	2.05 (1.1, 3.8)
Childhood conditions			
Had at least one episode of otitis media	1.32 (1.03, 1.7)	1.56 (1.1, 2.2)	1.56 (1.1, 2.2)
ENT operations			
Had tonsils removed	1.51 (1.04, 2.2)	1.86 (1.1, 3.2)	1.87 (1.1, 3.2)
Parental phenotypes			
Mother asthmatic	3.12 (2.4, 4.1)	5.31 (3.4, 8.5)	5.32 (3.4, 8.6)
Father asthmatic	2.65 (1.9, 3.6)	4.36 (2.6, 7.4)	4.38 (2.6, 7.4)
Socio, economic status			
Bottom 10% of index of disadvantage	2.20 (1.2, 4.0)	3.35 (1.4, 8.1)	3.35 (1.4, 8.1)
Bottom 10% of index of economic resources	4.08 (1.6, 10.4)	8.68 (1.5, 53.4)	8.68 (1.4, 54.2)
Number of smokers in household			
None	1	1	1
One	1.18 (.9, 1.6)	1.17 (.8, 1.8)	1.17 (.8, 1.8)
Two or more	1.06 (.8, 1.4)	1.15 (.7, 1.8)	1.15 (.7, 1.8)
Variance components			
Additive genetic		10.8 (3.8, 21.2)	1.82 (-4.2, 7.5)
Common family		3.03 (-8.4, .9)	
Genetic dominance			5.95 (-1.9, 16.6)

6.12 Variance components models for twin families

6.12.1 Model specification

The models were based on the GLMM with a parameterisation extended from that described previously by Burton et al. (1999), and were easily extended to incorporate nuclear twin family data. The model incorporates an extra component of variance, C_s, defined as the common sibling environment. It recognises the different pattern of shared environment that parents and children in the same family experience; parents share their environment only for the period of time that they co-habit, whereas children share a common environment for their entire lives. A brief description of model development as outlined in Burton's paper is given below.

For a continuous phenotype Q_{ij}, the model can be expressed as:

$$Q_{ij} = \mathbf{Z}_{ij}\beta + A_{ij} + C_{ij} + C_{sij} + E_{ij} \qquad \text{Model 1}$$

where the subjects j and i refer to the j^{th} member of the i^{th} nuclear family;
Q_{ij} is the observed value of a continuous trait in the ij^{th} individual (assumed to be multivariate Normal within a family);
$\mathbf{Z}_{ij}$ is a vector of observed covariates;
β is a corresponding vector of fixed regression coefficients.

The random effects, A_{ij}, C_{ij} and Cs_{ij} are each drawn from an appropriate multivariate Normal distribution (Burton et al. 1999), and represent additive polygenic effects (A_{ij}), common family environment (C_{ij}), and common sibling environment (Cs_{ij}).

Model 1 can be re-parameterized in terms of three functions F(), G() and H(), to give separate terms for the mothers, fathers and children, namely:

$$\text{Fathers:} \quad Q_{ij} = \mathbf{Z}_{ij}\beta + F_i + G_i + R^P{}_{ij} \qquad \text{Model 2}$$

$$\text{Mothers:} \quad Q_{ij} = \mathbf{Z}_{ij}\beta + F_i - Gi + R^P{}_{ij}$$

$$\text{Children:} \quad Q_{ij} = \mathbf{Z}_{ij}\beta + F_i + H_i + R^C{}_{ij}$$

where F_i is a random effect shared by all members of the i^{th} family:
$F_i \sim N(0,\{½\ \sigma^2{}_A + \sigma^2{}_C\})$.
G_i is shared by both parents in the i^{th} family: $G_i \sim N(0,½\sigma^2{}_A)$. It is *added* to the expected phenotype in fathers but *subtracted* in mothers.

H_i is shared by all children in the i^{th} family: $H_i \sim N(0, \sigma^2_{Cs})$.

R^P_{ij} is the residual error term when the j^{th} person in the i^{th} family is a parent: $R^P_{ij} \sim N(0, \sigma^2_{RP})$, $\sigma^2_{RP} = \sigma^2_{Cs} + \sigma^2_{EP}$.

R^C_{ij} is the residual error term when the j^{th} person in the i^{th} family is a child: $R^C_{ij} \sim N(0, \sigma^2_{RC})$, $\sigma^2_{RP} = \frac{1}{2}\sigma^2_A + \sigma^2_{EC}$.

Model 2 can be expressed in a mathematically equivalent form (Model 2*):

	Prediction equation	**Distributional equation**	
Fathers:	$E\{Q_{ij}\} = \mathbf{Z_{ij}\beta} + F_i + G_i$	$Q_{ij} \sim N(E\{Q_{ij}\}, \sigma^2_{RP})$	Model 2*
Mothers:	$E\{Q_{ij}\} = \mathbf{Z_{ij}\beta} + F_i - G_i$	$Q_{ij} \sim N(E\{Q_{ij}\}, \sigma^2_{RP})$	
Children:	$E\{Q_{ij}\} = \mathbf{Z_{ij}\beta} + F_i + H_i$	$Q_{ij} \sim N(E\{Q_{ij}\}, \sigma^2_{RC})$	

where $E\{Q_{ij}\}$ denotes the expected value of the phenotype in the j^{th} member of the i^{th} family given the fixed and random effects. The specified model is a generalized linear mixed model (GLMM) with a *multivariate Normal covariance structure*, a *Normal error*, and an *identity link* (Breslow & Clayton 1993, McCullagh & Nelder 1989).

Model 2* may now be generalized to model a binary phenotype D (D_{ij}=1 if the j^{th} member of the i^{th} family has disease D; D_{ij}=0 otherwise):

	Prediction equation	**Distributional equation**	
Fathers:	$\text{logit}(E\{P_{ij}\}) = \mathbf{Z}_{ij}\beta + F_i + G_i$	$D_{ij} \sim B(E\{P_{ij}\}, 1)$	Model 3
Mothers:	$\text{logit}(E\{P_{ij}\}) = \mathbf{Z}_{ij}\beta + F_i - G_i$	$D_{ij} \sim B(E\{P_{ij}\}, 1)$	
Children:	$\text{logit}(E\{P_{ij}\}) = \mathbf{Z}_{ij}\beta + F_i + H_i$	$D_{ij} \sim B(E\{P_{ij}\}, 1)$	

where: $B(E\{P_{ij}\}, 1)$ is a Bernouilli distribution; and $E\{P_{ij}\}$ is the expected probability of a positive response. This is a GLMM with a *logit link*, a *Bionomial error* and the joint distribution of higher order random effects is multivariate Normal (Breslow & Clayton 1993, McCullagh & Nelder 1989).

Modelling of twin family data is based on the covariance structure between members of the family as shown in Table 6.10.

Table 6.10:
Conditional within-family covariance matrices for twin families

	FATHER	MOTHER	MZ TWIN	ALL OTHER CHILDREN
Additive genetic effect (V_A)				
Father	σ^2_A	0	$\frac{1}{2}\sigma^2_A$	$\frac{1}{2}\sigma^2_A$
Mother	0	σ^2_A	$\frac{1}{2}\sigma^2_A$	$\frac{1}{2}\sigma^2_A$
MZ twin	$\frac{1}{2}\sigma^2_A$	$\frac{1}{2}\sigma^2_A$	σ^2_A	$\frac{1}{2}\sigma^2_A$
All other children	$\frac{1}{2}\sigma^2_A$	$\frac{1}{2}\sigma^2_A$	$\frac{1}{2}\sigma^2_A$	$\frac{1}{2}\sigma^2_A$
Common family environment (V_C)				
Father	σ^2_C	σ^2_C	σ^2_C	σ^2_C
Mother	σ^2_C	σ^2_C	σ^2_C	σ^2_C
MZ twin	σ^2_C	σ^2_C	σ^2_C	σ^2_C
All other children	σ^2_C	σ^2_C	σ^2_C	σ^2_C
Common sibling environment (V_{CS})				
Father	σ^2_{Cs}	0	0	0
Mother	0	σ^2_{Cs}	0	0
MZ twin	0	0	σ^2_{Cs}	σ^2_{Cs}
All other children	0	0	σ^2_{Cs}	σ^2_{Cs}

By decomposing the variance into the components σ^2_A, σ^2_C and σ^2_{Cs} as described, the simplest biological model implied (Hopper 1993) requires that the conditional variance in every individual includes the component $\sigma^2_A+\sigma^2_C+\sigma^2_{Cs}$ (Burton *et al* 1999). Under models 2* and 3, parents lack a component of σ^2_{Cs} and DZ and other siblings, $\frac{1}{2}\sigma^2_A$. To overcome this problem, Normally-distributed random effects with variances $\frac{1}{2}\sigma^2_A$ and σ^2_{Cs} were added to the linear predictor in DZ twins and singleton children, and parents, respectively (Burton *et al* 1999).

Corresponding parameterization can be developed for the ACD model, an example of which is contained in Appendix 9. The only problem with model interpretation is that the residual binomial variance is not analogous to σ^2_E, and it is therefore inappropriate to use it in calculations of, say, heritability. Instead,

inferences should be based upon the individual variance components alone, or on the proportion of the covariance accounted for by genetic effects, that is $\sigma^2_A/(\sigma^2_A+\sigma^2_C+\sigma^2_{Cs})$ for MZ twins and $\frac{1}{2}\sigma^2_A/(\frac{1}{2}\sigma^2_A+\sigma^2_C+\sigma^2_{Cs})$ for DZ twins and singleton siblings.

All models were fitted in WinBUGS 1.3 (Spiegelhalter *et al* 2000), and were subject to the following standard assumptions: Hardy-Weinberg equilibrium, no epistasis, no gene-environment interactions, random mating and additive genetic effects arose from autosomal genes (Burton *et al* 1999, Scurrah *et al* 2000).

As in the previous work of Burton et al. (1999), and Scurrah et al. (2000), the influence of prior assumptions was minimized by using flat Normal (mean=0, variance=10^6) prior distributions for unbounded fixed effects. Pareto (1, m^{-1}) distributions were used as priors for the precision (variance^{-1}). This is equivalent (Spiegelhalter et al. 1995) to specifying a uniform prior distribution for the variance of each random effect on the interval (0, m). When modelling a continuous trait with an inherently large variance (on whatever scale it is being considered) it is important to ensure that m is chosen to be large enough so that the interval (0, m) does not constrain the estimate of the variance. In all cases, I ensured that m was so large that the MCMC process estimating the corresponding variance never came close to approaching its upper boundary (at m).

6.12.2 Simulations

6.12.2.1 Continuous data

Simulated data sets were created in S-Plus 2000, comprising 2000 families of two parents and three children, two of which were twins. The model is a GLMM with a multivariate Normal covariance structure, a Normal error and an identity link as described in Burton *et al*., 1999 (Burton *et al* 1999). Covariates included an overall mean (mu), fixed effects for binary and continuous covariates (bvar and qvar, with coefficients bvbeta and qvbeta respectively), and random effects associated with variance components for additive polygenic effects (sigma2a), common family environment (sigma2c), and either common sibling

environment (sigma2cs) in the ACC_s model, or genetic dominance (sigma2d) in the ACD model. Different values for the covariates and variance components were given in each of 10 simulations (Table 6.11).

Table 6.11:
Results of simulations for continuous twin family data- ACD model

SIMULATION NUMBER	**1**	**2**	**3**	**4**	**5**
	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI
Covariates					
mu	*-2* ***-1.77*** -2.2, -1.4	*1* ***1.10*** .8, 1.4	*-1* ***-0.85*** -1.2, -.5	*2* ***1.57*** 1.1, 2.0	*-3* ***-3.07*** -3.5, -2.7
bvbeta	*0.5* ***0.58*** .2, .9	*1* ***1.07*** .82, 1.33	*1* ***1.01*** .7, 1.3	*1.5* ***1.53*** 1.2, 1.8	*-2.5* ***-2.54*** -2.9, -2.2
qvbeta	*-0.3* ***-0.29*** -.3, -.3	*1* ***1.01*** 1.001, 1.02	*-0.1* ***-.098*** -.11, -.09	*0.1* ***.099*** .09, .11	*-1* ***-1.0*** -1.01, -.98
Variance components					
A	*2* ***1.53*** .07, 4.1	*1* ***0.74*** .04, 2.08	*0.5* ***1.08*** .05, 2.8	*1.5* ***2.12*** .4, 4.4	*2.5* ***4.58*** 2.2, 7.0
C	*2.5* ***2.51*** 1.2, 3.8	*1* ***1.13*** .45, 1.79	*1.3* ***1.29*** .4, 2.2	*3* ***3.26*** 1.9, 4.7	*2* ***1.38*** .001, 2.8
D	*3* ***3.02*** .5, 6.6	*1* ***1.83*** .48, 3.97	*2.5* ***2.42*** .5, 5.2	*2* ***1.99*** .3, 4.5	*1.5* ***1.34*** .06, 3.7
E	*2* ***1.79*** 1.6, 2.0	*1* ***0.81*** .60, 1.14	*2* ***1.88*** 1.3, 2.9	*1.3* ***1.26*** .9, 1.8	*1.7* ***1.42*** 1.0, 1.9

Table 6.11 - continued

SIMULATION NUMBER	6	7	8	9	10
Covariates					
	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI
Covariates					
mu	*-1.5* ***-1.68*** -2.1, -1.2	*1.5* ***1.57*** 1.1, 2.0	*-0.1* ***0.14*** -.2, .5	*2* ***2.26*** 1.9, 2.6	*0.1* ***0.006*** -.3, .3
bvbeta	*0.7* ***0.90*** .50, .3	*-1* ***-0.61*** -1.0, -.2	*0.5* ***0.36*** .05, .7	*-0.1* ***-0.22*** -.6, .1	*-2* ***-2.19*** -2.5, -1.9
qvbeta	*-0.5* ***-0.51*** -.52, -.49	*1* ***1.0*** .98, 1.01	*-0.5* ***-0.5*** -.51, -.49	*2* ***2.01*** 1.997, 2.02	*2* ***2.00*** 1.98, 2.01
Variance components					
A	*1.5* ***2.23*** .3, 5.4	*2* ***2.55*** .06, 6.0	*1.2* ***2.38*** .6, 4.4	*0.5* ***0.45*** .01, 1.6	*3* ***3.34*** 1.2, 5.4
C	*3* ***2.21*** .6, 3.8	*2* ***1.36*** -.3, 2.9	*1* ***0.62*** -.4, 1.6	*1.7* ***1.24*** .3, 2.1	*0.5* ***-0.21*** -1.3, .8
D	*2.5* ***4.24*** .9, 9.4	*3* ***4.00*** 1.0, 8.6	*2.3* ***1.41*** .09, 3.5	*3* ***4.45*** 1.9, 8.1	*0.5* ***1.21*** .06, 3.6
E	*3* ***2.25*** 1.6, 3.3	*2* ***1.93*** 1.5, 2.9	*1.5* ***1.501*** 1.0, 2.2	*2.1* ***1.48*** 1.1, 2.2	*2.5* ***1.98*** 1.4, 2.7

6.12.2.2 Binary data

Analysis of simulated binary data in 2000 twin families, each with 2 parents and 3 children, two of which are twins was carried out in SPlus. The model is a GLMM with a binomial error and a logistic link as described in Burton *et al*., 1999 (Burton *et al* 1999). The model includes a grand mean (mu), fixed effects for binary and continuous covariates (bvar and qvar, with coefficients bvbeta and qvbeta respectively), and random effects associated with variance components for additive polygenic effects (sigma2a), common family environment (sigma2c), and genetic dominance (sigma2d). Different values for the covariates and variance components were given in each of 10 simulations (Table 6.12).

Table 6.12:
Results of simulations for binary twin family data

SIMULATION NUMBER	**1**	**2**	**3**	**4**	**5**
	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI
Covariates					
Mu	*-0.5* ***-0.51*** -.6, -.4	*-1.5* ***-1.55*** -1.9, -1.2	*1* ***1.17*** 1.0, 1.4	*2* ***2.46*** 1.9, 3.2	*1* ***0.92*** .8, 1.1
Bvbeta	*1.5* ***1.54*** 1.2, 1.9	*0.5* ***0.41*** .2, .6	*1* ***1.06*** .8, 1.3	*2* ***2.50*** 1.9, 3.2	*1* ***1.04*** .9, 1.3
qvbeta	*1.3* ***1.5*** 1.1, 1.9	*0.3* ***0.22*** -.03, .5	*0.7* ***0.93*** .6, 1.3	*2* ***1.92*** 1.4, 2.6	*1* ***1.00*** .8, 1.3
Variance components					
A	*1* ***0.53*** .1, 1.3	*2* ***1.96*** .6, 3.6	*3* ***4.70*** 2.9, 6.9	*3* ***4.08*** 1.4, 7.7	*1* ***0.45*** .1, 1.1
C	*1* ***1.26*** .7, 1.8	*1* ***0.80*** .2, 1.5	*2* ***1.77*** 1.0, 2.7	*2* ***3.82*** 2.0, 6.6	*1* ***1.02*** .7, 1.4
D	*2* ***2.40*** .05, 4.9	*3* ***2.97*** .8, 6.0	*1* ***1.19*** .1, 3.1	*3* ***7.44*** 2.7, 15.4	*2* ***1.54*** .5, 3.1

Table 6.12- continued

SIMULATION NUMBER	6	7	8	9	10
	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI
Covariates					
mu	*-2* ***-1.92*** -2.3, -1.6	*0.1* ***0.11*** .02, 0.2	*-3* ***-3.26*** -4.3, -2.6	*2* ***1.92*** 1.6, 2.3	*0.1* ***-0.087*** -4.3, -2.6
bvbeta	*0.5* ***0.63*** .4, .8	*1* ***1.19*** .1, 1.5	*-2.5* ***-2.80*** -3.7, -2.2	*-0.1* ***-0.04*** -.2, .1	*2* ***2.32*** 1.9, 2.8
qvbeta	*-0.3* ***-0.47*** -.8, -.2	*2.5* ***2.72*** 2.3, 3.3	*-1* ***-1.07*** -1.5, -.7	*2* ***2.00*** 1.6, 2.5	*2* ***2.32*** 1.9, 2.8
Variance components					
A	*2* ***1.41*** .3, 2.8	*1.1* ***0.70*** .1, 1.8	*2.5* ***1.01*** .1, 2.7	*0.5* ***1.3*** .2, 2.6	*3* ***4.8*** 3.0, 7.2
C	*2.5* ***2.53*** 1.6, 3.8	*0.7* ***1.27*** .7, 1.8	*2* ***2.90*** 1.7, 5.0	**1.7** ***1.17*** .6, 1.9	*0.5* ***0.57*** -.1, 1.3
D	*3* ***3.05*** 1.0, 6.2	*2.5* ***3.84*** 1.7, 7.1	*1.5* ***3.84*** .9, 9.1	*3* ***1.81*** .2, 4.6	*0.5* ***1.59*** .3, 3.9

6.13 Validation of methods using logistic models

Variables that were significant in the multivariate GEE analysis of DDA in twin families (section 5.12) were included as covariates in both the ACCs and ACD models, and analyzed in WinBUGS 1.3 (Spiegelhalter *et al* 2000). Results showed that additive genetic effects were important in the aetiology of asthma, as were common sibling environment (ACCs model) and genetic dominance (ACD model). Common family environment was not significantly associated with asthma in twin families (Table 6.13).

Table 6.13:
Comparison of GEE and BUGS models for DDA in twin families

VARIABLE	**GEE OR 95% CI**	**BUGS-ACCs OR 95% CI**	**BUGS-ACD OR 95% CI**
Child	1.20 (.7, 2.0)	1.47 (.6, 3.5)	1.48 (.6, 3.6)
Male gender	0.70 (.5, .9)	0.58 (.4, .9)	0.59 (.4, .9)
Child*sex interaction	1.81 (1.4, 2.4)	2.37 (1.5, 3.8)	2.38 (1.5, 3.8)
Lives in rural area	0.80 (.6, 1.0)	0.73 (.5, 1.0)	0.73 (.5, 1.0)
Had tonsils removed	1.58 (1.3-1.9)	1.96 (1.5, 2.6)	1.96 (1.5, 2.6)
Bottom 10% of index of disadvantage	1.72 (1.1, 2.6)	2.10 (1.0, 4.2)	2.09 (1.0, 4.2)
Bottom 10% of index of economic resources	2.33 (1.1, 4.9)	3.00 (0.7, 12.8)	3.01 (0.7, 12.5)
Smoking status			
Active smoker	1.02 (.8, 1.3)	1.06 (.7, 1.5)	1.06 (.7, 1.5)
Lives with 2+ smokers	1.04 (.9, 1.3)	1.06 (.7, 1.5)	1.06 (.7, 1.5)
Lives with 1 smoker	1.07 (.8, 1.3)	1.04 (.7, 1.5)	1.04 (.7, 1.5)
No passive or active smoke exposure	1	1	1
Variance components		**Estimate 95% CI**	**Estimate 95% CI**
Additive genetic		5.05 (2.6, 8.1)	5.06 (2.6, 8.1)
Common family		0.20 (-1.1, 1.3)	0.20 (-1.1, 1.3)
Common sibling		0.42 (0.02, 1.1)	
Genetic dominance			1.73 (.1, 4.3)

6.14 Testing the equal environments assumption

The previous BUGS programme was modified to add an additional term to test the validity of the EEA. The difference in phenotypic covariance between MZ and DZ twins was modelled as an extra component of variance, thereby enabling its significance to be tested directly (Appendix 10). Once again, ten simuations were performed, and as before, simulated data were produced in SPlus, comprising 2000 families of two parents and three children, two of which were twins. The model is a GLMM with a multivariate Normal covariance structure, a Normal error and an identity link as described in Burton et al. 1999. It includes a grand mean (mu), fixed effects for binary and continuous covariates (bvar and qvar with coefficients bvbeta and qvbeta respectively), and random effects associated with variance components for additive polygenic effects (sigma2a), common family environment (sigma2c), and genetic dominance (sigma2d). The extra term designed to measure the size of the extra environmental component of variance in the MZ twins was included as sigma2.mz.extra.environment.

Results of the simulations for continuous data are given in Table 6.14, and show that the model produced accurate and unbiased estimates of the covariates and variance components.

Table 6.14:
Results of simulations of continuous twin family data testing the EEA

SIMULATION NUMBER	**1**	**2**	**3**	**4**	**5**
	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI
Covariates					
mu	**-2** ***-2.07*** -2.2, -1.9	**1** ***1.10*** .8, 1.4	**-1.5** ***-1.32*** -1.6, -1.1	**1.5** ***1.68*** 1.4, 1.9	**-0.1** ***-0.20*** -.4, -.04
bvbeta	**0.5** ***0.46*** .4, .6	**1** ***1.07*** .8, 1.3	**0.7** ***0.62*** .5, .8	**-1** ***-1.02*** -1.2, -.9	**0.5** ***0.57*** .4, .7
qvbeta	**-0.3** ***-0.299*** -.302, -.297	**1** ***1.01*** 1.001, 1.02	**-0.5** ***-0.499*** -.504, -.493	**1** ***1.001*** .996, 1.006	**-0.5** ***-0.5005*** -.505, -.496
Variance components					
A	**2** ***2.49*** 1.8, 3.2	**1** ***0.74*** .04, 2.1	**1.5** ***0.94*** .09, 2.2	**2** ***2.20*** .9, 3.6	**1.2** ***1.83*** .8, 2.9
C	**2.5** ***2.50*** 2.1, 2.9	**1** ***1.13*** .5, 1.8	**3** ***3.60*** 2.9, 4.3	**2** ***2.03*** 1.4, 2.7	**1** ***0.80*** .3, 1.3
D	**3** ***2.85*** .2.2, 3.6	**1** ***1.83*** .5, 4.0	**2.5** ***2.18*** .7, 3.7	**3** ***2.56*** 1.2, 4.0	**2.3** ***2.03*** 1.0, 3.2
E	**2** ***1.79*** 1.6, 2.0	**1** ***0.81*** .6, 1.1	**3** ***3.31*** .2.6, 4.3	**2** ***1.85*** 1.5, 2.3	**1.5** ***1.47*** 1.2, 1.8
Extra environment	**2** ***1.4*** .7, 2.1	**1** ***0.84*** -1.2, 2.7	**2.5** ***3.44*** 1.8, 5.1	**1** ***1.1*** -.2, 2.4	**0.5** ***-0.05*** -.1, .8

Table 6.14 - continued

SIMULATION NUMBER	**6**	**7**	**8**	**9**	**10**
	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI	True value ***Estimate*** 95% CI
Covariates					
mu	**2** ***1.99*** 1.8, 2.2	**-2** ***-1.77*** -2.2, -1.4	**-1** ***-0.84*** -1.2, -.5	**2** ***1.57*** 1.1, 2.0	**-3** ***-3.07*** -3.5, -2.7
bvbeta	**-0.1** ***-0.12*** -.3, .02	**0.5** ***0.58*** .2, .9	**1** ***1.01*** .7, 1.3	**1.5** ***1.53*** 1.2, 1.8	**-2.5** ***-2.54*** -2.9, -2.2
qvbeta	**2** ***2.002*** 1.997,2.007	**-0.3** ***-0.294*** -.31, -.28	**-0.1** ***-0.097*** -.11, -.086	**0.1** ***0.099*** .088, .11	**-1** ***-0.995*** -1.01, -.98
Variance components					
A	**0.5** ***0.17*** .02, .6	**2** ***1.53*** .1, 4.1	**0.5** ***1.11*** .1, 2.9	**1.5** ***2.12*** .3, 4.5	**2.5** ***4.58*** 2.2, 7.0
C	**1.7** ***1.69*** 1.3, 2.1	**2.5** ***2.51*** 1.2, 3.8	**1.3** ***1.30*** .4, 2.2	**3** ***3.26*** 1.9, 4.7	**2** ***1.38*** -.01, 2.8
D	**3** ***3.22*** 2.0, 4.6	**3** ***3.02*** .5, 6.6	**2.5** ***2.34*** .3, 5.1	**2** ***1.99*** .3, 4.4	**1.5** ***1.34*** .1, 3.7
E	**2.1** ***1.89*** 1.5, 2.4	**2** ***1.95*** 1.3, 3.1	**2** ***1.90*** 1.3, 3.0	**1.3** ***1.26*** .9, 1.8	**1.7** ***1.42*** 1.0, 1.9
Extra environment	**1.3** ***1.62*** .2, 3.0	**2** ***4.10*** .2, 8.2	**0.5** ***0.32*** -2.2, 2.6	**1** ***1.64*** -07, 4.2	**0.7** ***-0.20*** -2.3, 2.0

The WATCH data did not contain a continuously-distributed asthma-related phenotype, so the binary phenotype DDA was used to test the above model. The final GEE model as determined by section 5.12 was modelled in BUGS and estimates of the variance components and the validity of the EEA assessed (Table 6.15).

Table 6.15:
Results of BUGS analysis testing the validity of the EEA

VARIABLE	**OR 95% CI**
Child	1.44 (.6, 3.4)
Male gender	0.58 (.4, .9)
Child*sex interaction	2.38 (1.5, 3.8)
Lives in rural area	0.72 (.5, 1.0)
Had tonsils removed	2.0 (1.5, 2.6)
Bottom 10% of index of disadvantage	2.08 (1.0, 4.2)
Bottom 10% of index of economic resources	3.01 (.7, 13.0)
Smoking status	
Active smoker	1.06 (.7, 1.5)
Lives with 2+ smokers	1.06 (.7, 1.5)
Lives with 1 smoker	1.04 (.7, 1.5)
No passive or active smoke exposure	1
Variance components	**Estimate 95% CI**
Additive genetic	5.10 (2.7, 8.2)
Common family	0.18 (-1.1, 1.3)
Genetic dominance	1.75 (.03, 4.5)
Extra environment	1.95 (-2.2, 7.2)

This model gives consistent and accurate estimates of the covariates compared with the GEE analysis. Additive genetic and genetic dominance play an important role in the aetiology of asthma in WA twin families, and the effect of common family environment is not important. The confidence interval around

the estimate of the extra coviance due to being a MZ twin contains 0, and hence there is no reason to doubt the validity of the EEA in this case.

6.15 Summary of results

This study of doctor-diagnosed asthma in WA twin families has shown that concordance was higher in MZ twins than DZ twins, and that heritability was estimated to be 78%. These results are similar to those found in numerous other studies of asthma in twins (Duffy *et al* 1990, Edfors-Lubs 1971, Harris *et al* 1997, Lichtenstein & Svartengren 1997, Skadhauge *et al* 1999). Biometric analysis of the asthma phenotype in WA twins and twin families also confirm results from previous studies that additive genetic effects play an important role in the aetiology of the disease, but the effects of common environment do not explain the familial nature of asthma in WA twins and their families.

The models developed to analyze twin and twin family data in BUGS produce accurate and unbiased estimates when using simulated data, for both continuous and binary outcomes, and by comparing the estimates with those obtained from the GEE analyses. By including the additional term to estimate the extra covariance to being a MZ twin, the validity of the EEA was tested directly. The models developed do not rely on identifying the trait-relevant environments that are likely to impact whether MZ and DZ twins are equally correlated, and was not significant for doctor-diagnosed asthma in WA twin families.

CHAPTER 7
DISCUSSION AND CONCLUSIONS

The principal aim of this study was to establish the WA Twin Register (WATR) comprising all multiple births to have occurred in WA since 1980. Supplementary aims were to use the WATR to conduct the WA Twin Child Health (WATCH) study into the genetic and environmental contributions to asthma and atopy in twins and their families, and to describe the genetic epidemiology of asthma in twin families by developing new methods of analyzing twin-family data. Finally, these new methods were extended to allow the validity of the classic twin method, and the equal environments assumption (EEA) in particular, to be tested.

The main conclusions that can be drawn are:

1. it is possible to establish a population-based register of multiple births using record linkage techniques of birth and other databases.
2. the WATR is a complete population-based register of all multiple births to have occurred in WA from 1980 to 1997 inclusive. It is the only population-based twin register in Australia, and one of only a few anywhere in the world.
3. the WATCH study used the WATR to study the genetic and environmental contributions to asthma and atopy in WA twins and their families. It is one of only a few twin-family studies to have been established.
4. the unique nature of the data contained in the WATR has allowed the characteristics of those who participated in the WATCH study to be directly compared with those who did not. Such examination showed that WATCH study families were no different from other twin families with respect to parental age at the first birth for the mother and racial origin.
5. by using the SEIFA codes, it was shown that WATCH families were not significantly different from the general WA population with respect to indices of advantage/disadvantage and economic resources.
6. compared with their singleton siblings, twins were lighter at birth, and born at shorter gestation. They were more likely than their siblings to have:
    a. been delivered by caesarean section;

b. spent time in the neonatal intensive care unit, or special care nursery;
c. been breast fed for less than six months, and to have had other milk products before the age of four months;
d. displayed behaviours characteristic of ADHD;
e. had at least one accident in the previous year;
f. spent more time absent from school in the previous year;
g. attended a remedial class at school;
h. been deemed by their parents to require extra school work in all areas examined; and
i. consulted a paediatrician, visited both a hospital emergency department and out-patient clinic, and required the services of a physiotherapist, speech pathologist and occupational therapist.

7. exposure to environmental tobacco smoke (ETS) was not associated with asthma or atopy in twins and twin families;
8. the most consistent risk factor for asthma and atopy in twins was having at least one parent with the same phenotype;
9. having no older siblings increased the risk of asthma in twins;
10. living in rural areas decreased the risk of asthma in twins;
11. with respect to asthma, WATCH study twins were representative of the WA population from which they were drawn;
12. MZ twins had higher concordance than DZ twins for all asthma and atopy end-points examined;
13. the heritability of asthma was estimated to be 78%;
14. shared family environment was not significantly associated with asthma in twin families;
15. additive genetic effects, and either genetic dominance or shared sibling environment, were significantly associated with asthma in twin families;
16. in WATCH study families, the extra component of variance due to being a MZ twin was not significant, and it can therefore be concluded that the the EEA, and hence the classic twin method, is valid in this instance.

7.1 The WA twin register

The WA twin register was established using the Maternal and Child Health Research database (MCHRDB), which is housed at and maintained by the Telethon Institute for Child Health research. The MCHRDB was established (Stanley *et al* 1994) using the statutory collection of data from the Midwives' Notification of case attended form, birth registration forms, death certificates and hospital inpatient morbidity data. All children born in WA, who attained at least 20 weeks gestation or weighed at least 400g at birth, are included. Cause of death was recorded from 20 weeks of gestation. Record linkage of these four data sets, together with data from the birth defects and cerebral palsy registers, allow a composite record for each birth to be constructed. Multiple births were identified using a specific code for plurality, and confirmed using probabilistic record linkage of sib-ships (Croft *et al* 2002). The MCHRDB, and consequently the WATR, contains data on maternal physical and socio-demographic characteristics, complications of pregnancy, perinatal details including infant birth weight and gestational age, all causes of death, and information on hospital in-patient morbidity (Stanley *et al* 1994). The completeness of these population-based data sets avoids the issues of selection and recall bias which can be a problem in epidemiological studies.

Comparison with data released by the WA Department of Health (Gee 1992, Gee & O'Neill 2001) shows that the WA Twin Register is complete for multiples born between 1980 and 1997 inclusive. A total of 11,189 children were identified as having being part of a multiple birth during the period, comprising 5,340 twin pairs and 166 sets of higher order multiples. Six hundred and thirty six multiple birth children from 506 families died during the perinatal period. The WATR is the only population-based register of multiple births in Australia, and complements the NHMRC-funded Australian Twin Registry (ATR) which registers about 13% of twins of all ages across all Australian states and territories (Clifford & Hopper 1986).

Some twin registers, notably those in Scandinavia, contain data on twins ascertained from the routine examination of birth records (Lichtenstein *et al* 2002, Skytthe *et al* 2002), while others enrol twins immediately after birth (Boomsma *et al* 2002b, Loos *et al* 1998). The Swedish Twin register identified

twins born between 1963 and 1990 using the Medical Birth Registry (Lichtenstein *et al* 2002), and the Danish twin register is reported to be complete for all liveborn pairs since 1968 (Skytthe *et al* 2002). The East Flanders Prospective Twin survey, which enrols twins within 24 hours of birth, has the advantage of accurate zygosity and chorionicity data which are collected by the routine examination of placental membranes, blood groups and DNA if necessary (Derom *et al* 2002, Loos *et al* 1998). About 50% of all newborn twins are currently registered on the Netherlands twin register (Boomsma *et al* 2002b). Some of these registers collect basic perinatal data (Derom *et al* 2002, Loos *et al* 1998), and data on deaths and cancer (Skytthe *et al* 2002), but none contains the amount of data available to the WATR. The MCHRDB, and consequently the WATR, is a unique and comprehensive collection of data pertaining to the pregnancy, birth, and perinatal period of every birth in WA since 1980. By routinely linking to the hospital morbidity database, the death register, CP and birth defects registries, the WATR is able to assemble a range of data on morbidity and mortality in childhood that is not available to any other twin register. It will facilitate future research into possible perinatal and childhood antecedents to adult disease as these children move through their lives.

It was important to establish that the WATCH study data was representative of the WA population from which it was drawn, to eliminate the effects of selection bias resulting from non-random sampling. This is particularly a problem in volunteer registers. It is known that female twins are more likely to volunteer to participate in research than male twins, as are MZ twins compared with DZ twins (Lykken *et al* 1987, Lykken *et al* 1978), and volunteer samples can under-represent twins from the lower socio-economic groups (Neale *et al* 1989). While this may not be a problem when studying conditions that vary little with SES, it is likely to give misleading results for the analysis of conditions highly dependent on SES, such as occupational or educational achievement (Heath *et al* 1989a). Non-random sampling is more of a concern when recruiting adult twins, as quite often the non-participation of either twin will exclude the twin pair from the sample. For conditions such as cognitive ability, individuals falling below a certain threshold have been shown to be less likely to volunteer (Lykken *et al* 1987). If the MZ twins are more concordant for this condition than DZ

twins, this will result in a greater loss of DZ twin pairs than MZ pairs. Under these conditions, it has been shown that there is a resultant differential attenuation of the MZ and DZ twin correlations, with DZ correlation being the more highly attenuated (Neale *et al* 1989).

7.2 The WATCH study

Families of twins born in WA between 1980 and 1992, and whose twins were not known to have died, were contacted and invited to participate in the WA Twin Child Health (WATCH) study. A total of 9,247 individuals from 1,758 families provided data on a wide range of health, behavioural and educational outcomes. Data collected by the WATCH study has enabled a comprehensive picture of WA twin families to be described. It includes information on a number of characteristics of the parents, including education and occupation, health conditions, accidents in the previous year, and the history of multiple births in their families. Asking the mother to complete a table outlining the relationship of every family member to both herself and the twins has enabled the degree of genetic relatedness of each family member to be determined.

The unique nature of the data contained in each composite record on the MCHRDB, and consequently also the WATR, allowed the representativeness of the WATCH study families to be assessed. No difference between participants and non-participants was found with respect to parental age and racial origin (Hansen *et al* 2000). The original questionnaires were designed to be used as a potential sampling tool for future studies into a wide range of health-related outcomes. Of necessity, they were very long and families estimated that it took over two hours to complete them (Hansen *et al* 2004). This resulted in a sub-optimal response rate of 57%. Despite this, over 90% of families were able to be traced to a current address up to 17 years after the birth of their twins. Response was found to be associated with the length of the questionnaires and the number of responses required by the parents (Hansen *et al* 2004). However, no resultant bias was introduced with respect to parental characteristics of participants and non-participants (Hansen *et al* 2000). There was concern that the nature and length of the questionnaires could bias the response towards the more highly educated families, and while there was no difference in the

proportion of parents who had completed a tertiary qualification compared with the WA population (ABS 2000), WATCH families had a lower mean score for the SEIFA index of education and occupation (ABS 1998) than the general population.

The WATCH study was able to confirm the results from many previous studies that, compared with single-born children, twins were, on average, lighter at birth, and born at shorter gestation (Alexander *et al* 1998, Liu & Blair 2002). For all gestational ages between 28 and 40 weeks, WA twins had lower mean birth weight than their singleton siblings. Data from twins did not include those pairs where one or both of them were known to have died, and parents tended also not to provide this information for any of their singleton children who had died. Therefore, while the data on birth weight is not complete, it still showed the same pattern as many other studies (Alexander *et al* 1998, Glinianaia *et al* 2000, Kiely 1990, Luke 1996, Luke *et al* 1991, Skjaerven *et al* 2000). Mothers experienced more complications during the pregnancy with her twins than with her singleton children. Others have found higher rates of pre-eclampsia (Cooperstock *et al* 2000), urinary tract infection (Conde-Agudelo *et al* 2000), and hypertension (Conde-Agudelo *et al* 2000, Senat & Ancel 1998) during a multiple pregnancy than during a singleton pregnancy. Although this and other studies (Scher *et al* 2002) found that twins are more likely than singletons to be delivered by caesarean section, review of the literature shows no optimal mode of delivery for twin pregnancies, when assessed by stillbirth and neonatal mortality rates (Haest *et al* 2005, Hogle *et al* 2003).

A difference of 127g was found between the average birth weight of male and female WATCH study twins. This finding is consistent with previous studies (Buckler & Green 1994, Rydhstroem 1992), but both the average birth weights and the amount of the difference were higher than those found in Canadian (Arbuckle *et al* 1993) and Australian (Roberts & Lancaster 1999) twins. The WATCH study data did not include those twins who were either stillborn or died during the neonatal period, both of which are known to be associated with low birth weight (Kilpatrick *et al* 1996, Liapis *et al* 1997, Nielson *et al* 1997), and so it was not unexpected that the average birth weights of WATCH study twins was higher than other population averages.

WATCH study twins and their singleton siblings have different birth weight distributions. Using the standard definition of "normal" birth weight range with the 2½ and 97½ percentiles as cut-off points, the range for twins was 1300g-3500g, while for their siblings it was 2400g-4400g. Similarly the "normal" range for gestation was 29-37 weeks for twins, and 35-40 weeks for siblings. These results are similar to those found in other studies (Alexander *et al* 1998, Cheung *et al* 2000, Min *et al* 2000). It is clear that the definitions of low birth weight and preterm delivery that are used for singleton deliveries do not have the same inferences for twin births, and that plurality-specific birth weight and gestational distributions should be used to identify any deviation from "normal" fetal growth patterns in twins and singletons.

In the WATCH study, the number of parents who believed their twins to be MZ was under-represented at 26%. Analysis of the nine percent of twin pairs whose mothers were unsure of their zygosity using Cohen's discriminant analysis (Cohen *et al* 1973, 1975), shows that 90% of them were more likely to be MZ than DZ. Taking this into account, the proportion of MZ twins in the WATCH study, therefore, is estimated to be 33.4%, which is the expected percentage obtained by the Weinberg method (Parisi 1995). This method is valid if the ratio of male to female births is 1:1. In the WATCH study it was 1.04:1. Thirty-six percent of twins in the East Flanders Prospective twin survey were MZ, of which the majority (71%) were monochorionic (Derom *et al* 2002, Loos *et al* 1998). The zygosity of twins not participating in the WATCH study is unknown, but the proportion of unsame sex twins on the WATR was 35%, slightly higher than the expected percentage of 33%. Male same sex twins were under-represented at 31%. The reason for this is unknown, especially as 51% of the twin children were male.

Twins in the WATCH study were more likely than their siblings to have experienced at least one accident in the last year, which may be the result of their tendency to display more impulsive behaviour (Bijur *et al* 1988, Lalloo *et al* 2003). Other studies have found that twins experience a higher risk of ADHD than singletons, which is associated with increased impulsivity (Hay & Preedy 2006). In turn, these behaviours are linked to increased speech and language

delay (Levy & Hay 1996) as well as reading problems and poor educational outcomes (Hack *et al* 2002, Henderson-Smart 1995). Low gestational age at birth is a strong predictor of child development, motor function, language and mental development (Akerman & Thomassen 1992, Stauffer *et al* 1988), which are all more common in twins than singletons. This study has also demonstrated that twins are likely to have poorer educational outcomes than their singleton siblings, as measured by having attended a remedial class, by the amount of extra school work their parents thought was required, and their increased rate of consultation with speech and occupational therapists.

Low gestational age is more common in twins than singletons, and its effect is not confined to the resultant adverse mortality. Gestational age is strongly related to cerebral palsy (CP) (Petterson *et al* 1993) and to child development (Zubrick *et al* 2000). The WATCH study is not representative in terms of the prevalence of CP in twins. Families who were known to have experienced the death of one or more of their multiples were not contacted, and as the surviving co-twin is at increased risk of CP compared with when both twins survive (Bergh *et al* 1999, Bonellie *et al* 2005, Grether *et al* 1993, Petterson *et al* 1993, Scher *et al* 2002), these data were not available.

It is possible that the increasing numbers of twins that occur as a result of fertility treatments arrive with a different demographic profile than spontaneous twin conceptions (Kiely *et al* 1992). The majority of patients attending the fertility clinics in WA have private health insurance, and are therefore less likely to belong to lowest SES groups. Evidence of this being the case was that parents who sought medical treatment before the pregnancy of their twins were more likely than those who did not do so, to be in the top 10% of the SEIFA indices of disadvantage (35% vs. 21%, $p<.0001$), advantage/disadvantage (27% vs. 17%, $p<.0001$), economic resources (18% vs. 14%, $p=.033$) and education and occupation (23% vs. 16%, $p=.0002$)

There was always a concern when recruiting families for the WATCH study that its focus on asthma and atopy would result in an “asthma-rich” sample. This was not the case, as there was no difference in the the prevalence of asthma in WATCH twin children and singleton WA children who either were part of the

Raine study (Joseph-Bowen *et al* 2004), or the Perth centre of the ISAAC study (Asher *et al* 1998). In addition there was no difference in the prevalence of asthma between WATCH twins and their singleton siblings, or between MZ and DZ twins.

The most consistent risk factor for asthma and atopy in WA twins was having at least one parent with the same phenotype. After adjusting for all other covariates, the risk of asthma in WA twins increased if their mother and father were asthmatic, with the mother conferring a slightly higher risk than the father (3.1 vs. 2.6), and the risk of asthma was increased by eight times if both parents were asthmatic. This analysis supports the importance of a positive family history in the development of asthma and atopy in children shown by previous studies (Litonjua *et al* 1998, Moffatt & Cookson 1996).

In WA children, males had a significantly higher prevalence of asthma than females, but the opposite was true for their parents. These result are in agreement with those determined by many previous studies (Gergen *et al* 1988, Mannino *et al* 2002, Schwartz *et al* 1990, Weitzman *et al* 1990a, Weitzman *et al* 1992). The results from this study appear to support the hypothesis that the reversal of the association of asthma prevalence with sex occurs during adolescence as suggested by other researchers (Gergen *et al* 1988, Manfreda *et al* 1993, Norrman *et al* 1994, Venn *et al* 1998).

Despite twins having a significantly higher rate of most of the potential individual risk factors for asthma than their siblings, there was no association between these factors and the prevalence of asthma, and there was no difference between the prevalence of asthma in twins and either their siblings, or in the singleton population of WA children. Various complications of pregnancy, such as antepartum haemorrhage, preterm contractions and placental problems, have been associated with an increased risk of asthma in children (McKeever *et al* 2001). These associations are confounded by the mother's asthma status as asthmatic mothers have more complications during pregnancy than non-asthmatic mothers (Kallen *et al* 2000, Wen *et al* 2001). Maternal asthma during pregnancy has been associated with impaired respiratory function and decreased oxygenation of the foetus (Bracken *et al* 2002). This may affect

asthma development in the offspring by altering the architecture of the foetus' airways, and explain why maternal asthma confers more risk than paternal asthma (Holberg *et al* 1998, Litonjua *et al* 1998). The only infection during pregnancy on the questionnaires was urinary tract infection (UTI), which was not associated with asthma in WA twins. Other studies showed a relationship between different types of infection during pregnancy and increased risk of asthma in the child, including vaginitis and febrile infections (Xu *et al* 1999), respiratory tract infections in general (Hughes *et al* 1999), episodes of influenza (Calvani *et al* 2004), candida (McKeever *et al* 2002) and UTI (Nafstad *et al* 2003).

WATCH study twins were more likely than their singleton siblings to have been delivered by caesarean section which has been identified as a risk factor for developing asthma by a number of studies (Burney *et al* 1990, Kero *et al* 2002, Remes *et al* 1996, Robertson *et al* 1991). The reasons for this association are not clear. Some have suggested that asthmatic mothers are more likely to have a caesarean delivery (Perlow *et al* 1992), or that mothers delivering by this mode experience more allergies themselves (Perlow *et al* 1992), but others have refuted these suggestions (Kero *et al* 2002). It is thought that being delivered vaginally aids in the maturation of the immune system and promotes lung function (Thilaganathan *et al* 1994).

Low birth weight, as defined for singleton populations, is associated with an increased risk of asthma during childhood in some studies (Braback & Hedberg 1998, Seidman *et al* 1991) but not others (Fergusson *et al* 1997, Lewis *et al* 1995). With such a high proportion of WA twins weighing less than 2500g at birth, a higher rate of asthma in twins would be expected if indeed birth weight itself was a cause of asthma. However, at any given gestation, twins weighed less than singletons, meaning that the lung development of twins would be more advanced than that of a singleton of the same weight (Amiel-Tison & Gluck 1995, Leveno *et al* 1984). Premature infants who require ventilation in a neonatal intensive care unit have also been shown to be at increased risk of wheezing illness (Frischer *et al* 1993, von Mutius *et al* 1993). Although being admitted to the NICU and requiring treatment from a respirator was related to the prevalence of asthma in WA twins in the univariate analysis, they failed to

reach significance after adjusting for all other covariates in the multivariate analysis.

The WATCH study found no association between any of the smoking variables examined and the prevalence of asthma and atopy in twin families. In particular there was no association between exposure to environmental tobacco smoke (ETS) and asthma in children. The reasons for this result are unclear. Smoking status as ascertained by questionnaire is affected by bias, and in the case of adolescent children, parents do not always know that they have taken up the habit. Where possible, I tried to eliminate any possible effect of active smoking on asthma and atopy in twins by excluding twin pairs where one or both of them were reported by their parents as ever having smoked. In 2002, a WA Department of Health survey showed that 15% of 12-15 year-olds had smoked (Fairthorne *et al* 2003), whereas only 2% of WATCH study children in this age group were reported by their parents as having ever smoked. Over the last few decades, the prevalence of asthma has increased at the same time that exposure to passive smoking has decreased. In 1990 the government in WA legislated to ban the advertising of tobacco products and in 1991 the WA Health Promotion Foundation (Healthway) was established (see www.healthway.wa.gov.au). Healthway provided the initial funding for the establishment of the WATR. Since then, legislation has been passed to prohibit smoking in restaurants and bars, in the work place, on public transport and in other public places such as sporting events (Government of Western Australia 2006). Whilst this has reduced the potential for children to be exposed to ETS outside the home, no such legislation exists to control smoking within the family home. The two questions on the parents questionnaire (Q93 and Q96) concerning household smoking rules, were subject to sometimes answered other than by the “Yes” or “No” options provided. Parents wrote that either they did not need such rules because they did not smoke, and no-one they knew smoked, or that people visiting their home already knew not to smoke inside. Exposure to passive smoking in the WATCH study was measured by the number of smokers living in the same house at the twins, and was therefore a very imprecise measure of their ETS exposure. Other than collecting urine samples to measure cotinine levels, all other measures of possible exposure to tobacco smoke are subject to measurement error.

It is well known that both active and passive smoking are associated with asthma. Compared with non-smokers with asthma, asthmatics who smoke have more symptoms, worse asthma control (Siroux *et al* 2000), poorer lung function (Lange *et al* 1998), more airway inflammation (Chalmers *et al* 2001) and less beneficial response to treatment (Chalmers *et al* 2002, Pedersen *et al* 1996). The 2001 National Health survey found that the proportion of smokers among asthmatics was higher than the proportion of smokers among people without asthma (ABS 2002). It also reported that among children with asthma, over 40% of them lived with one or more regular smokers.

Exposure to ETS in childhood is a recognised risk factor for the development of asthma symptoms and for the worsening of pre-existing asthma (AIHW 2005). International studies confirm that parental smoking is associated with more severe asthma in children (Strachan & Cook 1998), and that exposure to ETS after birth is a likely cause of wheezing or other acute respiratory illness in children (Strachan & Cook 1997). Cohort studies have shown that children with pre-existing asthma exposed to ETS have increased morbidity and asthma symptoms (Murray & Morrison 1989), more frequent exacerbations (Chilmonczyk *et al* 1993), and more severe symptoms (Chilmonczyk *et al* 1993, Murray & Morrison 1989). They are also more likely to attend hospital emergency departments with asthma (Evans *et al* 1987). Prevention of indoor smoking leads to a reduction in hospital admission in children with asthma (Gurken *et al* 2000).

The association between maternal smoking in pregnancy and early wheezing has been well documented (Gold *et al* 1999, Hanrahan *et al* 1992, Weitzman *et al* 1990b, Yuan *et al* 2003). It is often difficult to disentangle the separate effects of *in utero* exposure to maternal smoking and ETS exposure during childhood as they are highly correlated. Studies of neonates and infants of mothers who smoked during pregnancy suggest that *in utero* exposure is associated with reduced lung function in the perinatal period (Hanrahan *et al* 1992, Stick *et al* 1996). These studies excluded the effects of post-natal ETS exposure by measuring lung function near birth.

The rise in the prevalence and incidence of asthma during the 1980s and 1990s in the developed world was thought to be due, in part, to the adoption of a more hygienic environment, the tendency of parents to have fewer children and the more universal uptake of vaccination against common childhood illnesses such as measles and mumps. As a result in children were being exposed to fewer infections in early childhood. The observed increase in asthma in developing countries as they increasing adopt a Western lifestyle is further evidence that early exposure to infection may play a role in the aetiology of asthma. Data collected prospectively as each infection occurs would be the only way to determine whether the number of infections experienced by each child is related to the subsequent development of asthma and atopy.

It was not possible in this study to examine the hygiene hypothesis directly as no data on childhood infections, vaccinations or day care attendance were collected. One possible surrogate measures of exposure to childhood infections, namely having older siblings, showed an association with the prevalence of asthma; after adjusting for all other factors, having no older siblings increased the risk. Results from other research of the presence of older siblings and the risk of asthma do not produce consistent results. A review article found that nearly all of the studes reviewed showed an inverse relationship between hay fever and eczema and the number of siblings, but only two-thirds showed the same relationship with asthma (Karmaus & Botezan 2002). It seems that, where an inverse relationship was found, it was the number of older siblings, rather than family size *per se* that was important (Westergaard *et al* 2005). Since there was no difference in the rate of asthma between twins and their siblings, being part of a multiple birth itself had no association with the risk of asthma and atopy in WA children and adolescents, as was found by other researchers (Varjonen *et al* 1992). Living in an urban area is associated with many of the potential risk factors for asthma, and include increased exposure to passive smoking, increased time indoors, and differential access to health care (Aligne *et al* 2000, Evans 1992, Platts-Mills & Carter 1997). Living on a farm, with the association of having animals near the house, has also been found to be protective against asthma (Asher *et al* 1998, von Ehrenstein *et al* 2000). The "rural" variable in this analysis was based on the postcode of residence at the time the data were provided, and was defined as not living in the Perth

metropolitan area. It therefore incorporates many of these aspects of a rural lifestyle.

Most exacerbations of asthma are precipitated by viral infections, such as influenza and bronchiolitis. The timing of exposure to infections seems to be crucial in the aetiology of asthma and atopy (Bager *et al* 2002, Illi *et al* 2001). Early exposure before asthma becomes evident is thought to be protective, whereas exposure later in life may exacerbate symptoms once the disease is established (Calvani *et al* 2002). Early exposure to infections is thought to alter the natural history of asthma by causing the immature immune system to retain its allergic profile present at birth, thus increasing the risk of allergy in later life (Holt *et al* 1997, Martinez & Holt 1999, Prescott *et al* 1999).

The question of the definition of asthma has not been resolved. Asthma has many phenotypes (Kauffmann *et al* 1997) and there is no gold standard for defining it. Most definitions highlight variable airflow obstruction or inflammation (GINA 2006, NIH 1995) and are used in clinical practice to diagnose asthma. These definitions are, however, more descriptions of the characteristics of asthma and do not allow clear guidelines for separating asthmatics and non-asthmatics. The diagnosis of asthma is usually made by a clinician after performing a series of physiological measurements such as lung function tests, bronchial challenge tests to methacholine or histamine, skin test reactivity to a number of known allergens and measurement of serum IgE levels, which provide evidence of allergy. It is impossible to diagnose asthma from a questionnaire.

The purpose of an epidemiological study of asthma, such as this one, is not to make a clinical diagnosis but to examine the differences between asthmatics and non-asthmatics or differences in the prevalence and incidence of asthma in different groups of subjects. The occurrence of asthma is compared in populations not in individuals. An epidemiological study, therefore, needs an instrument that can accurately distinguish between asthmatic and non-asthmatic subjects, which does not involve clinical assessment by a physician. Doctor-diagnosed asthma (DDA) has been used as a reponse variable in many epidemiological studies of asthma (Luyt *et al* 1994, O'Connor & Weiss 1994),

including the large-scale international comparisons of disease prevalence (Asher *et al* 1998, ECRHS 1997). A high correlation with an actual physician diagnosis of the condition has been reported (McGill *et al* 1998, Wolf *et al* 1999). A diagnosis of asthma in a child is a memorable event for a parent and therefore they are likely to give a valid response to the question. It is not the "gold standard" for the definition of asthma; skin prick tests, quantification of serum IgE levels, lung function tests and bronchial hyperreactivity are preferred methods of diagnosis. However, as in most large epidemiological studies of asthma, extensive physiological testing is impractical and could further impair response rates.

7.3 The genetic epidemiology of asthma

Estimates of concordance and heritability for asthma for WATCH study twins are consistent with numerous other studies that have been conducted on twins of all ages throughtout the world. MZ twins were more concordant for each end point examined compared with DZ twins, in agreement with numerous other studies (Duffy *et al* 1990, Edfors-Lubs 1971, Harris *et al* 1997, Hopper *et al* 1990, Lichtenstein & Svartengren 1997, Skadhauge *et al* 1999). Correlation for asthma between family members increased as the degree of genetic relatedness increased. MZ twins had the highest correlation; DZ twins and parent-child correlations were approximately equal, with the least correlation between parents. Male MZ twins had a higher concordance than female DZ twins, but the opposite was true for DZ twins.

Heritability is defined as the proportion of phenotypic variance attributable to genetic effects. In this study of children aged between 6 and 18 years, heritability was estimated at 78%. This compares with estimates of 75% in Finnish adults (Harris *et al* 1997), 79% in Finnish adolescents (Rasanen 2000), 73% from Swedish children (Lichtenstein & Svartengren 1997) and 73% in Danes aged 12 to 41 years (Skadhauge *et al* 1999). Heritability is formally defined for quantitative traits (Hopper 2002b), and for binary traits a hypothetical construct known as "liability" is used. Liability is an underlying, unobservable, Normally-distributed trait and illness occurs if an individual's liability exceeds a pre-determined threshold level (Falconer 1965, Hopper 1993,

2002b, Khoury *et al* 1993). The heritability of a binary trait does not have a clear meaning and is prone to confusion. It is not the extent to which a trait is caused by genetic factors, as models do not allow the proportion of variance due to unshared environment, that is σ^2_E, is not directly equivalent to residual binomial variance. It is therefore preferable to quote the magnitude of the variance components individually, or on the proportion of covariance accounted for by genetic effects (Burton *et al* 1999). While there are problems with its estimation and interpretation, heritability is still a useful construct for researchers. Knowledge of the fact that a trait has high heritability can give extra weight for proceeding with a study which proposes to explore the genetic determinants of that trait. On the other hand, if heritability is low, genetic effects might be difficult to find, and those contemplating any study of the trait need to carefully consider the nature of the trait before embarking on it.

The main purpose of variance components analysis is to determine whether there is a genetic component to the phenotype of interest. If there is, then the next phase of genetic epidemiology can be embarked on with confidence, that is, segregation analysis, linkage analysis and association studies. Variance components analysis requires no information about genotypes or measured environmental determinants. No blood needs to be taken for DNA analysis. However, if information is available about specific genes and environmental determinants, it can be added to the analysis.

All biometric analyses of WATCH study twin and twin family data showed that shared environmental effects did not explain the familial patterns seen for asthma, and that the genetic effects are mainly additive. Once again, these results confirm those from Australia (Duffy *et al* 1990), Denmark (Skadhauge *et al* 1999), Finland (Rasanen 2000), Norway (Harris *et al* 1997) and Sweden (Lichtenstein & Svartengren 1997). This finding seems to be in direct contradiction to the results from epidemiological studies, who have shown that viruses, tobacco smoke exposure, and exposure to indoor air pollution, including house dust mites (HDMs) and moulds which are all shared by family members, to be risk factors for the development of asthma. One explanation for this is that these exposures only lead to asthma in genetically predisposed individuals, and this type of genotype-environment interaction will therefore be

"hidden" in the genetic component of the disease. This will result in an an higher estimate of heritability and no evidence of shared environment (Hopper 2000).

A twin-family study, such as the WATCH study, has advantages over both a twin study and a family study. A study of twins reared together cannot model genetic dominance and common environment together as they are confounded (Eaves *et al* 1978, Martin *et al* 1978). One study suggested that a twin-family study for the segregation analysis of a single gene was as efficient as a traditional family study four times its size (Duffy & Eaves 1991). A family study cannot estimate heritability as it is unable to distinguish between the genetic and environmental contributions to disease (Bracken *et al* 2002).

Structural equation modelling (SEM) is the most popular statistical tool in genetic epidemiology. The genetic and environmental effects are modelled as latent variables on the phenotypic difference of a measured trait within individuals. The contributions of the latent variables are estimated as regression coefficients in the linear regression of the measured trait on the latent variable. Nearly all biometrical twin analyses of psychiatric disorders are based on the liability-threshold model (Falconer 1965) and the correlation of liability between relatives is referred to as the tetrachoric correlation (Falconer 1965, Hopper 1993, 2002b, Khoury *et al* 1993). It is usually left to the analyst to decide upon which value of the threshold distinguishes between disease and no disease. The appropriate model, that is, AE, ACE or CE, is then fitted to the tetrachoric correlations to determine which variance components are important in the aetiology of the trait being studied.

BUGS analysis makes no assumptions about a hypothetical liability to disease, and once a working model has been established, it is relatively easy to extend it to fit other models. It is subject to certain assumptions, however, namely Hardy-Weinberg equilibrium, no epistasis, no gene-environment interactions, random mating and additive genetic effects arise from genes on autosomal chromosomes (Burton *et al* 1999, Scurrah *et al* 2000). The effect of violation of each assumption will vary between traits and will depend on the focus of the scientific interest (Hopper 1993). The Bayesian approach has a number of

advantages; model parameters are directly estimated, fixed and random effects are estimated simultaneously, a saturated variance components structure can be fitted to the data and the method can be extended to other phenotypes, different types of data, and multi-generational data (Palmer *et al* 2001). When analyzing twin data, it was shown that Bayesian methods outperformed SEMs for traits with high heritability (Kuhnert & Do 2003). While SEMs still remain more appropriate for modelling problems that assume bi-variate Normality, Bayesian methods are more suited for modeling complex phenotypes (Kuhnert & Do 2003).

7.4 The classic twin method

One of the strengths of this study is its ability to examine the validity of the classic twin method, which is based on three crucial assumptions; firstly, that the two different types of twins, MZ and DZ, can be reliably distinguished; secondly, that the twin sample is representative of the population from which it is drawn; and most importantly, that the equal environments assumption is true, that is, that MZ and DZ are equally correlated with respect to their environments.

The only reliable way to distinguish between MZ and DZ twins is via analysis of their DNA. In large population-based studies, this is often not feasible due to the prohibitive cost of the tests, estimated at $100-$200 per pair, ethical considerations and the practicality of collecting the specimens (Cohen *et al* 1975). In the 1970s Cohen and his colleagues developed an alternative method of determining zygosity, based on discriminant analysis of a set of questions answered by parents about the perceived degree of physical similarity and identity/confusion between the twins (Cohen *et al* 1973, 1975). They tested their methods on a group of twins who had their zygosity known through DNA analysis, and found high correlation between the methods ($\rho>0.97$). Other researchers using these methods have also reported high agreement between the results and actual zygosity ascertained from genetic testing (Eaves *et al* 1989, Rietveld *et al* 2000). This set of questions was included in the twins' questionnaire in the WATCH study (Appendix 2). There was over 90% agreement between parental assessment of zygosity and that determined by the

discriminant analysis. MZ twins showed higher averages on each question compared with DZ twins, with nearly all MZ twins being confused with each other by relatives other than the parents, and strangers. Twins of unknown zygosity were more likely to be MZ than DZ, which then makes the proportion of MZ twins in the WATCH study no different from that expected.

It has been shown that, for this study of asthma and atopy, the WATCH study twins are representative of the WA population of children with respect to the prevalence of doctor-diagnosed asthma (Asher *et al* 1998, Joseph-Bowen *et al* 2004), and there was no difference in asthma prevalence between twins and their siblings, and between MZ and DZ twins. The WATR is a complete collection of twin and other multiple births data assembled from the routine and statutory collection of birth registration data, which was then combined numerous other data sources to form a composite record for each birth. Families participating in the WATCH have been shown to be representative of all WA multiple birth families with respect to parental age and racial origin (Hansen *et al* 2000), and representative of the WA population with respect to socio-economic status (SES).

The methods of analyzing of twin family data developed using the winBUGS software (Spiegelhalter *et al* 2000) and described in section 6.12, have allowed the validity of the EEA to be tested directly, by including an extra component of variance to estimate the size of the effect due to being a MZ twin. The validity or otherwise of the EEA has been the subject of much debate, supported on the one hand by Kenneth Kendler, and refuted by Jay Joseph. Joseph argued that researchers turned to twin studies to estimate the effect of genetic factors on the condition of interest, as family studies were unable to prove such an effect exists because of confounding by the fact that family members share a common environment. He concluded that the EEA was untenable and the twin method measured nothing more than the greater environmental similarity of MZ versus DZ twins. He supported his arguments with numerous examples from the field of the psychological and behavioural sciences (Joseph 1998, 2000, 2001, 2002). On the other hand, Kendler argued that the validity of the EEA had been tested in numerous ways, and none of them produced any evidence to suggest that it was not true (Kendler *et al* 1993, Kendler *et al* 1994). The popularity of the

method diminished in the 1960s after Don Jackson published a critique of a series of five papers on schizophrenia, where he pointed out that female twins were more concordant than male twins, and DZ twins were more concordant than ordinary siblings (Joseph 2001). This led to the suspension of the methodology and an alternative definition of the EEA, the trait-relevant EEA, to be developed. The trait-relevant EEA implies that MZ and DZ twins are equally correlated for their exposure to environmental influences that are of aetiological relevance to the trait under study (Kendler *et al* 1993).

Intuitively, the assumption of EEA as it applies to asthma in WATCH study twins seems reasonable to assume as these childhood twins were reared together in a common environment and were exposed to potential risk factors for asthma, for example, parental smoking and indoor allergens, at the same time and to the same extent. A greater concordance for trait-relevant environmental exposures in twin pairs would be expected in twin pairs who are still living together than in those who are living apart (Heath *et al* 1989a). *In utero*, monochorionic MZ twins may experience a more adverse intrauterine environment due to competition compared with dichorionic twins, but no difference was found in the prevalence of asthma or wheezing between single-placenta MZ twins and DZ twins (Duffy 1993).

For asthma, the three most important environmental effects are exposure to ETS, infections and indoor air pollution. In the present study, there is no reason to believe that MZ twins would experience these exposures any differently from DZ twins. The WATCH study is a study of childhood twins, who have been reared together and have spent all their lives up to the time of the survey living in the same household. They are concordant (as far as I can determine) for smoking status, their household environment with respect to pets, floor coverings, heating and cooking sources is the same, and their propensity to be exposed to childhood infections would be the same, in that they either both have older siblings, or they both do not. It could also reasonably be assumed that they would either both attend child care at the same age, or both not attend child care. When studying the risk of asthma in adult twins, the EEA could be invalid as MZ twins are known to be more concordant for smoking status than DZ twins (Carmelli *et al* 1992). Although numerous researchers have attempted to resolve

the issue of the EEA by adjusting for the similarity of environment in the analysis, or stratifying twin correlations by indices of the amount of shared environment (Kendler & Gardner 1998), none, to my knowledge, have been able to estimate any difference between MZ and DZ environments directly. By doing this, any perceived problems with the validity of the EEA can be resolved.

The BUGS methods developed to analyze twin-family data described in this thesis present a general model for analyzing both continuous and binary phenotypic data in twin families. The models are not specific to any end point, and it is a simple process to adapt them to any situation without having to identify the trait-relevant environmental factors which may be important in the aetiology of the condition being studied. Introducing a common sibling environment in addition to the common family environment allows different environments for the generations to be modelled. It recognizes that parents only experience a common environment for the portion of their lives for which they co-habit, whereas all children experience a common environment for the entire length of their lives. Assessing the significance or otherwise of the term which estimates the extra environment due to being a MZ twin, that is, sigma2.mz.extra.environment, allows the researcher to conclude that the EEA is valid, or not valid. If it is found not to be valid, then the model need not be discarded all together as statistical methods are available to determine the role of genetic and other familial environmental factors, adjusting for these violations (Kendler *et al* 1993).

7.5 Strengths and weaknesses of the WATR and the WATCH study

The main strength of the WATR and the WATCH study is the nature of the data contained in WA twin register. The WATR is a complete population-based register of childhood multiples born in WA between 1980 and 1997, including those children who were stillborn, or who died during the neonatal period or later during childhood. It contains comprehensive data on the characteristics of the mother and her children, and includes information relating to each viable pregnancy the mother has. Sibships have been established which link a mother with each of her children. Regular linkage to both the WA death registration

system and WA hospital morbidity database will allow future studies into morbidity and mortality in WA multiple birth families to be conducted.

It has been shown that the WATCH study is representative of the WA population, by comparing characteristics of the parents and the SES of the family with those in all WA families. It is also representative to the population of all WA children with respect to diagnosed asthma, as there was no difference in prevalence of asthma between WATCH study children and WA children participating in other studies.

The main weaknesses of this study are those that are common to all epidemiological studies. The response rate was lower than was hoped for at 57% but it did not affect the representativeness of the study. Figures of this magnitude, however, are not uncommon; the Danish twin registry has reportedly enrolled 56% of all known twins on to their register (Skytthe *et al* 2002). Having a higher response rate does not necessarily ensure the complete elimination of bias (Berry & Kanouse 1987, Guadagnoli & Cunningham 1989). Zygosity and asthma were determined from answers given on self- and parent-completed questionnaires. It was financially and practically impossible to collect this information by the preferred methods, that is, by DNA analysis and clinical assessment by a physician, respectively. Both these questionnaire-based methods of data collection have been shown to have good agreement with the "gold standards" (Cohen *et al* 1973, 1975, Wolf *et al* 1999). Collection of smoking information by questionnaire is invariably subject to measurement error as smokers tend to under-report the amount that they smoke. Added factors in this study were that parents were asked to report on their children's smoking habit as well as their own, and mothers were asked about their smoking during pregnancy. The prevalence of teenage smoking is possibly much higher than that reported by parents in this study (Fairthorne *et al* 2003), and smoking during pregnancy would be subject to recall bias as the questionnaires were completed many years after most pregnancies. When assessing exposure to ETS, the questions about household rules regarding smoking in the house and car were ambiguous to some families and could have been worded differently. The number of smokers living in the same household as the children was used as the measure of likely exposure to ETS. Other methods could have been developed,

such as calculating pack-years of smoking and modelling exposure to ETS as either a continuous or categorical variable. But given the limitations in collecting smoking history as described above, it is not certain that this would have produced a more reliable estimate of passive smoking exposure than the one used.

To be able to examine the hygiene hypothesis in relation to the development of asthma it would have been useful to have information about child care attendance, use of vaccinations and childhood infections. The first two could easily have been incorporated into the questionnaires. It would, however, have been almost impossible to collect reliable data on the number and type of infections experienced by the children in the first few years of life. Some families completed the questionnaires at the time that their twins were 17 years old and their other children even older, and substantial recall bias would have been introduced.

7.6 Future work

The results presented in this thesis examining the genetic and environmental contributions to asthma in WA twin families represent the completion of the first stage of this study of the genetic epidemiology of asthma. The next phase was the "WATCH for Asthma" study which collected detailed phenotypic data on a subset of the WATCH study families to investigate and describe the genetic and environmental familial aggregation of childhood asthma and associated "intermediate" phenotypes. These "intermediate" phenotypes included serum IgE levels, exhaled nitric oxide (eNO), skin test reactions to a number of standard allergens, baseline spirometry and bronchial hyperresponsiveness to methacholine. Free zygosity testing was offered to parents who were unsure of their twins' zygosity. Families participating in the "WATCH for Asthma" study are an intensively-phenotyped subset of the WATR for future genetic epidemiological and molecular studies of childhood asthma and associated traits. In addition a blood sample was collected from each participant and DNA stored for the genotyping of candidate genes. The "WATCH for Asthma" study will also allow the comparison of asthma and zygosity information collected by questionnaire with those from physiological measurements and DNA analysis,

respectively. By conducting a questionnaire survey on the population of interest – the WATCH study - and then following it up with intensive clinical measurements on a sub-sample – the "WATCH for Asthma" study has been shown to be the most efficient method of testing a survey instrument against the so-called "gold standard" clinical diagnosis (Pekkanen & Pearce 2002).

The analytical methods described in this thesis will be used to model the "intermediate" phenotypic data from the "WATCH for Asthma" study to determine their relationship with asthma and calculate their genetic and environmental components. A study examining the genetic epidemiology of eNO has been completed (Coleman 2004) using the Fisher software programme (Lange *et al* 1988). Results from that study will be compared with results from a BUGS analysis. Genotyping of the DNA for 11 candidate genes has commenced and the relationship between the PHF11 gene and asthma has been described (McClenaghan 2006). Analysis of a further 10 candidate genes will be undertaken. It will then be possible to procede to linkage, segregation and linkage analysis of the asthma and atopy phenotypes.

7.7 Conclusions

This thesis has described the WA Register of multiple births and how it was used to conduct the WA Twin Child Health (WATCH) study. The WATR is a subset of the Maternal and Child Health Research database (MCHRDB) which was established from the statutory collection of data from the WA birth registration system and the Midwives' Notification form. It contains a unique collection of data relating to maternal physical and socio-demographic characteristics, complications of pregnancy, and perinatal details including infant birth weight and gestational age. Mortality and morbidity data from the WA death registration system and the WA hospital morbidity database are added on a regular basis when they become available. The WATR contains data on every multiple birth to have occurred in WA from 1980 to 1997. It is the only population-based twin register in Australia, and one of a few to have been established in the world.

The aim of the WATCH study was to study to genetic and environment contributions to childhood asthma and atopy, and to examine the role that exposure to ETS plays in the aetiology of these diseases. WATCH study twin families have been shown to be representative of the population from which they were drawn, and the prevalence of asthma in WATCH study twins was no different from that in their siblings, or in the general population of WA children of the same age. Data collected by the WATCH study was not restricted to asthma and atopy and has enabled a comprehensive picture of the health, behaviour and education of WA twin and their families to be described. It can be used as a sampling frame for future research into a number of conditions important to all WA families.

Living in the same household as one or more smoker conferred an 11% increased risk of asthma in twins. However, this effect was not significant. In children, boys had a higher prevalence of asthma than girls, but in parents, the rate in females was higher than that in males. There was no difference in the prevalence of asthma with sex for adolescents, suggesting that this period may be the point at which the change in the relationship between asthma and sex occurs. The most important risk factor for asthma in twins was having asthmatic parents.

MZ twins were more concordant for asthma than DZ twins, and heritability was estimated to be 78%. Additive genetic effects were important in the development of asthma as were the effects due to either genetic dominance or common sibling environment. In all models, common or shared environmental effects did not explain the familial pattern of asthma in WA twin families.

Methods of analyzing twin and twin family data were shown to produce consistent results with both simulated data, and results from the logistic regression analysis performed on real data. Extensions to the models allowed the component of variance due to being a MZ twin to be tested directly, providing a method of testing the validity of the equal environments assumption was valid.

The classic twin method used for analyzing the asthma phenotype data from the WATCH study is valid. I have demonstrated that MZ and DZ twins can be distinguished from each other, the WATCH study twins are representative of the population from which they were drawn, in terms of SES, asthma prevalence and maternal characteristics, and MZ and DZ are equally correlated with respect to their environmental exposures.

It is hoped that the data collected by the WATR and the WATCH study may ultimately lead to the discovery and/or explanation of the genetic and environmental factors associated with asthma and its related phenotypes. This could then lead to the development of diagnostic tools to predict which individual are at risk of developing asthma, more effective treatments and interventions for those with asthma, and a better understanding of what is required to prevent the disease from developing. The potential benefits will be felt across the whole community, and are particularly relevant in Australia, which has one of the highest prevalences of asthma in the world.

CHAPTER 8

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APPENDIX 1:

PUBLISHED PAPER

APPENDIX 2:

TWINS QUESTIONNAIRE

WATCH

QUESTIONNAIRE

THIS QUESTIONNAIRE IS ABOUT YOUR TWINS

APPENDIX 3:

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CODE FOR BUGS PROGRAMME FOR TWIN-FAMILY ACC_S MODEL

APPENDIX 10:

CODE FOR BUGS PROGRAMME FOR TESTING THE VALIDITY OF THE EEA

APPENDIX 1:

PUBLISHED PAPER

THE WESTERN AUSTRALIAN TWIN CHILD HEALTH (WATCH) STUDY: WORK IN PROGRESS

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ABSTRACT

The aim of the Western Australian Twin Child Health (WATCH) study was to establish the WA Twin Register, comprising all multiple births in WA between 1980 and 1992. Of the 3,314 eligible families with multiple birth children born in WA during that time, 2,779 families (84%) have been contacted and invited to join. To December 31, 1999, 1,925 families (69%) have accepted the invitation to join the Register and have been sent questionnaires to complete. So far, completed questionnaires from 1,569 families (59%) have been received. This is equivalent to data on over 8,600 individuals.

242 families (8%) have decline the invitation to participate, and a further 532 families have not responded to 3 contacts (letter of invitation, follow-up letter and newsletter) and are assumed to have declined the invitation, resulting in a non-participation rate 27%.

Extensive tracing procedures have been formulated and adopted in an attempt to contact the 441 eligible families not yet invited to join the Registry, the 108 families for whom we had an incorrect address, and the 85 families who have not responded to the initial invitation and have yet to be sent a follow-up letter.

Data from all twin families has been entered and preliminary analysis shows the following: there appears to be no difference between mothers participating in the study and those not participating in the study, with respect to place of residence (metro vs rural), age at first birth and aboriginality.

Concordancy for asthma for monozygous twins is higher than for dizygous twins (73% vs 28%). Significant risk factors for asthma in twins include being breast fed for less than 6 months and having an asthmatic mother. Twins with doctor-diagnosed asthma are more likely to have been exposed to environmental tobacco smoke than non-asthmatic twins. Measures of passive smoking include: maternal smoking, paternal smoking, having a smoke-free house and having a smoke-free car.

INTRODUCTION

Twin registries are a major source of genetic epidemiological studies of a wide range of phenotypes. [1]; [2] Many twin registries have been established around the world, including those in Sweden [3], England [4], Norway [5], Finland [6], Denmark [7], Monte Carlo [8], Italy [9] and the USA [10]. Most are population-based, while others rely on the registration of volunteers [4]. Some have been established to study a specific sub-population, for example, war veterans [10], [11]. Twin registries have been used to study the genetic and environmental contributions of a number of health conditions [2], including asthma [12], behavioural disorders [13], psychiatric disorders [14], [15], epilepsy [16], alcoholism [17] and arthritis [18], but none has used a *twin-family* design to study asthma and atopy. Australia already has a national database of twins, the NHMRC-funded Australian Twin Registry (ATR) [19] which is an invaluable research tool and has been used to study a large number of different conditions, leading to an impressive list of publications in international journals. However, it is volunteer-based and covers only 12.5% of Australian twins of all ages [20]. Although this is not a problem in many areas of application, it can lead to problems for studies in some areas of Public Health. This is because many of the outcomes and exposures of interest to public health are strongly influence by factors such as social class, which also modulate response to voluntary data collection. There is therefore scientific merit in complementing the national volunteer registry, the ATR, with a population-based register. The value of establishing twin registries at both state and national levels in the US has been established by Kirby [21]. The complementary relationship of WATCH to the ATR is enhanced by its twin-family design (the ATR is based on twins alone).

The Western Australian Twin Child Health (WATCH) study commenced in 1997 using a grant from the WA Health Promotion Foundation (Healthway). The aim of the WATCH study was to set up the Western Australian (WA) Twin Registry, a population-based register of all multiples born in WA since 1980, and to determine the representativeness of that register with respect to the population of all WA children born since 1980. This will enable the relative contributions of genetics and environmental factors in childhood diseases and the patterns of

behaviour in WA children, with the first focus of interest being childhood asthma and atopy and exposure to environmental tobacco smoke.

METHODS

Establishing the WA Twin Register

In order to establish the Western Australian (WA) Twin Register, all multiples born in WA between 1980 and 1992 were identified using the WA Maternal and Child Health Research database (MCHRDB) maintained at the TVW Telethon Institute for Child Health research (ICHR). This is a unique population-based data resource of all births in WA from 1980 onwards which is constantly updated and linked to other health-related databases, such as the hospital morbidity database, the birth defects register, the cerebral palsy register, and to the WA death register. Multiple births were identified using previously developed computerised record linkage techniques which establishes sibships on this database (Croft, PhD thesis, in progress). All potential multiple births so identified were checked manually, and any links still unresolved were then checked against the original birth record held at the Registrar General's Office in Perth.

Baseline Data

Maternal identifiers from the MCHRDB were then linked to the electronic State Electoral Roll at the Department of Public Health, the University of Western Australia (UWA), to identify the birth mother's current address. Mothers so traced were sent a letter inviting her and her family to join the Registry. Non-responders to the introductory letter were contacted by telephone after one month. If no telephone number was available, a reminder follow-up letter was sent. New addresses were sought for those letters returned undelivered and the same follow-up procedures were followed. In November 1998 a newsletter was sent to all families that had been contacted to join the study. For those who had replied neither to the initial letter or the follow-up letter, a further invitation to join the study was included with the newsletter. If there was no reply within one month, the family were treated as non-participants and no attempt was made to contact them again.

Families who agreed to participate were sent self-completion questionnaires covering the multiples, their parents and all of their siblings. The questionnaires covered aspects of medical history and relevant health behaviours before, during and after the pregnancy of each child. There were also a series of demographic questions specifically for the parents to determine ethnicity, nationality and socio-economic status. Questions aimed at determining zygosity, family history of multiple births and nuclear family structure were also included. In addition to questions on asthma and atopy, data on a number of other conditions (for example, birth defects, attention deficit hyperactivity disorder (ADHD), epilepsy, physical disabilities, health service utilisation, educational difficulties, perceived need for extra services etc.)

Families for whom we did not receive completed questionnaires within two months were contacted to find out whether they were having any problems. Contact was maintained monthly until the completed questionnaires were received, or families decided that they no longer wished to participate. A number of families decided to withdraw from the study because they felt that the questionnaires were too long and they did not have the spare time to fill them in. Therefore, in June 1998 a much shorter questionnaire was developed and offered to families who felt this way. Follow-up was made after one month, and then monthly thereafter.

Asthma and atopic disease

Questions pertaining to respiratory and atopic symptoms and family history were based upon validated questions in a modified American Thoracic Society Questionnaire [22]. Questions relating to the active smoking habits of all members of the household were based upon the questionnaire of the National Heart Foundation Risk Factor study. Additional questions, some based on a local passive smoking questionnaire (Pregnancy and Infancy Survey) were used to quantify the child's habitual exposure to tobacco smoke both in the home and elsewhere. Cook et al. (1993) found that a questionnaire-based assessment of smoke exposure within and outside the home was as good a predictor of lung function in a child as a salivary cotinine measurement. [23]

The primary end-point of interest was doctor-diagnosed asthma (DDA), hay fever and eczema before the age of 15 years. DDA is often used as a response variable in epidemiological studies of asthma [24]. Because a doctor-diagnosis of asthma is memorable, it was anticipated that valid information would be obtained from parents and siblings as well as from multiples themselves. In the past, DDA often led to restricted physical activity at school and we expect that parents to be able to date the diagnosis fairly accurately. By restricting the age of DDA to less than 15 years, we reduced the confusion of passive smoking with active smoking. It is not claimed that DDA is a gold standard for asthma/atopy and in many non-epidemiological studies, skin prick tests, quantification of IgE levels and tests of lung function are used to comprehensively assess atopic status. However, as in most large epidemiological studies of asthma, extensive physiological testing would be impractical and might well impair response rates. It has been shown that asthma questionnaire items determining wheeze are valid instruments for measuring current asthma in children and adults in epidemiological studies [25]. There is good evidence that DDA is a useful correlate of the long-term prognosis of wheezing [26]; [27]. As is standard in studies of asthma/atopy, a number of secondary end-points will also be investigated.

These will include:

(i) DDA or any wheeze after the age of 1 year;

(ii) Doctor diagnosed atopy (asthma, eczema or hayfever) before age 15; and

(iii) Any symptoms of wheeze, cough or breathlessness in the last 12 months

Assessing representativeness of Register

Multiples not on the Register

All multiple birth children ascertained by the record linkage procedures described above will be linked to the Hospital Morbidity database, Birth Defects' Register and the Cerebral Palsy Register to determine whether there are any differences in prevalence and severity of known major health conditions, birth defects (eg, Down Syndrome, spina bifida etc) and cerebral palsy between those children who agreed to participate in the study, and those who did not. Any differences may bias results.

Singleton Children

To be able to assess the representativeness of the WA Twin Register of the general population of WA children, prevalence of the outcomes of interest, for example doctor-diagnosed asthma (DDA) after the age of 1, doctor diagnosis of atopy before the age of 15, among multiple birth children, will be compared with singleton children. To do this, data already collected from several large WA studies will be used. These include the Raine Study and the Pregnancy and Infancy survey carried out by TICHR, and the Busselton Health Study conducted by various research bodies, including the Department of Public Health, UWA, Princess Margaret Hospital and Sir Charles Gairdner Hospital.

Data analysis

Preliminary analysis was carried out using SAS. Data was manipulated to allow information of the risk factors among parents (eg. DDA, mother's smoking during pregnancy, parental smoking, household rules governing smoking etc) to be incorporated along with the data from the twins. Tetrachoric correlation was used as the measure of concordancy of various measures of asthma and atopy between the twins, as part of the PROC FREQ command in SAS.

Early Speech and Language Study

Mothers of multiples born in WA between September 1997 and August 1998, and a random selection of singleton controls born during the same period, have been contacted near the time of the children's first and second birthday to collect data on their early speech and language development, and temperament. Children with delayed speech at the age of 2 have been identified and informed of speech pathology services they may wish to access. It is hoped to follow-up these children in the future. No results of this part of the study are presented in this paper.

RESULTS

Participation rate

Between 1980 and 1992, 3,679 families were identified as having had a multiple birth in Western Australia (WA); at total of 7,524 children, comprising 3,602 twin pairs and 105 sets of higher order multiples , that is, triplets, quadruplets or quintuplets. Three hundred and sixty five families experienced the death of

one or more of their multiples and were not contacted as part of the WATCH study. Of the 3,314 eligible families, 84% (2,779) were traced to a current address and invited to join the WATCH study. Of these, 1,925 (69%) have agreed to participate, and completed questionnaires have been received from 59% (1,653). This gives an effective participation rate of 50% of eligible families.

Only 40% of families contacted responded to the initial letter with no follow-up. The most effective method of follow-up to non-responders to the letter of invitation was by telephone (Table 1)

Table 1: Response and follow-up to the initial letter of invitation.

Method of contact	Number of families contacted	Number (%) of replies	Number (%) of participants
Mailed Initial letter	2779	1111 (40)	1003 (36)
Telephone follow up	938	845 (90)	765 (82)
Mail follow-up	608	180 (30)	127 (21)
Telephone and mail follow-up	93	38 (41)	30 (32)

Of the 1,653 completed questionnaires received, 777 (47%) families had no follow-up, 496 (30%) families had one follow-up contact, and 314 (19%) families were sent at least one more set of questionnaires. After receiving questionnaires, 166 (10%) families decided that they no longer wished to participate and withdrew from the study. In summary, of the 2,779 families contacted, only 465 (17%) replied to the introductory letter and returned completed questionnaires without requiring some follow-up.

The mean age of the mother at the time of the birth of their multiples was 28.6 years with a range of 14-45 years. Mothers under 20 years old at the time of the birth of their multiples were more likely to experience the death of one of their multiples (Table 2)

Table 2: Mother's age at birth of multiples

Age of mother (yrs)	Number	Number (%) of deaths
Under 20	125	24 (19)*
20-29	1990	190 (10)
30-39	1498	144 (10)
40+	60	7 (12)

*p<0.001

Representativeness of the Register

When comparing participants with non-participants, there was no difference in mother's place of residence –57% of mothers living in metropolitan Perth agreed to participate compared with 60% of mothers living in rural WA (the corresponding non-participation rates were 7% for Perth metropolitan area and 4% for rural WA).There was no difference in demographic characteristics of the parents when comparing study participants with non-participants (Table 3).

Table 3: Parental characteristics (%) of study participants and non-participants

	Participant (n=1925)	Non-participant (n=249)
Mother <25 years old	29	33
Father <25 years old	14	13
Mother non-Aboriginal	96	91

Preliminary results for asthma and atopy

Asthma and atopy was reported to be a common condition among multiple birth families (Table 4)

Table 4: Prevalence (%)of reported asthma and atopy in WA multiple birth families.

End point of interest	Twins (n=2866)	Siblings* (n=1209)	Parents# (n=2745)
Doctor diagnosed asthma	27	25	15
Ever wheezed	27	25	20
Wheezed in last 12 months	13	14	12
Wheezed 3 or more times since 1st birthday	29	20	17
Doctor diagnosed hay fever	16	17	30
Doctor diagnosed eczema	18	17	13

*year of birth 1980-1992
#birth parents

Length of breast-feeding and parental asthma appeared to be the only risk factors which had a significant effect of the diagnosis of asthma in twins. Parental smoking appears to have no significant effect (Table 5).

Table 5: Prevalance (%) of possible risk factors for asthma in twins

Risk factor of interest	Asthmatic twins	Non-asthmatic twins	p-value
Maternal smoking during pregnancy	18	19	0.49
Mother ever smoked	55	51	0.083
Father ever smoked	64	61	0.12
Non smoking house	76	74	0.53
Non smoking car	84	84	0.69
Mother diagnosed with asthma	31	13	<0.001
Father diagnosed with asthma	21	9	<0.001
Breast fed for less than 6 months	64	59	0.013
Other milk introduced before 4 months	73	70	0.10

Monozygous (MZ) showed higher concordance than dizygous (DZ) twins for all end points of interest (Table 6).

Table 6: Concordancy between MZ and DZ twins for asthma and atopy end-points

End point of interest	MZ twins	DZ twins
Doctor diagnosed asthma	0.88	0.54
Doctor diagnosed hay fever	0.91	0.58
Doctor diagnosed eczema	0.84	0.47

FUTURE WORK

Multivariate analysis taking correlated structure into account

The first step in ascribing a genetic component to the aetiology of a human disease is to demonstrate that the phenotype of interest clusters in families [28]. Having demonstrated clustering, the next logical step entails extended variance-components analysis to disentangle variation arising from environmental sources from that due to genetic causes [29]; [28]. For binary phenotypes or censored survival time, analysis will be based on Gibbs sampling methods as described by Burton et al. [30] Analyses based on generalised estimating equations will be undertaken using conventional GEE methods [31] as implemented by the GEE() function in S-Plus.

Zygosity will be assessed as part of the questionnaire using standard questions [32]. In those cases where zygosity is indeterminate, it will be treated as unknown and the relevant twins excluded from all analysis where zygosity is critical. MZ twins share 100% of their genes by inheritance while DZ share only 50%. If, as is standard, it is assumed that the sharing of environment is similar in MZ and DZ twins, a greater concordancy in the disease status (phenotype) of MZ twins compared with DZ twins is suggestive of a genetic contribution to an aetiological mechanism. As a robust test of whether passive smoking causes or triggers asthma directly or indirectly (only in individuals who are genetically susceptible), the following procedure will be adopted:

1. calculate $\Delta \rho_{(smoke+)} = \rho_{MZ(smoke+)} - \rho_{DZ(smoke+)}$
2. calculate $\Delta \rho_{(smoke-)} = \rho_{MZ(smoke-)} - \rho_{DZ(smoke-)}$

3. compare $\Delta \rho_{(smoke+)}$ with $\Delta \rho_{(smoke-)}$

where ρ represents concordancy for asthma

smoke+ represents exposure to ETS

smoke- represents non-exposure to ETS

MZ - monozygous twins

DZ – dizygous twins

Further analysis incorporating the additional information provided by parents and siblings will permit the sources of phenotypic variation to be resolved more completely and more powerfully. For example, variation will be able to be attributed to the combined additive effect of many genes, other non-additive genetic effects and to shared family environment [29].

Genetic modelling will be undertaken using Gibbs Sampling (BUGS 0.6), generalised estimating equations (Stata) and structural equation modelling (Mx). Using a twin-family structure, we will be able to estimate separate variance components; viz. common additive polygenic genetic effects (σ_A^2), common family environmental effects (σ_C^2), and common sibling environmental effects (σ_{Cs}^2), dominance polygenic effects (σ_D^2) and unshared (error) determinants (σ_E^2).

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INSTRUCTIONS

- We would like these questions to be answered by the twins' mother as they are about things that may have happened before, during and since the twins were born. If this is not possible, then the closest relative should answer the questions.

- At first sight, the questionnaire may seem long but you will probably be able to skip many of the questions as they do not apply to you.

- Most answers require a ✓ in a box or a short answer written in the space provided. Some questions allow you to ✓more than one box. When this is the case, clear instructions are given with each question.

- For some questions you will see an → with a number after the box you have ticked. This means that the next few questions do not apply to you. Please follow the instructions that are given and go to the question number stated after the →. Where there is no → please go to the next question.

- When you have finished, please answer the questionnaire about yourself and your partner.

- If you have any problems completing the questionnaire or have any other questions, please ring (08) 9382 2007 or 1800 819 684 between 9am and 5pm Monday to Friday.

ALL THE INFORMATION YOU GIVE US IN THIS QUESTIONNAIRE WILL REMAIN

CONFIDENTIAL

Firstly, some general questions about your twins.
For each question, please ✓ one box or write your answer in the space provided.

		TWIN 1	TWIN 2
1	**What is his/her first name?**	_____	_____
2	**What is his/her sex?**		
	Female	0 ❑	0 ❑
	Male	1 ❑	1 ❑
3	**What is his/her date of birth?**	___/____/19___	___/____/19___
4	**What was his/her birthweight?**	_____ grams or ____lbs _____oz	_____ grams or ____lbs _____oz
5	**What was the length of the pregnancy?**	_____ weeks or _____months	

What is the name of the person completing this questionnaire?

_____

What is your relationship to the twins?

_____

We have made every effort to determine whether any of the children in this study have died in Western Australia, but we do not know about children who have died elsewhere in Australia or overseas, and realise that we may not know about some of those who died in WA. We appreciate that it may be difficult for you to answer questions about your child who is no longer alive, but we would very much appreciate it if you could answer the three questions below.

It is not necessary to complete the rest of the questionnaire about a child who has died, but you may do so if you wish. Of course, we would certainly find any extra information extremely useful.

	TWIN 1	**TWIN 2**
When did he/she die?	___/____/19___	___/____/19___
Where did he/she die?	_____	_____
What was the cause of death?	_____	_____
	_____	_____

In the future, we may want to study families where one or both twins have died. Would you like to be contacted if we decide to do this?

No	0	❑
Yes	1	❑
Unsure at the moment	2	❑

We appreciate you giving us this information.

Thank you very much.

These questions will help us determine whether your twins are identical or non-identical.

6 **Are the twins the same sex?**

No	0	❑	**→Q20**
Yes	1	❑	

7 **I believe the twins to be:** (✓one box)

Genetically identical *(one egg, monozygotic)*	1	❑
Genetically non-identical *(two eggs, dizygotic)*	2	❑
Not sure	9	❑

To what extent do you think your twins are similar *AT THIS TIME* for the following features? Please ✓ one box for each question.

8 **Height**

Not at all similar	1	❑
Somewhat similar	2	❑
Exactly similar	3	❑

9 **Weight**

Not at all similar	1	❑
Somewhat similar	2	❑
Exactly similar	3	❑

10 **Facial appearance**

Not at all similar	1	❑
Somewhat similar	2	❑
Exactly similar	3	❑

11 **Hair colour**

Not at all similar	1	❑
Somewhat similar	2	❑
Exactly similar	3	❑

12 **Eye Colour**

Not at all similar	1	❑
Somewhat similar	2	❑
Exactly similar	3	❑

13 **Complexion**

Not at all similar	1	❑
Somewhat similar	2	❑
Exactly similar	3	❑

14 **Do they look as alike as two peas in a pod?**

No	0	❑
Yes	1	❑

15 **Does their mother ever mistake one for the other?**

No 0 ❑
Yes 1 ❑

16 **Does their father ever mistake one for the other?**

No 0 ❑
Yes 1 ❑

17 **Are they sometimes mistaken for each other by other relatives?**

No 0 ❑
Yes 1 ❑

18 **Is it hard for strangers to tell them apart?**

No 0 ❑
Yes 1 ❑

19 **Do they have very similar personalities?**

No 0 ❑
Yes 1 ❑

These questions are about your pregnancy with the twins.
If you have difficulty remembering, please give the best answers you can.
For each question, please ✓ one or more boxes, or write your answer in the space provided.

20 ***BEFORE THIS PREGNANCY*, for how long were you trying to conceive?**

Not specifically trying 0 ❑

Months or years *(please specify)* _____ mths or ___ yrs

21 ***BEFORE THIS PREGNANCY*, did you or your partner seek medical advice or have any investigations, treatments or operations for difficulty becoming pregnant or to assist you in becoming pregnant?**

No 0 ❑
Yes *(please give details below)* 1 ❑

_____
_____
_____
_____

Don't know/can't remember 9 ❑

22 **Did you take any of the following vitamin supplements *IN THE MONTH BEFORE* you became pregnant?**

Multivitamins	1	❑
Multivitamins with folic acid	2	❑
Folic acid	3	❑
FeFol or FGF	4	❑
Other vitamins *(please specify)*	5	❑

_____

I took vitamins but not sure which ones	6	❑
I can't remember if I took vitamins or not	9	❑
I did not take vitamin supplements	0	❑

23 **Did you take any of the following vitamin supplements in the *FIRST THREE MONTHS* of pregnancy?**

Multivitamins	1	❑
Multivitamins with folic acid	2	❑
Folic acid	3	❑
FeFol or FGF	4	❑
Other vitamins *(please specify)*	5	❑

_____

I took vitamins but not sure which ones	6	❑
I can't remember if I took vitamins or not	9	❑
I did not take vitamin supplements	0	❑

24 **Did you smoke during this pregnancy?**

No, I have never smoked	0	❑	→**Q27**
No, I gave up smoking before I was pregnant	1	❑	→**Q26**
No, I gave up smoking when I was trying to become pregnant	2	❑	→**Q26**
No, I gave up smoking as soon as I knew I was pregnant	3	❑	→**Q26**
Yes, but I gave up during the pregnancy	4	❑	→**Q25**
Yes, I smoked during this pregnancy	5	❑	→**Q25**

25 ***COMPARED WITH BEFORE YOU BECAME PREGNANT*, how much did you smoke during this pregnancy?**

Less than before	0	❑
About the same amount as before	1	❑
More than before	2	❑
Don't know/can't remember	9	❑

26 **Did you start smoking again after the children were born?**

No	0	❑
Yes	1	❑

27 **Did you take any medicines for epilepsy during pregnancy?**

No	0	❑
Yes	1	❑

28 **Did you have any fits or seizures during pregnancy?**

No	0	❑
Yes	1	❑

29 **Did you have any complications during pregnancy?** (✓ if yes)

Bleeding serious enough to require bed rest	01	❑
Threatened miscarriage (*under 20 weeks*)	02	❑
Urinary tract infection	03	❑
Pre-eclampsia *(toxaemia)*	04	❑
High blood pressure	05	❑
Diabetes	06	❑
Placenta praevia	07	❑
Premature rupture of membranes	08	❑
Other *(please specify)*	09	❑
_____		
Don't know/can't remember	99	❑
No complications	00	❑

30 ***ON THE ADVICE OF YOUR DOCTOR*, how long did you rest in bed during the pregnancy?**

In hospital	days	_____	OR, wks	_____
At home	days	_____	OR, wks	_____
Don't know/can't remember	9	❑		
I did not spend any time resting in bed during the pregnancy	0	❑		

31 **In the first few months after the twins were born were you more depressed than usual?**

No	0	❑
Yes	1	❑
Don't know/can't remember	9	❑

This section asks about your twins soon after they were born.
You may find it helpful to write the child's name in the space at the top of each page.
Please use one column for each child, and for each question, ✓ one or more boxes as directed, or write your answer in the space provided.

	TWIN 1	TWIN 2
Name	_____	_____

32 **What was the type of delivery?**

Normal	1	❑	1	❑
Breech	2	❑	2	❑
Forceps	3	❑	3	❑
Vacuum extraction	4	❑	4	❑
Booked caesarean section	5	❑	5	❑
Emergency caesarean section	6	❑	6	❑

33 **Did he/she have any of the following conditions *IMMEDIATELY* after birth?** *that is, while he/she was still in hospital)* (✓ if yes)

Respiratory distress	1	❑	1	❑
Convulsions	2	❑	2	❑
Other serious condition (*please specify*)	3	❑	3	❑
_____			_____	
Don't know/can't remember	9	❑	9	❑
None of these conditions	0	❑	0	❑

		TWIN 1	TWIN 2
	Name	_____	_____
34	**Did he/she have any of the following treatments *IMMEDIATELY* after birth?** *that is, while he/she was still in hospital)* (✓ if yes)		
	Respirator/ventilator	1 ❑	1 ❑
	Fed through a naso-gastric tube	2 ❑	2 ❑
	Other treatment *(please specify)*	3 ❑	3 ❑
		_____	_____
	Don't know/can't remember	9 ❑	9 ❑
	None of these treatments	0 ❑	0 ❑
35	**How long did he/she spend in neonatal intensive care unit, or special care nursery?**		
	Was not in neonatal intensive care OR special care nursery	0 ❑	0 ❑
	Time in intensive care unit	___ days or ____wks	___ days or ____wks
	Time in special care nursery	___ days or ____wks	___ days or ____wks
	Total time in neonatal intensive care and special care nursery *(but I can't remember how long in each section)*	___ days or ____wks	___ days or ____wks
36	**How long was he/she breastfed?** (✓ one box)		
	Was not breastfed	0 ❑	0 ❑
	Was breastfed for less than 3 months after birth	1 ❑	1 ❑
	Was breastfed for at least 3 months but less than 6 months after birth	2 ❑	2 ❑
	Was breastfed for at least 6 months but less than 9 months after birth	3 ❑	3 ❑
	Was breastfed for at least 9 months but less than 12 months after birth	4 ❑	4 ❑
	Was breastfed for 12 months or longer after birth	5 ❑	5 ❑
	Don't know/can't remember	9 ❑	9 ❑
37	**At what age was he/she first given any milk *OTHER THAN BREAST MILK*?**		
	He/she was given "comp" feeds in hospital	0 ❑	0 ❑
	Age at which he/she was first given milk other than breast milk *AT HOME*	_____ mths	_____ mths

Next, some questions about your children NOW.
Please answer each question by putting a ✓ in one or more boxes as directed, or by writing in the space provided.

		TWIN 1	TWIN 2
	Name	_____	_____
38	**How much does he/she weigh *NOW*?**		
		_____ kgs	_____ kgs
39	**How tall is he/she *NOW*?**		
		_____ cms	_____ cms
40	**Does he/she have difficulty keeping attention on work or games?**		
	Not at all	0 ❑	0 ❑
	Just a little/sometimes	1 ❑	1 ❑
	Pretty much/often	2 ❑	2 ❑
	Very much/very often	3 ❑	3 ❑
41	**Does he/she lose things necessary for tasks at home, school or work?** *(for example, toys, pencils, tools)*		
	Not at all	0 ❑	0 ❑
	Just a little/sometimes	1 ❑	1 ❑
	Pretty much/often	2 ❑	2 ❑
	Very much/very often	3 ❑	3 ❑
42	**Does he/she have difficulty organising tasks and activities?**		
	Not at all	0 ❑	0 ❑
	Just a little/sometimes	1 ❑	1 ❑
	Pretty much/often	2 ❑	2 ❑
	Very much/very often	3 ❑	3 ❑
43	**Does he/she fidget with hands or feet, or squirm in his/her seat?**		
	Not at all	0 ❑	0 ❑
	Just a little/sometimes	1 ❑	1 ❑
	Pretty much/often	2 ❑	2 ❑
	Very much/very often	3 ❑	3 ❑
44	**Does he/she have difficulty waiting his/her turn?**		
	Not at all	0 ❑	0 ❑
	Just a little/sometimes	1 ❑	1 ❑
	Pretty much/often	2 ❑	2 ❑
	Very much/very often	3 ❑	3 ❑
45	**Does he/she interrupt or intrude on others?** *(for example, butts into conversations or games)*		
	Not at all	0 ❑	0 ❑
	Just a little/sometimes	1 ❑	1 ❑
	Pretty much/often	2 ❑	2 ❑
	Very much/very often	3 ❑	3 ❑

These questions are about your children's health.
Please answer each question by putting a ✓ in one or more boxes as directed, or by writing in the space provided.

		TWIN 1	TWIN 2	
	Name	_____	_____	
46	**Has he/she *EVER* had any of the following health conditions *CONFIRMED BY A DOCTOR*?** (✓ if yes)			
	Heart problems	01 ❑	01 ❑	
	Febrile convulsions	02 ❑	02 ❑	
	Epilepsy	03 ❑	03 ❑	
	Kidney disease	04 ❑	04 ❑	
	Arthritis or rheumatism	05 ❑	05 ❑	
	Diabetes	06 ❑	06 ❑	
	Asthma or allergies	07 ❑	07 ❑	
	Cancer including leukaemia	08 ❑	08 ❑	
	Any blood disorder	09 ❑	09 ❑	
	Migraine or severe headaches	10 ❑	10 ❑	
	Cystic fibrosis	11 ❑	11 ❑	
	Cerebral palsy	12 ❑	12 ❑	
	Down syndrome	13 ❑	13 ❑	
	Fragile X	14 ❑	14 ❑	
	Spina bifida	15 ❑	15 ❑	
	Intellectual disability	16 ❑	16 ❑	
	Developmental delay or lag	17 ❑	17 ❑	
	Any condition, including birth defects, present since birth (eg. club foot, cleft palate and lip missing fingers and toes), *(please specify)*	18 ❑ _____	18 ❑ _____	
	Any other condition *(please specify)*	19 ❑ _____	19 ❑ _____	
	None of the above	00 ❑	00 ❑	
47	**Has he/she *EVER* had wheezing?** (by "wheezing", we mean a whistling sound coming from within the chest)			
	No	0 ❑	0 ❑	**→Q55**
	Yes	1 ❑	1 ❑	
48	**In the *LAST 12 MONTHS* has he/she wheezed?**			
	No	0 ❑	0 ❑	
	Yes	1 ❑	1 ❑	
49	**Has he/she had three or more episodes of wheeze since his/her *FIRST* birthday?**			
	No	0 ❑	0 ❑	
	Yes	1 ❑	1 ❑	
50	**How old was your child when he/she *FIRST* wheezed?**			
		_____yrs_____mths	_____yrs_____mths	
51	**In the *LAST 12 MONTHS*, how often has he/she wheezed?** (✓ one box)			
	Not at all	0 ❑	0 ❑	
	Less often than twice a month	1 ❑	1 ❑	
	Twice a month or more	2 ❑	2 ❑	
	Twice a week or more	3 ❑	3 ❑	
	Most days	4 ❑	4 ❑	

		TWIN 1		TWIN 2	
	Name	_____		_____	
52	**How old was your child when he/she *LAST* wheezed?**				
		_____yrs_____mths		_____yrs_____mths	
53	**Has he/she *EVER* had an attack of wheezing that has made him/her feel short of breath?**				
	No	0 ❑		0 ❑	
	Yes	1 ❑		1 ❑	
54	**In the *LAST 12 MONTHS*, how often has he/she woken at night with wheezing or shortness of breath?** (✓ one box)				
	Not at all	0 ❑		0 ❑	
	Less often than twice a month	1 ❑		1 ❑	
	Twice a month or more	2 ❑		2 ❑	
	Twice a week or more	3 ❑		3 ❑	
	Most nights	4 ❑		4 ❑	
55	**Has he/she *EVER* had asthma?** (✓ one box)				
	No	0 ❑	**→Q60**	0 ❑	**→Q60**
	Yes, I was told by a doctor	1 ❑		1 ❑	
	Yes, I was told by someone other than a doctor	2 ❑		2 ❑	
56	**How old was your child when he/she *FIRST* had asthma?**				
		_____yrs_____mths		_____yrs_____mths	
57	**Does he/she still have asthma?**				
	No	0 ❑		0 ❑	
	Yes	1 ❑	**→Q59**	1 ❑	**→Q59**
58	**How old was your child when he/she *LAST* had an episode of asthma?**				
		_____yrs_____mths		_____yrs_____mths	
59	**In the *LAST 12 MONTHS*, how often has he/she had an episode of asthma?**				
	Not at all	0 ❑		0 ❑	
	Less often than twice a month	1 ❑		1 ❑	
	Twice a month or more	2 ❑		2 ❑	
	Twice a week or more	3 ❑		3 ❑	
	Most days	4 ❑		4 ❑	
60	**Has his/her asthma or wheezing *EVER* limited any of the following activities?** (✓ if yes)				
	Sports	1 ❑		1 ❑	
	Running	2 ❑		2 ❑	
	Walking up hills or stairs	3 ❑		3 ❑	
	Usual daily activity	4 ❑		4 ❑	
	None of the above	0 ❑		0 ❑	
	Never had asthma or wheezing	8 ❑	**→Q66**	8 ❑	**→Q66**
61	**Are there any times of the year when his/her asthma or wheezing is/was particularly bad?** (✓ if yes)				
	No, same all year round	0 ❑		0 ❑	
	Spring	1 ❑		1 ❑	
	Summer	2 ❑		2 ❑	
	Autumn	3 ❑		3 ❑	
	Winter	4 ❑		4 ❑	
	Different each year	5 ❑		5 ❑	
	Not sure	9 ❑		9 ❑	

		TWIN 1	TWIN 2
	Name		

62 **In the *LAST 12 MONTHS*, about how many days of school or work has he/she missed because of asthma, wheezing or breathing problems?**

	TWIN 1	TWIN 2
None	0 ❑	0 ❑
1 or 2 days	1 ❑	1 ❑
3 to 5 days	2 ❑	2 ❑
6 to 10 days	3 ❑	3 ❑
11 days or more	4 ❑	4 ❑
Doesn't go to school or work	8 ❑	8 ❑
Don't know/can't remember	9 ❑	9 ❑

63 ***IN TOTAL*, how many times has he/she been admitted to hospital because of asthma or wheezing?**

	TWIN 1	TWIN 2
None	0 ❑	0 ❑
Number of times *(please specify)*	_____	_____

64 **In the *LAST 12 MONTHS*, how many times has he/she been admitted to hospital because of asthma or wheezing?**

	TWIN 1	TWIN 2
None	0 ❑	0 ❑
Number of times *(please specify)*	_____	_____

65 **Have any of the following *EVER* made him/her wheezy or brought on an asthma attack?** (✓ if yes)

	TWIN 1	TWIN 2
Cold weather	01 ❑	01 ❑
Change in temperature	02 ❑	02 ❑
Cats	03 ❑	03 ❑
Dogs	04 ❑	04 ❑
Other animals *(please specify)*:	05 ❑	05 ❑
	_____	_____
Dusty parts of the house	06 ❑	06 ❑
Exercise	07 ❑	07 ❑
Colds or chest infections	08 ❑	08 ❑
Feathers	09 ❑	09 ❑
Closeness to flowering plants	10 ❑	10 ❑
Cigarette smoke	11 ❑	11 ❑
Foods or drinks *(please specify)*:	12 ❑	12 ❑
	_____	_____
Other *(please specify)*:	13 ❑	13 ❑
	_____	_____
None of the above	00 ❑	00 ❑

66 **Does he/she *USUALLY* have a cough?**
(usually means as much as 4 to 6 times a day on 4 or more days of the week)

	TWIN 1	TWIN 2
No	0 ❑	0 ❑
Yes	1 ❑	1 ❑

67 **Has he/she *EVER* had a troublesome, dry cough at night which went on for *MORE THAN 3 WEEKS* and was not associated with a cold or 'flu?**

	TWIN 1	TWIN 2
No	0 ❑	0 ❑
Yes	1 ❑	1 ❑

		TWIN 1		TWIN 2	
	Name	_____		_____	
68	**In the *LAST 12 MONTHS*, how often has he/she had a troublesome, dry cough *AT NIGHT*?** (✓ one box)				
	Not at all	0 ❑		0 ❑	
	Less often than twice a month	1 ❑		1 ❑	
	Twice a month or more	2 ❑		2 ❑	
	Twice a week or more	3 ❑		3 ❑	
	Most nights	4 ❑		4 ❑	
69	***APART FROM ASTHMA*, has he/she *EVER* had any serious chest illness or illnesses?**				
	No	0 ❑	**→Q71**	0 ❑	**→Q71**
	Yes	1 ❑		1 ❑	
70	**What was the serious chest illness/es he/she had?**				
		_____		_____	
		_____		_____	
71	**Has he/she *EVER* had hay fever or nasal allergies?** (that is, sneezing, runny or blocked nose, with or without itchy eyes, ***not*** associated with a cold)				
	No	0 ❑	**→Q76**	0 ❑	**→Q76**
	Yes	1 ❑		1 ❑	
72	**Was his/her hay fever or nasal allergy confirmed by a doctor?**				
	No	0 ❑		0 ❑	
	Yes	1 ❑		1 ❑	
73	**How old was the child when he/she *FIRST* had hay fever or nasal allergies?**				
		_____yrs_____mths		_____yrs_____mths	
74	**In the *LAST 12 MONTHS*, has he/she had hay fever or nasal allergies?**				
	No	0 ❑		0 ❑	
	Yes	1 ❑	**→Q76**	1 ❑	**→Q76**
75	**How old was the child when he/she *LAST* had hay fever or nasal allergies?**				
		_____yrs_____mths		_____yrs_____mths	
76	**Has your child *EVER* had a skin problem which occurred in his/her skin creases?** (that is, in front of the elbows, behind the knees, on the front of the ankles, around the neck, or around the ears or eyes)				
	No	0 ❑		0 ❑	
	Yes	1 ❑		1 ❑	
77	**Has he/she *EVER* had eczema?** (eczema is an itchy, dry rash on the face, arms or legs)				
	No	0 ❑	**→Q82**	0 ❑	**→Q82**
	Yes	1 ❑		1 ❑	
78	**Was his/her eczema confirmed by a doctor?**				
	No	0 ❑		0 ❑	
	Yes	1 ❑		1 ❑	
79	**How old was the child when he/she *FIRST* had eczema?**				
		_____yrs_____mths		_____yrs_____mths	

		TWIN 1		TWIN 2	

	Name_____	_____

80 **In the *LAST 12 MONTHS*, has he/she had eczema?**

No	0 ❑		0 ❑		
Yes	1 ❑	→Q82	1 ❑		→Q82

81 **How old was the child when he/she *LAST* had eczema?**

_____yrs_____mths _____yrs_____mths

82 ***IN TOTAL*, how many middle ear infections (otitis media) has he/she had?**

None	0 ❑	0 ❑
1 or 2	1 ❑	1 ❑
3 to 5	2 ❑	2 ❑
6 to 10	3 ❑	3 ❑
11 or more	4 ❑	4 ❑
So many that I've lost count	5 ❑	5 ❑
Don't know/can't remember	9 ❑	9 ❑

83 **Has he/she had any of the following operations?** (✓ if yes)

Tonsils removed	1 ❑	1 ❑
Adenoids removed	2 ❑	2 ❑
Grommets inserted	3 ❑	3 ❑
None of the above	0 ❑	0 ❑

We would now like to know about accidents.
Please answer each question by carefully following the instructions given.

	TWIN 1	TWIN 2
Name	_____	_____

84 **In the *LAST 12 MONTHS* how many *accidents* has he/she had that resulted in a reduction in the amount or level of daily activity, or required medical advice or treatment?**

None	0 ❑	→Q89	0 ❑		→Q89
Number of accidents *(please specify)*	_____		_____		

85 **Of the accidents you reported above, how many required:**
(please specify number for each of the following)

Admission to hospital	_____	_____
Treatment at a hospital accident and emergency department	_____	_____
Treatment by a health professional	_____	_____
Advice from a health professional	_____	_____
Minor treatment at home	_____	_____
No treatment or advice required	_____	_____

		TWIN 1	TWIN 2
	Name	_____	_____
86	**Of the accidents you reported above, how many required:** (please specify number for each of the following)		
	Staying in bed	_____	_____
	Some time off school or work	_____	_____
	Reduced activity only	_____	_____
	No change in amount or level of daily activity	_____	_____
87	**Of the accidents you reported above, how many resulted in the following injuries?** (please specify number for each type of injury)		
	Head injury with loss of consciousness	_____	_____
	Broken bones or fractures *(not a head injury)*	_____	_____
	Dislocations	_____	_____
	Sprains or strains	_____	_____
	Broken teeth or teeth knocked out	_____	_____
	Internal injuries	_____	_____
	External wound needing stitches	_____	_____
	External bruising	_____	_____
	Burns and scalds	_____	_____
	Poisoning	_____	_____
	Other	_____	_____
	(please specify)	_____	_____

	TWIN 1	TWIN 2
Name	_____	_____

88 **Of the accidents you reported above, how many happened in the following places?**
(please specify number for each different place)

	TWIN 1	TWIN 2
Inside own home	_____	_____
Inside someone else's home	_____	_____
In your own garden	_____	_____
In someone else's garden	_____	_____
At work, school or playgroup	_____	_____
In a park or playground	_____	_____
Playing sport	_____	_____
In a road traffic crash *as a driver of a vehicle*	_____	_____
In a road traffic crash *as a passenger in a vehicle*	_____	_____
In a road traffic crash *as a pedestrian*	_____	_____
Bike riding	_____	_____
Other	_____	_____
(please specify)	_____	_____

89 ***Other than in the LAST 12 MONTHS,*** **how many head injuries with loss of consciousness has he/she had?**

	TWIN 1	TWIN 2
None	0 ❑	0 ❑
Number of head injuries (*please specify*)	_____	_____

90 ***IN THE LAST 12 MONTHS,*** **on about how many days has he/she stayed away from school or work because of illness, injury or medical condition?**

	TWIN 1	TWIN 2
None	0 ❑	0 ❑
1 or 2 days	1 ❑	1 ❑
3 to 5 days	2 ❑	2 ❑
6 to 10 days	3 ❑	3 ❑
11 days or more	4 ❑	4 ❑
Doesn't go to school or work	8 ❑	8 ❑
Don't know/can't remember	9 ❑	9 ❑

This section is about your children's eyesight and hearing, and any help they may need at home.
Please answer each question by putting a ✓ in one box only, unless otherwise instructed.

		TWIN 1		TWIN 2	
	Name	_____		_____	
91	**Does he/she have:** (✓ one box)				
	Normal vision in both eyes	1 ❑		1 ❑	
	Vision corrected by prescription glasses or contact lenses	2 ❑		2 ❑	
	Impaired vision	3 ❑		3 ❑	
	Blind	4 ❑		4 ❑	
92	**Is he/she:** (✓ one box)				
	Able to hear with both ears *(that is, have normal hearing)*	1 ❑		1 ❑	
	Deaf in one ear	2 ❑		2 ❑	
	Deaf in both ears	3 ❑		3 ❑	
93	**Does he/she need any help to walk?** (✓ one box)				
	Supervision only	1 ❑		1 ❑	
	Assistance from one person	2 ❑		2 ❑	
	Assistance from 2 people	3 ❑		3 ❑	
	Unable to walk	4 ❑		4 ❑	
	No help required	0 ❑	**→Q96**	0 ❑	**→Q96**
94	**For how long is he/she likely to need this help?** (✓ one box)				
	For less than 12 months	1 ❑		1 ❑	
	For more than 12 months	2 ❑		2 ❑	
	For their lifetime	3 ❑		3 ❑	
	Don't know	9 ❑		9 ❑	
95	***OTHER THAN FOR REASONS OF AGE*, does he/she need physical help with any of the following?** (✓ if yes)				
	Getting on and off the toilet, wiping themselves	1 ❑		1 ❑	
	Washing themselves *(either sitting or standing)*	2 ❑		2 ❑	
	Upper body dressing	3 ❑		3 ❑	
	Lower body dressing	4 ❑		4 ❑	
	Using cutlery to bring food to the mouth	5 ❑		5 ❑	
	No help needed	0 ❑		0 ❑	

These questions are about some medicines that your twins may be taking now or may have taken in the past.
Please answer each question by putting a ✓ in one box only, unless otherwise instructed.

		TWIN 1		TWIN 2	
	Name	_____		_____	
96	**Has he/she *EVER* taken any medicine for asthma or wheezing?** (medicine includes inhalers, liquids, tablets, syrups, nebulisers)				
	No	0 ❑	**→Q102**	0 ❑	**→Q102**
	Yes	1 ❑		1 ❑	
97	**How old was your child when he/she *FIRST* took medicine for asthma or wheezing?**				
		_____yrs_____mths		_____yrs_____mths	

	TWIN 1	TWIN 2
Name	_____	_____

98 ***IN THE LAST 12 MONTHS*, what medicines has he/she taken when he/she gets an attack of asthma or wheezing?** (✓ as many as you need to)

	TWIN 1	TWIN 2
None	0 ❑	0 ❑
Anti-histamines	1 ❑	1 ❑
Ventolin, bricanyl, berotec, alupent, respolin or asmol by inhaler	2 ❑	2 ❑
Ventolin, bricanyl, berotec, alupent, respolin or asmol by nebuliser	3 ❑	3 ❑
Serevent	4 ❑	4 ❑
Atrovent	5 ❑	5 ❑
Theophylline tablets or syrup (such as theo-dur, nuelin, austyn or cardophylline)	6 ❑	6 ❑
Intal, intal forte or tilade	7 ❑	7 ❑
Inhaled steroid sprays (such as becotide, aldecin, becloforte, pulmicort or flixotide)	8 ❑	8 ❑
Prednisone/prednisolone (such as sone, deltasone, panafort, deltasolone, panafcortelone, solone or dexamethasone)	9 ❑	9 ❑

99 **Does he/she take medicines for asthma or wheezing on *MOST DAYS* or *EVERY DAY*?**

	TWIN 1		TWIN 2	
No	0 ❑	→Q101	0 ❑	→Q101
Yes, most days	1 ❑		1 ❑	
Yes, every day	2 ❑		2 ❑	

100 **Which medicines does he/she take on *MOST DAYS* or *EVERY DAY*?** (✓ as many as you need to)

	TWIN 1	TWIN 2
Anti-histamines	1 ❑	1 ❑
Ventolin, bricanyl, berotec, alupent, respolin or asmol by inhaler	2 ❑	2 ❑
Ventolin, bricanyl, berotec, alupent, respolin or asmol by nebuliser	3 ❑	3 ❑
Serevent	4 ❑	4 ❑
Atrovent	5 ❑	5 ❑
Theophylline tablets or syrup (such as theo-dur, nuelin, austyn or cardophylline)	6 ❑	6 ❑
Intal, intal forte or tilade	7 ❑	7 ❑
Inhaled steroid sprays (such as becotide, aldecin, becloforte, pulmicort or flixotide)	8 ❑	8 ❑
Prednisone/prednisolone (such as sone, deltasone, panafort, deltasolone, panafcortelone, solone or dexamethasone)	9 ❑	9 ❑

101 **In the *LAST 12 MONTHS*, how many courses of oral steroids *(taken by mouth)* has he/she taken for attacks of asthma or wheezing?**

	TWIN 1	TWIN 2
None	0 ❑	0 ❑
Number of courses *(please specify)*	_____	_____

102 **Has he/she *EVER* taken any medication for fits or seizures?**

	TWIN 1		TWIN 2	
No	0 ❑	→Q104	0 ❑	→Q104
Yes	1 ❑		1 ❑	

		TWIN 1		TWIN 2	
	Name	_____		_____	
103	**What is the name of this medication?**	_____		_____	
104	**Has he/she *EVER* taken any medication for hyperactivity or attention/learning problems?**				
	No	0 ❑	→Q109	0 ❑	→Q109
	Yes	1 ❑		1 ❑	
105	**What was/is the name of the medication?** (you may ✓ more than one box)				
	Ritalin *(methylphenidate)*	1 ❑		1 ❑	
	Dexamphetamine	2 ❑		2 ❑	
	Clonidine	3 ❑		3 ❑	
	Other *(please specify)*	4 ❑		4 ❑	
		_____		_____	
	Don't know/can't remember	9 ❑		9 ❑	
106	**How old was your child when he/she *FIRST* took this medication?**				
		_____yrs_____mths		_____yrs_____mths	
107	**Is he/she *CURRENTLY* on any medication for hyperactivity or attention problems?**				
	No	0 ❑		0 ❑	
	Yes	1 ❑	→Q109	1 ❑	→Q109
108	**How long was he/she on this medication?**				
	Years	_____		_____	

We would like to know about your children's education.
Please answer each question by putting a ✓ in one box only, unless otherwise instructed.

		TWIN 1		TWIN 2	
	Name	_____		_____	
109	**What year (grade) is he/she in at school?** (✓one box)				
	Pre-school/pre-primary	00 ❑		00 ❑	
	Year (grade) (*please specify*)	_____		_____	
	Ungraded class	13 ❑		13 ❑	
	Has left school	14 ❑		14 ❑	
	Too young to go to school	88 ❑	→Q114	88 ❑	→Q114
	Has never been to school	99 ❑	→Q114	99 ❑	→Q114
110	**Has he/she *EVER* repeated or failed a school year/grade?**				
	No	0 ❑		0 ❑	
	Yes	1 ❑		1 ❑	
	First year in school	2 ❑		2 ❑	
111	**Has he/she *EVER* had been in any of the following classes?**(✓ if yes)				
	General remedial class	1 ❑		1 ❑	
	Remedial English	2 ❑		2 ❑	
	Remedial Reading	3 ❑		3 ❑	
	Remedial Mathematics	4 ❑		4 ❑	
	Special class for gifted and talented children *(eg, PEAC, ATP)*	7 ❑		7 ❑	
	None of the above	0 ❑		0 ❑	

	TWIN 1	TWIN 2
Name	_____	_____

112 **In your opinion, does he/she require remedial instruction in any of the following?** (✓ if yes)

Speech and oral language	1 ❑	1 ❑
Written language	2 ❑	2 ❑
Reading	3 ❑	3 ❑
Number work	4 ❑	4 ❑
Motor skills	5 ❑	5 ❑
No extra work needed	0 ❑	0 ❑

113 **How satisfied are you with his/her progress with educational and learning skills?**

Very satisfied	1 ❑	1 ❑
Satisfied	2 ❑	2 ❑
Neither	3 ❑	3 ❑
Dissatisfied	4 ❑	4 ❑
Very dissatisfied	5 ❑	5 ❑

These questions ask you to say how satisfied you are with certain areas of your child's progress.

	TWIN 1	TWIN 2
Name	_____	_____

114 **How satisfied are you with his/her progress in getting on with his/her twin?** (✓ one box)

Very satisfied	1 ❑	1 ❑
Satisfied	2 ❑	2 ❑
Neither	3 ❑	3 ❑
Dissatisfied	4 ❑	4 ❑
Very dissatisfied	5 ❑	5 ❑
Not applicable	8 ❑	8 ❑

115 **How satisfied are you with his/her progress in getting on with his/her *OTHER* brothers and/or sisters?**

Very satisfied	1 ❑	1 ❑
Satisfied	2 ❑	2 ❑
Neither	3 ❑	3 ❑
Dissatisfied	4 ❑	4 ❑
Very dissatisfied	5 ❑	5 ❑
Not applicable	8 ❑	8 ❑

116 **How satisfied are you with his/her progress in getting on with other children?**

Very satisfied	1 ❑	1 ❑
Satisfied	2 ❑	2 ❑
Neither	3 ❑	3 ❑
Dissatisfied	4 ❑	4 ❑
Very dissatisfied	5 ❑	5 ❑

117 **How satisfied are you with his/her general behaviour?**

Very satisfied	1 ❑	1 ❑
Satisfied	2 ❑	2 ❑
Neither	3 ❑	3 ❑
Dissatisfied	4 ❑	4 ❑
Very dissatisfied	5 ❑	5 ❑

	TWIN 1	TWIN 2
Name	_____	_____

118 **How satisfied are you with his/her progress with physical development and coordination?**

Very satisfied	1 ❑	1 ❑
Satisfied	2 ❑	2 ❑
Neither	3 ❑	3 ❑
Dissatisfied	4 ❑	4 ❑
Very dissatisfied	5 ❑	5 ❑

These questions are about smoking and may not apply to your children because of their age. *If this is the case, please go to question 128*

If *ANY* of your children smoke, please ✓ one box, or write your answer in the space.

	TWIN 1	TWIN 2
Name	_____	_____

119 **Has he/she *EVER* smoked cigarettes, cigars or a pipe?**

No	0 ❑	→Q128	0 ❑	→Q128
Yes	1 ❑		1 ❑	
Don't know	9 ❑		9 ❑	

120 **How old was the child when he/she *FIRST* started smoking?**

Age started smoking (*years*)	_____	_____

121 **Does he/she still smoke?**

No	0 ❑	→Q124	0 ❑	→Q124
Yes	1 ❑		1 ❑	
Don't know	9 ❑		9 ❑	

122 **How much does he/she smoke *NOW*?**

Number of cigarettes *PER DAY*	_____	_____
Number of cigars *PER DAY*	_____	_____
Grams of tobacco *PER WEEK* *(a 2 ounce pouch of tobacco equals 50 grams)*	_____	_____
Don't know	9 ❑	9 ❑

123 **In the *LAST 12 MONTHS*, has he/she changed the amount smoked?**

No, he/she smokes about the same amount	0 ❑	→Q126	0 ❑	→Q126
Yes, he/she smoke less than before	1 ❑	→Q126	1 ❑	→Q126
Yes, he/she smokes more than before	2 ❑	→Q126	2 ❑	→Q126
Don't know	9 ❑	→Q126	9 ❑	→Q126

124 **When did he/she give up smoking?**

Year	19 _____		19 _____	
OR, age *(years)*	_____		_____	
Don't know	9 ❑		9 ❑	

	TWIN 1	TWIN 2
Name	_____	_____

125 **How much *DID* he/she smoke?**

Number of cigarettes *PER DAY*	_____	_____
Number of cigars *PER DAY*	_____	_____
Grams of tobacco *PER WEEK* *(a 2 oz pouch of tobacco equals 50 grams)*	_____	_____
Don't know	9 ❑	9 ❑

126 **When he/she is with your *OTHER* children, either in the house or in the car, which of the following does he/she do *NOW* or *WHEN THEY USE TO SMOKE*?**

Not smoke at all	0 ❑	0 ❑
Smoke less than normal	1 ❑	1 ❑
Smoke about the same	2 ❑	2 ❑
Smoke more than normal	3 ❑	3 ❑
Difficult to say	4 ❑	4 ❑

127 **How often does or did he/she smoke when your children are/were present?**

Never	0 ❑	0 ❑
Some of the time	1 ❑	1 ❑
Most of the time	2 ❑	2 ❑
Always	3 ❑	3 ❑

Next, we would like to know the type and frequency of health-related services your children have used.

	Name	TWIN 1 _____				TWIN 2 _____			
128	**How often has he/she used the following health services?** On every line, please ✓one box for each child								
		Never	**Once only**	**2-5 times**	**More than 5 times**	**Never**	**Once only**	**2-5 times**	**More than 5 times**
	Paediatrician	❑	❑	❑	❑	❑	❑	❑	❑
	Hospital emergency department	❑	❑	❑	❑	❑	❑	❑	❑
	Hospital out-patient department or clinic	❑	❑	❑	❑	❑	❑	❑	❑
	Admitted to hospital for at least one night	❑	❑	❑	❑	❑	❑	❑	❑
	Dentist	❑	❑	❑	❑	❑	❑	❑	❑
	Orthodontist	❑	❑	❑	❑	❑	❑	❑	❑
	Physiotherapist	❑	❑	❑	❑	❑	❑	❑	❑
	Speech therapist	❑	❑	❑	❑	❑	❑	❑	❑
	Occupational therapist	❑	❑	❑	❑	❑	❑	❑	❑
	School guidance officer or school psychologist	❑	❑	❑	❑	❑	❑	❑	❑
	Child & adolescent mental health clinic	❑	❑	❑	❑	❑	❑	❑	❑
	Other psychologist	❑	❑	❑	❑	❑	❑	❑	❑
	Other psychiatrist	❑	❑	❑	❑	❑	❑	❑	❑
	Other specialist doctor, eg. Neurologist, Oral Surgeon *(please specify)*								
_____		❑	❑	❑	❑	❑	❑	❑	❑
_____		❑	❑	❑	❑	❑	❑	❑	❑
_____		❑	❑	❑	❑	❑	❑	❑	❑
	Disabilities services commission	❑	❑	❑	❑	❑	❑	❑	❑
	Family and children's services	❑	❑	❑	❑	❑	❑	❑	❑
	Some other organisation or person *(please specify)*	❑	❑	❑	❑	❑	❑	❑	❑

129 **Finally, is he/she part of any other study conducted by the TVW Telethon Institute for Child Health Research, Princess Margaret Hospital, King Edward Memorial Hospital, Curtin University or the Australian Twin Registry?**

No	0	❑ →Q131
Yes	1	❑
Don't know	9	❑ →Q131

130 **What is the name of the study? (Please specify)**

Twin 1:_____

Twin 2:_____

131

If there is anything else you would like to tell us about your children, or if there are any special services you think he/she needs, please write in the space below.

Twin 1:

_____

_____

_____

_____

_____

_____

_____

Twin 2:

_____

_____

_____

_____

_____

_____

_____

This is the end of the questionnaire about your twins.
Now, please fill in questionnaire 2 about yourself and your partner.
Thank you for your cooperation.

INSTRUCTIONS

- Please fill out the information for your partner, even if he is not the father of your twins, triplets, quadruplets or quintuplets.

- If you do not have a partner at present, please answer the questions about yourself anyway.

- At first sight, the questionnaire may seem long but you will probably be able to skip many of the questions as they do not apply to you.

- Most answers require a ✓ in a box or a short answer written in the space provided. Some questions allow you to ✓more than one box. When this is the case, clear instructions are given with each question.

- For some questions you will see an → with a number after the box you have ticked. This means that the next few questions do not apply to you. Please follow the instructions that are given and go to the question number stated after the →. Where there is no → please go to the next question.

- When you have finished, please fill in questionnaire 3 about other children in your family, if you have any. If you do not have any other children, please put both questionnaires and the signed consent forms in the reply-paid envelope and post it back to us.

Please remember to get each member of the family to sign a separate consent form.

If you have any problems completing the questionnaire or have any other questions, please ring (08) 9382 2007 or 1800 819 684 between 9am and 5pm Monday to Friday.

ALL THE INFORMATION YOU GIVE US IN THIS QUESTIONNAIRE WILL REMAIN

CONFIDENTIAL

1. Following the example below, please list all members of your immediate family *(that is, yourself, your current partner and any children either of you have)*, their relationship to you and the "twins", their first names and date of birth. Please list your children in order from oldest to youngest.

Example

	Name	Date of birth	Relationship to me	Relationship to the "twins"
Yourself	Freda	01/11/1958	myself	mother
Your partner	Fred	28/02/1955	my partner	step-father
Child 1	Paul	15/06/1981	my partner's son from his first marriage	step-brother
Child 2	John	01/01/1982	twins from my first marriage	twin 1
Child 3	Jane	01/01/1982	twins from my first marriage	twin 2
Child 4	Anne	30/04/1997	our son from this marriage	half-sister

Your answer

	Name	Date of birth	Relationship to me	Relationship to the "twins"
Yourself		____/____/19___	myself	
Your partner		____/____/19___		
Child 1		____/____/19___		
Child 2		____/____/19___		
Child 3		____/____/19___		
Child 4		____/____/19___		
Child 5		____/____/19___		
Child 6		____/____/19___		
Child 7		____/____/19___		

Firstly, some general questions about yourself and your current partner.
For each question, please ✓ one box or write your answer in the space provided.

		YOURSELF	YOUR PARTNER
2	**How much do you weigh?**	_____ kgs or ____stone _____lbs	_____ kgs or ____stone _____lbs
3	**How tall are you?**	_____ cms or ____ft _____ins	_____ cms or ____ft _____ins
4	**In which country were you born?** (if born in Australia, please give the State)	_____	_____
5	**If you were not born in Australia, in what year did you first arrive here?**	19 ____	19 ____
6	**What is the main language you speak at home?**		
	English	1 ❑	1 ❑
	Aboriginal language	2 ❑	2 ❑
	Italian	3 ❑	3 ❑
	Greek	4 ❑	4 ❑
	Cantonese	5 ❑	5 ❑
	Mandarin	6 ❑	6 ❑
	Arabic	7 ❑	7 ❑
	German	8 ❑	8 ❑
	Other (*please specify*)	9 ❑	9 ❑
		_____	_____
7	**With which national group do you identify?**	_____	_____
8	**What is your marital status?** (✓ more than one if necessary)		
	Never married	1 ❑	1 ❑
	In first marriage	2 ❑	2 ❑
	Remarried	3 ❑	3 ❑
	Separated but not divorced	4 ❑	4 ❑
	Divorced	5 ❑	5 ❑
	Widowed	6 ❑	6 ❑
	De facto	7 ❑	7 ❑

		YOURSELF	YOUR PARTNER
9	**What is your current occupation, or previous occupation if you are currently unemployed?**		
	Work at home	1 ❑	1 ❑
	Student	2 ❑	2 ❑
	Professional employment *(eg, lawyer, engineer, teacher)*	3 ❑	3 ❑
	Semi-professional employment *(eg, salesperson, clerical)*	4 ❑	4 ❑
	Skilled trades *(eg, plumber, electrician, mechanic)*	5 ❑	5 ❑
	Unskilled trades *(eg, labourer)*	6 ❑	6 ❑
	Other (*please specify*)	7 ❑	7 ❑
10	**What is the highest level of education you have reached?**		
	Never went to school	1 ❑	1 ❑
	Primary school	2 ❑	2 ❑
	Some high school	3 ❑	3 ❑
	Finished high school *(Year 12 or equivalent)*	4 ❑	4 ❑
	Apprenticeship or trade qualifications	5 ❑	5 ❑
	University degree	6 ❑	6 ❑
	Other (*please specify*)	7 ❑	7 ❑

This section asks some questions about your health.
Please answer each question by putting a ✓ in one or more boxes as directed, or by writing in the space provided.

		YOURSELF	YOUR PARTNER
11	**Have you *EVER* had any of the following health conditions *CONFIRMED BY A DOCTOR*?** (✓ if yes)		
	Heart problems	1 ❑	1 ❑
	Febrile convulsions	2 ❑	2 ❑
	Epilepsy	3 ❑	3 ❑
	Kidney disease	4 ❑	4 ❑
	Arthritis or rheumatism	5 ❑	5 ❑
	Diabetes	6 ❑	6 ❑
	Asthma or allergies	7 ❑	7 ❑
	Cancer including leukaemia	8 ❑	8 ❑
	Any blood disorder	9 ❑	9 ❑
	Migraine or severe headaches	10 ❑	10 ❑
	Cystic fibrosis	11 ❑	11 ❑
	Cerebral palsy	12 ❑	12 ❑
	Down syndrome	13 ❑	13 ❑
	Fragile X	14 ❑	14 ❑
	Spina bifida	15 ❑	15 ❑
	Intellectual disability	16 ❑	16 ❑
	Developmental delay or lag	17 ❑	17 ❑
	Any condition, including birth defects, present since birth *(eg. club foot, cleft palate and lip, missing fingers and toes)* (*please specify*)	18 ❑ _____	18 ❑ _____
	Any other condition (*please specify*)	19 ❑ _____	19 ❑ _____
	None of the above	00 ❑	00 ❑
12	**Have you *EVER* had wheezing?** (by "wheezing", we mean a whistling sound coming from within the chest)		
	No	0 ❑ **→Q20**	0 ❑ **→Q20**
	Yes	1 ❑	1 ❑
13	**In the *LAST 12 MONTHS* have you wheezed?**		
	No	0 ❑	0 ❑
	Yes	1 ❑	1 ❑
14	**Have you had three or more episodes of wheeze since your *FIRST* birthday?**		
	No	0 ❑	0 ❑
	Yes	1 ❑	1 ❑
15	**How old were you when you *FIRST* wheezed?**		
	Years	_____	_____

		YOURSELF			YOUR PARTNER		
16	**In the *LAST 12 MONTHS*, how often have you wheezed?** (✓ one box)						
	Not at all	0	❑		0	❑	
	Less often than twice a month	1	❑		1	❑	
	Twice a month or more	2	❑		2	❑	
	Twice a week or more	3	❑		3	❑	
	Most days	4	❑		4	❑	
17	**How old were you when you *LAST* wheezed?**						
	Years	_____			_____		
18	**Have you *EVER* had an attack of wheezing that has made you feel short of breath?**						
	No	0	❑		0	❑	
	Yes	1	❑		1	❑	
19	**In the *LAST 12 MONTHS*, how often have you woken at night with wheezing or shortness of breath?** (✓ one box)						
	Not at all	0	❑		0	❑	
	Less often than twice a month	1	❑		1	❑	
	Twice a month or more	2	❑		2	❑	
	Twice a week or more	3	❑		3	❑	
	Most nights	4	❑		4	❑	
20	**Have you *EVER* had asthma?** (✓ one box)						
	No	0	❑	**→Q25**	0	❑	**→Q25**
	Yes, I was told by a doctor	1	❑		1	❑	
	Yes, I was told by someone *other than a doctor*	2	❑		2	❑	
21	**How old were you when you *FIRST* had asthma?**						
	Years	_____			_____		
22	**Do you still have asthma?**						
	No	0	❑		0	❑	
	Yes	1	❑	**→Q24**	1	❑	**→Q24**
23	**How old were you when you *LAST* had an episode of asthma?**						
	Years	_____			_____		
24	**In the *LAST 12 MONTHS*, how often have you had an episode of asthma?** (✓ one box)						
	Not at all	0	❑		0	❑	
	Less often than twice a month	1	❑		1	❑	
	Twice a month or more	2	❑		2	❑	
	Twice a week or more	3	❑		3	❑	
	Most days	4	❑		4	❑	

		YOURSELF		YOUR PARTNER	
25	**Has your asthma or wheezing *EVER* limited any of the following activities?** (✓ if yes)				
	Sports	1 ❑		1 ❑	
	Running	2 ❑		2 ❑	
	Walking up hills or stairs	3 ❑		3 ❑	
	Usual daily activity	4 ❑		4 ❑	
	None of the above	0 ❑		0 ❑	
	Never had asthma or wheezing	8 ❑	**→Q31**	8 ❑	**→Q31**
26	**Are there any times of the year when your asthma or wheezing is/was particularly bad?** (✓ if yes)				
	No, same all year round	0 ❑		0 ❑	
	Spring	1 ❑		1 ❑	
	Summer	2 ❑		2 ❑	
	Autumn	3 ❑		3 ❑	
	Winter	4 ❑		4 ❑	
	Different each year	5 ❑		5 ❑	
	Not sure	9 ❑		9 ❑	
27	**In the *LAST 12 MONTHS*, about how many days of work or study have you missed because of asthma, wheezing or breathing problems?** (✓ one box)				
	None	0 ❑		0 ❑	
	1 or 2 days	1 ❑		1 ❑	
	3 to 5 days	2 ❑		2 ❑	
	6 to 10 days	3 ❑		3 ❑	
	11 days or more	4 ❑		4 ❑	
	Don't go to work/do not study	8 ❑		8 ❑	
	Don't know/can't remember	9 ❑		9 ❑	
28	***IN TOTAL*, how many times have you been admitted to hospital because of asthma or wheezing?**				
	None	0 ❑		0 ❑	
	Number of times *(please specify)*	_____		_____	
29	**In the *LAST 12 MONTHS*, how many times have you been admitted to hospital because of asthma or wheezing?**				
	None	0 ❑		0 ❑	
	Number of times *(please specify)*	_____		_____	

	YOURSELF	YOUR PARTNER

30 **Have any of the following *EVER* made you wheezy or brought on an asthma attack?**
(✓ if yes)

	YOURSELF	YOUR PARTNER
Cold weather	01 ❑	01 ❑
Change in temperature	02 ❑	02 ❑
Cats	03 ❑	03 ❑
Dogs	04 ❑	04 ❑
Other animals (*please specify*)	05 ❑	05 ❑
Dusty parts of the house	06 ❑	06 ❑
Exercise	07 ❑	07 ❑
Colds or chest infections	08 ❑	08 ❑
Feathers	09 ❑	09 ❑
Closeness to flowering plants	10 ❑	10 ❑
Cigarette smoke	11 ❑	11 ❑
Foods or drinks (*please specify*)	12 ❑	12 ❑
Other (*please specify*)	13 ❑	13 ❑
None of the above	00 ❑	00 ❑

31 **Do you *USUALLY* have a cough?**
(usually means as much as 4 to 6 times a day on 4 or more days of the week)

	YOURSELF	YOUR PARTNER
No	0 ❑	0 ❑
Yes	1 ❑	1 ❑

32 **Have you *EVER* had a troublesome, dry cough at night which went on for *MORE THAN 3 WEEKS* and was not associated with a cold or 'flu?**

	YOURSELF	YOUR PARTNER
No	0 ❑	0 ❑
Yes	1 ❑	1 ❑

33 **In the *LAST 12 MONTHS*, how often have you had a troublesome, dry cough *AT NIGHT*?**
(✓ one box)

	YOURSELF	YOUR PARTNER
Not at all	0 ❑	0 ❑
Less often than twice a month	1 ❑	1 ❑
Twice a month or more	2 ❑	2 ❑
Twice a week or more	3 ❑	3 ❑
Most nights	4 ❑	4 ❑

34 ***APART FROM ASTHMA*, have you *EVER* had any serious chest illness or illnesses?**

	YOURSELF		YOUR PARTNER	
No	0 ❑	**→Q36**	0 ❑	**→Q36**
Yes	1 ❑		1 ❑	

35 **What was the serious chest illness/es you had?**

		YOURSELF			YOUR PARTNER		
36	**Have you *EVER* had hay fever or nasal allergies?** (that is, sneezing, runny or blocked nose, with or without itchy eyes, ***not*** associated with a cold)						
	No	0	❑	→**Q41**	0	❑	→**Q41**
	Yes	1	❑		1	❑	
37	**Was your hay fever or nasal allergy confirmed by a doctor?**						
	No	0	❑		0	❑	
	Yes	1	❑		1	❑	
38	**How old were you when you *FIRST* had hayfever or nasal allergies?**						
	Years	_____			_____		
39	**In the *LAST 12 MONTHS*, have you had hay fever or nasal allergies?**						
	No	0	❑		0	❑	
	Yes	1	❑		1	❑	
40	**How old were you when you *LAST* had hay fever or nasal allergies?**						
	Years	_____			_____		
41	**Have you *EVER* had a skin problem which occurred in your skin creases?** (that is, in front of the elbows, behind the knees, on the fronts of the ankles, around the neck, or around the ears or eyes)						
	No	0	❑		0	❑	
	Yes	1	❑		1	❑	
42	**Have you *EVER* had eczema?** (eczema is an itchy, dry rash on the face, arms or legs)						
	No	0	❑	→**Q47**	0	❑	→**Q47**
	Yes	1	❑		1	❑	
43	**Was your eczema confirmed by a doctor?**						
	No	0	❑		0	❑	
	Yes	1	❑		1	❑	
44	**How old were you when you *FIRST* had eczema?**						
	Years	_____			_____		
45	**In the *LAST 12 MONTHS*, have you had eczema?**						
	No	0	❑		0	❑	
	Yes	1	❑	→**Q47**	1	❑	→**Q47**
46	**How old were you when you *LAST* had eczema?**						
	Years	_____			_____		
47	***IN TOTAL*, how many middle ear infections (otitis media) have you had?**						
	None	0	❑		0	❑	
	1 or 2	1	❑		1	❑	
	3 to 5	2	❑		2	❑	
	6 to 10	3	❑		3	❑	
	11 or more	4	❑		4	❑	
	So many that I've lost count	5	❑		5	❑	
	Don't know/can't remember	9	❑		9	❑	

		YOURSELF	YOUR PARTNER
48	**Have you had any of the following operations?** (✓ if yes)		
	Tonsils removed	1 ❑	1 ❑
	Adenoids removed	2 ❑	2 ❑
	Grommets inserted	3 ❑	3 ❑
	None of the above	0 ❑	0 ❑

We would now like to know about accidents.
Please answer each question by carefully following the instructions given.

		YOURSELF	YOUR PARTNER
49	**In the *LAST 12 MONTHS* how many *accidents* have you had that resulted in a reduction in the amount or level of daily activity, or required medical advice or treatment?**		
	None	0 ❑ **→Q54**	0 ❑ **→Q54**
	Number of accidents (*please specify*)	_____	_____
50	**Of the accidents you reported above, how many required:** (please the specify number of accidents for each of the following)		
	Admission to hospital	_____	_____
	Treatment at a hospital accident and emergency department	_____	_____
	Treatment by a health professional	_____	_____
	Advice from a health professional	_____	_____
	Minor treatment at home	_____	_____
	No treatment or advice required	_____	_____
51	**Of the accidents you reported above, how many required:** (please specify number of accidents for each of the following)		
	Staying in bed	_____	_____
	Some time off work or study	_____	_____
	Reduced activity only	_____	_____
	No change in amount or level of daily activity	_____	_____

	YOURSELF	YOUR PARTNER

52 **Of the accidents you reported above, how many resulted in the following injuries?**
(please specify number for each injury)

Head injury with loss of consciousness	_____	_____
Broken bones or fractures *(not a head injury)*	_____	_____
Dislocations	_____	_____
Sprains or strains	_____	_____
Broken teeth or teeth knocked out	_____	_____
Internal injuries	_____	_____
External wound needing stitches	_____	_____
External bruising	_____	_____
Burns and scalds	_____	_____
Poisoning	_____	_____
Other *(please specify)*	_____	_____
	_____	_____

53 **Of the accidents you reported above, how many happened in the following places?**
(please specify number for each place)

Inside own home	_____	_____
Inside someone else's home	_____	_____
In your own garden	_____	_____
In someone else's garden	_____	_____
At work or while studying	_____	_____
Playing sport	_____	_____
In a road traffic crash *as the driver of a vehicle*	_____	_____
In a road traffic crash *as a passenger in a vehicle*	_____	_____
In a road traffic crash *as a pedestrian*	_____	_____
Bike riding	_____	_____
Other *(please specify)*	_____	_____
	_____	_____

54 ***Other than in the LAST 12 MONTHS,*** **how many head injuries with loss of consciousness have you had?**

None	0 ❑	0 ❑
Number of head injuries (*please specify*)	_____	_____

These questions ask about your eyesight and hearing, and whether you need any help at home.
Please answer each question by putting a ✓ in one box only, unless otherwise instructed.

		YOURSELF			YOUR PARTNER		
55	**Do you have:** (✓ one box)						
	Normal vision in both eyes	1	❑		1	❑	
	Your vision corrected by prescription glasses or contact lenses	2	❑		2	❑	
	Impaired vision	3	❑		3	❑	
	Blind	4	❑		4	❑	
56	**Are you:** (✓ one box)						
	Able to hear with both ears *(that is, have normal hearing)*	1	❑		1	❑	
	Deaf in one ear	2	❑		2	❑	
	Deaf in both ears	3	❑		3	❑	
57	**Do you need any help to walk?** (✓ one box)						
	Supervision only	1	❑		1	❑	
	Assistance from one person	2	❑		2	❑	
	Assistance from 2 people	3	❑		3	❑	
	Unable to walk	4	❑		4	❑	
	No help required	0	❑	**→Q59**	0	❑	**→Q59**
58	**For how long are you likely to need this help?** (✓ one box)						
	For less than 12 months	1	❑		1	❑	
	For more than 12 months	2	❑		2	❑	
	For your lifetime	3	❑		3	❑	
	Don't know	9	❑		9	❑	
59	**Do you need physical help with:** (✓ if yes)						
	Getting on and off the toilet, wiping yourself	1	❑		1	❑	
	Washing yourself (*either sitting or standing*)	2	❑		2	❑	
	Upper body dressing	3	❑		3	❑	
	Lower body dressing	4	❑		4	❑	
	Using cutlery to bring food to the mouth	5	❑		5	❑	
	No help needed	0	❑		0	❑	

We would now like to ask you about some medicines that you may be taking now or may have taken in the past.
Please answer each question by putting a ✓ in one box only, unless otherwise instructed.

		YOURSELF			YOUR PARTNER		
60	**Have you *EVER* taken any medicine for asthma or wheezing?** (medicine includes inhalers, liquids, tablets, syrups, nebulisers)						
	No	0	❑	**→Q66**	0	❑	**→Q66**
	Yes	1	❑		1	❑	
61	**How old were you when you *FIRST* took medicine for asthma or wheezing?**						
	Years	_____			_____		
62	**What medicines do you take when you get an attack of asthma or wheezing?** (✓ as many as you need to)						
	None	0	❑		0	❑	
	Anti-histamines	1	❑		1	❑	
	Ventolin, bricanyl, berotec, alupent, respolin or asmol *by inhaler*	2	❑		2	❑	
	Ventolin, bricanyl, berotec, alupent, respolin or asmol *by nebuliser*	3	❑		3	❑	
	Serevent	4	❑		4	❑	
	Atrovent	5	❑		5	❑	
	Theophylline tablets or syrup *(such as theo-dur, nuelin, austyn or cardophylline)*	6	❑		6	❑	
	Intal, intal forte or tilade	7	❑		7	❑	
	Inhaled steroid sprays (*such as becotide, aldecin, becloforte, pulmicort or flixotide)*	8	❑		8	❑	
	Prednisone/prednisolone *(such as sone, deltasone, panafort, deltasolone, panafcortelone, solone or dexamethasone)*	9	❑		9	❑	
63	**Do you take medicines for asthma or wheezing on *MOST DAYS* or *EVERY DAY*?**						
	No	0	❑	**→Q65**	0	❑	**→Q65**
	Yes, most days	1	❑		1	❑	
	Yes, every day	2	❑		2	❑	

		YOURSELF			YOUR PARTNER		
64	**Which medicines do you take on *MOST DAYS* or *EVERY DAY*?** (✓ as many as you need to)						
	Anti-histamines	1	❑		1	❑	
	Ventolin, bricanyl, berotec, alupent, respolin or asmol *by inhaler*	2	❑		2	❑	
	Ventolin, bricanyl, berotec, alupent, respolin or asmol *by nebuliser*	3	❑		3	❑	
	Serevent	4	❑		4	❑	
	Atrovent	5	❑		5	❑	
	Theophylline tablets or syrup *(such as theo-dur, nuelin, austyn or cardophylline)*	6	❑		6	❑	
	Intal, intal forte or tilade	7	❑		7	❑	
	Inhaled steroid sprays (*such as becotide, aldecin, becloforte, pulmicort or flixotide)*	8	❑		8	❑	
	Prednisone/prednisolone *(such as sone, deltasone, panafort, deltasolone, panafcortelone, solone or dexamethasone)*	9	❑		9	❑	
65	**In the *LAST 12 MONTHS*, how many courses of oral steroids (taken by mouth) have you taken for attacks of asthma or wheezing?**						
	None	0	❑		0	❑	
	Number of courses *(please specify)*		_____			_____	
66	**Have you *EVER* taken any medication for fits or seizures?**						
	No	0	❑	**→Q68**	0	❑	**→Q68**
	Yes	1	❑		1	❑	
67	**What is the name of this medication?**						
		_____			_____		
		_____			_____		

We would like to know if there are any other twins in your family.
Please answer each question by putting a ✓ in one or more boxes as directed, or by writing in the space provided.

		YOURSELF			YOUR PARTNER		
68	**How many brothers and sisters do you have?** *(please include any brothers and sisters who are no longer alive)*						
	None	0	❑	**→Q72**	0	❑	**→Q72**
	Number of sisters *(write '0' if you have no sisters)*		_____			_____	
	Number of brothers *(write '0' if you have no brothers)*		_____			_____	
69	**Are there twins or other multiples among you and your brothers and sisters?**						
	No	0	❑	**→Q71**	0	❑	**→Q71**
	Yes	1	❑		1	❑	
70	**Below, ✓ any box below where the answer is YES**						
	I have a sister who is *MY IDENTICAL* twin	01	❑		01	❑	
	I have a brother who is *MY IDENTICAL* twin	02	❑		02	❑	
	I have a sister who is *MY NON-IDENTICAL* twin	03	❑		03	❑	
	I have a brother who is *MY NON-IDENTICAL* twin	04	❑		04	❑	
	I am a triplet, quad or quin	05	❑		05	❑	
	I have two sisters who are *IDENTICAL* twins	06	❑		06	❑	
	I have two sisters who are *NON-IDENTICAL* twins	07	❑		07	❑	
	I have two brothers who are *IDENTICAL* twins	08	❑		08	❑	
	I have two brothers who are *NON-IDENTICAL* twins	09	❑		09	❑	
	I have a brother and a sister who are *NON-IDENTICAL* twins	10	❑		10	❑	
	I have brothers and/or sisters who are triplets, quads or quins	11	❑		11	❑	
71	**Do any of your brothers or sisters have children who are twins or other multiples?**						
	No	0	❑		0	❑	
	Yes *(please give details below)*	1	❑		1	❑	

(e.g., my sister has identical twin girls, my brother has a boy and a girl who are twins

Your brothers and sisters:

_____

_____

_____

Your partner's brothers and sisters:

_____

_____

_____

		YOURSELF			YOUR PARTNER		
72	**How many brothers and sisters does *YOUR MOTHER* have?** ***(please include any brothers and sisters who are no longer alive)***						
	None	0	❑	**→Q76**	0	❑	**→Q76**
	Number of sisters *(write '0' if your mother has no sisters)*		_____			_____	
	Number of brothers *(write '0' if your mother has no brothers)*		_____			_____	
73	**Are/were there twins or other multiples among your mother and her brothers and sisters?**						
	No	0	❑	**→Q75**	0	❑	**→Q75**
	Yes	1	❑		1	❑	
74	**Below, ✓ any box below where the answer is YES**						
	My mother has a sister who is HER IDENTICAL twin	01	❑		01	❑	
	My mother has a sister who is HER NON-IDENTICAL twin	02	❑		02	❑	
	My mother has a brother who is HER twin	03	❑		03	❑	
	My mother is a triplet, quad or quin	04	❑		04	❑	
	My mother has two sisters who are IDENTICAL twins	05	❑		05	❑	
	My mother has two sisters who are NON-IDENTICAL twins	06	❑		06	❑	
	My mother has two brothers who are IDENTICAL twins	07	❑		07	❑	
	My mother has two brothers who are NON-IDENTICAL twins	08	❑		08	❑	
	My mother has a brother and a sister who are NON-IDENTICAL twins	09	❑		09	❑	
	My mother has brothers and/or sisters who are triplets, quads or quins	10	❑		10	❑	
75	**Do any of your *MOTHER'S* brothers or sisters have children who are twins or other multiples?**						
	No	0	❑		0	❑	
	Yes *(please give details below)*	1	❑		1	❑	

(e.g., my mother's brother has non-identical twin boys)

Your mother's brothers and sisters:

_____

_____

_____

Your partner's mother's brothers and sisters:

_____

_____

_____

		YOURSELF			YOUR PARTNER		
76	**How many brothers and sisters does *YOUR FATHER* have?** ***(please include any brothers and sisters who are no longer alive)***						
	None	0	❑	**→Q80**	0	❑	**→Q80**
	Number of sisters *(write '0' if your father has no sisters)*		_____			_____	
	Number of brothers *(write '0' if your father has no brothers)*		_____			_____	
77	**Are/were there twins or other multiples among your father and his brothers and sisters?**						
	No	0	❑	**→Q79**	0	❑	**→Q79**
	Yes	1	❑		1	❑	
78	**Below, ✓ any box below where the answer is YES**						
	My father has a brother who is HIS IDENTICAL twin	01	❑		01	❑	
	My father has a brother who is HIS NON-IDENTICAL twin	02	❑		02	❑	
	My father has a sister who is HIS twin	03	❑		03	❑	
	My father is a triplet, quad or quin	04	❑		04	❑	
	My father has two sisters who are IDENTICAL twins	05	❑		05	❑	
	My father has two sisters who are NON-IDENTICAL twins	06	❑		06	❑	
	My father has two brothers who are IDENTICAL twins	07	❑		07	❑	
	My father has two brothers who are NON-IDENTICAL twins	08	❑		08	❑	
	My father has a brother and a sister who are NON-IDENTICAL twins	09	❑		09	❑	
	My father has brothers and/or sisters who are triplets, quads or quins	10	❑		10	❑	
79	**Do any of your *FATHER'S* brothers or sisters have children who are twins or other multiples?**						
	No	0	❑		0	❑	
	Yes *(please give details below)*	1	❑		1	❑	

(e.g., my father's sister has identical twin boys)

Your father's brothers and sisters:

_____

_____

Your partner's father's brothers and sisters:

_____

_____

80 **If there are any other twins or other multiples in your family, please give details below** ***(e.g., my cousin on my father's side has identical twin boys)***

_____

We now ask you some questions about smoking.
Please answer each question by putting a ✓ in one or more boxes as directed, or by writing in the space.

		YOURSELF			YOUR PARTNER		
81	**Have you *EVER* smoked cigarettes, cigars or a pipe?**						
	No	0	❑	**→Q90**	0	❑	**→Q90**
	Yes	1	❑		1	❑	
82	**How old were you when you *FIRST* started smoking?**						
	Years						
83	**Do you still smoke?**						
	No	0	❑	**→Q86**	0	❑	**→Q86**
	Yes	1	❑		1	❑	
84	**How much do you smoke *NOW*?**						
	Number of cigarettes *PER DAY*		_____			_____	
	Number of cigars *PER DAY*		_____			_____	
	Grams of tobacco *PER WEEK* *(a 2 ounce pouch of tobacco equals 50 grams)*		_____			_____	
85	**In the *LAST 12 MONTHS*, have you changed the amount you smoke?**						
	No, I smoke about the same amount	0	❑	**→Q88**	0	❑	**→Q88**
	Yes, I smoke less than I used to	1	❑	**→Q88**	1	❑	**→Q88**
	Yes, I smoke more than I used to	1	❑	**→Q88**	1	❑	**→Q88**
86	**When did you give up smoking?**						
	Year	19	_____		19	_____	
	or, age		_____	years		_____	years
87	**How much *DID* you smoke?**						
	Number of cigarettes *PER DAY*		_____			_____	
	Number of cigars *PER DAY*		_____			_____	
	Grams of tobacco *PER WEEK* (*a 2 ounce pouch of tobacco equals 50 grams*)		_____			_____	

	YOURSELF	YOUR PARTNER

88 **When you are/were with your children, either in the house or in the car, which of the following do you do *NOW* or *WHEN YOU USED TO SMOKE*?**

Not smoke at all	0 ❑	0 ❑
Smoke less than normal	1 ❑	1 ❑
Smoke about the same	2 ❑	2 ❑
Smoke more than normal	3 ❑	3 ❑
Difficult to say	4 ❑	4 ❑

89 **How often do/did you smoke when your children are/were present?**

Never	0 ❑	0 ❑
Some of the time	1 ❑	1 ❑
Most of the time	2 ❑	2 ❑
Always	3 ❑	3 ❑

Next, some questions about other people living in your house.
Please ✓ one box, or write your answer in the space provided.

90 **Does any other person who lives in your house smoke regularly?**

No other people live in the house	0 ❑	→**Q93**
No, the other person/people in the house does/do not smoke	1 ❑	→**Q93**
Yes, the other person/people in the house smokes	2 ❑	

91 ***IN TOTAL*, how many cigarettes, cigars etc are smoked by other people living in your house?**

Number of cigarettes *PER DAY* _____

Number of cigars *PER DAY* _____

Grams of tobacco *PER WEEK*
(a 2 ounce pouch of tobacco equals 50 grams) _____

92 **How often does the other person/people living in your house smoke when your children are present?**

Never	0 ❑
Some of the time	1 ❑
Most of the time	2 ❑
Always	3 ❑

Do you have household rules about smoking, such as:

93 **No smoking inside the home?**

No	0	❑	
Yes	1	❑	→**Q96**

94 **Smoke-free rooms in the home?**

No	0	❑
Yes	1	❑

95 **No smoking allowed inside when children are present?**

No	0	❑
Yes	1	❑

96 **Having a smoke-free car?**

No	0	❑
Yes	1	❑

97 **How often do you ask visitors not to smoke in your home?**

Never	0	❑
Some of the time	1	❑
Most of the time	2	❑
Always	3	❑

98 **How often do you choose non-smoking areas in restaurants, cafes and other public places when your children are with you?**

Never	0	❑
Some of the time	1	❑
Most of the time	2	❑
Always	3	❑

99 **How often do other people smoke when they are with your children?**

Never	0	❑
Some of the time	1	❑
Most of the time	2	❑
Always	3	❑

100 **Do your children regularly spend time outside the home where people smoke cigarettes in the presence of your children?**

No	0	❑
Yes	1	❑

We would like to know the type and frequency of health-related services you used
FOR YOURSELF AND YOUR PARTNER BEFORE THE TWINS WERE BORN.

101 **How often did you and your partner use the following health services *BEFORE YOUR TWINS WERE BORN*?**
(for each person, ✓ one box on every line)

	YOURSELF				YOUR PARTNER			
	Never	**Once only**	**2-5 times**	**More than 5 times**	**Never**	**Once only**	**2-5 times**	**More than 5 times**
Specialist doctor *(for example, neurologist, gastroenterologist, cardiologist)* *(please specify type of specialist below)*								
_____	❑	❑	❑	❑	❑	❑	❑	❑
_____	❑	❑	❑	❑	❑	❑	❑	❑
_____	❑	❑	❑	❑	❑	❑	❑	❑
_____	❑	❑	❑	❑	❑	❑	❑	❑
_____	❑	❑	❑	❑	❑	❑	❑	❑
Routine maternity check-up	❑	❑	❑	❑	❑	❑	❑	❑
Hospital emergency department	❑	❑	❑	❑	❑	❑	❑	❑
Hospital out-patient department or clinic	❑	❑	❑	❑	❑	❑	❑	❑
Admitted to hospital for at least one night *(other than for the delivery of a baby)*	❑	❑	❑	❑	❑	❑	❑	❑
Dentist	❑	❑	❑	❑	❑	❑	❑	❑
Physiotherapist	❑	❑	❑	❑	❑	❑	❑	❑
Speech therapist	❑	❑	❑	❑	❑	❑	❑	❑
Occupational therapist	❑	❑	❑	❑	❑	❑	❑	❑
Psychologist	❑	❑	❑	❑	❑	❑	❑	❑
Psychiatrist	❑	❑	❑	❑	❑	❑	❑	❑
Some other organisation or person *(please specify)*	❑	❑	❑	❑	❑	❑	❑	❑
	_____				_____			

We would like to know the type and frequency of health-related services you used *FOR YOURSELF AND YOUR PARTNER SINCE THE TWINS WERE BORN.*

102 **How often did you and your partner use the following health services *SINCE YOUR TWINS WERE BORN*?**
(for each person, ✓one box on every line)

	YOURSELF				YOUR PARTNER			
	Never	**Once only**	**2-5 times**	**More than 5 times**	**Never**	**Once only**	**2-5 times**	**More than 5 times**
Specialist doctor *(for example, neurologist, gastroenterologist, cardiologist)* *(please specify type of specialist below)*								
_____	❑	❑	❑	❑	❑	❑	❑	❑
_____	❑	❑	❑	❑	❑	❑	❑	❑
_____	❑	❑	❑	❑	❑	❑	❑	❑
_____	❑	❑	❑	❑	❑	❑	❑	❑
_____	❑	❑	❑	❑	❑	❑	❑	❑
Routine maternity check-up	❑	❑	❑	❑	❑	❑	❑	❑
Hospital emergency department	❑	❑	❑	❑	❑	❑	❑	❑
Hospital out-patient department or clinic	❑	❑	❑	❑	❑	❑	❑	❑
Admitted to hospital for at least one night *(other than for the delivery of a baby)*	❑	❑	❑	❑	❑	❑	❑	❑
Dentist	❑	❑	❑	❑	❑	❑	❑	❑
Physiotherapist	❑	❑	❑	❑	❑	❑	❑	❑
Speech therapist	❑	❑	❑	❑	❑	❑	❑	❑
Occupational therapist	❑	❑	❑	❑	❑	❑	❑	❑
Psychologist	❑	❑	❑	❑	❑	❑	❑	❑
Psychiatrist	❑	❑	❑	❑	❑	❑	❑	❑
Some other organisation or person *(please specify)*	❑	❑	❑	❑	❑	❑	❑	❑
	_____				_____			

We would like some information about the reproductive history of the mother.
Please ✓ one box or write your answer in the space provided.

103 ***APART FROM THE TWINS*, have you had any other pregnancies?**

No 0 ❑ →**Q107**
Yes 1 ❑

104 ***IN TOTAL*, how many pregnancies have you had?**

_____

105 ***IN TOTAL*, how many *MULTIPLE* pregnancies have you had?**

_____

106 ***IN TOTAL*, how many children have you had?**

Now living _____
Born alive, but no longer living _____
Stillborn _____

107

It is possible that we may need to contact you again about this study. We would be grateful if you could give us the names, addresses and telephone numbers of two friends or relatives who do not live with you, whom we can contact if we are not able to reach you at any time.

Name: _____ Name: _____
Address: _____ Address: _____
_____ _____
Phone No: _____ Phone no: _____

We welcome your comments below and would like to know if there are any services for multiple birth families that you would like to see developed which are not available now. We are also especially interested in hearing about what you hope to get from us as a result of your participation in this study. Thank you very much for your help.

This is the end of the questionnaire about you and your partner.
Now, please fill in questionnaire 3 about any other children you have.

If you do not have any other children, please put the two questionnaires you have completed, together with the signed consent forms, in the reply-paid envelope and send it back to us.

Thank you very much for taking the time to give us this valuable information.

INSTRUCTIONS

- We would like these questions to be answered by the children's mother as they are about things that may have happened before, during and since the children were born. If this is not possible, then the closest relative should answer the questions.

- At first sight, the questionnaire may seem long but you will probably be able to skip many of the questions as they do not apply to you.

- Most answers require a ✓ in a box or a short answer written in the space provided. Some questions allow you to ✓more than one box. When this is the case, clear instructions are given with each question.

- For some questions you will see an → with a number after the box you have ticked. This means that the next few questions do not apply to you. Please follow the instructions that are given and go to the question number stated after the →. Where there is no → please go to the next question.

- When you have finished, please put the questionnaires and the signed consent forms in the reply-paid envelope and post it back to us.

Please remember to get each member of the family to sign a separate consent form.

- If you have any problems completing the questionnaire or have any other questions, please ring (08) 9382 2007 or 1800 819 684 between 9am and 5pm Monday to Friday.

ALL THE INFORMATION YOU GIVE US IN THIS QUESTIONNAIRE WILL REMAIN

CONFIDENTIAL

Firstly, some general questions about your children.
For each question, please ✓ one box or write your answer in the space provided.

		CHILD 1	CHILD 2
1	**What is his/her first name?**	_____	_____
2	**What is his/her sex?**		
	Female	0 ❑	0 ❑
	Male	1 ❑	1 ❑
3	**What is his/her date of birth?**	___/____/19___	___/____/19___
4	**In which country was he/she born?** (if born in Australia, please give the State)	_____	_____
5	**What was his/her birthweight?**	_____ grams or ____lbs _____oz	_____ grams or ____lbs _____oz
6	**What was the length of the pregnancy?**	_____ weeks or _____months	_____ weeks or _____months

What is the name of the person completing this questionnaire?

_____

What is your relationship to the children?

Child 1: _____

Child 2: _____

We have made every effort to determine whether any of the children in this study have died in Western Australia, but we do not know about children who have died elsewhere in Australia or overseas, and realise that we may not know about some of those who died in WA. We appreciate that it may be difficult for you to answer questions about your child who is no longer alive, but we would very much appreciate it if you could answer the three questions below.

It is not necessary to complete the rest of the questionnaire about a child who has died, but you may do so if you wish. Of course, we would certainly find any extra information extremely useful.

	CHILD 1	**CHILD 2**
When did he/she die?	___/____/19___	___/____/19___
Where did he/she die?	_____	_____
What was the cause of death?	_____	_____
	_____	_____

In the future, we may want to study families where one or both children have died. Would you like to be contacted if we decide to do this?

No	0	❑
Yes	1	❑
Unsure at the moment	2	❑

We appreciate you giving us this information.

Thank you very much.

Questions 7 to 24 are about the pregnancy and birth of these children.
If you have difficulty remembering, please give the best answers you can.
For each question, ✓ one or more boxes, or write your answer in the space.

If you do not know any information about the pregnancy and birth of either or both of these children, please go to Q 25 for that child or those children.

	CHILD 1	CHILD 2
Name	_____	_____

7 ***BEFORE THIS PREGNANCY*, for how long were you trying to conceive?**

Not specifically trying	0 ❑	0 ❑
Months or years *(please specify)*	_____ mths or ___yrs	_____ mths or ___yrs

8 ***BEFORE THIS PREGNANCY*, did you or your partner seek medical advice or have any investigations, treatments or operations for difficulty becoming pregnant or to assist you in becoming pregnant?**

No	0 ❑	0 ❑
Yes *(please give details below)*	1 ❑	1 ❑

child 1:_____
_____

child 2:_____
_____

9 **Did you take any of the following vitamin supplements in the *MONTH BEFORE* you became pregnant?**

Multivitamins	1 ❑	1 ❑
Multivitamins with folic acid	2 ❑	2 ❑
Folic acid	3 ❑	3 ❑
FeFol or FGF	4 ❑	4 ❑
Other vitamins *(please specify)*	5 ❑	5 ❑
	_____	_____
I took vitamins but not sure which ones	6 ❑	6 ❑
I can't remember if I took vitamins or not	9 ❑	9 ❑
I did not take vitamin supplements	0 ❑	0 ❑

10 **Did you take any of the following vitamin supplements in the *FIRST THREE MONTHS* of pregnancy?**

Multivitamins	1 ❑	1 ❑
Multivitamins with folic acid	2 ❑	2 ❑
Folic acid	3 ❑	3 ❑
FeFol or FGF	4 ❑	4 ❑
Other vitamins *(please specify)*	5 ❑	5 ❑
	_____	_____
I took vitamins but not sure which ones	6 ❑	6 ❑
I can't remember if I took vitamins or not	9 ❑	9 ❑
I did not take vitamin supplements	0 ❑	0 ❑

		CHILD 1		CHILD 2	
	Name	_____		_____	
11	**Did you smoke during this pregnancy?**				
	No, I have never smoked	0 ❑	**→Q14**	1 ❑	**→Q14**
	No, I gave up smoking before I was pregnant	1 ❑	**→Q13**	2 ❑	**→Q13**
	No, I gave up smoking when I was trying to become pregnant	2 ❑	**→Q13**	3 ❑	**→Q13**
	No, I gave up smoking as soon as I knew I was pregnant	3 ❑	**→Q13**	1 ❑	**→Q13**
	Yes, but I gave up during the pregnancy	4 ❑	**→Q12**	2 ❑	**→Q12**
	Yes, I smoked during this pregnancy	5 ❑	**→Q12**	3 ❑	**→Q12**
12	***COMPARED WITH BEFORE YOU BECAME PREGNANT*, how much did you smoke during this pregnancy?**				
	Less than before	0 ❑		0 ❑	
	About the same amount as before	1 ❑		1 ❑	
	More than before	2 ❑		2 ❑	
	Don't know/can't remember	9 ❑		9 ❑	
13	**Did you start smoking again after the children were born?**				
	No	0 ❑		0 ❑	
	Yes	1 ❑		1 ❑	
14	**Did you take any medicines for epilepsy during pregnancy?**				
	No	0 ❑		0 ❑	
	Yes	1 ❑		1 ❑	
15	**Did you have any fits or seizures during pregnancy?**				
	No	0 ❑		0 ❑	
	Yes	1 ❑		1 ❑	
16	**Did you have any complications during pregnancy?** (✓ if yes)				
	Bleeding serious enough to require bed rest	01 ❑		01 ❑	
	Threatened miscarriage *(under 20 weeks)*	02 ❑		02 ❑	
	Urinary tract infection	03 ❑		03 ❑	
	Pre-eclampsia *(toxaemia)*	04 ❑		04 ❑	
	High blood pressure	05 ❑		05 ❑	
	Diabetes	06 ❑		06 ❑	
	Placenta praevia	07 ❑		07 ❑	
	Premature rupture of membranes	08 ❑		08 ❑	
	Other *(please specify)*	09 ❑		09 ❑	
		_____		_____	
	Don't know/can't remember	99 ❑		99 ❑	
	No complications	00 ❑		00 ❑	
17	***ON THE ADVICE OF YOUR DOCTOR*, how long did you rest in bed during the pregnancy?**				
	In hospital	____ days or ___wks		____ days or ___wks	
	At home	____ days or ___wks		____ days or ___wks	
	Don't know/can't remember	9 ❑		9 ❑	
	I did not spend any time resting in bed during the pregnancy	0 ❑		0 ❑	

		CHILD 1	CHILD 2
	Name	_____	_____
18	**In the first few months after the children were born were you more depressed than usual?**		
	No	0 ❑	0 ❑
	Yes	1 ❑	1 ❑
	Don't know/can't remember	9 ❑	9 ❑
19	**What was the type of delivery?**		
	Normal	1 ❑	1 ❑
	Breech	2 ❑	2 ❑
	Forceps	3 ❑	3 ❑
	Vacuum extraction	4 ❑	4 ❑
	Booked caesarean section	5 ❑	5 ❑
	Emergency caesarean section	6 ❑	6 ❑
20	**Did he/she have any of the following conditions *IMMEDIATELY* after birth?** *(that is, while he/she was still in hospital)* (✓ if yes)		
	Respiratory distress	1 ❑	1 ❑
	Convulsions	2 ❑	2 ❑
	Other serious condition *(please specify)*	3 ❑	3 ❑
		_____	_____
	Don't know/can't remember	9 ❑	9 ❑
	None of these conditions	0 ❑	0 ❑
21	**Did he/she have any of the following treatments *IMMEDIATELY* after birth?** *(that is, while he/she was still in hospital)* (✓ if yes)		
	Respirator/ventilator	1 ❑	1 ❑
	Fed through a naso-gastric tube	2 ❑	2 ❑
	Other treatment *(please specify)*	3 ❑	3 ❑
		_____	_____
	Don't know/can't remember	9 ❑	9 ❑
	None of these treatments	0 ❑	0 ❑
22	**How long did he/she spend in neonatal intensive care unit, or special care nursery?**		
	Was not in neonatal intensive care OR special care nursery	0 ❑	0 ❑
	Time in intensive care unit:	___ days or ____wks	___ days or ____wks
	Time in special care nursery	___ days or ____wks	___ days or ____wks
	Total time in neonatal intensive care **and** special care nursery *(but I can't remember how long in each section)*	___ days or ____wks	___ days or ____wks
23	**How long was he/she breastfed?** (✓ one box)		
	Was not breastfed	0 ❑	0 ❑
	Was breastfed for less than 3 months after birth	1 ❑	1 ❑
	Was breastfed for at least 3 months but less than 6 months after birth	2 ❑	2 ❑
	Was breastfed for at least 6 months but less than 9 months after birth	3 ❑	3 ❑
	Was breastfed for at least 9 months but less than 12 months after birth	4 ❑	4 ❑
	Was breastfed for 12 months or longer after birth	5 ❑	5 ❑
	Don't know/can't remember	9 ❑	9 ❑

	CHILD 1	CHILD 2
Name	_____	_____

24 **At what age was he/she first given any milk *OTHER THAN BREAST MILK*?**

He/she was given "comp" feeds in hospital	0 ❑	0 ❑
Age at which he/she was first given milk other than breast milk *AT HOME*	_____ mths	_____ mths

Next, some questions about your children NOW.
Please answer each question by putting a ✓ in one box, or by writing in the space provided.

	CHILD 1	CHILD 2
Name	_____	_____

25 **How much does he/she weigh *NOW*?**

_____ kgs _____ kgs

26 **How tall is he/she *NOW*?**

_____ cms _____ cms

27 **Does he/she have difficulty keeping attention on work or games?**

Not at all	0 ❑	0 ❑
Just a little/sometimes	1 ❑	1 ❑
Pretty much/often	2 ❑	2 ❑
Very much/very often	3 ❑	3 ❑

28 **Does he/she lose things necessary for tasks at home, school or work?**
(for example, toys, pencils, tools)

Not at all	0 ❑	0 ❑
Just a little/sometimes	1 ❑	1 ❑
Pretty much/often	2 ❑	2 ❑
Very much/very often	3 ❑	3 ❑

29 **Does he/she have difficulty organising tasks and activities?**

Not at all	0 ❑	0 ❑
Just a little/sometimes	1 ❑	1 ❑
Pretty much/often	2 ❑	2 ❑
Very much/very often	3 ❑	3 ❑

30 **Does he/she fidget with hands or feet, or squirm in his/her seat?**

Not at all	0 ❑	0 ❑
Just a little/sometimes	1 ❑	1 ❑
Pretty much/often	2 ❑	2 ❑
Very much/very often	3 ❑	3 ❑

31 **Does he/she have difficulty waiting his/her turn?**

Not at all	0 ❑	0 ❑
Just a little/sometimes	1 ❑	1 ❑
Pretty much/often	2 ❑	2 ❑
Very much/very often	3 ❑	3 ❑

	CHILD 1	CHILD 2
Name	_____	_____

32 **Does he/she interrupt or intrude on others?** *(for example, butts into conversations or games)*

Not at all	0 ❑	0 ❑
Just a little/sometimes	1 ❑	1 ❑
Pretty much/often	2 ❑	2 ❑
Very much/very often	3 ❑	3 ❑

These questions are about your children's health.
Please answer each question by putting a ✓ in one or more boxes as directed, or by writing in the space provided.

	CHILD 1	CHILD 2
Name	_____	_____

33 **Has he/she *EVER* had any of the following health conditions *CONFIRMED BY A DOCTOR*?** (✓ if yes)

Heart problems	01 ❑	01 ❑
Febrile convulsions	02 ❑	02 ❑
Epilepsy	03 ❑	03 ❑
Kidney disease	04 ❑	04 ❑
Arthritis or rheumatism	05 ❑	05 ❑
Diabetes	06 ❑	06 ❑
Asthma or allergies	07 ❑	07 ❑
Cancer including leukaemia	08 ❑	08 ❑
Any blood disorder	09 ❑	09 ❑
Migraine or severe headaches	10 ❑	10 ❑
Cystic fibrosis	11 ❑	11 ❑
Cerebral palsy	12 ❑	12 ❑
Down syndrome	13 ❑	13 ❑
Fragile X	14 ❑	14 ❑
Spina bifida	15 ❑	15 ❑
Intellectual disability	16 ❑	16 ❑
Developmental delay or lag	17 ❑	17 ❑
Any condition, including birth defects, present since birth *(eg. club foot, cleft palate and lip missing fingers and toes), (please specify)*	18 ❑	18 ❑
	_____	_____
Any other condition *(please specify)*	19 ❑	19 ❑
	_____	_____
None of the above	00 ❑	00 ❑

34 **Has he/she *EVER* had wheezing?**
(by "wheezing", we mean a whistling sound coming from within the chest)

No	0 ❑	**→Q42**	0 ❑	**→Q42**
Yes	1 ❑		1 ❑	

35 **In the *LAST 12 MONTHS* has he/she wheezed?**

No	0 ❑	0 ❑
Yes	1 ❑	1 ❑

		CHILD 1		CHILD 2	
	Name	_____		_____	
36	**Has he/she had three or more episodes of wheeze since his/her *FIRST* birthday?**				
	No	0 ❑		0 ❑	
	Yes	1 ❑		1 ❑	
37	**How old was your child when he/she *FIRST* wheezed?**				
		_____yrs_____mths		_____yrs_____mths	
38	**In the *LAST 12 MONTHS*, how often has he/she wheezed?** (✓ one box)				
	Not at all	0 ❑		0 ❑	
	Less often than twice a month	1 ❑		1 ❑	
	Twice a month or more	2 ❑		2 ❑	
	Twice a week or more	3 ❑		3 ❑	
	Most days	4 ❑		4 ❑	
39	**How old was your child when he/she *LAST* wheezed?**				
		_____yrs_____mths		_____yrs_____mths	
40	**Has he/she *EVER* had an attack of wheezing that has made him/her feel short of breath?**				
	No	0 ❑		0 ❑	
	Yes	1 ❑		1 ❑	
41	**In the *LAST 12 MONTHS*, how often has he/she woken at night with wheezing or shortness of breath?** (✓ one box)				
	Not at all	0 ❑		0 ❑	
	Less often than twice a month	1 ❑		1 ❑	
	Twice a month or more	2 ❑		2 ❑	
	Twice a week or more	3 ❑		3 ❑	
	Most nights	4 ❑		4 ❑	
42	**Has he/she *EVER* had asthma?** (✓ one box)				
	No	0 ❑	**→Q47**	0 ❑	**→Q47**
	Yes, I was told by a doctor	1 ❑		1 ❑	
	Yes, I was told by someone *other than a doctor*	2 ❑		2 ❑	
43	**How old was your child when he/she *FIRST* had asthma?**				
		_____yrs_____mths		_____yrs_____mths	
44	**Does he/she still have asthma?**				
	No	0 ❑		0 ❑	
	Yes	1 ❑	**→Q46**	1 ❑	**→Q46**
45	**How old was your child when he/she *LAST* had an episode of asthma?**				
		_____yrs_____mths		_____yrs_____mths	
46	**In the *LAST 12 MONTHS*, how often has he/she had an episode of asthma?**				
	Not at all	0 ❑		0 ❑	
	Less often than twice a month	1 ❑		1 ❑	
	Twice a month or more	2 ❑		2 ❑	
	Twice a week or more	3 ❑		3 ❑	
	Most days	4 ❑		4 ❑	

		CHILD 1		CHILD 2	
	Name	_____		_____	
47	**Has his/her asthma or wheezing *EVER* limited any of the following activities?** (✓ if yes)				
	Sports	1 ❑		1 ❑	
	Running	2 ❑		2 ❑	
	Walking up hills or stairs	3 ❑		3 ❑	
	Usual daily activity	4 ❑		4 ❑	
	None of the above	0 ❑		0 ❑	
	Never had asthma or wheezing	8 ❑	**→Q53**	8 ❑	**→Q53**
48	**Are there any times of the year when his/her asthma or wheezing is/was particularly bad?** (✓ if yes)				
	No, same all year round	0 ❑		0 ❑	
	Spring	1 ❑		1 ❑	
	Summer	2 ❑		2 ❑	
	Autumn	3 ❑		3 ❑	
	Winter	4 ❑		4 ❑	
	Different each year	5 ❑		5 ❑	
	Not sure	9 ❑		9 ❑	
49	**In the *LAST 12 MONTHS*, about how many days of school or work has he/she missed because of asthma, wheezing or breathing problems?**				
	None	0 ❑		0 ❑	
	1 or 2 days	1 ❑		1 ❑	
	3 to 5 days	2 ❑		2 ❑	
	6 to 10 days	3 ❑		3 ❑	
	11 days or more	4 ❑		4 ❑	
	Doesn't go to school or work	8 ❑		8 ❑	
	Don't know/can't remember	9 ❑		9 ❑	
50	***IN TOTAL*, how many times has he/she been admitted to hospital because of asthma or wheezing?**				
	None	0 ❑		0 ❑	
	Number of times *(please specify)*	_____		_____	
51	**In the *LAST 12 MONTHS*, how many times has he/she been admitted to hospital because of asthma or wheezing?**				
	None	0 ❑		0 ❑	
	Number of times *(please specify)*	_____		_____	

		CHILD 1	CHILD 2
	Name	_____	_____
52	**Have any of the following *EVER* made him/her wheezy or brought on an asthma attack?** (✓ if yes)		
	Cold weather	01 ❑	01 ❑
	Change in temperature	02 ❑	02 ❑
	Cats	03 ❑	03 ❑
	Dogs	04 ❑	04 ❑
	Other animals *(please specify)*	05 ❑	05 ❑
		_____	_____
	Dusty parts of the house	06 ❑	06 ❑
	Exercise	07 ❑	07 ❑
	Colds or chest infections	08 ❑	08 ❑
	Feathers	09 ❑	09 ❑
	Closeness to flowering plants	10 ❑	10 ❑
	Cigarette smoke	11 ❑	11 ❑
	Foods or drinks *(please specify)*	12 ❑	12 ❑
		_____	_____
	Other (*please specify*):	13 ❑	13 ❑
		_____	_____
	None of the above	00 ❑	00 ❑
53	**Does he/she *USUALLY* have a cough?** (usually means as much as 4 to 6 times a day on 4 or more days of the week)		
	No	0 ❑	0 ❑
	Yes	1 ❑	1 ❑
54	**Has he/she *EVER* had a troublesome, dry cough at night which went on for *MORE THAN 3 WEEKS* and was not associated with a cold or 'flu?**		
	No	0 ❑	0 ❑
	Yes	1 ❑	1 ❑
55	**In the *LAST 12 MONTHS*, how often has he/she had a troublesome, dry cough *AT NIGHT*?** (✓ one box)		
	Not at all	0 ❑	0 ❑
	Less often than twice a month	1 ❑	1 ❑
	Twice a month or more	2 ❑	2 ❑
	Twice a week or more	3 ❑	3 ❑
	Most nights	4 ❑	4 ❑
56	***APART FROM ASTHMA*, has he/she *EVER* had any serious chest illness or illnesses?**		
	No	0 ❑ **→Q58**	0 ❑ **→Q58**
	Yes	1 ❑	1 ❑
57	**What was the serious chest illness/es he/she had?**		
		_____	_____
		_____	_____
58	**Has he/she *EVER* had hayfever or nasal allergies?** (that is, sneezing, runny or blocked nose, with or without itchy eyes, ***not*** associated with a cold)		
	No	0 ❑ **→Q63**	0 ❑ **→Q63**
	Yes	1 ❑	1 ❑

		CHILD 1		CHILD 2	
	Name	_____		_____	
59	**Was his/her hay fever or nasal allergy confirmed by a doctor?**				
	No	0 ❑		0 ❑	
	Yes	1 ❑		1 ❑	
60	**How old was the child when he/she *FIRST* had hay fever or nasal allergies?**				
		_____yrs_____mths		_____yrs_____mths	
61	**In the *LAST 12 MONTHS*, has he/she had hay fever or nasal allergies?**				
	No	0 ❑		0 ❑	
	Yes	1 ❑	**→Q63**	1 ❑	**→Q63**
62	**How old was the child when he/she *LAST* had hay fever or nasal allergies?**				
		_____yrs_____mths		_____yrs_____mths	
63	**Has your child *EVER* had a skin problem which occurred in his/her skin creases?** (that is, in front of the elbows, behind the knees, on the front of the ankles, around the neck, or around the ears or eyes)				
	No	0 ❑		0 ❑	
	Yes	1 ❑		1 ❑	
64	**Has he/she *EVER* had eczema?** (eczema is an itchy, dry rash on the face, arms or legs)				
	No	0 ❑	**→Q69**	0 ❑	**→Q69**
	Yes	1 ❑		1 ❑	
65	**Was his/her eczema confirmed by a doctor?**				
	No	0 ❑		0 ❑	
	Yes	1 ❑		1 ❑	
66	**How old was the child when he/she *FIRST* had eczema?**				
		_____yrs_____mths		_____yrs_____mths	
67	**In the *LAST 12 MONTHS*, has he/she had eczema?**				
	No	0 ❑		0 ❑	
	Yes	1 ❑	**→Q69**	1 ❑	**→Q69**
68	**How old was the child when he/she *LAST* had eczema?**				
		_____yrs_____mths		_____yrs_____mths	
69	***IN TOTAL*, how many middle ear infections (otitis media) has he/she had?**				
	None	0 ❑		0 ❑	
	1 or 2	1 ❑		1 ❑	
	3 to 5	2 ❑		2 ❑	
	6 to 10	3 ❑		3 ❑	
	11 or more	4 ❑		4 ❑	
	So many that I've lost count	5 ❑		5 ❑	
	Don't know/can't remember	9 ❑		9 ❑	
70	**Has he/she had any of the following operations?** (✓ if yes)				
	Tonsils removed	1 ❑		1 ❑	
	Adenoids removed	2 ❑		2 ❑	
	Grommets inserted	3 ❑		3 ❑	
	None of the above	0 ❑		0 ❑	

We would now like to know about accidents.

Please answer each question by carefully following the instructions given.

		CHILD 1	CHILD 2
	Name	_____	_____
71	**In the *LAST 12 MONTHS* how many *accidents* has he/she had that resulted in a reduction in the amount or level of daily activity, or required medical advice or treatment?**		
	None	0 ❑ **→Q76**	0 ❑ **→Q76**
	Number of accidents *(please specify)*	_____	_____
72	**Of the accidents you reported above, how many required:** (please specify number for each of the following)		
	Staying in bed	_____	_____
	Some time off school or work	_____	_____
	Reduced activity only	_____	_____
	No change in amount or level of daily activity	_____	_____
73	**Of the accidents you reported above, how many required:** (please specify number for each of the following)		
	Admission to hospital	_____	_____
	Treatment at a hospital accident and emergency department	_____	_____
	Treatment by a health professional	_____	_____
	Advice from a health professional	_____	_____
	Minor treatment at home	_____	_____
	No treatment or advice required	_____	_____

	CHILD 1	CHILD 2
Name	_____	_____

74 **Of the accidents you reported above, how many resulted in the following injuries?**
(please specify number for each type of injury)

Head injury with loss of consciousness	_____	_____
Broken bones or fractures *(not a head injury)*	_____	_____
Dislocations	_____	_____
Sprains or strains	_____	_____
Broken teeth or teeth knocked out	_____	_____
Internal injuries	_____	_____
External wound needing stitches	_____	_____
External bruising	_____	_____
Burns and scalds	_____	_____
Poisoning	_____	_____
Other	_____	_____
(please specify)	_____	_____

75 **Of the accidents you reported above, how many happened in the following places?**
(please specify number for each place)

Inside your own home	_____	_____
Inside someone else's home	_____	_____
In your own garden	_____	_____
In someone else's garden	_____	_____
At work, school or playgroup	_____	_____
In a park or playground	_____	_____
Playing sport	_____	_____
In a road traffic crash *as a driver of a vehicle*	_____	_____
In a road traffic crash *as a passenger in a vehicle*	_____	_____
In a road traffic crash *as a pedestrian*	_____	_____
Bike riding	_____	_____
Other	_____	_____
(please specify)	_____	_____

		CHILD 1		CHILD 2	
	Name	_____		_____	
76	***Other than in the LAST 12 MONTHS,*** **how many head injuries with loss of consciousness has he/she had?**				
	None	0 ❑		0 ❑	
	Number of head injuries (*Please specify*):	_____		_____	
77	***IN THE LAST 12 MONTHS,*** **on about how many days has he/she stayed away from school or work because of illness, injury or medical condition?**				
	None	0 ❑		0 ❑	
	1 or 2 days	1 ❑		1 ❑	
	3 to 5 days	2 ❑		2 ❑	
	6 to 10 days	3 ❑		3 ❑	
	11 days or more	4 ❑		4 ❑	
	Doesn't go to school or work	8 ❑		8 ❑	
	Don't know/can't remember	9 ❑		9 ❑	

This section is about your children's eyesight and hearing, and any help they may need at home.
Please answer each question by putting a ✓ in one box only, unless otherwise instructed.

		CHILD 1		CHILD 2	
	Name	_____		_____	
78	**Does he/she have:** (✓ one box)				
	Normal vision in both eyes	1 ❑		1 ❑	
	Vision corrected by prescription glasses or contact lenses	2 ❑		2 ❑	
	Impaired vision	3 ❑		3 ❑	
	Blind	4 ❑		4 ❑	
79	**Is he/she:** (✓ one box)				
	Able to hear with both ears *(that is, normal hearing)*	1 ❑		1 ❑	
	Deaf in one ear	2 ❑		2 ❑	
	Deaf in both ears	3 ❑		3 ❑	
80	**Does he/she need any help to walk?** (✓ one box)				
	Supervision only	1 ❑		1 ❑	
	Assistance from one person	2 ❑		2 ❑	
	Assistance from 2 people	3 ❑		3 ❑	
	Unable to walk	4 ❑		4 ❑	
	No help required	0 ❑	**→Q82**	0 ❑	**→Q82**
81	**For how long is he/she likely to need this help?** (✓ one box)				
	For less than 12 months	1 ❑		1 ❑	
	For more than 12 months	2 ❑		2 ❑	
	For their lifetime	3 ❑		3 ❑	
	Don't know	9 ❑		9 ❑	
82	***OTHER THAN FOR REASONS OF AGE*****, does he/she need physical help with any of the following?** (✓ if yes)				
	Getting on and off the toilet, wiping themselves	1 ❑		1 ❑	
	Washing themselves *(either sitting or standing)*	2 ❑		2 ❑	
	Upper body dressing	3 ❑		3 ❑	
	Lower body dressing	4 ❑		4 ❑	
	Using cutlery to bring food to the mouth	5 ❑		5 ❑	
	No help needed	0 ❑		0 ❑	

These questions are about some medicines that your children may be taking now or may have taken in the past.
Please answer each question by putting a ✓ in one box only, unless otherwise instructed.

		CHILD 1		CHILD 2	
	Name	_____		_____	
83	**Has he/she *EVER* taken any medicine for asthma or wheezing?** (medicine includes inhalers, liquids, tablets, syrups, nebulisers)				
	No	0 ❑	**→Q89**	0 ❑	**→Q89**
	Yes	1 ❑		1 ❑	
84	**How old was your child when he/she *FIRST* took medicine for asthma or wheezing?**				
		_____yrs_____mths		_____yrs_____mths	
85	***IN THE LAST 12 MONTHS*, what medicines has he/she taken when he/she gets an attack of asthma or wheezing?** (✓ as many as you need to)				
	None	0 ❑		0 ❑	
	Anti-histamines	1 ❑		1 ❑	
	Ventolin, bricanyl, berotec, alupent, respolin or asmol by inhaler	2 ❑		2 ❑	
	Ventolin, bricanyl, berotec, alupent, respolin or asmol by nebuliser	3 ❑		3 ❑	
	Serevent	4 ❑		4 ❑	
	Atrovent	5 ❑		5 ❑	
	Theophylline tablets or syrup *(such as theo-dur, nuelin, austyn or cardophylline)*	6 ❑		6 ❑	
	Intal, intal forte or tilade	7 ❑		7 ❑	
	Inhaled steroid sprays *(such as becotide, aldecin, becloforte, pulmicort or flixotide)*	8 ❑		8 ❑	
	Prednisone/prednisolone *(such as sone, deltasone, panafort, deltasolone, panafcortelone, solone or dexamethasone)*	9 ❑		9 ❑	
86	**Does he/she take medicines for asthma or wheezing on *MOST DAYS* or *EVERY DAY*?**				
	No	0 ❑	**→Q87**	0 ❑	**→Q87**
	Yes, most days	1 ❑		1 ❑	
	Yes, every day	2 ❑		2 ❑	
87	**Which medicines does he/she take on *MOST DAYS* or *EVERY DAY*?** (✓ as many as you need to)				
	Anti-histamines	1 ❑		1 ❑	
	Ventolin, bricanyl, berotec, alupent, respolin or asmol by inhaler	2 ❑		2 ❑	
	Ventolin, bricanyl, berotec, alupent, respolin or asmol by nebuliser	3 ❑		3 ❑	
	Serevent	4 ❑		4 ❑	
	Atrovent	5 ❑		5 ❑	
	Theophylline tablets or syrup *(such as theo-dur, nuelin, austyn or cardophylline)*	6 ❑		6 ❑	
	Intal, intal forte or tilade	7 ❑		7 ❑	
	Inhaled steroid sprays *(such as becotide, aldecin, becloforte, pulmicort or flixotide)*	8 ❑		8 ❑	
	Prednisone/prednisolone *(such as sone, deltasone, panafort, deltasolone, panafcortelone, solone or dexamethasone)*	9 ❑		9 ❑	

		CHILD 1			CHILD 2	
	Name	_____			_____	
88	**In the *LAST 12 MONTHS*, how many courses of oral steroids (taken by mouth) has he/she taken for attacks of asthma or wheezing?**					
	None	0 ❑			0 ❑	
	Number of courses *(please specify)*	_____			_____	
89	**Has he/she *EVER* taken any medication for fits or seizures?**					
	No	0 ❑	**→Q91**		0 ❑	**→Q91**
	Yes	1 ❑			1 ❑	
90	**What is the name of this medication?**					
		_____			_____	
91	**Has he/she *EVER* taken any medication for hyperactivity or attention/learning problems?**					
	No	0 ❑	**→Q96**		0 ❑	**→Q96**
	Yes	1 ❑			1 ❑	
92	**What was/is the name of the medication?** (you may ✓ more than one box)					
	Ritalin *(methylphenidate)*	1 ❑			1 ❑	
	Dexamphetamine	2 ❑			2 ❑	
	Clonidine	3 ❑			3 ❑	
	Other *(please specify)*	4 ❑			4 ❑	
		_____			_____	
	Don't know/can't remember	9 ❑			9 ❑	
93	**How old was your child when he/she *FIRST* took this medication?**					
		_____yrs_____mths			_____yrs_____mths	
94	**Is he/she *CURRENTLY* on any medication for hyperactivity or attention problems?**					
	No	0 ❑			0 ❑	
	Yes	1 ❑	**→Q96**		1 ❑	**→Q96**
95	**How long was he/she on this medication?**					
	Years	_____			_____	

We would like to know about your children's education.
Please answer each question by putting a ✓ in one box only, unless otherwise instructed.

		CHILD 1		CHILD 2	
	Name	_____		_____	
96	**What grade (year) is he/she in at school?** (✓one box)				
	Pre-school/pre-primary	00 ❑		00 ❑	
	Grade (year) (*please specify*)	_____		_____	
	Ungraded class	13 ❑		13 ❑	
	Has left school	14 ❑		14 ❑	
	Too young to go to school	88 ❑	→**Q101**	88 ❑	→**Q101**
	Has never been to school	99 ❑	→**Q101**	99 ❑	→**Q101**
97	**Has he/she *EVER* repeated or failed a school year/grade?**				
	No	0 ❑		0 ❑	
	Yes	1 ❑		1 ❑	
	First year in school	2 ❑		2 ❑	
98	**Has he/she *EVER* had been in any of the following classes?**(✓ if yes)				
	General remedial class	1 ❑		1 ❑	
	Remedial English	2 ❑		2 ❑	
	Remedial Reading	3 ❑		3 ❑	
	Remedial Mathematics	4 ❑		4 ❑	
	Special class for gifted and talented children *(eg, PEAC, ATP)*	7 ❑		7 ❑	
	None of the above	0 ❑		0 ❑	
99	**In your opinion, does he/she require remedial instruction in any of the following?** (✓ if yes)				
	Speech and oral language	1 ❑		1 ❑	
	Written language	2 ❑		2 ❑	
	Reading	3 ❑		3 ❑	
	Number work	4 ❑		4 ❑	
	Motor skills	5 ❑		5 ❑	
	No extra work needed	0 ❑		0 ❑	
100	**How satisfied are you with his/her progress with educational and learning skills?**				
	Very satisfied	1 ❑		1 ❑	
	Satisfied	2 ❑		2 ❑	
	Neither	3 ❑		3 ❑	
	Dissatisfied	4 ❑		4 ❑	
	Very dissatisfied	5 ❑		5 ❑	

These questions ask you to say how satisfied you are with certain areas of your child's progress.

		CHILD 1	CHILD 2
	Name	_____	_____
101	**How satisfied are you with his/her progress in getting on with the multiples?** (✓ one box)		
	Very satisfied	1 ❑	1 ❑
	Satisfied	2 ❑	2 ❑
	Neither	3 ❑	3 ❑
	Dissatisfied	4 ❑	4 ❑
	Very dissatisfied	5 ❑	5 ❑
	Not applicable	8 ❑	8 ❑
102	**How satisfied are you with his/her progress in getting on with his/her *OTHER* brothers and/or sisters?**		
	Very satisfied	1 ❑	1 ❑
	Satisfied	2 ❑	2 ❑
	Neither	3 ❑	3 ❑
	Dissatisfied	4 ❑	4 ❑
	Very dissatisfied	5 ❑	5 ❑
	Not applicable	8 ❑	8 ❑
103	**How satisfied are you with his/her progress in getting on with other children?**		
	Very satisfied	1 ❑	1 ❑
	Satisfied	2 ❑	2 ❑
	Neither	3 ❑	3 ❑
	Dissatisfied	4 ❑	4 ❑
	Very dissatisfied	5 ❑	5 ❑
104	**How satisfied are you with his/her general behaviour?**		
	Very satisfied	1 ❑	1 ❑
	Satisfied	2 ❑	2 ❑
	Neither	3 ❑	3 ❑
	Dissatisfied	4 ❑	4 ❑
	Very dissatisfied	5 ❑	5 ❑
105	**How satisfied are you with his/her progress with physical development and coordination?**		
	Very satisfied	1 ❑	1 ❑
	Satisfied	2 ❑	2 ❑
	Neither	3 ❑	3 ❑
	Dissatisfied	4 ❑	4 ❑
	Very dissatisfied	5 ❑	5 ❑

These questions are about smoking and may not apply to your children because of their age. *If this is the case, please and go to question 115*
If *ANY* of your children smoke, please ✓ one box, or write your answer in the space.

		CHILD 1		CHILD 2	
	Name				
106	**Has he/she *EVER* smoked cigarettes, cigars or a pipe?**				
	No	0 ❑	→Q115	0 ❑	→Q115
	Yes	1 ❑		1 ❑	
	Don't know	9 ❑		9 ❑	
107	**How old was the child when he/she *FIRST* started smoking?**				
	Age started smoking (*years*)	_____		_____	
108	**Does he/she still smoke?**				
	No	0 ❑	→Q111	0 ❑	→Q111
	Yes	1 ❑		1 ❑	
	Don't know	9 ❑		9 ❑	
109	**How much does he/she smoke *NOW*?**				
	Number of cigarettes *PER DAY*	_____		_____	
	Number of cigars *PER DAY*	_____		_____	
	Grams of tobacco *PER WEEK* *(a 2 ounce pouch of tobacco equals 50 grams)*	_____		_____	
	Don't know	99 ❑		99 ❑	
110	**In the *LAST 12 MONTHS*, has he/she changed the amount smoked?**				
	No, he/she smokes about the same amount	0 ❑	→Q113	0 ❑	→Q113
	Yes, he/she smoke less than before	1 ❑	→Q113	1 ❑	→Q113
	Yes, he/she smokes more than before	2 ❑	→Q113	2 ❑	→Q113
	Don't know	9 ❑	→Q112	9 ❑	→Q112
111	**When did he/she give up smoking?**				
	Year	_____		_____	
	or, age	_____		_____	
	Don't know	99 ❑		99 ❑	
112	**How much *DID* he/she smoke?**				
	Number of cigarettes *PER DAY*	_____		_____	
	Number of cigars *PER DAY*	_____		_____	
	Grams of tobacco *PER WEEK* *(a 2 ounce pouch of tobacco equals 50 grams)*	_____		_____	
	Don't know	99 ❑		99 ❑	

	Name	CHILD 1	CHILD 2
113	**When he/she is with your *OTHER* children, either in the house or in the car, which of the following does he/she do *NOW* or *WHEN THEY USE TO SMOKE*?**		
	Not smoke at all	0 ❑	0 ❑
	Smoke less than normal	1 ❑	1 ❑
	Smoke about the same	2 ❑	2 ❑
	Smoke more than normal	3 ❑	3 ❑
	Difficult to say	4 ❑	4 ❑
114	**How often does or did he/she smoke when your children are/were present?**		
	Never	0 ❑	0 ❑
	Some of the time	1 ❑	1 ❑
	Most of the time	2 ❑	2 ❑
	Always	3 ❑	3 ❑

Next, we would like to know the type and frequency of health-related services your children have used.

	Name	CHILD 1 _____				CHILD 2 _____			
115	**How often has he/she used the following health services?** On every line, please ✓one box for each child	**Never**	**Once only**	**2-5 times**	**More than 5 times**	**Never**	**Once only**	**2-5 times**	**More than 5 times**
	Paediatrician	❑	❑	❑	❑	❑	❑	❑	❑
	Hospital emergency department	❑	❑	❑	❑	❑	❑	❑	❑
	Hospital out-patient department or clinic	❑	❑	❑	❑	❑	❑	❑	❑
	Admitted to hospital for at least one night	❑	❑	❑	❑	❑	❑	❑	❑
	Dentist	❑	❑	❑	❑	❑	❑	❑	❑
	Orthodontist	❑	❑	❑	❑	❑	❑	❑	❑
	Physiotherapist	❑	❑	❑	❑	❑	❑	❑	❑
	Speech therapist	❑	❑	❑	❑	❑	❑	❑	❑
	Occupational therapist	❑	❑	❑	❑	❑	❑	❑	❑
	School guidance officer or school psychologist	❑	❑	❑	❑	❑	❑	❑	❑
	Child & adolescent mental health clinic	❑	❑	❑	❑	❑	❑	❑	❑
	Other psychologist	❑	❑	❑	❑	❑	❑	❑	❑
	Other psychiatrist	❑	❑	❑	❑	❑	❑	❑	❑
	Other specialist doctor, eg. Neurologist, Oral Surgeon *(please specify)*								
	_____	❑	❑	❑	❑	❑	❑	❑	❑
	_____	❑	❑	❑	❑	❑	❑	❑	❑
	_____	❑	❑	❑	❑	❑	❑	❑	❑
	Disabilities services commission	❑	❑	❑	❑	❑	❑	❑	❑
	Family and children's services	❑	❑	❑	❑	❑	❑	❑	❑
	Some other organisation or person *(please specify)*	❑	❑	❑	❑	❑	❑	❑	❑

116 **Finally, is he/she part of any other study conducted by the TVW Telethon Institute for Child Health Research, Princess Margaret Hospital, King Edward Memorial Hospital, Curtin University or the Australian Twin Registry?**

No	0	❑	→**Q118**
Yes	1	❑	
Don't know	9	❑	→**Q118**

117 **What is the name of the study? (Please specify)**

Child 1:_____

Child 2:_____

118

If there is anything else you would like to tell us about your children, or if there are any special services you think he/she needs, please write in the space below.

Child 1:

_____

_____

_____

_____

_____

_____

Child 2:

_____

_____

_____

_____

_____

_____

This is the end of the questionnaire about other children in your family.

Please make sure that you have completed questionnaire 1 about your multiples, and questionnaire 2 about yourself and your partner. Then put all the questionnaires, together with the signed consent forms, in the reply-paid envelope and send it back to us.

Thank you very much for taking the time to give us this valuable information.

INSTRUCTIONS

- This questionnaire asks about things that may have happened in your family before, during and since your twins were born.

- It is probably best if this questionnaire is answered by one of the parents, but if this is not possible, then the closest relative should answer the questions.

- Most answers require a ✓ in a box or a short answer written in the space provided. Some questions allow you to ✓more than one box. When this is the case, clear instructions are given.

- For some questions you will see an → with a number after the box you have ticked. This means that the next few questions do not apply to you. Please follow the instructions that are given and go to the question number stated after the →. Where there is no → please go to the next question.

- If you have any difficulty remembering any of the information, please give the best answer you can, or leave that question blank. Anything that you are able to tell us will be extremely useful.

- If you have any problems completing the questionnaire or have any other questions, please ring (08) 9382 2007 or (freecall) 1800 819 684 between 9am and 5pm Monday to Friday, where a member of staff will help you.

- When you have finished the questionnaire, please send it back to us, together with the signed consent form, in the reply-paid envelope provided. ***No stamp is needed.***

- ***If possible, please try to send it back to us within ONE MONTH.***

ALL THE INFORMATION YOU GIVE US IN THIS QUESTIONNAIRE

WILL REMAIN CONFIDENTIAL

Thank you for your assistance

Firstly, please complete the following:

What is the name of the person completing this questionnaire?_____

What is your relationship to the twins? _____

Date on which questionnaire was completed: ____/____/19____

Following the example below, please list all members of your immediate family *(that is, yourself, your partner and any children either of you have)*, their relationship to you and the twins, their first names and date of birth. Please list the children in order from oldest to youngest.

Example

	Name	Date of birth	Relationship to me	Relationship to the twins
Myself	Freda	01/11/1958		mother
My partner	Fred	28/02/1955	my partner	step-father
Child 1	Paul	15/06/1981	my partner's son from his first marriage	step-brother
Child 2	John	01/01/1982	my twin son	twin 1
Child 3	Jane	01/01/1982	my twin daughter	twin 2
Child 4	Anne	30/04/1991	my daughter from my first marriage	half-sister

Your answer

	Name	Date of birth	Relationship to me	Relationship to the twins
Myself		____/____/19____		
My partner		____/____/19____		
Child 1		____/____/19____		
Child 2		____/____/19____		
Child 3		____/____/19____		
Child 4		____/____/19____		
Child 5		____/____/19____		
Child 6		____/____/19____		
Child 7		____/____/19____		
Child 8		____/____/19____		

Questions 1 to 30 are about things that may have happened before, during and since the birth of your twins. For each question, please follow the instructions carefully.

Questions 1 to 14 will help us determine whether your twins are identical or non-identical.
Please ✓ one box for each question.

1. Are the twins the same sex?

No ❑$_0$→**Q15**
Yes ❑$_1$

---

2. I believe the twins to be: (✓ one box)

Identical (one egg, monozygous) ❑$_1$
Non-identical (two eggs, dizygous) ❑$_2$
Not sure ❑$_9$

To what extent do you think your twins are similar *AT THIS TIME* for the following features?

3. Height

Not at all similar ❑$_1$
Somewhat similar ❑$_2$
Exactly similar ❑$_3$

---

4. Weight

Not at all similar ❑$_1$
Somewhat similar ❑$_2$
Exactly similar ❑$_3$

---

5. Facial appearance

Not at all similar ❑$_1$
Somewhat similar ❑$_2$
Exactly similar ❑$_3$

---

6. Hair colour

Not at all similar ❑$_1$
Somewhat similar ❑$_2$
Exactly similar ❑$_3$

7. Eye colour

Not at all similar ❑$_1$
Somewhat similar ❑$_2$
Exactly similar ❑$_3$

---

8. Complexion

Not at all similar ❑$_1$
Somewhat similar ❑$_2$
Exactly similar ❑$_3$

---

9. Do they look as alike as two peas in a pod?

No ❑$_0$
Yes ❑$_1$

---

10. Does their mother ever mistake one for the other?

No ❑$_0$
Yes ❑$_1$

---

11. Does their father ever mistake one for the other?

No ❑$_0$
Yes ❑$_1$

---

12. Are they sometimes mistaken for each other by other relatives?

No ❑$_0$
Yes ❑$_1$

---

13. Is it hard for strangers to tell them apart?

No ❑$_0$
Yes ❑$_1$

---

14. Do they have very similar personalities?

No ❑$_0$
Yes ❑$_1$

Questions 15 to 23 are about your pregnancy with the twins.
(✓ one box for each question, or write your answer in the space provided)

15. *BEFORE THIS PREGNANCY*, for how long were you trying to conceive?

Not specifically trying ❑ 00

Months _____ or, years _____

---

16. *BEFORE THIS PREGNANCY*, did you or your partner seek medical advice or have any investigations, treatments or operations for difficulty becoming pregnant or to assist you in becoming pregnant?

No ❑ 0
Yes ❑ 1

(please give details below)

_____
_____
_____
_____
_____
_____
_____
_____

---

17. Did you smoke during this pregnancy?

No, I have never smoked ❑ 0 →**Q20**
No, I gave up before I was pregnant ❑ 1 →**Q19**
No, I gave up when I was trying to become pregnant ❑ 2 →**Q19**
No, I gave up as soon as I knew I was pregnant ❑ 3 →**Q19**
Yes, but I gave up during pregnancy ❑ 4 →**Q18**
Yes, I smoked during this pregnancy ❑ 5 →**Q18**

---

18. *COMPARED WITH BEFORE YOU BECAME PREGNANT*, how much did you smoke during this pregnancy?

Less than before ❑ 1
About the same as before ❑ 2
More than before ❑ 3
Don't know/can't remember ❑ 9

19. Did you start smoking again after the twins were born?

No ❑ 0
Yes ❑ 1

---

20. *ON THE ADVICE OF YOUR DOCTOR*, how long did you rest in bed during this pregnancy?

No time resting in bed ❑ 00

At home: days _____ or weeks _____

In hospital: days _____ or weeks _____

Don't know/can't remember ❑ 99

---

21. Did you have complications during this pregnancy? (✓ if yes)

Bleeding serious enough for bed rest ❑ 01
Threatened miscarriage *(under 20 weeks)* ❑ 02
Urinary tract infection ❑ 03
Pre-eclampsia *(toxaemia)* ❑ 04
High blood pressure ❑ 05
Diabetes or pregnancy-related *(gestational)* diabetes ❑ 06
Placenta praevia ❑ 07
Premature rupture of membranes ❑ 08
Twin-to-twin transfusion syndrome ❑ 09
Other *(please specify below)* ❑ 10

_____
_____
_____
_____

Don't know/can't remember ❑ 99
No complications ❑ 00

---

22. What was the length of this pregnancy?

weeks _____ or months _____

---

23. In the first few months after the twins were born, did you feel more depressed than usual?

No ❑ 0
Yes ❑ 1
Don't know/can't remember ❑ 9

This section asks about your twins since their birth.
Please use one column for each child, and for each question, ✓ one or more boxes as directed, or write your answer in the space provided.

		TWIN 1	TWIN 2
	Name		
24	**What was the type of delivery?**		
	Normal	❑ 1	❑ 1
	Breech	❑ 2	❑ 2
	Forceps	❑ 3	❑ 3
	Vacuum	❑ 4	❑ 4
	Booked caesarean section	❑ 5	❑ 5
	Emergency caesarean section	❑ 6	❑ 6
25	**What was his/her birthweight?**	grams _____	grams _____
		or lbs oz	lbs oz
26	**Did he/she have any of the following conditions *IMMEDIATELY* after birth?** *(that is, while he/she was still in hospital)* (✓ if yes)		
	Respiratory distress	❑ 1	❑ 1
	Convulsions, fits or seizures	❑ 2	❑ 2
	Other serious condition (*please specify*):	❑ 3	❑ 3
	Twin 1:		
	Twin 2:		
	Don't know/can't remember	❑ 9	❑ 9
	None of these conditions	❑ 0	❑ 0
27	**Did he/she have any of the following treatments *IMMEDIATELY* after birth?** *(that is, while he/she was still in hospital)* (✓ if yes)		
	Respirator/ventilator	❑ 1	❑ 1
	Fed through a naso-gastric tube	❑ 2	❑ 2
	Other treatment (*please specify below*):	❑ 3	❑ 3
	Twin 1:		
	Twin 2:		
	Don't know/can't remember	❑ 9	❑ 9
	None of these treatments	❑ 0	❑ 0
28	**How long did he/she spend in neonatal intensive care unit, or special care nursery?**		
	Was not in neonatal intensive care OR special care nursery	❑ 0 0	❑ 0 0
	Time in intensive care unit:	___ days or ____wks	___ days or ____wks
	Time in special care nursery:	___ days or ____wks	___ days or ____wks
	Total time in neonatal intensive care **and** special care nursery *(but I can't remember how long in each section)*	___ days or ____wks	___ days or ____wks
29	**For how long was he/she breastfed?** (✓ one box)		
	Was not breastfed	❑ 0	❑ 0
	Less than 3 months	❑ 1	❑ 1
	At least 3 months but less than 6 months	❑ 2	❑ 2
	At least 6 months but less than 9 months	❑ 3	❑ 3
	At least 9 months but less than 12 months	❑ 4	❑ 4
	12 months or longer	❑ 5	❑ 5
	Don't know/can't remember	❑ 9	❑ 9

30 **At what age was he/she first given any milk *OTHER THAN BREAST MILK*?**

Twin 1 _____ days _____ or weeks _____ or months

Twin 2 _____ days _____ or weeks _____ or months

Questions 31 to 36 relate to yourself and your partner.
For each question, please ✓ one box or write your answers in the space provided.

	YOURSELF	YOUR PARTNER
31 In which country were you born? (if born in Australia, please give the State)	_____	_____
32 If you were NOT born in Australia, in what year did you first arrive here?	_____	_____
33 What is the main language you speak at home?		
English	❑1	❑1
Aboriginal language	❑2	❑2
Italian	❑3	❑3
Greek	❑4	❑4
Other *(please specify)*	❑5	❑5
	_____	_____
34 What is your marital status? *(✓ more than one box if necessary)*		
Never married	❑1	❑1
In first marriage	❑2	❑2
Remarried	❑3	❑3
Separated but not divorced	❑4	❑4
Divorced	❑5	❑5
Widowed	❑6	❑6
De facto	❑7	❑7
35 What is your current occupation, or previous occupation if you are currently unemployed?		
Work at home	❑1	❑1
Student	❑2	❑2
Professional employment *(eg lawyer, engineer, teacher)*	❑3	❑3
Semi-professional employment *(eg salesperson, clerical)*	❑4	❑4
Skilled trades *(eg plumber, electrician, mechanic)*	❑5	❑5
Unskilled trades *(eg labourer)*	❑6	❑6
Other *(please specify)*	❑7	❑7
	_____	_____
36 What is the highest level of education you have reached?		
Never went to school	❑1	❑1
Primary school	❑2	❑2
Some high school	❑3	❑3
Finished high school *(Year 12 or equivalent)*	❑4	❑4
Apprenticeship, TAFE or trade qualifications	❑5	❑5
University degree	❑6	❑6
Other *(please specify)*	❑7	❑7
	_____	_____

Questions 37 to 56 relate to smoking.
For each question, please ✓ one box or write your answers in the space provided.

		YOURSELF	YOUR PARTNER
37	**Have you *EVER* smoked cigarettes, cigars or a pipe?**		
	No	❑0 →**Q46**	❑0 →**Q46**
	Yes	❑1	❑1
38	**How old were you when you *FIRST* started smoking?**		
	Years	_____	_____
39	**Do you still smoke?**		
	No	❑0 →**Q42**	❑0 →**Q42**
	Yes	❑1	❑1
40	**How much do you smoke *NOW*?**		
	Number of cigarettes *PER DAY*	_____	_____
	Number of cigars *PER DAY*	_____	_____
	Grams of tobacco *PER WEEK* (*a 2 ounce pouch of tobacco equals 50 grams*)	_____	_____
41	**In the *LAST 12 MONTHS*, have you changed the amount you smoke?**		
	No, I smoke about the same amount	❑0 →**Q44**	❑0 →**Q44**
	Yes, I smoke less than I used to	❑1 →**Q44**	❑1 →**Q44**
	Yes, I smoke more than I used to	❑2 →**Q44**	❑2 →**Q44**
42	**When did you give up smoking?**		
	Year	19 _____	19 _____
	or, age	_____ years	_____ years
43	**How much *DID* you smoke?**		
	Number of cigarettes *PER DAY*	_____	_____
	Number of cigars *PER DAY*	_____	_____
	Grams of tobacco *PER WEEK* (*a 2 ounce pouch of tobacco equals 50 grams*)	_____	_____
44	**When you are/were with your children, either in the house or in the car, which of the following do you do *NOW* or *WHEN YOU USED TO SMOKE*?**		
	Not smoke at all	❑0	❑0
	Smoke less than normal	❑1	❑1
	Smoke about the same	❑3	❑3
	Smoke more than normal	❑4	❑4
	Difficult to say	❑5	❑5
45	**How often do/did you smoke when your children are/were present?**		
	Never	❑0	❑0
	Some of the time	❑1	❑1
	Most of the time	❑3	❑3
	Always	❑4	❑4

These questions are about other people living in your house.
Please ✓ one box, or write your answer in the space provided.

46 Does any other person who lives in your house smoke regularly?

No other person live in the house ❑$_0$→**Q49**

No, the other person/people in the house does/do not smoke ❑$_0$→**Q49**

Yes, the other person/people in the house smokes ❑$_0$→**Q49**

---

47 *IN TOTAL*, how many cigarettes, cigars etc. are smoked by the other people living in your house

Number of cigarettes *PER DAY* ____

Number of cigars *PER DAY* ____

Grams of tobacco *PER WEEK* ____

(a 2oz. pouch of tobacco equals 50 grams)

---

48 How often does the other person/people living in your house smoke when your children are present?

Never ❑$_0$
Some of the time ❑$_1$
Most of the time ❑$_2$
Always ❑$_3$

Do you have household rules about smoking, such as:

49 No smoking inside the home?

No ❑$_0$
Yes ❑$_0$→**Q52**

---

50 Smoke-free rooms in the home?

No ❑$_0$
Yes ❑$_1$

51 No smoking allowed inside when the children are present?

No ❑$_0$
Yes ❑$_1$

---

52 Having a smoke-free car?

No ❑$_0$
Yes ❑$_1$

53 How often do you ask visitors not to smoke in your home?

Never ❑$_0$
Some of the time ❑$_1$
Most of the time ❑$_2$
Always ❑$_3$

---

54 How often do you choose non-smoking areas in restaurants, cafes and other public places when your children are with you?

Never ❑$_0$
Some of the time ❑$_1$
Most of the time ❑$_2$
Always ❑$_3$

---

55 How often do other people smoke when they are with your children?

Never ❑$_0$
Some of the time ❑$_1$
Most of the time ❑$_2$
Always ❑$_3$

---

56 Do your children regularly spend time outside the home where people smoke in the presence of your children?

No ❑$_0$
Yes ❑$_1$

Questions 57 to 60 about *EVERY* member of your family, that is, your twins, yourself, your partner and any other children you have. For each question, please ✓ one or more boxes as directed, or write your answer in the space provided.

57 How much does each person weight *NOW*?

Yourself: _____ kgs, or _____ stone _____pounds

Your partner _____ kgs, or _____ stone _____pounds

Twin 1: _____ kgs, or _____ stone _____pounds

Twin 2: _____ kgs, or _____ stone _____pounds

Child 1: _____ kgs, or _____ stone _____pounds

Child 2: _____ kgs, or _____ stone _____pounds

Child 3: _____ kgs, or _____ stone _____pounds

Child 4: _____ kgs, or _____ stone _____pounds

---

58 How tall is each person *NOW*?

Yourself: _____ cms, or _____ feet_____inches

Your partner _____ cms, or _____ feet_____inches

Twin 1: _____ cms, or _____ feet_____inches

Twin 2: _____ cms, or _____ feet_____inches

Child 1: _____ cms, or _____ feet_____inches

Child 2: _____ cms, or _____ feet_____inches

Child 3: _____ cms, or _____ feet_____inches

Child 4: _____ cms, or _____ feet_____inches

59 Has any member of your family *EVER* had any of the following health conditions *CONFIRMED BY A DOCTOR* (✓ if yes)

	YOURSELF	YOUR PARTNER	TWIN 1	TWIN 2
High blood pressure	❑ 01	❑ 01	❑ 01	❑ 01
Heart problems	❑ 02	❑ 02	❑ 02	❑ 02
Febrile convulsions	❑ 03	❑ 03	❑ 03	❑ 03
Other convulsions, fits or seizures, or epilepsy	❑ 04	❑ 04	❑ 04	❑ 04
Arthritis or rheumatism	❑ 05	❑ 05	❑ 05	❑ 05
Kidney disease	❑ 06	❑ 06	❑ 06	❑ 06
Diabetes	❑ 07	❑ 07	❑ 07	❑ 07
Bronchiolitis	❑ 08	❑ 08	❑ 08	❑ 08
Asthma	❑ 09	❑ 09	❑ 09	❑ 09
Hay fever	❑ 10	❑ 10	❑ 10	❑ 10
Eczema	❑ 11	❑ 11	❑ 11	❑ 11
Other allergies	❑ 12	❑ 12	❑ 12	❑ 12
Migraine or severe headaches	❑ 13	❑ 13	❑ 13	❑ 13
Cancer, including leukaemia	❑ 14	❑ 14	❑ 14	❑ 14
Attention deficit disorder (ADHD)	❑ 15	❑ 15	❑ 15	❑ 15
Cystic fibrosis	❑ 16	❑ 16	❑ 16	❑ 16
Cerebral palsy	❑ 17	❑ 17	❑ 17	❑ 17
Down syndrome	❑ 18	❑ 18	❑ 18	❑ 18
Fragile X	❑ 19	❑ 19	❑ 19	❑ 19
Spina bifida	❑ 20	❑ 20	❑ 20	❑ 20
Intellectual disability	❑ 21	❑ 21	❑ 21	❑ 21
Developmental delay or lag	❑ 22	❑ 22	❑ 22	❑ 22
Any condition, including birth defects, present since birth (eg club foot, cleft palate & lip, missing fingers and toes) *(please specify)*	❑ 23	❑ 23	❑ 23	❑ 23

Yourself: _____

Your partner: _____

Twin 1: _____

Twin 2: _____

Any other condition or disability *(please specify)*	❑ 24	❑ 24	❑ 24	❑ 24

Yourself: _____

Your partner: _____

Twin 1: _____

Twin 2: _____

None of these conditions	❑ 00	❑ 00	❑ 00	❑ 00

(question 39 continues over the page for your other children)

YOUR OTHER CHILDREN (please write their names below)				
Name:	CHILD 1 _____	CHILD 2 _____	CHILD 3 _____	CHILD 4 _____
High blood pressure	❑ 01	❑ 01	❑ 01	❑ 01
Heart problems	❑ 02	❑ 02	❑ 02	❑ 02
Febrile convulsions	❑ 03	❑ 03	❑ 03	❑ 03
Other convulsions, fits or seizures, or epilepsy	❑ 04	❑ 04	❑ 04	❑ 04
Arthritis or rheumatism	❑ 05	❑ 05	❑ 05	❑ 05
Kidney disease	❑ 06	❑ 06	❑ 06	❑ 06
Diabetes	❑ 07	❑ 07	❑ 07	❑ 07
Bronchiolitis	❑ 08	❑ 08	❑ 08	❑ 08
Asthma	❑ 09	❑ 09	❑ 09	❑ 09
Hay fever	❑ 10	❑ 10	❑ 10	❑ 10
Eczema	❑ 11	❑ 11	❑ 11	❑ 11
Other allergies	❑ 12	❑ 12	❑ 12	❑ 12
Migraine or severe headaches	❑ 13	❑ 13	❑ 13	❑ 13
Cancer, including leukaemia	❑ 14	❑ 14	❑ 14	❑ 14
Attention deficit disorder (ADHD)	❑ 15	❑ 15	❑ 15	❑ 15
Cystic fibrosis	❑ 16	❑ 16	❑ 16	❑ 16
Cerebral palsy	❑ 17	❑ 17	❑ 17	❑ 17
Down syndrome	❑ 18	❑ 18	❑ 18	❑ 18
Fragile X	❑ 19	❑ 19	❑ 19	❑ 19
Spina bifida	❑ 20	❑ 20	❑ 20	❑ 20
Intellectual disability	❑ 21	❑ 21	❑ 21	❑ 21
Developmental delay or lag	❑ 22	❑ 22	❑ 22	❑ 22
Any condition, including birth defects, present since birth (eg club foot, cleft palate & lip, missing fingers and toes) *(please specify)*	❑ 23	❑ 23	❑ 23	❑ 23

Child 1: _____

Child 2: _____

Child 3: _____

Child 4: _____

Any other condition or disability *(please specify)*	❑ 24	❑ 24	❑ 24	❑ 24

Child 1: _____

Child 2: _____

Child 3: _____

Child 4: _____

None of these conditions	❑ 00	❑ 00	❑ 00	❑ 00

60 **At the present time, do any members of your family *(that is, yourself, your partner or any of your children)* take any medicines *REGULARLY*?** *(for example, for asthma, high blood pressure, epilepsy, ADHD etc)*

No ❑0
Yes ❑1

(please give details in the table below)

Name of family member	Name of medicine	Condition it is used to treat

Finally, we would like to know how you feel about completing this questionnaire, as it is the first time we have used it.

61 **Approximately how long did it take you to complete the questionnaire?** _____ minutes

---

62 **Did you find any of the questions confusing or difficult to answer?** No ❑$_0$ Yes ❑$_1$

please comment _____

_____

_____

---

63 **Did you find any of the questions upsetting or offensive?** No ❑$_0$ Yes ❑$_1$

please comment _____

_____

_____

---

64 **A short report from the study will be available when the study has finished. Would you like us to send you a copy?**

No ❑$_0$ Yes ❑$_1$

---

65 **Please write below any comments concerning this questionnaire, the other questionnaires in this package, the research or anything else you would like to tell us about.**

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

This is the end of the questionnaire.
Thank you very much for taking the time to give us this valuable information.

APPENDIX 6:

SUPPLEMENTARY TABLES OF THE UNIVARIATE GEE ANALYSIS OF TWIN DATA

Wheezing ever

Table 1:
Effect of individual and family characteristics on wheezing in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Individual variables		
Male gender	1.64 (1.4-2.0)	<.0001
Overweight	1.00 (.8-1.3)	.97
Twin variables		
Zygosity (MZ vs DZ)	1.14 (.9-1.5)	.35
Has older siblings	0.84 (.7-1.1)	.15
Season of birth		
Spring	1	
Summer	0.95 (.7-1.3)	.75
Autumn	1.09 (.8-1.5)	.60
Winter	0.90 (.6-1.3)	.55
Family variables		
Lives in rural area	0.77 (.6-1.0)	.04

Table 1:
Effect of prenatal, birth and perinatal factors on wheezing in WA twins age 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Conceived using assisted reproduction	0.91 (.8-1.1)	.38
Periconceptional multivitamin use		
In month before pregnancy	1.18 (.9-1.6)	.30
In first 3 months of pregnancy	1.14 (.9-1.4)	.27
Complications of pregnancy		
Bleeding serious enough for bed rest	1.30 (.9-1.9)	.17
Threatened miscarriage under 20 weeks	1.42 (1.0-2.1)	.07
Urinary tract infection	1.48 (.9-2.5)	.15
Toxaemia	1.18 (.8-1.8)	.41
Gestational diabetes	1.91 (.8-4.4)	.13
Placenta praevia	1.48 (.6-3.6)	.40
Premature rupture of membranes	1.47 (1.0-2.2)	.08
Birth variables		
Delivered by caesarean section	1.28 (1.0-1.6)	.05
Birth weight – Q1 vs. Q4	1.56 (1.2-2.1)	.0030
Gestation over 32 weeks	0.90 (.8-1.0)	.065
Was admitted to NICU	1.35 (1.1-1.7)	.0071
Was admitted to SCN	1.40 (1.1-1.7)	.0026
Had respiratory distress after birth	1.41 (1.1-1.8)	.0058
Needed respirator after birth	1.42 (1.1-1.8)	.0091
Fed through naso-gastric tube after birth	1.59 (1.3-2.0)	.0001

Table 2:
Effect of infant feeding on wheezing in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Length of breast feeding		
Was not breast fed	1	
Less than 3 months	0.77 (.5-1.1)	.16
At least 3 months but less than 6 months	0.88 (.6-1.3)	.54
At least 6 months	1.02 (.7-1.4)	.90
Other milk introduced before 4 months	1.15 (.9-1.5)	.23

Table 3:
Effects of childhood conditions on wheezing in WA twins, aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Behaviour disorders		
ADHD (hyperactive type)	1.29 (1.0-1.7)	.081
ADHD (inattentive type)	0.98 (.7-1.4)	.92
Conditions diagnosed by a doctor		
Febrile convulsions	1.43 (.8-2.5)	.20
Migraines	1.38 (.9-2.1)	.15
At least one episode of otitis media	1.32 (1.0-1.7)	.023
ENT operations		
Had tonsils removed	1.50 (1.0-2.2)	.039
Had adenoids removed	1.36 (1.0-2.0)	.096
Had grommets inserted	1.04 (.7-1.5)	.84

Table 4:
Effects of parental variables on wheezing in WA twins, aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Maternal variables		
Post natal depression	1.06 (1.0-1.1)	.096
Ever wheezed	3.30 (2.6-4.3)	<.0001
In professional occupation	1.08 (.9-1.4)	.51
Has tertiary qualifications	1.29 (1.0-1.7)	.084
Under 25 when twins born	1.02 (.7-1.4)	.93
Paternal variables		
Ever wheezed	2.79 (2.1-3.7)	<.0001
In professional occupation	1.13 (.9-1.4)	.32
Has tertiary qualifications	1.19 (.9-1.6)	.21
Under 25 when twins born	1.06 (.7-1.6)	.79

Table 5:
Effects of passive smoking exposure on wheezing in WA twins, aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Number of smokers in household		
None	1	
One	1.24 (.9-1.6)	.12
Two or more	0.92 (.7-1.6)	.61
Other smoking variables		
Mother smoked during pregnancy	0.81 (.6-1.1)	.20
Mother ever smoked	0.90 (.7-1.2)	.44
Father ever smoked	0.97 (.8-1.2)	.82
No smoking rules in house	1.24 (.9-1.6)	.13
No smoking rules in car	1.22 (.9-1.7)	.25

Current wheeze

Table 6:
Effect of individual and family characteristics on current wheeze in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Individual variables		
Male gender	0.89 (.6-1.3)	.52
Overweight	1.05 (.7-1.6)	.83
Twin variables		
Zygosity (MZ vs DZ)	0.70 (.4-1.1)	.097
Has older siblings	0.94 (.6-1.4)	.74
Season of birth		
Spring	1	
Summer	1.50 (.9-2.5)	.12
Autumn	0.87 (.5-1.4)	.59
Winter	1.27 (.8-2.1)	.36
Family variables		
Lives in rural area	0.97 (.6-1.4)	.87

Table 7:
Effect of prenatal, birth and perinatal factors on current wheeze in WA twins age 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Conceived using assisted reproduction	1.01 (.8-1.2)	.90
Periconceptional multivitamin use		
In month before pregnancy	1.34 (.8-2.2)	.25
In first 3 months of pregnancy	1.20 (.8-1.7)	.34
Complications of pregnancy		
Bleeding serious enough for bed rest	0.83 (.5-1.5)	.54
Threatened miscarriage under 20 weeks	0.56 (.3-1.0)	.056
Urinary tract infection	1.16 (.6-2.4)	.68
Toxaemia	1.37 (.7-2.6)	.33
Gestational diabetes	1.72 (.6-4.7)	.30
Placenta praevia	3.66 (.8-17.6)	.11
Premature rupture of membranes	1.16 (.6-2.4)	.68
Birth variables		
Delivered by caesarean section	1.10 (.8-1.6)	.61
Birth weight – Q1 vs. Q4	1.37 (.8-2.3)	.22
Gestation over 32 weeks	0.99 (.8-1.2)	.93
Was admitted to NICU	1.12 (.8-1.6)	.54
Was admitted to SCN	0.98 (.7-1.4)	.91
Had respiratory distress after birth	1.44 (1.0-2.2)	.077
Needed respirator after birth	1.23 (.8-1.9)	.36
Fed through naso-gastric tube after birth	1.01 (.7-1.5)	.95

Table 8:
Effect of infant feeding on current wheezing in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Length of breast feeding		
Was not breast fed	1	
Less than 3 months	0.75 (.4-1.3)	.34
At least 3 months but under 6 months	0.66 (.4-1.2)	.20
At least 6 months	0.53 (.3-.9)	.027
Other milk introduced before 4 months	1.83 (1.2-2.8)	.0038

Table 9:
Effect of childhood conditions on current wheeze in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Behaviour disorders		
ADHD (hyperactive type)	1.13 (.7-1.9)	.62
ADHD (inattentive type)	1.76 (1.0-3.3)	.074
Conditions diagnosed by a doctor		
Febrile convulsions	1.59 (.7-3.6)	.28
Migraines	1.40 (.8-2.6)	.27
At least one episode of otitis media	1.02 (.7-1.5)	.93
ENT operations		
Had tonsils removed	1.17 (.7-2.0)	.59
Had adenoids removed	1.22 (.7-2.1)	.48
Had grommets inserted	0.79 (.4-1.4)	.45

Table 10:
Effect of parental variables on current wheeze in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Maternal variables		
Post natal depression	0.93 (.8-1.0)	.18
Ever wheezed	1.56 (1.1-2.3)	.023
In professional occupation	0.99 (.7-1.4)	.95
Has tertiary qualifications	1.55 (1.0-2.4)	.054
Under25 when twins born	0.83 (.5-1.4)	.51
Paternal variables		
Ever wheezed	1.63 (1.1-2.4)	.018
In professional occupation	1.15 (.8-1.7)	.48
Has tertiary qualifications	1.44 (.9-2.2)	.11
Under25 when twins born	0.74 (.3-1.6)	.44

Table 11:
Effect of passive smoking exposure on current wheeze in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Number of current smokers in household		
None	1	
One	1.31 (.8-2.1)	.26
Two or more	1.20 (.6-2.2)	.56
Other smoking variables		
Mother smoked during pregnancy	0.82 (.5-1.4)	.44
Mother ever smoked	0.78 (.5-1.2)	.26
Mother current smoker	0.83 (.5-1.4)	.51
Father ever smoked	1.21 (.8-1.8)	.33
Father current smoker	1.45 (.9-2.3)	.11
No smoking rules in house	0.80 (.5-1.3)	.35
No smoking rules in car	0.79 (.5-1.3)	.38

Table 12:
Effect of socio-economic status on current wheeze in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Index of disadvantage		
Bottom 10%	1.30 (.5-3.2)	.57
Top 10%	1.20 (.8-1.8)	.42
Index of advantage/disadvantage		
Bottom 10%	1.35 (.6-3.2)	.49
Top 10%	1.22 (.8-1.9)	.39
Index of economic resources		
Bottom 10%		
Top 10%	1.34 (.8-2.2)	.24
Index of education and employment		
Bottom 10%	1.62 (.7-3.6)	.22
Top 10%	1.20 (.8-1.9)	.40

Doctor-diagnosed asthma

Table 13:
Effect of individual and family characteristics on asthma in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Individual variables		
Male gender	1.36 (1.1-1.7)	.0041
Overweight	1.13 (.9-1.4)	.34
Individual variables		
Zygosity (MZ vs DZ)	0.98 (.8-1.3)	.91
Has older siblings	0.68 (.6-.9)	.0006
Season of birth		
Spring	1	
Summer	0.96 (.7-1.3)	.82
Autumn	1.08 (.8-1.5)	.62
Winter	0.81 (.6-1.1)	.20
Individual variables		
Lives in rural area	0.77 (.6-1.0)	.038

Table 14:
Effect of prenatal, birth and perinatal factors on asthma in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Conceived using assisted reproduction	0.95 (.8-1.2)	.62
Complications of pregnancy		
Bleeding serious enough for bed rest	1.48 (1.0-2.1)	.028
Threatened miscarriage under 20 weeks	1.65 (1.1-2.4)	.0074
Urinary tract infection	1.25 (.7-2.1)	.412
Toxaemia	1.28 (.9-1.9)	.19
Gestational diabetes	1.36 (.6-3.1)	.46
Placenta praevia	1.34 (.6-3.0)	.49
Premature rupture of membranes	1.41 (.9-2.1)	.11
Birth variables		
Delivered by caesarean section	1.33 (1.1-1.7)	.016
Birth weight – Q1 vs. Q4	1.64 (1.2-2.2)	.0011
Gestation over 32 weeks	0.86 (.8-1.0)	.0088
Spent time in NICU	0.75 (.6-.9)	.0068
Spent time in SCN	0.76 (.6-.9)	.0095
Had respiratory distress after birth	1.44 (1.1-1.8)	.0030
Needed respirator after birth	1.41 (1.1-1.8)	.0088
Fed through naso-gastric tube after birth	1.49 (1.2-1.9)	.0005

Table 15:
Effect of infant feeding on asthma in WA twins aged 6-18 years, after adjusting for age.

VARIABLE	OR 95% CI	P-VALUE
Length of breast feeding		
Was not breast fed	1	
Less than 3 months	0.75 (.5-1.1)	.13
At least 3 months but less than 6 months	0.90 (.6-1.3)	.60
At least 6 months	1.04 (.7-1.5)	.83
Other milk introduced before 4 months	0.98 (.8-1.2)	.88

Table 16:
Effect of childhood conditions on asthma in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Behaviour disorders		
ADHD (hyperactive type)	1.16 (.9-1.6)	.33
ADHD (inattentive type)	0.94 (.6-1.4)	.75
Conditions diagnosed by a doctor		
Febrile convulsions	1.50 (.8-2.8)	.20
Migraines	1.75 (1.1-2.7)	.010
At least one episode of otitis media	1.37 (1.1-1.7)	.0058
ENT operations		
Had tonsils removed	1.66 (1.2-2.4)	.0046
Had adenoids removed	1.64 (1.2-2.3)	.0055
Had grommets inserted	1.26 (.9-1.8)	.20

Table 17:
Effect of parental variables on asthma in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Maternal variables		
Post natal depression	1.06 (1.0-1.1)	.056
Asthmatic	3.10 (2.4-4.0)	<.0001
In professional occupation	1.05 (.8-1.3)	.69
Has tertiary qualifications	0.96 (.7-1.3)	.79
Under 25 when twins born	1.36 (1.0-1.9)	.056
Paternal variables		
Asthmatic	2.54 (1.9-3.4)	<.0001
In professional occupation	1.00 (.8-1.2)	.99
Has tertiary qualifications	0.98 (.8-1.3)	.91
Under 25 when twins born	1.21 (.8-1.8)	.34

Table 18:
Effect of passive smoking exposure on asthma in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Number of smokers in household		
None	1	
One	1.12 (.8-1.5)	.44
Two or more	1.11 (.8-1.5)	.47
Other smoking variables		
Mother smoked during pregnancy	1.06 (.8-1.4)	.68
Mother ever smoked	1.14 (.9-1.5)	.31
Father ever smoked	1.17 (.9-1.5)	.18
No smoking rules in house	1.02 (.8-1.3)	.87
No smoking rules in car	1.04 (.8-1.4)	.82

Table 19:
Effect of socio-economic status on asthma in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Index of disadvantage		
Bottom 10%	2.26 (1.3-3.8)	.0027
Top 10%	0.88 (.7-1.1)	.32
Index of advantage/disadvantage		
Bottom 10%	1.12 (.6-2.0)	.71
Top 10%	0.93 (.7-1.2)	.59
Index of economic resources		
Bottom 10%	2.61 (.9-7.6)	.078
Top 10%	1.12 (.8-1.5)	.44
Index of education and employment		
Bottom 10%	1.24 (.7-2.1)	.43
Top 10%	1.05 (.8-1.4)	.76

Current asthma

Table 20:
Effect of individual and family characteristics on current asthma in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Individual variables		
Male gender	1.06 (.8-1.5)	.75
Overweight	0.88 (.6-1.3)	.56
Twin variables		
Zygosity (MZ vs DZ)	0.79 (.5-1.2)	.31
Has older siblings	1.33 (.9-1.9)	.14
Season of birth		
Spring	1	
Summer	1.63 (1.0-2.7)	.068
Autumn	0.99 (.6-1.7)	.96
Winter	1.51 (.9-2.6)	.14
Family variables		
Lives in rural area	0.91 (.6-1.4)	.65

Table 21:
Effect of prenatal, birth and perinatal factors on current asthma in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Conceived using assisted reproduction	1.12 (.8-1.5)	.43
Periconceptional multivitamin use		
In month before pregnancy	1.19 (.7-1.9)	.49
In first 3 months of pregnancy	0.83 (.6-1.2)	.34
Complications of pregnancy		
Bleeding serious enough for bed rest	0.78 (.4-1.4)	.41
Threatened miscarriage under 20 weeks	0.49 (.3-.9)	.020
Urinary tract infection	1.56 (.7-3.5)	.28
Toxaemia	1.38 (.8-2.5)	.31
Gestational diabetes	1.65 (.5-5.9)	.44
Placenta praevia	2.71 (.8-9.1)	.11
Premature rupture of membranes	1.42 (.7-2.9)	.33
Birth variables		
Delivered by caesarean section	1.03 (.7-1.5)	.90
Birth weight – Q1 vs. Q4	1.56 (.9-2.6)	.085
Gestation over 32 weeks	1.01 (.8-1.2)	.91
Was admitted to NICU	1.31 (.9-1.9)	.15
Was admitted to SCN	1.23 (.8-1.8)	.27
Had respiratory distress after birth	1.99 (1.3-3.1)	.0015
Needed respirator after birth	1.46 (.9-2.3)	.089
Fed through naso-gastric tube after birth	1.29 (.9-1.9)	.20

Table 22:
Effect of infant feeding on current asthma in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Length of breast feeding		
Was not breast fed	1	
Less than 3 months	0.93 (.5-1.7)	.82
At least 3 months, but less than 6 months	0.63 (.3-1.2)	.17
At least 6 months	0.60 (.3-1.1)	.081
Other milk introduced before 4 months	1.79 (1.2-2.7)	.0057

Table 23:
Effect of childhood conditions on current asthma in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Behaviour disorders		
ADHD (hyperactive type)	1.10 (.7-1.8)	.72
ADHD (inattentive type)	1.76 (1.0-3.2)	.065
Conditions diagnosed by a doctor		
Febrile convulsions	1.96 (.8-4.8)	.14
Migraines	1.07 (.6-1.9)	.81
At least one episode of otitis media	1.14 (.8-1.7)	.52
ENT operations		
Had tonsils removed	1.17 (.7-2.1)	.60
Had adenoids removed	1.24 (.7-2.2)	.46
Had grommets inserted	0.85 (.5-1.6)	.59

Table 24:
Effect of parental variables characteristics on current asthma in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Maternal variables		
Post natal depression	0.96 (.9-1.1)	.43
Asthmatic	1.60 (1.1-2.4)	.027
In professional occupation	0.94 (.6-1.4)	.75
Has tertiary qualifications	2.06 (1.2-3.4)	.0043
Under 25 when twins born	0.66 (.4-1.1)	.12
Paternal variables		
Asthmatic	1.96 (1.2-3.1)	.0053
In professional occupation	1.20 (.8-1.8)	.37
Has tertiary qualifications	1.78 (1.1-2.8)	.017
Under 25 when twins born	0.66 (.3-1.5)	.31

Table 25:
Effect of passive smoking exposure on current asthma in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Number of current smokers in household		
None	1	
One	1.02 (.6-1.6)	.92
Two or more	0.85 (.5-1.5)	.57
Other smoking variables		
Mother smoked during pregnancy	0.69 (.4-1.1)	.13
No smoking rules in house	1.14 (.7-1.8)	.56
Mother ever smoked	0.74 (.5-1.1)	.16
Mother current smoker	0.67 (.4-1.1)	.12
Father ever smoked	1.04 (.7-1.5)	.85
Father current smoker	1.05 (.7-1.6)	.83
No smoking rules in car	1.22 (.7-2.0)	.45

Table 26:
Effect of socio-economic status on current asthma in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Index of disadvantage		
Bottom 10%	1.34 (.6-3.1)	.49
Top 10%	1.44 (.9-2.3)	.11
Index of advantage/disadvantage		
Bottom 10%	1.26 (.5-3.2)	.61
Top 10%	1.58 (1.0-2.5)	.056
Index of economic resources		
Bottom 10%	5.29 (.6-42.7)	.12
Top 10%	1.41 (.9-2.3)	.17
Index of education and employment		
Bottom 10%	1.45 (.6-3.2)	.37
Top 10%	1.47 (.9-2.4)	.11

Hay fever

Table 27:
Effect of individual and family characteristics on hay fever in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Individual variables		
Male gender	1.24 (1.0-1.5)	.03
Overweight	1.20 (.9-1.5)	.12
Twin variables		
Zygosity (MZ vs DZ)	1.16 (.9-1.5)	.26
Has older siblings	0.64 (.5-.8)	.0001
Season of birth		
Spring	1	
Summer	0.96 (.7-1.3)	.77
Autumn	1.00 (.7-1.4)	.99
Winter	0.94 (.7-1.3)	.69
Family variables		
Lives in rural area	0.58 (.4-.7)	<.0001

Table 28:
Effect of prenatal, birth and perinatal factors on hay fever in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Conceived using assisted reproduction	1.03 (.9-1.2)	.75
Complications of pregnancy		
Bleeding serious enough for bed rest	1.23 (.9-1.8)	.26
Threatened miscarriage under 20 weeks	1.45 (1.0-2.1)	.052
Urinary tract infection	1.23 (.7-2.1)	.45
Toxaemia	1.60 (1.1-2.3)	.011
Gestational diabetes	0.89 (.4-2.2)	.80
Placenta praevia	1.54 (.8-3.2)	.24
Premature rupture of membranes	1.48 (1.0-2.3)	.079
Birth variables		
Delivered by caesarean section	1.37 (1.1-1.7)	.0071
Birth weight – Q1 vs. Q4	1.37 (1.0-1.8)	.034
Gestation over 32 weeks	0.93 (.8-1.0)	.21
Was admitted to NICU	1.29 (1.0-1.6)	.014
Was admitted to SCN	1.23 (1.0-1.5)	.049
Had respiratory distress after birth	1.02 (.8-1.3)	.85
Needed respirator after birth	1.06 (.8-1.4)	.68
Fed through naso-gastric tube after birth	1.34 (1.1-1.7)	.0099

Table 29:
Effect of infant feeding on hay fever in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Length of breast feeding		
Was not breast fed	1	
Less than 3 months	0.97 (.7-1.4)	.88
At least 3 months, but less than 6 months	0.76 (.5-1.1)	.15
At least 6 months	0.86 (.6-1.2)	.38
Other milk introduced before 4 months	0.96 (.8-1.2)	.72

Table 30:
Effect of childhood conditions on hay fever in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Behaviour disorders		
ADHD (hyperactive type)	1.12 (.8-1.5)	.50
ADHD (inattentive type)	1.28 (.9-1.8)	.18
Conditions diagnosed by a doctor		
Febrile convulsions	1.29 (.7-2.3)	.38
Migraines	1.76 (1.2-2.6)	.0051
At least one episode of otitis media	1.49 (1.2-1.9)	.0004
ENT operations		
Had tonsils removed	1.57 (1.1-2.2)	.0075
Had adenoids removed	1.80 (1.3-2.5)	.0005
Had grommets inserted	1.57 (1.1-2.2)	.0080

Table 31:
Effect of parental variables on hay fever in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Maternal variables		
Post natal depression	1.04 (1.0-1.1)	.26
Has hay fever	2.69 (2.1-3.4)	<.0001
In professional occupation	1.02 (.8-1.3)	.88
Has tertiary qualifications	1.38 (1.1-1.8)	.016
Under 25 when twins born	1.12 (.8-1.5)	.46
Paternal variables		
Has hay fever	2.70 (2.2-3.4)	<.0001
In professional occupation	1.36 (1.1-1.7)	.0061
Has tertiary qualifications	1.44 (1.1-1.9)	.0067
Under 25 when twins born	0.85 (.5-1.4)	.52

Table 32:
Effect of passive smoking exposure on hay fever in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Number of smokers in household		
None	1	
One	0.77 (.6-1.0)	.055
Two or more	0.94 (.7-1.2)	.67
Other smoking variables		
Mother smoked during pregnancy	0.82 (.6-1.1)	.20
Mother ever smoked	0.88 (.7-1.1)	.33
Father ever smoked	0.93 (.7-1.2)	.51
No smoking rules in house	1.09 (.8-1.4)	.5265
No smoking rules in car	1.31 (1.0-1.8)	.0892

Table 33:
Effect of socio-economic status on hay fever in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Index of disadvantage		
Bottom 10%	1.73 (1.0-3.1)	.066
Top 10%	1.27 (1.0-1.6)	.055
Index of advantage/disadvantage		
Bottom 10%	0.86 (.5-1.6)	.61
Top 10%	1.38 (1.1-1.8)	.017
Index of economic resources		
Bottom 10%	0.66 (.2-2.3)	.51
Top 10%	1.20 (.9-1.6)	.25
Index of education and employment		
Bottom 10%	0.84 (.5-1.5)	.54
Top 10%	1.43 (1.1-1.9)	.012

Current hay fever

Table 34:
Effect of individual and family characteristics on current hay fever in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Individual variables		
Male gender	0.72 (.5-1.1)	.13
Overweight	0.99 (.6-1.7)	.95
Individual variables		
Zygosity (MZ vs DZ)	1.13 (.6-2.0)	.67
Has older siblings	0.88 (.5-1.4)	.60
Season of birth		
Spring	1	
Summer	1.08 (.6-2.2)	.82
Autumn	1.51 (.8-3.1)	.25
Winter	1.10 (.6-2.2)	.77
Family variables		
Live in rural area	0.80 (.5-1.4)	.41

Table 35:
Effect of prenatal, birth and perinatal factors on current hay fever in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Conceived using assisted reproduction	1.88 (1.0-3.6)	.050
Complications of pregnancy		
Bleeding serious enough for bed rest	0.91 (.4-2.0)	.80
Threatened miscarriage under 20 weeks	1.83 (.7-5.0)	.24
Urinary tract infection	0.98 (.3-3.1)	.97
Toxaemia	1.41 (.6-3.3)	.43
Gestational diabetes	1.94 (.3-12.8)	.49
Placenta praevia	0.41 (.1-1.2)	.10
Premature rupture of membranes	1.97 (.6-6.8)	.28
Birth variables		
Delivered by caesarean section	1.65 (1.0-2.8)	.061
Birth weight – Q1 vs. Q4	1.28 (.7-2.4)	.46
Gestation over 32 weeks	0.97 (.8-1.2)	.78
Spent time in NICU	0.77 (.6-.95)	.014
Spent time in SCN	0.81 (.7-1.0)	.050
Has respiratory distress after birth	1.12 (0.6-2.0)	.70
Needed respirator after birth	1.04 (0.6-1.9)	.90
Fed through naso-gastric tube after birth	1.30 (0.8-2.2)	.32

Table 36:
Effect of infant feeding on current hay fever in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Length of breast feeding		
Was not breast fed	1	
Less than 3 months	0.93 (04-2.1)	.87
At least 3 months, but less than 6 months	0.77 (.3-1.7)	.52
At least 6 months	0.93 (.4-1.9)	.85
Other milk introduced before 4 months	0.78 (.5-1.3)	.34

Table 37:
Effect of childhood conditions on current hay fever in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Behaviour disorders		
ADHD (hyperactive type)	0.78 (.4-1.5)	.43
ADHD (inattentive type)	0.88 (.4-1.8)	.73
Conditions diagnosed by a doctor		
Febrile convulsions	1.77 (.4-7.8)	.45
Migraines	1.02 (.4-2.3)	.98
At least one episode of otitis media	1.42 (.9-2.3)	.16
ENT operations		
Had tonsils removed	1.10 (.6-2.1)	.79
Had adenoids removed	0.78 (.4-1.4)	.41
Had grommets inserted	1.05 (.5-2.1)	.89

Table 38:
Effect of parental variables on current hay fever in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Maternal variables		
Post natal depression	1.06 (.9-1.3)	.54
Ever had hay fever	1.14 (.7-2.0)	.62
In professional occupation	1.55 (.9-2.6)	.087
Has tertiary qualifications	1.12 (.6-2.0)	.72
Under 25 when twins born	0.98 (.5-2.0)	.95
Paternal variables		
Ever had hay fever	0.94 (.6-1.6)	.82
In professional occupation	1.50 (.9-2.5)	.11
Has tertiary qualifications	1.18 (.6-2.2)	.59
Under 25 when twins born	0.50 (.2-1.1)	.082

Table 39:
Effect of passive smoking exposure on current hay fever in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Number of current smokers in household		
None	1	
One	1.23 (.5-2.8)	.62
Two or more	1.36 (.6-2.9)	.41
Other smoking variables		
Mother smoked during pregnancy	1.09 (.6-2.1)	.80
Mother ever smoked	0.99 (.6-1.7)	.96
Mother current smoker	0.87 (.4-1.7)	.70
Father ever smoked	0.83 (.5-1.4)	.48
Father current smoker	0.74 (.4-1.3)	.30
No smoking rules in house	0.67 (.4-1.3)	.22
No smoking rules in car	0.62 (.3-1.4)	.24

Table 40:
Effect of socio-economic status on current hay fever in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Index of disadvantage		
Bottom 10%	0.38 (.1-1.1)	.075
Top 10%	0.83 (.5-1.4)	.49
Index of advantage/disadvantage		
Bottom 10%	0.57 (.2-2.0)	.65
Top 10%	0.63 (.4-1.1)	.092
Index of economic resources		
Bottom 10%	0.33 (.03-3.8)	.38
Top 10%	0.98 (.5-1.9)	.95
Index of education and employment		
Bottom 10%	0.73 (.2-2.7)	.64
Top 10%	0.81 (.5-1.5)	.47

Eczema

Table 41:
Effect of individual and family characteristics on eczema in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Individual variables		
Male gender	1.01 (0.8-1.3)	.95
Overweight	1.02 (.8-1.3)	.87
Twin variables		
Zygosity (MZ vs DZ)	1.15 (.9-1.5)	.31
Has older siblings	0.97 (.8-1.2)	.81
Season of birth		
Spring	1	
Summer	0.86 (.6-1.2)	.39
Autumn	0.97 (.7-1.4)	.85
Winter	0.95 (.7-1.3)	.77
Family variables		
Lives in rural area	0.80 (.6-1.0)	.082

Table 42:
Effect of prenatal, birth and perinatal factors on eczema in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Conceived using assisted reproduction	0.83 (.7-1.0)	.10
Periconceptional multivitamin use		
In month before pregnancy	1.12 (.8-1.5)	.50
In first 3 months of pregnancy	1.06 (.8-1.3)	.65
Complications of pregnancy		
Bleeding serious enough for bed rest	1.10 (.7-1.6)	.64
Threatened miscarriage under 20 weeks	0.98 (.6-1.5)	.93
Urinary tract infection	1.50 (.9-2.5)	.11
Toxaemia	1.30 (.9-1.9)	.19
Gestational diabetes	1.62 (.6-4.0)	.30
Placenta praevia	2.30 (1.0-5.3)	.053
Premature rupture of membranes	0.57 (.3-1.0)	.047
Birth variables		
Delivered by caesarean section	1.07 (.8-1.4)	.61
Birth weight – Q1 vs. Q4	0.94 (.9-1.1)	.70
Gestation over 32 weeks	1.11 (1.0-1.3)	.080
Was admitted to NICU	0.97 (.8-1.2)	.82
Was admitted to SCN	0.94 (.8-1.2)	.63
Had respiratory distress after birth	0.84 (.6-1.1)	.26
Needed respirator after birth	0.99 (.7-1.4)	.96
Fed through naso-gastric tube after birth	0.90 (.7-1.2)	.41

Table 43:
Effect of infant feeding on eczema in WA twins aged 6-18 years, after adjusting for age.

VARIABLE	OR 95% CI	P-VALUE
Length of breast feeding		
Was not breast fed	1	
Less than 3 months	1.05 (.7-1.5)	.79
At least 3 months, but less than 6 months	1.21 (.8-1.8)	.35
At least 6 months	1.14 (.8-1.6)	.46
Other milk introduced before 4 months	1.20 (.9-1.5)	.16

Table 44:
Effect of childhood conditions on eczema in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Behaviour disorders		
ADHD (hyperactive type)	1.30 (1.0-1.8)	.089
ADHD (inattentive type)	1.09 (.7-1.6)	.69
Conditions diagnosed by a doctor		
Febrile convulsions	1.29 (.7-2.4)	.43
Migraines	1.62 (1.0-2.5)	.036
At least one episode of otitis media	1.15 (.9-1.5)	.27
ENT operations		
Had tonsils removed	1.32 (.9-2.0)	.16
Had adenoids removed	1.08 (.7-1.6)	.72
Had grommets inserted	0.98 (.7-1.4)	.92

Table 45:
Effect of parental variables on eczema in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Maternal variables		
Post natal depression	1.04 (1.0-1.1)	.23
Has eczema	2.12 (1.6-2.8)	<.0001
In professional occupation	0.99 (.8-1.3)	.93
Has tertiary qualifications	0.83 (.6-1.1)	.23
Under 25 when twins born	1.09 (.8-1.6)	.64
Paternal variables		
Has eczema	2.67 (1.9-3.8)	<.0001
In professional occupation	0.96 (.8-1.2)	.73
Has tertiary qualifications	0.64 (.5-.9)	.0038
Under 25 when twins born	1.21 (.8-1.8)	.36

Table 46:
Effect of passive smoking exposure on eczema in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Number of smokers in household		
None	1	
One	1.10 (.8-1.5)	.51
Two or more	1.07 (.8-1.4)	.66
Other smoking variables		
Mother smoked during pregnancy	1.02 (.8-1.4)	.88
Mother smoked	1.11 (.8-1.4)	.45
Father smoked	1.02 (.8-1.3)	.90
No smoking rules in house	1.07 (.8-1.4)	.65
No smoking rules in car	0.96 (.7-1.3)	.79

Table 47:
Effect of socio-economic status on eczema in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Index of disadvantage		
Bottom 10%	1.44 (0.8-2.6)	.22
Top 10%	1.13 (.9-1.5)	.38
Index of advantage/disadvantage		
Bottom 10%	1.24 (.7-2.1)	.45
Top 10%	1.12 (.8-1.5)	.46
Index of economic resources		
Bottom 10%	0.45 (.1-1.8)	.26
Top 10%	1.10 (.8-1.5)	.54
Index of education and employment		
Bottom 10%	1.31 (.8-2.2)	.28
Top 10%	1.04 (.8-1.4)	.79

Current eczema

Table 48:
Effect of individual and family characteristics on current eczema in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Individual variables		
Male gender	0.67 (.4-1.0)	.044
Overweight	0.76 (.5-1.2)	.24
Twin variables		
Zygosity (MZ vs DZ)	1.37 (.9-2.2)	.18
Has older siblings	1.27 (.8-1.9)	.25
Season of birth		
Spring	1	
Summer	0.73 (.4-1.3)	.28
Autumn	0.45 (.2-.8)	.0071
Winter	.68 (.4-1.2)	.18
Family variables		
Lives in rural area	0.71 (.4-1.1)	.13

Table 49:
Effect of prenatal, birth and perinatal factors on current eczema in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Conceived using assisted reproduction	0.84 (.5-1.4)	.48
Periconceptional multivitamin use		
In month before pregnancy	1.09 (.6-1.9)	.76
In first 3 months of pregnancy	0.77 (.5-1.2)	.22
Complications of pregnancy		
Bleeding serious enough for bed rest	0.72 (.4-1.4)	.31
Threatened miscarriage under 20 weeks	1.15 (.6-2.4)	.70
UTI	2.85 (1.1-7.5)	.034
Toxaemia	1.16 (.6-2.2)	.66
Gestational diabetes	2.71 (.8-9.3)	.11
Placenta praevia	0.48 (.2-1.3)	.16
Premature rupture of membranes	1.93 (.7-5.4)	.21
Birth variables		
Delivered by caesarean section	1.25 (.8-1.9)	.31
Birth weight – Q1 vs. Q4	1.31 (.8-2.3)	.33
Gestation over 32 weeks	1.10 (.9-1.3)	.39
Was admitted to NICU	0.99 (.7-1.5)	.95
Was admitted to SCN	1.00 (.7-1.5)	.98
Had respiratory distress after birth	0.91 (.6-1.5)	.71
Needed respirator after birth	0.87 (.5-1.4)	.58
Fed through naso-gastric tube after birth	1.02 (.7-1.6)	.91

Table 50:
Effect of birth weight and gestation on current eczema in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Birth weight		
Under 2100 g	1.21 (.8-1.9)	.43
Under 1750 g	0.79 (.4-1.7)	.53
By 100 g increase	1.00 (.96-1.03)	.76
Lowest quartile vs. highest quartile	1.31 (.8-2.3)	.33
Gestation over 32 weeks	1.10 (.9-1.3)	.39

Table 51:
Effect of childhood conditions on current eczema in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Behaviour disorders		
ADHD (hyperactive type)	0.76 (.4-1.4)	.36
ADHD (inattentive type)	0.90 (.4-1.9)	.79
Conditions diagnosed by a doctor		
Febrile convulsions	1.02 (.4-3.0)	.96
Migraines	0.46 (.2-1.0)	.049
At least one episode of otitis media	1.12 (.7-1.7)	.62
ENT operations		
Had tonsils removed	1.09 (.6-2.0)	.78
Had adenoids removed	0.97 (.5-1.8)	.91
Had grommets inserted	1.43 (.7-2.8)	.28

Table 52:
Effect of parental variables on current eczema in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Maternal variables		
Post natal depression	1.04 (.9-1.2)	.53
Has eczema	1.34 (.8-2.1)	.21
In professional occupation	0.98 (.7-1.5)	.93
Has tertiary qualifications	0.86 (.5-1.4)	.56
Under 25 when twins born	0.74 (.4-1.4)	.32
Paternal variables		
Has eczema	0.82 (.5-1.4)	.47
In professional occupation	1.15 (.8-1.7)	.51
Has tertiary qualifications	1.64 (.9-2.9)	.080
Under 25 when twins born	0.46 (.2-1.0)	.042

Table 53:
Effect of passive smoking exposure on current eczema in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Number of current smokers in household		
None	1	
One	0.80 (.5-1.3)	.36
Two or more	1.06 (.5-2.1)	.88
Other smoking variables		
Mother smoked during pregnancy	1.09 (.6-1.8)	.74
Mother ever smoked	0.94 (.6-1.5)	.79
Mother current smoker	0.81 (.5-1.4)	.45
Father ever smoked	1.25 (.8-1.9)	.28
Father current smoker	1.09 (.7-1.7)	.72
No smoking rules in house	0.88 (.6-1.4)	.61
No smoking rules in car	0.88 (.5-1.5)	.62

Table 54:
Effect of socio-economic status on current eczema in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Index of disadvantage		
Bottom 10%	1.28 (.5-3.1)	.58
Top 10%	0.98 (.6-1.5)	.93
Index of advantage/disadvantage		
Bottom 10%	0.96 (.4-2.5)	.93
Top 10%	1.02 (.6-1.7)	.93
Index of economic resources		
Bottom 10%	1.23 (.1-19.1)	.88
Top 10%	1.28 (.7-2.2)	.38
Index of education and employment		
Bottom 10%	0.63 (.3-1.5)	.30
Top 10%	1.38 (.8-2.3)	.22

Atopy

Table 55:
Effect of individual and family characteristics on atopy in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Individual variables		
Male gender	1.26 (1.1-1.5)	.012
Overweight	1.16 (.9-1.4)	.17
Twin variables		
Zygosity (MZ vs DZ)	1.19 (.9-1.5)	.16
Has older siblings	0.67 (.6-.8)	.0001
Season of birth		
Spring	1	
Summer	0.93 (.7-1.2)	.64
Autumn	1.15 (.9-1.5)	.36
Winter	.93 (.7-1.2)	.60
Family variables		
Lives in rural area	0.69 (.6-.8)	.0006

Table 56:
Effect of prenatal, birth and perinatal factors on atopy in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Conceived using assisted reproduction	0.91 (.8-1.1)	.31
Complications of pregnancy		
Bleeding serious enough for bed rest	1.15 (.8-1.6)	.42
Threatened miscarriage under 20 weeks	1.16 (.8-1.7)	.41
Urinary tract infection	1.82 (1.1-3.1)	.026
Toxaemia	1.79 (1.2-2.6)	.0020
Gestational diabetes	1.16 (.5-2.6)	.72
Placenta praevia	1.61 (.8-3.3)	.19
Premature rupture of membranes	1.19 (.8-1.8)	.42
Birth variables		
Delivered by caesarean section	1.20 (1.0-1.5)	.086
Birth weight – Q1 vs. Q4	1.37 (1.1-1.8)	.02
Gestation over 32 weeks	0.94 (.8-1.0)	.25
Was admitted to NICU	1.26 (1.04-1.5)	.019
Was admitted to SCN	1.21 (1.0-1.5)	.048
Has respiratory distress after birth	1.10 (.9-1.4)	.43
Needed respirator after birth	1.14 (.9-1.5)	.29
Fed through naso-gastric tube after birth	1.37 (1.1-1.7)	.0031

Table 57:
Effect of infant feeding on atopy in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Length of breast feeding		
Was not breast fed	1	
Less than 3 months	0.91 (.7-1.3)	.58
At least 3 months, but less than 6 months	0.88 (.6-1.2)	.48
At least 6 months	1.02 (.8-1.4)	.92
Other milk introduced before 4 months	1.15 (.9-1.4)	.19

Table 58:
Effect of childhood conditions on atopy in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Behaviour disorders		
ADHD (hyperactive type)	1.10 (.8-1.5)	.50
ADHD (inattentive type)	0.98 (.7-1.5)	.91
Conditions diagnosed by a doctor		
Febrile convulsions	1.67 (.9-3.0)	.084
Migraines	1.59 (1.1-2.4)	.025
At least one episode of otitis media	1.42 (1.2-1.7)	.0007
ENT operations		
Had tonsils removed	1.64 (1.2-2.3)	.0029
Had adenoids removed	1.58 (1.1-2.2)	.0058
Had grommets inserted	1.31 (.9-1.8)	.10

Table 59:
Effect of parental variables on atopy in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Maternal variables		
Post natal depression	1.04 (1.0-1.1)	.21
Atopic	2.61 (2.1-3.2)	<.0001
In professional occupation	1.09 (.9-1.3)	.40
Has tertiary qualifications	1.07 (.8-1.4)	.63
Under 25 when twins born	1.10 (.8-1.5)	.51
Paternal variables		
Atopic	2.26 (1.8-2.8)	<.0001
In professional occupation	1.20 (1.0-1.5)	.079
Has tertiary qualifications	1.00 (.8-1.3)	.99
Under 25 when twins born	1.05 (.7-1.5)	.78

Table 60:
Effect of passive smoking exposure on atopy in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Number of smokers in household		
None	1	
One	0.96 (.8-1.2)	.72
Two or more	1.09 (.8-1.4)	.49
Other smoking variables		
Mother smoked during pregnancy	0.99 (.8-1.3)	.95
Mother ever smoked	1.05 (.8-1.3)	.68
Father ever smoked	1.08 (.9-1.3)	.48
No smoking rules in house	1.06 (.8-1.3)	.64
No smoking rules in car	1.07 (.8-1.4)	.65

Table 61:
Effect of socio-economic status on atopy in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Index of disadvantage		
Bottom 10%	1.78 (1.0-3.1)	.044
Top 10%	1.08 (.9-1.4)	.53
Index of advantage/disadvantage		
Bottom 10%	0.93 (.6-1.6)	.79
Top 10%	1.17 (.9-1.5)	.21
Index of economic resources		
Bottom 10%	1.16 (.4-3.1)	.77
Top 10%	1.06 (.8-1.4)	.70
Index of education and employment		
Bottom 10%	1.06 (.6-1.7)	.82
Top 10%	1.20 (.9-1.6)	.16

Current atopy

Table 62
Effect of individual and family characteristics on current atopy in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Individual variables		
Male gender	0.91 (.7-1.2)	.4558
Overweight	0.87 (.6-1.2)	.3582
Twin variables		
Zygosity (MZ vs DZ)	1.06 (.8-1.5)	.7304
Has older siblings	0.97 (.7-1.3)	.8089
Season of birth		
Spring	1	
Summer	0.98 (.6-1.5)	.9291
Autumn	0.74 (.5-1.1)	.4173
Winter	0.80 (.5-1.2)	.2888
Family variables		
Lives in rural area	0.70 (.5-.96)	.0270

Table 63:
Effect of prenatal, birth and perinatal factors on current atopy in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Conceived using assisted reproduction	1.29 (1.0-1.7)	.087
Periconceptional multivitamin use		
In month before pregnancy	0.93 (.6-1.4)	.70
In first 3 months of pregnancy	0.80 (.6-1.1)	.14
Complications of pregnancy		
Bleeding serious enough for bed rest	0.95 (.6-1.5)	.82
Threatened miscarriage under 20 weeks	1.53 (.9-2.6)	.12
UTI	1.13 (.6-2.2)	.73
Toxaemia	1.34 (.8-2.2)	.23
Gestational diabetes	2.29 (.6-8.2)	.20
Placenta praevia	0.72 (.3-1.7)	.45
Premature rupture of membranes	1.73 (.9-3.3)	.090
Birth variables		
Gestation over 32 weeks	0.98 (.8-1.1)	.77
Delivered by caesarean section	1.37 (1.0-1.9)	.046
Birth weight – Q1 vs. Q4	1.61 (1.1-2.4)	.015
Gestation over 32 weeks	0.98 (.8-1.1)	.77
Was admitted to NICU	1.25 (.9-1.7)	.13
Was admitted to SCN	1.21 (.9-1.6)	.19
Had respiratory distress after birth	1.24 (.9-1.8)	.22
Needed respirator after birth	1.13 (.8-1.6)	.52
Fed through naso-gastric tube after birth	1.30 (1.0-1.8)	.094

Table 64:
Effect of infant feeding on current atopy in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Length of breast feeding		
Was not breast fed	1	
Less than 3 months	0.96 (.6-1.5)	.88
At least 3 months, but less than 6 months	0.68 (.4-1.1)	.12
At least 6 months	0.66 (.4-1.01)	.058
Other milk introduced before 4 months	0.62 (.4-.8)	.0016

Table 65:
Effect of childhood conditions on current atopy in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Behaviour disorders		
ADHD (hyperactive type)	0.99 (.7-1.5)	.96
ADHD (inattentive type)	1.18 (.7-1.9)	.49
Conditions diagnosed by a doctor		
Febrile convulsions	1.08 (.5-2.2)	.84
Migraines	1.12 (.7-1.8)	.64
At least one episode of otitis media	1.27 (1.0-1.7)	.11
ENT operations		
Had tonsils removed	1.25 (.8-2.0)	.33
Had adenoids removed	1.23 (.8-1.9)	.35
Had grommets inserted	1.37 (.9-2.2)	.17

Table 66:
Effect of parental variables on current atopy in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Maternal variables		
Post natal depression	1.01 (.9-1.1)	.88
Atopic	1.55 (1.2-2.1)	.0046
In professional occupation	1.04 (.8-1.4)	.77
Has tertiary qualifications	1.26 (.9-1.8)	.21
Under 25 when twins born	0.89 (.6-1.5)	.58
Paternal variables		
Atopic	1.45 (1.1-1.9)	.011
In professional occupation	1.21 (.9-1.6)	.20
Has tertiary qualifications	1.54 (1.1-2.2)	.023
Under 25 when twins born	0.47 (.3-.8)	.0020

Table 67:
Effect of passive smoking exposure on current atopy in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Number of current smokers in household		
None	1	
One	0.86 (.6-1.2)	.42
Two or more	0.74 (.5-1.2)	.18
Other smoking variables		
Mother smoked during pregnancy	0.84 (.6-1.2)	.35
Mother ever smoked	0.89 (.7-1.2)	.49
Mother current smoker	0.72 (.5-1.1)	.10
Father ever smoked	0.94 (.7-1.3)	.66
Father current smoker	0.90 (.6-1.3)	.53
No smoking rules in house	0.99 (.7-1.4)	.96
No smoking rules in car	1.02 (.7-1.5)	.94

Table 68:
Effect of socio-economic status on current atopy in WA twins aged 6-18 years, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Index of disadvantage		
Bottom 10%	0.79 (.4-1.6)	.53
Top 10%	1.26 (.9-1.7)	.17
Index of advantage/disadvantage		
Bottom 10%	0.71 (.3-1.5)	.37
Top 10%	1.16 (.8-1.6)	.41
Index of economic resources		
Bottom 10%	1.15 (.2-6.1)	.87
Top 10%	1.40 (.9-2.1)	.10
Index of education and employment		
Bottom 10%	0.68 (.3-1.4)	.27
Top 10%	1.40 (1.0-2.0)	.078

APPENDIX 7:

SUPPLEMENTARY TABLES OF THE UNIVARIATE GEE ANALYSIS OF TWIN-FAMILY DATA

Doctor-diagnosed asthma

Table 69:
Effect of individual and family characteristics on DDA in WA twin families, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Individual variables		
Child	1.18 (.7, 2.0)	.53
Male gender	0.67 (.5, .9)	.0011
Child*sex interaction	1.88 (.1.4, 2.5)	<.001
Overweight	1.17 (1.0, 1.4)	.074
Family variables		
Lives in rural area	0.80 (.7, 1.0)	.028

Table 70:
Effect of health conditions on DDA in WA twin families, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Migraines	1.34 (1.1, 1.7)	.017
At least one episode of otitis media	1.22 (1.0, 1.5)	.031
Had tonsils removed	1.61 (1.3, 2.0)	<.001
Had adenoids removed	1.64 (1.3, 2.0)	<.001
Had grommets inserted	1.23 (.9, 1.6)	.15

Table 71:
Effect of smoking on DDA in WA twin families, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
No active or passive smoke exposure	1	
Lives with one smoker	1.07 (.8, 1.4)	.55
Lives with two or more smokers	1.04 (.8, 1.3)	.74
Active smoker	0.96 (.8, 1.2)	.76
No smoking rules in home	1.11 (.9, 1.4)	.35
No smoking rules in car	0.98 (.8, 1.2)	.88

Table 72:
Effect of socio-economic status on DDA in WA twin families, after adjusting for age

VARIABLE	OR 95% CI	P-VALUE
Index of disadvantage		
Bottom 10%	1.74 (1.2, 2.6)	.0088
Top 10%	0.96 (.8, 1.2)	.69
Index of advantage/disadvantage		
Bottom 10%	1.34 (.9, 2.0)	.15
Top 10%	1.03 (.8-1.3)	.78
Index of economic resources		
Bottom 10%	2.12 (1.0, 4.6)	.046
Top 10%	1.11 (.9-1.4)	.40
Index of education and employment		
Bottom 10%	1.31 (.9, 1.9)	.16
Top 10%	1.11 (.9-1.4)	.39

APPENDIX 8:

CODE FOR BUGS PROGRAMME FOR TWIN AD MODEL

FILE NAME: dda.AD.twin.pair.GEE.mu.odc

-real data, twins only
-phenotypic data from both parents
-AD model
-includes intercept
-fixed effects: covariates as determined by GEE models

SEED

3879401

MODEL

```
model
{
#dummy use of input variables

temp1<-fam[1];
temp2<-fam.orig[1];
temp3<-id[1];
temp4<-dda[1];
temp5<-mz[1];
temp6<-age[1];
temp7<-int[1] ;
temp8<-passive1[1];
temp9<-passive2[1];
temp10<-sex[1] ;
temp11<-older[1];
temp12<-rural[1];
temp13<-miscarr[1];
temp14<-om[1] ;
temp15<-tonsil[1] ;
temp16<-mdda[1];
temp17<-fdda[1] ;
temp18<-disadvlo[1];
temp19<-econlo[1];


#input transformations
for(k in 1:NUMSUBS)
{
phenobin[k]<-equals(dda[k],1);
}

#create random effects
for(j in 1:NUMFAMS)
{
mz.set1[j]~dnorm(0,taumz);
```

```
dz.set1[j]~dnorm(0,taudz);

}

for(k in 1:NUMSUBS)
{
UNSHARED.dz[k]~dnorm(0,taua2.cut);
}

for(k in 1:NUMSUBS)
{
fixed[k]<-mu + beta.age*age[k]+ beta.int*int[k]+ beta.passive1*passive1[k]+
          beta.passive2*passive2[k]+ beta.sex*sex[k]+ beta.older*older[k]+
          beta.rural*rural[k]+ beta.miscarr*miscarr[k]+ beta.om*om[k]+
          beta.tonsil*tonsil[k]+beta.mdda*mdda[k]+ beta.fdda*fdda[k]+
          beta.disadvlo*disadvlo[k]+ beta.econlo*econlo[k];


random.mz[k]<-equals(id[k],21)*equals(mz[k],1)*mz.set1[fam[k]]+
              equals(id[k],22)*equals(mz[k],1)*mz.set1[fam[k]];

random.dz1[k]<-equals(id[k],21)*equals(mz[k],0)*dz.set1[fam[k]]+
               equals(id[k],22)*equals(mz[k],0)*dz.set1[fam[k]];

random.dz2[k]<-equals(id[k],21)*equals(mz[k],0)*UNSHARED.dz[k]+
               equals(id[k],22)*equals(mz[k],0)*UNSHARED.dz[k];


logit(lp[k])<-fixed[k]+random.mz[k]+random.dz1[k]+random.dz2[k];

phenobin[k]~dbern(lp[k]);
}

#priors
taumz ~ dgamma(0.0001,0.0001);
taudz ~  dgamma(0.0001,0.0001);

sigma2mz<-1/taumz;
sigma2dz<-1/taudz;
sigma2a<-4*sigma2dz-sigma2mz;
sigma2d<-2*sigma2mz-4*sigma2dz;

sigma2unshared<-SIGMA2A/2 + 3*SIGMA2D/4;
taua2.cut<-1/sigma2unshared;

mu ~ dnorm(0,1.0E-6);
beta.age ~ dnorm(0,1.0E-6);
beta.int ~ dnorm(0,1.0E-6);
beta.passive1 ~ dnorm(0,1.0E-6);
beta.passive2 ~ dnorm(0,1.0E-6);
beta.sex ~ dnorm(0,1.0E-6);
beta.older ~ dnorm(0,1.0E-6);
beta.rural ~ dnorm(0,1.0E-6);
```

```
beta.miscarr ~ dnorm(0,1.0E-6);
beta.om ~ dnorm(0,1.0E-6);
beta.tonsil ~ dnorm(0,1.0E-6);
beta.mdda ~ dnorm(0,1.0E-6);
beta.fdda ~ dnorm(0,1.0E-6);
beta.disadvlo ~ dnorm(0,1.0E-6);
beta.econlo ~ dnorm(0,1.0E-6);

#Output transformations
main[1]<-mu;
main[2]<-beta.age;
main[3]<-beta.int;
main[4]<-beta.passive1;
main[5]<-beta.passive2;
main[6]<-beta.sex;
main[7]<-beta.older;
main[8]<-beta.rural
main[9]<-beta.miscarr;
main[10]<-beta.om;
main[11]<-beta.tonsil;
main[12]<-beta.mdda;
main[13]<-beta.fdda;
main[14]<-beta.disadvlo;
main[15]<-beta.econlo;
main[16]<-sigma2a;
main[17]<-sigma2d;
}
```

DATA

```
list(NUMSUBS=2034,NUMFAMS=1017,SIGMA2A=0.0001,SIGMA2D=0.0001)

<data file here>
```

INITS

```
list(mu=0, beta.age=0, beta.int=0, beta.passive1=0, beta.passive2=0,
    beta.sex=0, beta.older=0, beta.rural=0, beta.miscarr=0, beta.om=0,
    beta.tonsil=0, beta.mdda=0, beta.fdda=0, beta.disadvlo=0, beta.econlo=0,
    taumz=0.5, taudz=1);
```

APPENDIX 9:

CODE FOR BUGS PROGRAMME FOR TWIN-FAMILY ACC_S MODEL

FILE NAME: dda.ACCs.family.GEE.new

-uses complete family data
-zygosity known
-one set of twins per family
-twins + mother + father + sibs

SEED
285472

MODEL

model

{

#dummy use of input vectors
temp1<-fam[1];
temp2<-fam.orig[1];
temp3<-id[1];
temp4<-dda[1];
temp5<-age[1];
temp6<-agelnage[1];
temp7<-mz[1];
temp8<-passive[1];
temp9<-passive0[1];
temp10<-passive1[1];
temp11<-passive2[1];
temp12<-passive3[1];
temp13<-child[1];
temp14<-sex[1];
temp15<-childsex[1];
temp16<-rural[1];
temp17<-tonsil[1];
temp18<-disadvlo[1];
temp19<-econlo[1];

#input transformations
for(k in 1:NUMSUBS)

{

phenobin[k]<-equals(dda[k],1);

}

```
#Create random effects
for(j in 1:NUMFAMS)

{
f[j]~dnorm(0,tauf);
g[j]~dnorm(0,taug);
h[j]~dnorm(0,tauh);
mz.extra[j]~dnorm(0,taumzextra);

}

for(k in 1:NUMSUBS)

{
UNSHARED.PARENT[k]~dnorm(0,TAUPARENT.cut);
UNSHARED.CHILD[k]~dnorm(0,TAUCHILD.cut);

}

for(k in 1:NUMSUBS)

{
#fixed effects
fixed[k]<-          mu + beta.age*age[k] + beta.lnage*agelnage[k] +
                    beta.passive3*passive3[k] + beta.passive2*passive2[k] +
                    beta.passive1*passive1[k] + beta.child*child[k] +
                    beta.sex*sex[k] +       beta.childsex*childsex[k] +
                    beta.rural *rural[k] +  eta.tonsil*tonsil[k] +
                    beta.disadvlo*disadvlo[k] +beta.econlo*econlo[k];

#random effects
random1[k]<-        f[fam[k]]+
                    step(id[k]-20)*h[fam[k]]+
                    equals(id[k],10)*(-g[fam[k]]+UNSHARED.PARENT[k])+
                    equals(id[k],11)*( g[fam[k]]+UNSHARED.PARENT[k]);

random2[k]<-        equals(id[k],21)*equals(mz[k],1)*(mz.extra[fam[k]])+
                    equals(id[k],22)*equals(mz[k],1)*(mz.extra[fam[k]]);

random3[k]<-        equals(id[k],21)*equals(mz[k],0)*(UNSHARED.CHILD[k])+
                    equals(id[k],22)*equals(mz[k],0)*(UNSHARED.CHILD[k]);

random4[k]<-        step(id[k]-60)*(UNSHARED.CHILD[k]);

logit(pmean[k])<-fixed[k] + random1[k] + random2[k] + random3[k] +
                    random4[k];


phenobin[k]~dbern(pmean[k]);

}
```

#Priors

```
mu ~ dnorm(0,1.0E-6);
beta.age ~ dnorm(0,1.0E-6);
beta.lnage ~ dnorm(0,1.0E-6);
beta.sex ~ dnorm(0,1.0E-6);
beta.child ~ dnorm(0,1.0E-6);
beta.childsex ~ dnorm(0,1.0E-6);
beta.passive1 ~ dnorm(0,1.0E-6);
beta.passive2 ~ dnorm(0,1.0E-6);
beta.passive3 ~ dnorm(0,1.0E-6);
beta.tonsil ~ dnorm(0,1.0E-6);
beta.rural ~ dnorm(0,1.0E-6);
beta.disadvlo ~ dnorm(0,1.0E-6);
beta.econlo ~ dnorm(0,1.0E-6);

tauf~dpar(1,0.01);
taug~dpar(1,0.01);
tauh~dpar(1,0.01);
taumzextra~dpar(1,0.01);

sigma2a<-2/taug;
sigma2c<-1/tauf - sigma2a/2;
sigma2cs<-1/tauh;
sigma2mzextra<-1/taumzextra;

sigma2unsharedparent<-sigma2cs;
sigma2unsharedchild<-sigma2a/2;

tauparent<-1/sigma2unsharedparent;
tauchild<-1/sigma2unsharedchild;

TAUPARENT.cut<-cut(tauparent);
TAUCHILD.cut<-cut(tauchild);
```

#Output transformations

```
main[1]<-mu
main[2]<-beta.age
main[3]<-beta.lnage
main[4]<-beta.passive3
main[5]<-beta.passive2
main[6]<-beta.passive1
main[7]<-beta.child
main[8]<-beta.sex
main[9]<-beta.childsex
main[10]<-beta.rural
main[11]<-beta.tonsil
main[12]<-beta.disadvlo
main[13]<-beta.econlo
main[14]<-sigma2a
main[15]<-sigma2c
main[16]<-sigma2cs

}
```

```
DATA
list(NUMSUBS=5346,NUMFAMS=1107)
<data file here>

INITS
list(mu=0, beta.age=0, beta.lnage=0, beta.sex=0, beta.child=0,
beta.childsex=0,
      beta.passive1=0, beta.passive2=0, beta.passive3=0,  beta.tonsil=0,
      beta.rural=0, beta.disadvlo=0, beta.econlo=0, tauf=1,taug=1,tauh=1,
      taumzextra=2);
```

APPENDIX 10:

CODE FOR BUGS PROGRAMME FOR TESTING THE VALIDITY OF THE EEA

FILE NAME: dda.ACD.family.GEE.eea
-uses complete family data
-zygosity known
-one set of twins per family
-twins + mother + father + sibs
-add in sigma2mz.environment to test the EEA

SEED
397546

MODEL

```
model
{
#dummy use of input vectors
temp1<-fam[1];
temp2<-fam.orig[1];
temp3<-id[1];
temp4<-dda[1];
temp5<-age[1];
temp6<-agelnage[1];
temp7<-mz[1];
temp8<-passive[1];
temp9<-passive0[1];
temp10<-passive1[1];
temp11<-passive2[1];
temp12<-passive3[1];
temp13<-child[1];
temp14<-sex[1];
temp15<-childsex[1];
temp16<-rural[1];
temp17<-tonsil[1];
temp18<-disadvlo[1];
temp19<-econlo[1];

#input transformations
for(k in 1:NUMSUBS)
{

phenobin[k]<-equals(dda[k],1);
}
```

```
#Create random effects
for(j in 1:NUMFAMS)
{
f[j]~dnorm(0,tauf);
g[j]~dnorm(0,taug);
h[j]~dnorm(0,tauh);
mz.extra[j]~dnorm(0,tau.mz.extra.total);

}

for(k in 1:NUMSUBS)
{
UNSHARED.PARENT[k]~dnorm(0,tauparent.cut);
UNSHARED.CHILD[k]~dnorm(0,tauchild.cut);

}

for(k in 1:NUMSUBS)
{

#fixed effects
fixed[k]<-      mu + beta.age*age[k] +beta.lnage*agelnage[k] +
                beta.passive3*passive3[k] + beta.passive2*passive2[k] +
                beta.passive1*passive1[k] + beta.child*child[k] +
                beta.sex*sex[k] + beta.childsex*childsex[k] +
                beta.rural*rural[k] + beta.tonsil*tonsil[k] +
                beta.disadvlo*disadvlo[k] +beta.econlo*econlo[k];

#random effects
random1[k]<- f[fam[k]]+
                step(id[k]-20)*h[fam[k]]+
                equals(id[k],10)*(-g[fam[k]]+UNSHARED.PARENT[k])+
                equals(id[k],11)*( g[fam[k]]+UNSHARED.PARENT[k]);

random2[k]<-equals(id[k],21)*equals(mz[k],1)*(mz.extra[fam[k]])+
                equals(id[k],22)*equals(mz[k],1)*(mz.extra[fam[k]]);

random3[k]<-equals(id[k],21)*equals(mz[k],0)*(UNSHARED.CHILD[k])+
                equals(id[k],22)*equals(mz[k],0)*(UNSHARED.CHILD[k]);

random4[k]<-step(id[k]-60)*(UNSHARED.CHILD[k]);

logit(pmean[k])<- fixed[k] + random1[k] + random2[k] + random3[k] +
                random4[k];


phenobin[k]~dbern(pmean[k]);

}
```

```
#Priors
mu ~ dnorm(0,1.0E-6);
beta.age~ dnorm(0,1.0E-6);
beta.lnage~ dnorm(0,1.0E-6);
beta.sex ~ dnorm(0,1.0E-6);
beta.child ~ dnorm(0,1.0E-6);
beta.childsex ~ dnorm(0,1.0E-6);
beta.passive1 ~ dnorm(0,1.0E-6);
beta.passive2 ~ dnorm(0,1.0E-6);
beta.passive3 ~ dnorm(0,1.0E-6);
beta.tonsil ~ dnorm(0,1.0E-6);
beta.rural ~ dnorm(0,1.0E-6);
beta.disadvlo ~ dnorm(0,1.0E-6);
beta.econlo~ dnorm(0,1.0E-6);

tauf~dpar(1,0.01);
taug~dpar(1,0.01);
tauh~dpar(1,0.01);
tau.mz.extra.total~dpar(1,0.01);

sigma2a<-2/taug;
sigma2c<-1/tauf - sigma2a/2;
sigma2d<-4/tauh;
sigma2.mz.extra.total<-1/tau.mz.extra.total;

sigma2unsharedparent.temp<-SIGMA2D;
sigma2unsharedchild.temp<-(SIGMA2A/2)+(3*SIGMA2D/4);

#remove negative traps
sigma2unsharedparent<-max(sigma2unsharedparent.temp, 0.0001);
sigma2unsharedchild<-max(sigma2unsharedchild.temp, 0.0001);

tauparent<-1/sigma2unsharedparent;
tauchild<-1/sigma2unsharedchild;

tauparent.cut<-cut(tauparent);
tauchild.cut<-cut(tauchild);

sigma2.mz.extra.environment<-sigma2.mz.extra.total - (sigma2a/2) -
(3*sigma2d/4);
```

```
#Output transformations
main[1]<-mu
main[2]<-beta.age
main[3]<-beta.lnage
main[4]<-beta.passive3
main[5]<-beta.passive2
main[6]<-beta.passive1
main[7]<-beta.child
main[8]<-beta.sex
main[9]<-beta.childsex
main[10]<-beta.rural
main[11]<-beta.tonsil
main[12]<-beta.disadvlo
main[13]<-beta.econlo
main[14]<-sigma2a
main[15]<-sigma2c
main[16]<-sigma2d
main[17]<-sigma2.mz.extra.total;
main[18]<-sigma2.mz.extra.environment;
}


DATA
list(NUMSUBS=5346,NUMFAMS=1107,SIGMA2A=0.0001,SIGMA2D=0.0001)

<data file here>


INITS
list(mu=0, beta.age=0, beta.lnage=0,beta.sex=0, beta.child=0, beta.childsex=0,
        beta.passive1=0, beta.passive2=0, beta.passive3=0,beta.tonsil=0,
        beta.rural=0, beta.disadvlo=0, beta.econlo=0, tauf=1, taug=1, tauh=1,
        tau.mz.extra.total=2);
```