Optimising strategies for the detection of familial hypercholesterolaemia

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This thesis is presented for the degree of Doctor of Philosophy of The University of Western Australia

School of Medicine and Pharmacology 2016
DECLARATION

I declare that this thesis is my own composition and that all sources have been acknowledged. For any work that has been published with other authors, I have obtained permission from all co-authors to include this work in my thesis.

Signed

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ABSTRACT

**Background:** Familial hypercholesterolaemia (FH) is an autosomal co-dominant disorder of lipoprotein metabolism characterised by elevated low-density lipoprotein cholesterol (LDL-cholesterol) and premature coronary artery disease (CAD). FH fulfils the screening criteria for a medical condition described by the World Health Organization. However, in spite of this, most countries have not implemented systematic screening programs for FH, and only a minority of individuals with FH are currently diagnosed.

**Hypothesis:** The care of individuals with FH can be enhanced by employing laboratory, primary care and specialist lipid services.

**Aims and scope:** To examine the hypothesis, a series of studies were conducted to explore the potential a community laboratory has to screen for FH and to investigate methods to improve FH detection. A survey of general practitioner (GP) awareness and knowledge of FH was conducted, and the GPs ability to accurately use the Dutch Lipid Clinic Network Criteria (DLCNC) to identify individuals at high and low risk of FH assessed. The spectrum of mutations causative of FH in Western Australia was described and assessed in relation to a phenotypic diagnosis of FH. Finally, a study was performed to determine the yield and effectiveness of genetic cascade screening for FH in Western Australia.

**Results:** Community laboratories perform large numbers of LDL-cholesterol measurements, predominantly requested by GPs (91.8%). An LDL-cholesterol of ≥6.5 mmol/L (present in 1:398 individuals) was selected to investigate methods to improve
FH detection. A secondary cause of elevated LDL-cholesterol was identified in 8.3% of these individuals. Laboratory interpretative comments highlighting the possibility of the diagnosis of FH were associated with significantly greater reductions in LDL-cholesterol than controls, and a trend to increased referral to a specialist. However, specialist referral rates were generally low, and only higher (11.5 vs. 1%, p<0.05) when referral was specifically included in the comment. A telephone call from the chemical pathologist to the requesting GP was associated with increased specialist referral rates (27% vs. 4%, p<0.0001). At follow-up, FH was present in 72% of individuals with an LDL-cholesterol of ≥6.5 mmol/L and 30% had identifiable mutations on genetic testing.

GP awareness of national guidelines and knowledge of hereditability, prevalence and diagnostic features of FH were found to be suboptimal, despite their perception that they were the most effective health practitioners for managing FH. The vast majority of GPs selected appropriate lipid-lowering therapy and preferred interpretative comments to alert them to the possibility of FH. There was good agreement (83.6%) in the DLCNC scores calculated by GPs and specialists. GPs accurately categorised individuals at high (86.7%) and low (94.0%) risk of FH, establishing that the DLCNC is a suitable means to augment FH detection in primary care and ensure appropriate specialist referral.

The mutation spectrum in Western Australia was similar to the UK and France. As expected, the mutation detection rate was highest in individuals with clinically definite (70%) or probable (29%) FH. Genetic cascade testing yielded two new cases of FH per index case, or three new cases if three or more family members were tested. Significant additional reductions in LDL-cholesterol (-25% overall) were achieved in individuals found to have FH despite almost half (48%) already taking statin therapy at diagnosis. Significant improvements were also seen in non-lipid CAD risk factors; 80%
of individuals with hypertension attained blood pressure targets and 40% of smokers ceased.

**Conclusions:** The studies in this thesis collectively demonstrate that laboratory, primary care and specialist lipid services can be employed to enhance the care of individuals with FH, by augmenting the detection and treatment of the condition. Interpretative comments were associated with significant LDL-cholesterol reductions, and a telephone call to the requesting GP of high-risk individuals significantly improved the detection of FH. Identifying individuals with FH remains effective and leads to additional reductions in LDL-cholesterol and improvements in non-lipid cardiovascular risk factors after specialist review. Additional research is required to further investigate methods for optimising the detection of FH and the translation of the findings into effective health policy.
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STATEMENT OF PERSONAL CONTRIBUTION

I was involved in the formulation, design, the investigation/intervention and analysis of all studies described in this thesis. I was primarily responsible for these processes for four studies: opportunistic screening for FH in a community laboratory, impact of interpretative commenting on FH detection and management, impact of a telephone call on FH detection and the effectiveness of genetic cascade screening for FH in Western Australia which are described in Chapters 3-5 and 9 of this thesis. I was responsible for the design and analysis of the study describing GPs knowledge of FH (Chapter 6), and for conducting the investigation and analysis of the study assessing GPs ability to accurately identify FH in primary care (Chapter 7).

I was also primarily responsible for writing the initial and subsequent drafts for seven of the manuscripts that form part of this thesis (publications 1, 3, 5-8 and 10 in the publication section), and for submitting these manuscripts for publication. I was co-author of the initial draft of the manuscript entitled “Screening for familial hypercholesterolaemia” that forms part of Chapter 1 with Mr R Bender, a Bachelor of Medical Science student I was co-supervising. I was also the co-author of the initial draft of the manuscript entitled “Optimising the Detection and Management of Familial Hypercholesterolaemia: Central Role of Primary Care and its Integration with Specialist Services” that forms part of Chapter 10 with Prof A Vickery.

My role in the study reported in Chapter 8, describing the mutations present in Western Australia, involved the assessment, counselling and referral of some of the patients for genetic testing, assisting with oversight of the genetic testing, validating some of the genetic results, as well as reviewing and improving drafts of the manuscript.
In addition to the above, my contribution also included:

1. Establishing and management of the community laboratory lipid database at St John of God Pathology.
2. Conducting the literature reviews for the studies.
3. I was involved with drafting the ethics approvals for the studies.
4. Programming the expert computer system to append the interpretative comments.
5. Performing statistical analysis, although more complex analysis was performed with the assistance of Ms Sally Burrows and Dr Jing Pang.
6. Performing the majority of the phone calls to the requesting general practitioners for the study in Chapter 5, Dr Glenn Edwards performed the rest of these calls.
7. Reviewing most of the patients referred to the lipid disorders clinic from the studies in Chapter 4 and 5, conducting the history and physical examination. I was also responsible for the investigation and management of these patients, as part of clinical service in my role as a consultant in the lipid disorders clinic.
8. Preparing and presenting some of this data as posters to the Cardiac Society of Australia and New Zealand meetings; namely
   a. Opportunistic detection of FH via a community laboratory.
   b. Impact of interpretative comments on laboratory reports on FH detection and LDL-cholesterol reduction.
   c. Detecting FH in the community: impact of a telephone call from the chemical pathologist to the requesting general practitioner of patients found to be at high risk.
   d. Using the Dutch lipid clinic network criteria to establish the likelihood of FH in primary care.
e. General practitioner’s knowledge and practices regarding FH in Australia.

9. Presenting some of these data at meetings of the Australasian Atherosclerosis Society’s FH Network, Australasian Association of Clinical Biochemists, Royal College of Pathologists of Australasia, and multiple local meetings at Royal Perth Hospital and at various Western Australia State Health symposia.
ACKNOWLEDGEMENTS

I am very grateful for the support and guidance of my supervisors Winthrop Professor Gerald Watts and Clinical Professor John Burnett. A PhD candidate could not hope for better supervisors. I would also specifically like to thank both supervisors for their advice and mentoring regarding my ongoing research and career.

I would like to thank the other clinical members of the Familial Hypercholesterolaemia Western Australia (FHWA) program; Clinical Associate Professor Tim Bates, Emeritus Professor Trevor Redgrave, Professor Peter O’Leary, Dr Simon Dimmitt, Dr Jing Pang, Ms Amanda Juniper, Ms Lynda Southwell, Ms Jackie Ryan and Ms Maria Vulin. I would especially like to acknowledge Dr Pang the FHWA database manager. I also acknowledge that this program and some of the data were acquired as part of clinical service at the Royal Perth Hospital lipid disorders clinic, and thank the other staff and management for allowing this. Additionally, I acknowledge the funding support for the FHWA program from the Australian Better Health Initiative, Royal Perth Hospital, PathWest Laboratory Medicine and the University of Western Australia. I would also like to thank the FH Primary care team; Professor Alistair Vickery, Dr Jacqueline Garton-Smith and Associate Professor Andrew Kirke. Furthermore, I appreciate the education, advice and assistance with genetic testing for familial hypercholesterolaemia from Prof Frank van Bockxmeer, Dr Amanda Hooper and Ms Lan Nguyen.

I would also like to thank St John of God Pathology for allowing me to undertake the community laboratory aspects of this research. I am grateful to Dr Glenn Edwards for his guidance and advice programing and using the expert computer system, LabWizard®.

XXX
I am grateful to the co-authors involved with this research, and look forward to further collaborations. I would specifically like to thank Ms Sally Burrows for all of her advice and guidance with the statistical analyses. I have enjoyed the conversations surrounding the pros and cons of various tests, which have rekindled my interest in mathematics.

Finally, I am indebted to my extended family and friends for their support and patience, as I would not have been able to complete this research without it. I would specifically like to thank my wife Katja, parents Jenny and Dave and children; Katharina, William and Henrietta - I look forward to regaining some free time to spend with you all.
# ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ABCA1</td>
<td>Adenosine triphosphate binding cassette transporter A1</td>
</tr>
<tr>
<td>ACAT</td>
<td>Acyl-coenzyme A cholesterol acyltransferase</td>
</tr>
<tr>
<td>ACC</td>
<td>American College of Cardiology</td>
</tr>
<tr>
<td>ADA</td>
<td>American Diabetes Association</td>
</tr>
<tr>
<td>ALP</td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>ApoB</td>
<td>Apolipoprotein B</td>
</tr>
<tr>
<td>ApoC</td>
<td>Apolipoprotein C</td>
</tr>
<tr>
<td>ApoE</td>
<td>Apolipoprotein E</td>
</tr>
<tr>
<td>AUD</td>
<td>Australian dollar</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>CETP</td>
<td>Cholesteryl ester transfer protein</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>CKD</td>
<td>Chronic kidney disease</td>
</tr>
<tr>
<td>Chol</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>CIMT</td>
<td>Carotid intima medial thickness</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>DLCNC</td>
<td>Dutch Lipid Clinic Network Criteria</td>
</tr>
<tr>
<td>DLCNCS</td>
<td>Dutch Lipid Clinic Network Criteria Score</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>FDB</td>
<td>Familial defective apolipoprotein B-100</td>
</tr>
<tr>
<td>FH</td>
<td>Familial hypercholesterolaemia</td>
</tr>
<tr>
<td>FRACGP</td>
<td>Fellowship of the Royal Australasian College of General Practitioners</td>
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</table>
GGT \(\gamma\) glutamyltransferase
GP General Practitioner
HbA1c Haemoglobin A1c, glycosylated haemoglobin
HDL High density lipoprotein
HMG-CoA 3-Hydroxy-3-methyl-glutaryl coenzyme A
IDL Intermediate density lipoprotein
IQR Interquartile range
LDL Low density lipoprotein
LDLR Low density lipoprotein receptor
LDLR \(LDLR\) Low density lipoprotein receptor gene
LDLRAp1 Low density lipoprotein receptor adaptor protein 1
Lp(a) Lipoprotein (a)
MED-PED Make Early Diagnosis – Prevent Early Death
MLPA Multiplex ligation-dependent probe amplification
MTP Microsomal triglyceride transfer protein
NCEP National Cholesterol Education Program
NCEP ATP III National Cholesterol Education Program Adult Treatment Panel III
NICE National Institute for Clinical Excellence
PCR Polymerase chain reaction
PCSK9 Proprotein convertase subtilisin/kexin type 9
PL Phospholipid
QALY Quality adjusted life year
RACGP Royal Australasian College of General Practitioners
SJGP St John of God Pathology
SREBP Sterol regulatory element binding protein
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>TG</td>
<td>Triglyceride</td>
</tr>
<tr>
<td>TSH</td>
<td>Thyroid stimulating hormone</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
</tr>
<tr>
<td>USPSTF</td>
<td>United States Preventive Services Task Force</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very low density lipoprotein</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WOSCOPS</td>
<td>West of Scotland Coronary Prevention Study</td>
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CHAPTER 1:
INTRODUCTION AND REVIEW OF LITERATURE

Aspects of this chapter have been published:


1.1 INTRODUCTION

Genetic, pathological and epidemiological studies have demonstrated that plasma concentrations of low density lipoprotein cholesterol (LDL-cholesterol) are directly related to the incidence of coronary events and cardiovascular deaths. Increased concentrations of plasma LDL-cholesterol are associated with an increased risk of developing atherosclerotic coronary artery disease (CAD). A small proportion of patients with very high plasma LDL-cholesterol concentrations have familial hypercholesterolaemia (FH).

FH is a relatively common autosomal dominant condition caused primarily by mutations in the LDL-receptor gene (LDLR), and characterised by markedly increased LDL-cholesterol concentrations from birth. However, worldwide the vast majority of individuals with FH are undetected and often inadequately treated. Early diagnosis and treatment of FH is important, as lipid-lowering drugs decrease atherosclerosis progression and reduce the risk of premature cardiovascular events. FH meets the World Health Organization (WHO) criteria for systematic screening, and a variety of screening approaches have been undertaken in different countries.

Australia does not have a systematic FH screening program. However, general cardiovascular screening is advocated in Australia – as it is in most western counties. This thesis will explore the potential to enhance the care of individuals with FH by effectively employing laboratory, primary care and specialist services. This chapter reviews FH from pathogenesis to the efficacy and cost-effectiveness of treatment, and then outlines the current guidelines for cardiovascular disease (CVD) screening in the general population.
1.2 FAMILIAL HYPERCHOLESTEROLAEMIA

1.2.1 Overview of FH

FH was first recognised as an inherited disorder by Müller in the late 1930s, where related individuals exhibited xanthomata, hypercholesterolaemia and premature CAD.\(^{(14)}\) Khachadurian in the 1960s demonstrated a gene-dosage effect, where homozygotes had a more severe phenotype than heterozygotes.\(^{(15)}\) A breakthrough in the understanding of the mechanism behind FH came in the mid 1970s, when Brown and Goldstein elucidated the LDL receptor pathway and showed that defects in the LDL receptor (LDLR) cause FH.

FH has been estimated to affect 1 in 500 individuals worldwide,\(^{(3)}\) but the prevalence has more recently been shown to be between 1 in 200\(^{(16,17)}\) and 1 in 300\(^{(18)}\). FH is more prevalent in certain populations, where founder effects have led to 1 in 70 Afrikaners, 1 in 170 Christian Lebanese, and 1 in 270 Québécois carrying an FH-causing mutation.\(^{(19,20)}\) The main biochemical abnormality observed in FH is an elevated LDL-cholesterol concentration, predominantly due to reduced function of the LDLR pathway, which removes LDL particles from the circulation into the liver. If untreated, patients are at high risk of premature atherosclerotic CAD, which develops in \(~50\%\) of males by age 50 and 30\% of females by age 60 years.\(^{(4)}\)

1.2.2 Pathophysiology of FH

1.2.2.1 Endogenous lipoprotein pathway

Lipids (cholesterol, triglyceride, phospholipids and cholesterol esters) circulate in the plasma in lipoproteins due to their hydrophobic nature. Lipoproteins are complexes of lipids with a central hydrophobic core surrounded by more polar lipids stabilised by apolipoproteins.\(^{(21)}\) Apolipoproteins also act as ligands for receptor mediated metabolic processes.\(^{(22-24)}\)
Lipoprotein transport can be separated into two pathways.\(^{25}\) The exogenous lipoprotein pathway transports dietary lipid (predominantly triglyceride) to the peripheral tissues, whereas the endogenous lipoprotein pathway transports hepatically synthesised lipid to the peripheral tissues for utilisation.\(^{25}\)

Low density lipoprotein is produced as part of the endogenous lipoprotein pathway (Figure 1). Very low density lipoprotein (VLDL) is synthesised in the liver as a lipid-poor nascent VLDL, which then acquires lipid through the interaction of microsomal triglyceride transfer protein (MTP) and apolipoprotein B100 (apoB100), apolipoprotein E (apoE) and apolipoprotein C (apoC - CI, CII and CIII).\(^{26}\) The VLDL contains \(~60\%\) triglyceride, \(15\%\) phospholipid, \(10\%\) cholesterol, \(10\%\) protein – predominantly apoB100 and \(5\%\) cholesterol ester. VLDL is hydrolysed in the circulation through the interaction of apoCII with lipoprotein lipase to release triglyceride to the peripheral tissues. Through this process VLDL is transformed into intermediate density lipoprotein (IDL). IDL is further hydrolysed by hepatic lipase to form LDL, which contains \(37\%\) cholesterol, \(10\%\) triglycerides and apoB100.\(^{26}\) There is one molecule of apoB100 on each of the lipoproteins in the endogenous pathway. ApoB100 is essential for the assembly and secretion of VLDL and provides the structural stability for LDL.\(^{26}\)
The endogenous lipoprotein pathway starts with the formation of nascent VLDL particles that contain apolipoprotein B100. Triglyceride, phospholipids and cholesterol are added to the nascent VLDL through the interaction of MTP and apoB100 to form mature VLDL. VLDL then enters the circulation and triglycerides are removed by interaction of lipoprotein lipase and apoCII, and the VLDL is converted into the smaller IDL. The majority of IDL is converted into LDL after further triglyceride is removed by hepatic lipase, although some is catabolised by the liver. LDL can be removed from the circulation via endocytosis as described in Figure 2.
1.2.2.2 LDL receptor

Goldstein and Brown co-discovered the LDLR while investigating the aetiology of FH.\textsuperscript{(22)} They also introduced three fundamental concepts to cell biology: receptor mediated endocytosis, receptor recycling and receptor feedback regulation.\textsuperscript{(22)} The LDLRs cluster on the cell membrane in clathrin coated pits.\textsuperscript{(27)} Circulating LDL binds with the LDLR via apoB, and the LDLR-LDL complex is then internalised by endocytosis. The endosomes then become acidic though the activity of adenosine triphosphate dependent proton pumps in the endosome wall. The lowered pH causes the LDLR – LDL receptor complex to dissociate.\textsuperscript{(22, 28)} The LDLR then leaves the endosome and migrates back to the cell membrane, while the LDL is delivered to the lysosome and is degraded and hydrolysed releasing free cholesterol.\textsuperscript{(28)} The accumulation of free cholesterol then inactivates the sterol regulatory element binding protein (SREBP), which is a transcription factor for the expression of genes responsible for both cholesterol and LDLR synthesis.\textsuperscript{(29, 30)} The LDLR pathway is represented in Figure 2.

1.2.2.3 Proprotein convertase subtilisin/kexin type 9

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a convertase that controls the degradation of the LDLR. PCSK9 is produced by the hepatocyte, and once secreted can bind to the extracellular epidermal growth factor domain A of the LDLR on the surface of the hepatocyte. The LDLR/PCSK9 complex is then internalised via clathrin dependant endocytosis. The bound PCSK9 prevents the recycling of the LDLR, and directs the receptor towards lysosomal degradation.\textsuperscript{(31)} The control of degradation explains why gain-of-function mutations in PCSK9 cause elevations in LDL-cholesterol,\textsuperscript{(32)} and loss-of-function mutations lower LDL-cholesterol and CAD risk.\textsuperscript{(33)}
FIGURE 2. LDL RECEPTOR PATHWAY

LDL – Low density lipoprotein, HMG Co A reductase – 3-Hydroxy-3-methyl-glutaryl coenzyme A reductase, ACAT – acyl-coenzyme A cholesterol acyltransferase.

Circulating LDL particles bind to the LDLR in the clathrin coated pits of the hepatocyte through an interaction between the LDLR and apoB100. The LDLR-LDL complex is then internalised by endocytosis. The endosome becomes acidic and the LDLR-LDL dissociate. The LDL particle is degraded and hydrolysed to release free cholesterol. The accumulation of free cholesterol then inactivates the sterol regulatory element binding protein (SREBP), which is a transcription factor for the expression of genes responsible for both cholesterol synthesis and the synthesis of the LDLR. Reproduced from Goldstein and Brown, 2009 with permission.\(^\text{22}\)
1.2.3 Molecular pathology

More than 1,000 mutations causing FH have been reported; these span the entire \textit{LDLR} gene, and range from single nucleotide substitutions through to large structural rearrangements.\(^2, 34-36\) While the majority of FH is caused by mutations in the \textit{LDLR}, amino acid changes in apoB-100 also lead to a form of FH called familial ligand-defective apoB-100 (FDB).\(^37\) As well as having an important structural role in LDL, apoB-100 acts as a ligand for the LDLR to facilitate clearance of LDL particles from plasma. Rare heterozygous gain-of-function mutations in the \textit{PCSK9} gene cause a severe form of FH by causing accelerated degradation of the LDLR, while mutations in the LDLR adaptor protein 1 (LDLRAP1) can lead to a rare form of autosomal recessive hypercholesterolaemia.\(^6, 38\)

1.2.3.1 Molecular testing for FH

More than 1200 variants have been reported in the \textit{LDLR} gene, although only 79\% are recognised to be pathogenic.\(^39\) Methods used for screening for FH mutations vary between laboratories, but usually involve either exon-by-exon sequencing analysis, mutation scanning techniques, or other methods to identify commonly occurring mutations in the target population, usually in combination with multiplex ligation-dependent probe amplification (MLPA). MLPA is used to detect large deletions and duplications that would usually escape detection by conventional DNA sequence analysis due to the PCR amplification of the remaining normal allele. Mutation scanning techniques such as denaturing high-performance liquid chromatography,\(^40, 41\) and high resolution melt analysis\(^42, 43\) with mutation confirmation by DNA sequencing can be used as cost-effective means of detecting mutations. Splice site mutations, which can cause exon skipping, retention of an intron, or activation of cryptic sites during mRNA splicing, can also be studied using cDNA sequence analysis.\(^44\)
Multiplex MassARRAY spectrometry\(^{(45)}\) and amplification refractory mutation system\(^{(46)}\) techniques have been reported that enable the rapid detection of 56 and 20 of the commonly occurring FH-causing mutations in the United Kingdom (UK), respectively. In addition, a DNA microarray (LIPOchip) is in use in Spain that rapidly detects 240 \(LDLR\) mutations, three \(APOB\) mutations and six \(PCSK9\) mutations, as well as \(LDLR\) copy number variation\(^{(47)}\). The LIPOchip has subsequently been refined for use in Europe\(^{(48)}\). Recently, other FH microarrays have been developed for use in combination with MLPA\(^{(49, 50)}\).

Currently, the reported rates of identifying a pathogenic mutation in genetic screening programs of patients with clinical FH range from 50% to 90%, depending on the methods of clinical diagnosis and genetic screening\(^{(11, 35, 46, 51, 52)}\). Once a mutation has been identified within an FH index case, family members can be easily screened; 50% of first-degree relatives would be expected to carry the mutation. The 10% to 50% of individuals with clinical or phenotypic FH in whom a mutation is not detected may have either an unidentified mutation, a mutation in another as yet undiscovered gene, or polygenic hypercholesterolaemia. Identifying individuals with an FH phenotype caused by the accumulation of multiple common variants that cause a small increase in LDL-cholesterol has been proposed using the LDL-cholesterol gene score, which calculates a weighted LDL-cholesterol gene score combining the known minor alleles\(^{(53, 54)}\).

Genetic testing for FH is recommended by most\(^{(11, 55-57)}\) but not all FH guidelines\(^{(58)}\). This is discussed more thoroughly in Chapters 8 and 9. However, in general FH genetic testing is of a very high quality\(^{(59)}\) and is acceptable to patients\(^{(60)}\), although there are issues around access to genetic screening, even within a single health system\(^{(61)}\).

Targeted or whole exome sequencing can be performed using next generation sequencing, which may offer further insights and efficiencies into FH detection\(^{(62, 63)}\).
However, the reports to-date have demonstrated relatively poor detection yields, either when used after traditional sequencing,\(^{(64)}\) or in a high-risk population.\(^{(65)}\) Further work in this field is required before any firm conclusions can be made.

**1.2.3.2 Pathogenicity of FH mutations**

FH is clinically classified by elevations in LDL-cholesterol, although individuals have been described with mutations in the \(LDLR\) but with an LDL-cholesterol below the \(75^{\text{th}}\) percentile of the normal population.\(^{(66)}\) This may be secondary to the inheritance of another variant that causes reduced LDL-cholesterol\(^{(67-70)}\) or inter-current illness, but it may signify that the mutation is not pathogenic – and does not cause elevated LDL-cholesterol.\(^{(71)}\) The pathogenicity of a mutation may be judged clinically by comparing the lipid profiles of individuals with and without the mutation. However, this relies on clinical judgment and a large (50+ member) pedigree.\(^{(71)}\) Huijgen et al describe a mutation as non-pathogenic if the mean LDL-cholesterol was not above the \(75^{\text{th}}\) percentile for age and gender, the LDL-cholesterol was not higher in carriers than non-carriers and the proportion of carriers on lipid-lowering therapy was not higher than non-carriers.\(^{(71)}\)

Establishing the pathogenicity of a variant is very important, as the CVD risk was higher in individuals with pathogenic mutations compared to non-pathogenic mutations.\(^{(72)}\) Indeed, individuals with non-pathogenic variants have a similar survival to family members without the variant, confirming their classification as non-pathogenic.\(^{(72)}\) There are web-based computer prediction tools (e.g. MutationTaster, PolyPhen, SIFT) to estimate the pathogenicity of mutations, which may be referred to as in silico analysis.\(^{(35, 71, 73)}\) However, these are not always accurate, and can falsely label mutations as likely or unlikely pathogenic.\(^{(72)}\) Mutations may be classified based on their phenotypic effects on the LDLR protein: Class 1 mutations fail to produce any LDLR protein (null alleles). Class 2 mutations affect the transport between the
endoplasmic reticulum and Golgi apparatus (transport defective). Class 3 mutations fail to bind LDL (binding defective). Class 4 mutations are unable to cluster in the clathrin-coated pits and internalised into the cell (internalisation defective) and Class 5 mutations fail to discharge the LDLR in the endosome and are not recycled to the cell surface (recycling defective).\(^{(19)}\) However, functional analysis has only been performed on the minority of mutations.\(^{(19)}\)

### 1.2.4 Clinical features of FH

Heterozygous FH patients often have ~2 fold elevations in plasma LDL-cholesterol concentrations. However, there is a marked overlap between the LDL-cholesterol concentration of individuals with and without FH, owing to a combination of genotypic variation in the mutations causing FH, lifestyle factors and polymorphisms in other lipid related genes such as the \(APOE\) gene.\(^{(74)}\) The overlap in LDL-cholesterol concentration makes identifying individuals with FH more difficult, and is discussed in Chapter 3. Cholesterol may be deposited in peripheral tissues, often leading to thickening of the Achilles and extensor tendons, and the presence of arcus corneae and xanthelasmata by middle age (Figure 3). However, these do not occur in all cases, tend to develop with age, and may regress with lipid lowering therapy. More importantly, cholesteryl ester accumulation in the arterial walls leads to the development of atheroma and atherosclerotic plaques. About half of men and one third of women with FH experience a coronary event by the age of 60 years,\(^{(4, 75, 76)}\) with FH homozygotes manifesting a more severe form of the disorder. Early atherosclerosis (observed as endothelial dysfunction and increased carotid intima-media thickness) can be seen in untreated FH children.\(^{(77, 78)}\)
Patients with FH often have a strong family history of hypercholesterolaemia and/or premature CAD, or physical signs as detailed above and represented in Figure 3. Specialist laboratories can perform genetic testing to determine whether a pathogenic DNA sequence variant is present. Because \textit{LDLR} loss-of-function mutations are inherited in a co-dominant pattern and have a gene dosage effect, the inheritance of two mutated alleles gives rise to the more severe, but far less prevalent homozygous FH or compound heterozygous FH. Homozygous FH and compound heterozygous FH are found in one in a million individuals in most populations.\textsuperscript{(19)} Although this may occur in 1:30,000 Afrikaners,\textsuperscript{(79)} 1:100,000 Christian Lebanese,\textsuperscript{(80)} and 1:270,000\textsuperscript{(81)} Québécois due to the increased mutation frequency in these communities.\textsuperscript{(82)}

Homozygous FH is characterised by large increases in LDL-cholesterol (in the order of 15-24 mmol/L) and severe cutaneous and tendinous xanthomas, with marked
hypercholesterolaemia present at birth and coronary atherosclerosis occurring in childhood.\textsuperscript{(83)} Without treatment, FH homozygotes do not usually survive beyond age 30 years.\textsuperscript{(83, 84)} These patients also develop aortic stenosis and exhibit atherosclerotic plaques in the aortic root and supravalvular regions.\textsuperscript{(85)} The diagnosis and treatment of homozygous FH has been recently reviewed.\textsuperscript{(83, 86)}

1.2.5 Criteria for the diagnosis of FH

A key challenge facing the care of FH is the systematic detection of index cases. It is extremely important that FH patients are identified early and commence cholesterol-lowering therapy to reduce their cardiovascular risk. Unfortunately, the majority of FH patients are either undiagnosed or diagnosed only after the primary coronary event. There is a large phenotypic heterogeneity observed with FH, thus there is a need for accurate clinical diagnostic criteria for the early diagnosis of FH in adults.

The clinical diagnosis of FH is based on personal and family history, physical examination and plasma cholesterol concentrations. However, there are no internationally agreed criteria for the phenotypic diagnosis of FH. There are three commonly used clinical tools for diagnosing FH; the Dutch Lipid Clinic Network Criteria (DLCNC), the Simon Broome Register criteria, and the Make Early Diagnosis - Prevent Early Death (MED-PED) criteria.\textsuperscript{(87-89)} These criteria differ in their need for DNA testing and in their diagnostic effectiveness.\textsuperscript{(90)}

The DLCNC is based on personal and family history of premature CVD, family history of hypercholesterolaemia, presence of tendon xanthomata or premature arcus cornealis, plasma LDL-cholesterol levels and detection of a pathogenic mutation known to cause FH (Table 1).\textsuperscript{(87)} Points are allocated for each criterion, with the total score classifying individuals as “Definite”, “Probable” or “Possible” FH.

The DLCNC are the preferred criteria in Australasia,\textsuperscript{(55)} as the integrated numeric scoring system incorporates aspects from the history, examination and the
plasma LDL-cholesterol level, thereby providing a more sensitive method for detecting index cases with FH. The clinical utility of age-and gender-specific plasma LDL-cholesterol cut-offs for the diagnosis of first-degree relatives with FH has yet to be established in Australasia.\(^{(91)}\) The phenotypic diagnosis of FH should be based on at least two fasting plasma LDL-cholesterol measurements. The value of taking a family history of hypercholesterolaemia and premature CAD is well established, but its use is often neglected in clinical practice.\(^{(92, 93)}\) Secondary causes of hypercholesterolaemia should be excluded (e.g. primary hypothyroidism, nephrotic syndrome, cholestasis).

Very recently, a modified diagnostic criteria based on the DLCNC has been described from Wales.\(^{(94)}\) The major differences were the Welsh criteria: increased the score from 2-6 for an individual if family members had a xanthomata, had an age weighted score for personal and family history of premature CAD, a negative score for elevated triglycerides and provided criteria to adjust LDL-cholesterol to pre-treated values. There was a strong correlation between the score and the presence of an identifiable mutation, although further work and comparison with other criteria are required.\(^{(94)}\)
### TABLE 1. DUTCH LIPID CLINIC NETWORK CRITERIA

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Family history</strong></td>
<td></td>
</tr>
<tr>
<td>First degree relative with known premature coronary and/or vascular disease (males &lt;55 years, females &lt;60 years), <strong>OR</strong></td>
<td>1</td>
</tr>
<tr>
<td>First degree relative with known LDL-cholesterol &gt;95(^{th}) percentile for age and sex</td>
<td></td>
</tr>
<tr>
<td>First degree relative with tendon xanthomas and/or arcus cornealis, <strong>OR</strong></td>
<td>2</td>
</tr>
<tr>
<td>Children aged &lt;18 years with LDL-cholesterol &gt;95(^{th}) percentile for age and sex</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical history</strong></td>
<td></td>
</tr>
<tr>
<td>Patient with premature coronary artery disease (age as above)</td>
<td>2</td>
</tr>
<tr>
<td>Patient with premature cerebral or peripheral vascular disease (age as above)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Physical examination</strong></td>
<td></td>
</tr>
<tr>
<td>Tendon xanthomata</td>
<td>6</td>
</tr>
<tr>
<td>Arcus cornealis at age &lt;45 years</td>
<td>4</td>
</tr>
<tr>
<td><strong>LDL-cholesterol</strong></td>
<td></td>
</tr>
<tr>
<td>LDL-cholesterol ≥8.5 mmol/L</td>
<td>8</td>
</tr>
<tr>
<td>LDL-cholesterol 6.5-8.4 mmol/L</td>
<td>5</td>
</tr>
<tr>
<td>LDL-cholesterol 5.0-6.4 mmol/L</td>
<td>3</td>
</tr>
<tr>
<td>LDL-cholesterol 4.0-4.9 mmol/L</td>
<td>1</td>
</tr>
<tr>
<td><strong>DNA analysis</strong>: functional mutation in the (LDLR, APOB) or (PCSK9) gene</td>
<td>8</td>
</tr>
<tr>
<td><strong>Stratification</strong></td>
<td><strong>Total score</strong></td>
</tr>
<tr>
<td>Definite FH</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Probable FH</td>
<td>6-8</td>
</tr>
<tr>
<td>Possible FH</td>
<td>3-5</td>
</tr>
<tr>
<td>Unlikely FH</td>
<td>&lt;3</td>
</tr>
</tbody>
</table>
However, it should be noted that the DLCNC are not applicable to children, and their diagnosis relies on either genetic testing (where there has been a recognised pathogenic mutation detected in the parent), or serial plasma LDL-cholesterol concentrations. Recommended cut-offs for further investigation are 75th percentile LDL-cholesterol levels, being 3.0 mmol/L for boys and 3.3 mmol/L for girls.\(^{(55)}\)

The Simon Broome Register criteria (Table 2)\(^{(88)}\) are similar to the DLCNC, but do not assign a numerical value to individual diagnostic parameters. While the Simon Broome criteria are appropriate for the diagnosis of index individuals, they are specifically not recommended for the diagnosis of relatives of those with FH, as they have a higher prior probability of FH, and age-and gender-specific plasma LDL-cholesterol cut-offs are recommended.\(^{(91)}\)

The MED-PED criteria are based on validated age-adjusted serum cholesterol cut-off points, with different cut-offs used for the general population and for relatives of known FH patients (Table 3).\(^{(95)}\) These criteria describe the total and LDL-cholesterol thresholds of the general population and 1\(^{st}\), 2\(^{nd}\) and 3\(^{rd}\) degree relatives, and can be applied to screen relatives, unlike the DLCNC.
### TABLE 2. SIMON BROOME CRITERIA

#### DEFINITE FH

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value</th>
<th>Age</th>
<th>Relative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol &gt;7.5 mmol/L or LDL-cholesterol &gt;4.9 mmol/L in an adult</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol &gt;6.7 mmol/L or LDL-cholesterol &gt;4.0 mmol/L in a child (&lt;16 years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tendon xanthomata in the patient, or in a first or second degree relative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA based evidence of an LDLR, APOB or PCSK9 mutation</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### POSSIBLE FH

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value</th>
<th>Age</th>
<th>Relative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol &gt;7.5 mmol/L or LDL-cholesterol &gt;4.9 mmol/L in an adult</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol &gt;6.7 mmol/L or LDL-cholesterol &gt;4.0 mmol/L in a child (&lt;16 years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AND one of the following:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of premature myocardial infarction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50 years in a first degree relative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60 years in a second degree relative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of raised cholesterol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult first or second degree relative: total cholesterol &gt;7.5 mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child (&lt;16 years) first or second degree relative: total cholesterol &gt;6.7 mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 3. MAKE EARLY DIAGNOSIS – PREVENT EARLY DEATH (MED-PED) CRITERIA

#### Total and LDL-cholesterol criteria for diagnosing probable FH

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; degree relative</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; degree relative</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; degree relative</th>
<th>General population</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>5.7 (4.0)</td>
<td>5.9 (4.3)</td>
<td>6.2 (4.4)</td>
<td>7.0 (5.2)</td>
</tr>
<tr>
<td>20-29</td>
<td>6.2 (4.4)</td>
<td>6.5 (4.6)</td>
<td>6.7 (4.8)</td>
<td>7.5 (5.7)</td>
</tr>
<tr>
<td>30-39</td>
<td>7.0 (4.9)</td>
<td>7.2 (5.2)</td>
<td>7.5 (5.4)</td>
<td>8.8 (6.2)</td>
</tr>
<tr>
<td>≥40</td>
<td>7.5 (5.3)</td>
<td>7.8 (5.6)</td>
<td>8.0 (5.8)</td>
<td>9.3 (6.7)</td>
</tr>
</tbody>
</table>

Total cholesterol (mmol/L). LDL-cholesterol is shown in brackets (mmol/L).

### 1.2.6 Australasian model of care for FH

A multidisciplinary group from Royal Perth Hospital, The University of Western Australia and the Western Australian Department of Health has developed and implemented a comprehensive model of care in Western Australia; an initiative funded by the Australian Better Health Initiative, PathWest, Royal Perth Hospital and the University of Western Australia. The FH model of care embodied the integrated opinion of the group, as well as advice from national and international sources of expertise in the field and has been in operation since 2007. The model of care builds on other Australian recommendations on the detection and management of FH,<sup>(96-98)</sup> and has now been extended to an FH Australasia Network model of care.<sup>(55)</sup>
An executive summary of the FH model of care has been reported\(^{99}\) and the key elements were as follows: The recommended diagnostic tool to diagnose FH in adults is the DLCNC. All index cases should be offered genetic testing for FH. Testing of close family relatives (or ‘cascade screening’) employing both phenotypic and genotypic approaches should be used. Whether classified as having “possible”, “probable” or “definite” FH, all patients should have a detailed clinical assessment to investigate other cardiovascular risk factors (e.g. smoking, obesity, diabetes and hypertension), presence of symptomatic or subclinical atherosclerosis, and to exclude secondary causes of hypercholesterolaemia.

After clinical assessment, all diagnosed cases of FH should be risk stratified based on the presence of other CVD risk factors and on symptomatic or asymptomatic evidence of atherosclerotic CAD (Table 4).\(^ {55}\) Traditional CVD risk scores are not reliable to guide management for individuals with FH, particularly for younger individuals, and should not be used. Non-invasive tests for atherosclerosis should be considered and individualised to specific clinical situations.

<table>
<thead>
<tr>
<th>Cardiovascular risk</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lowest</strong></td>
<td>No other cardiovascular risk factors (e.g. smoking, obesity, diabetes, and hypertension) and negative tests for subclinical atherosclerosis.</td>
</tr>
<tr>
<td><strong>Intermediate</strong></td>
<td>At least one other cardiovascular risk factor or subclinical evidence of early atherosclerosis.</td>
</tr>
<tr>
<td><strong>Highest</strong></td>
<td>A history of symptomatic cardiovascular disease (coronary, cerebral or peripheral) and/or a revascularisation procedure, or with subclinical evidence of more advanced atherosclerosis.</td>
</tr>
</tbody>
</table>
The LDL-cholesterol and apoB treatment targets recommended in Australasian FH model of care are described in Table 5. Lower LDL-cholesterol and apoB concentrations are recommended for individuals with higher CVD risk. Plasma apoB concentrations reflect the total number of atherogenic lipoprotein particles present in the circulation. The cornerstone of treatment for FH is pharmacotherapy, but diet and lifestyle should also be optimised. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are by far the most common and effective drugs to treat FH.(12, 13, 100-108) Statins decrease atherosclerotic CAD(12, 13, 104, 109) and have been shown to be cost-effective in the treatment of FH.(110, 111) Higher risk patients who require greater LDL-cholesterol and apoB reductions will require combination therapy to achieve therapeutic targets; especially ezetimibe,(112) but also niacin, fibrates and bile acid binding resins.(113) Therapeutic efficacy, safety, medication adherence and compliance should be monitored closely.(55)

<table>
<thead>
<tr>
<th>Cardiovascular risk</th>
<th>LDL-cholesterol (mmol/L)</th>
<th>ApoB (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest</td>
<td>&lt;4.0</td>
<td>&lt;1.3</td>
</tr>
<tr>
<td>Intermediate</td>
<td>&lt;3.0</td>
<td>&lt;1.1</td>
</tr>
<tr>
<td>Highest</td>
<td>&lt;2.0</td>
<td>&lt;0.9</td>
</tr>
</tbody>
</table>
1.2.7 Overview of treatment for FH

A diet low in animal fats and a healthy lifestyle remain the cornerstone treatment for lipid disorders in general.\(^{11, 55, 56, 58}\) Diet and lifestyle modifications are central to lipid-lowering in these FH guidelines, although the evidence for these recommendations is derived from general CVD.\(^{114-117}\) However, diet and lifestyle modifications are only usually associated with 10-15\% reductions in LDL-cholesterol.\(^{118, 119}\) Recent evidence suggests that a Mediterranean diet may have added benefit for CAD risk reduction.\(^{120}\)

It remains essential to treat the non-cholesterol cardiovascular risk factors, such as diabetes, smoking and hypertension, which remain important modifiable risk factors even for individuals with FH.\(^{121}\) Statins are the basis of pharmacologic therapy for severe hypercholesterolaemia, with evidence the reduced CVD events and mortality in individuals with\(^{13}\) and without FH.\(^{100}\) Ezetimibe lowers LDL-cholesterol by reducing cholesterol absorption via binding to the Niemann-Pick C1 like 1 receptor on the brush border of the gastrointestinal tract that results in up-regulation of the LDLR.\(^{122}\) However, whether ezetimibe reduces CVD endpoints remains to be determined, as it was not demonstrated to reduce carotid intima medial thickness in FH patients in addition to statins,\(^{123}\) although this may have related to study design. Niacin, fibrates, bile acid sequestrants and omega-3 fish oils may also play a role in lipid management.\(^{56}\)

LDL-cholesterol apheresis can lead to dramatic reductions in LDL-cholesterol via extracorporeal removal, although this is generally only reserved for individuals with severe hypercholesterolaemia and vascular disease or those with homozygous FH. An in-depth discussion of apheresis is beyond the scope of this thesis, although this has recently been reviewed.\(^{82, 124, 125}\) Furthermore, liver transplantation corrects the LDLR deficit and improves the lipid profile for patient with FH, although this is a very invasive procedure and necessitates lifelong use of immunosuppressive medication. The
role liver transplantation has in the treatment of FH is likely to diminish with the emergence of newer medications to lower LDL-cholesterol, although further research is required.\textsuperscript{(126)}

Gene therapy has been proposed for FH and was initially trailed in 1995, although this was not successful.\textsuperscript{(127)} However since that time there have been significant advances in vector stability and delivery, and also advances in knowledge of LDLR metabolism with the discovery of PCSK9. Recently there have been some encouraging results incorporating this technology in cultured hepatocytes and mice.\textsuperscript{(128)} However, a lot of questions remain and further research is required before gene therapy may become an option for FH.\textsuperscript{(129)}

1.2.7.1 LDL-cholesterol goals for individuals with FH

There is currently not an international consensus on the LDL-cholesterol targets for individuals with FH primarily due to a lack of clinical trial data. Thus different LDL-cholesterol targets are recommended by the published guidelines, as presented in Table 6. The guidelines also have different targets for children and adolescents, which tend to be less strict than the adult targets. This section will predominantly focus on the adult recommendations. The FH guidelines recommend assessment of cardiovascular risk and alter the LDL-cholesterol and apoB targets accordingly.

The Australasian guidelines recommend an LDL-cholesterol of $<$4.0 mmol/L for low-risk and $<$2.0 mmol/L for high-risk patients (Table 5 and 6).\textsuperscript{(55)} The European guidelines LDL-cholesterol targets are $<$3.5 mmol/L for children, $<$2.5 mmol/L for low-risk and $<$1.8 mmol/L for high-risk adults. The National Institute for Clinical Excellence (NICE) guidelines recommend a reduction in LDL-cholesterol of $\geq$50\%,\textsuperscript{(57)} as do the American, Canadian and International FH Foundation guidelines\textsuperscript{(56, 58, 130)} although absolute LDL-cholesterol recommendations are included (Table 6).
<table>
<thead>
<tr>
<th>Guideline</th>
<th>CAD risk category</th>
<th>LDL-cholesterol target</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Australasian</strong>&lt;sup&gt;(55)&lt;/sup&gt;</td>
<td>Low</td>
<td>&lt;4.0 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td>&lt;3.0 mmol/L</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>&lt;2.0 mmol/L</td>
</tr>
<tr>
<td><strong>European</strong>&lt;sup&gt;(11)&lt;/sup&gt;</td>
<td>General</td>
<td>&lt;2.5 mmol/L</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>&lt;1.8 mmol/L</td>
</tr>
<tr>
<td><strong>NICE</strong>&lt;sup&gt;(57)&lt;/sup&gt;</td>
<td>General</td>
<td>≥ 50% reduction from pre-treatment</td>
</tr>
<tr>
<td><strong>American (NLA)</strong>&lt;sup&gt;(58)&lt;/sup&gt;</td>
<td>General</td>
<td>≥ 50% reduction from pre-treatment</td>
</tr>
<tr>
<td></td>
<td>Secondary; general</td>
<td>&lt;4.1 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Secondary; high-risk</td>
<td>&lt;2.6 mmol/L</td>
</tr>
<tr>
<td><strong>International Foundation</strong>&lt;sup&gt;(56)&lt;/sup&gt;</td>
<td>General</td>
<td>≥ 50% reduction from pre-treatment</td>
</tr>
<tr>
<td></td>
<td>Secondary; general</td>
<td>&lt;2.5 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Secondary; high</td>
<td>&lt;1.8 mmol/L</td>
</tr>
<tr>
<td><strong>Canadian Cardiovascular Society</strong>&lt;sup&gt;(130)&lt;/sup&gt;</td>
<td>General</td>
<td>≥ 50% reduction from pre-treatment</td>
</tr>
<tr>
<td></td>
<td>Secondary</td>
<td>&lt;2.0 mmol/L</td>
</tr>
</tbody>
</table>
1.3 SCREENING FOR FH

1.3.1 Principles of screening

Wilson and Jungner described the principles and practice of screening for a medical condition in a World Health Organization guideline in 1968.\(^{(131)}\) The fundamental requirement was that the course of the disease must be modifiable or preventable by early detection and treatment or intervention. They described ten criteria that should be considered before implementing a screening program:

1. The condition sought should be an important health problem.
2. There should be an accepted treatment for patients with recognised disease.
3. Facilities for the diagnosis and treatment should be available.
4. There should be a recognisable latent or early asymptomatic stage.
5. There should be a suitable test or examination.
6. The test should be acceptable to the population.
7. The natural history of the condition, including the development from the latent to the declared disease, should be adequately understood.
8. There should be an agreed policy on whom to treat as patients.
9. The cost of case finding (including diagnosis and treatment) should be economically balanced in relation to total possible expenditure on medical care.
10. Case-finding should be a continuous process, and not a “once and for all” project.

The WHO clarified the criteria for non-communicable disease screening in 2005.\(^{(132)}\)
1.3.2 Principles of genetic screening

The WHO principles and criteria were described before genetic testing was available, and although these remain very relevant, the WHO proposed international guidelines on ethical issues in medical genetics in 1998. These guidelines reiterated the premise that the objective of genetic screening is to prevent disease or secure early diagnosis and treatment. They also reaffirm the voluntary use of genetic screening. Eight criteria were proposed:

1. Genetic screening should be voluntary, not mandatory.
2. Genetic screening should be preceded by adequate information about the purpose and possible outcomes of the screen or test and potential choices to be made.
3. Anonymous screening for epidemiological purposes may be conducted after notification of the population to be screened.
4. Results should not be disclosed to employers, insurers, schools, or others without the individual’s consent, in order to avoid possible discrimination.
5. In rare circumstances where disclosure may be in the best interests of the individual or public safety, the health provider may work with the individual towards a decision by him/herself.
6. Test results should be followed by genetic counselling, particularly when they are unfavourable.
7. If treatment or prevention exists or is available, this should be offered with a minimal delay.
8. Newborn screening should be mandatory and free of charge if the early diagnosis and treatment will benefit the newborn.
However, there are currently only a few genetic screening programs with sufficient evidence for application, and marked regional variation exists in European screening programs.\(^{(134)}\) The availability of genetic testing outside formal guidelines, which may be marketed directly to consumers or clinicians, is also a cause for considerable concern.\(^{(135)}\) Evidence-based classifications\(^{(136)}\) and frameworks\(^{(135)}\) have been proposed, and the National Institute of Health has established a registry of genetic tests.\(^{(137)}\)

FH fulfils the WHO criteria for systematic screening, as FH is a relatively common, life threatening condition that causes premature CAD, the diagnostic tests are reliable and acceptable, and pharmacotherapy with statins improves prognosis.\(^{(131)}\) Screening for FH involves assessment of LDL-cholesterol concentrations, accompanied by an inspection for physical signs of FH such as tendon xanthomata, and evaluation of family history.\(^{(97)}\) First-degree relatives of a patient with FH have a 50% likelihood of having the condition.

There is strong evidence to support genetic screening for FH, and it has been suggested that FH would meet the tier 1 evidence-based testing criteria proposed by Khoury et al.\(^{(56, 134, 136)}\) Genetic testing for FH is recommend by most,\(^{(11, 55-57, 87)}\) but not all guidelines; the National Lipid Association do not recommend routine genetic testing.\(^{(58)}\)

1.3.3 Detecting individuals with FH

1.3.3.1 Detecting the first member for a family with FH: “The index case”

A variety of strategies have been proposed to screen for FH in the population including: universal,\(^{(138-140)}\) opportunistic\(^{(57, 141, 142)}\) and targeted screening (Table 7). These are strategies discussed in Sections 1.3.3 - 1.3.8. Many of these methods require investigation to determine their effectiveness, acceptability and cost. Most strategies are healthcare driven, although the cascade screening for awareness and detection of FH (CASCADE FH) registry has direct patient access.\(^{(143)}\)
**TABLE 7. SCREENING STRATEGIES FOR FH**

<table>
<thead>
<tr>
<th><strong>Universal Screening</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Plasma cholesterol during childhood immunisation&lt;sup&gt;(138)&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>- Lipid profile on children aged 9-11 years&lt;sup&gt;(139)&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Opportunistic Screening</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Patients presenting to the GP for unrelated reasons&lt;sup&gt;(57, 141, 142)&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>- Incorporated into wellness checks</td>
<td></td>
</tr>
<tr>
<td>- Highlighting high-risk patients&lt;sup&gt;(55)&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>- Pharmacists screening people filling statin prescriptions&lt;sup&gt;(55, 144)&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Targeted Screening</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Patients presenting to hospital with premature vascular disease&lt;sup&gt;(55)&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>o Coronary care unit</td>
<td></td>
</tr>
<tr>
<td>o Stroke unit</td>
<td></td>
</tr>
<tr>
<td>o Vascular surgical ward</td>
<td></td>
</tr>
<tr>
<td>o Cardiothoracic units</td>
<td></td>
</tr>
<tr>
<td>- Review of electronic medical records&lt;sup&gt;(141, 145)&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Once the index case is identified, cascade screening can be performed on their family members. Cascade screening is a proven cost-effective method of detecting family members with FH<sup>(146)</sup> but the current challenge is the systematic identification of index cases, without which cascade screening cannot occur. Index patients should be sought amongst hospital patients with premature CVD admitted to coronary care, stroke cardiothoracic and vascular units, as well as by opportunistic and focused screening in ambulatory care settings, including general practice<sup>(55)</sup>. This is an area that requires major research, and is the focus of this thesis, see Chapter 2.
1.3.3.2 Cascade screening

Cascade screening is introduced in this section, but is discussed in detail in the following chapters referring to the benefit and cost-effectiveness of detecting FH. A succinct review is also presented in Chapters 8 and 9, which focus on genetic cascade screening in Australia.

Cascade screening is a way of identifying people at risk for a genetic condition by a process of systematic family tracing.\(^{(147)}\) Cascade screening is also referred to as cascade testing, involves communicating health information about an individual to their relatives so they become informed about a shared family risk. Cascade screening is used to find family members at higher \textit{a priori} risk of a genetically inherited condition such as FH so that they can choose to undertake diagnostic tests. The first step in cascade screening is to request permission from the individual diagnosed with FH to inform relatives. Where permission is granted, relatives are then informed either directly through health professionals or indirectly through their family member. Relatives are invited to a health clinic for screening. The patients’ perceptions of cascade screening and disease/wellbeing have generally been very positive from both adults and children.\(^{(148-152)}\)

The NICE guidelines from the UK recommends cascade screening of close biological relatives of people with a clinical diagnosis of FH in order to identify additional FH patients.\(^{(153)}\) The ultimate goal of this testing is to reduce morbidity and mortality from CAD in persons with FH through early diagnosis and effective disease management.
1.3.3.3 Detecting children with FH: universal screening

Detecting FH in the neonatal period has been proposed using the dried blood samples taken for universal screening for inborn errors of metabolism. It would be possible to screen for FH in this manner, although the feasibility of this method requires further investigation. FH testing does not need to be performed on Guthrie card, as there is no need to wait for metabolic profiles to form. However, LDL-cholesterol and lipoprotein concentrations are inversely associated with gestational age and birth weight,\(^{(154)}\) and influenced by environmental factors,\(^{(155)}\) which limit the neonatal period for universal FH screening. ApoB produced similarly disappointing results for neonatal FH screening.\(^{(156,157)}\)

The process of atherosclerosis begins in childhood, although the optimal screening approach for FH in children is less clear than in adults. A meta-analysis has shown that children aged 1-9 years are best for a universal screening strategy for FH.\(^{(138)}\) Using family history alone to determine the need for cholesterol screening in children may fail to detect FH.\(^{(158)}\) Universal screening during routine immunisation at ~15 months of age using plasma cholesterol has been proposed, children with a total cholesterol (or LDL-cholesterol) concentration >95\(^{th}\) percentile should have genetic testing or clinical investigations to confirm the diagnosis.\(^{(138)}\) A child-parent testing program could then identify adult family members with FH.

There are marked differences in the recommendations for detecting children with FH among the international FH guidelines, with some advocating universal screening between ages 1-9 years\(^{(159)}\) others 9-11 years\(^{(139)}\) and another guideline from Belgium limiting testing children to cascade screening only.\(^{(160)}\) However, on the basis of current evidence, the most cost-effective approach to identifying people with FH is case finding in the family of those known to have the disorder.\(^{(161,162)}\)
1.3.4 Detecting FH in primary care

1.3.4.1 Role of genetic testing in primary care

The general practitioner’s (GP’s) role in detecting genetic disorders is increasing, although there are some concerns over GP knowledge and confidence in clinical genetics.\(163,164\) Levels of knowledge and confidence with clinical genetics was associated with younger age (<40 years), genetics training, and having patients with genetic conditions under their care.\(163\) GPs were confident and willing to screen for cystic fibrosis, although only 30% were prepared to counsel cystic fibrosis carriers.\(165,166\) GPs generally accept they have a role in clinical genetics, but described this as mainly taking a detailed family history and gatekeeping referrals.\(167\) Further work is required to optimise and define the GP’s role in genetic testing.\(164,168,169\)

1.3.4.2 Identifying FH in primary care

GPs are well placed to opportunistically detect index cases in the community setting.\(142,170\) The majority of individuals see their GP yearly, and the GP often provides care to the family,\(170\) thus knowledge of a family history of premature CAD and/or severely elevated LDL-cholesterol could be used to raise their suspicion of FH in a kindred, and refer or perform FH testing of family members.\(142\)

However, most individuals with FH remain undiagnosed, even if they are registered with a GP.\(142,171\) The under diagnosis rates are higher in younger individuals,\(171\) who may have the most benefit from early detection and intervention.\(11,12,172\) The most effective method of ensuring these people are diagnosed with FH remains to be elucidated, and is the focus of this thesis, see Chapter 2.

It is possible to detect individuals with undiagnosed FH by searching a general practice database. In a review of a 5,200 patient general practice, Tyerman found 670 patients of 2,097 aged between 25 and 55 years with a positive family history for CAD.\(145\) Of those, 22 had a plasma cholesterol of >7.5 mmol/L; 16 of whom had an
LDL-cholesterol of >5.0 mmol/L, a 0.3% yield in this practice. The estimated cost per case identified using this method was £2,487 (~$3,830AUD).\(^{(145)}\)

An informatics approach to FH detection in primary care used an electronic general practice database to search for ischaemic heart disease (IHD), lipid disorder, statin prescription and cholesterol >7.0 mmol/L in a 12,100 patient practice.\(^{(141)}\) They identified 402 (3.3%) patients with possible FH and after reviewing their notes 12 cases of definite FH were found, of which only two were known to have FH by the practice. They also found eight probable cases of FH, with only one previously known to the practice. Of interest, two (17%) of the definite and four (50%) of the probable FH cases were unknown to the regional lipid clinic. There were 216 (54%) individuals identified with possible FH by the above searches, and after specialist review of the clinic notes, 47 (12%) were deemed to merit recall for a detailed family history and clinical examination for tendon xanthomata.

Although the use of electronic patient records makes the identification of index cases with FH easier, accuracy of the case notes is paramount to the detection process. For example, the age of IHD recorded in the database did not match that in the notes in 18.5% of cases.\(^{(141)}\) Of more concern, six (30%) of the individuals identified as probable or definite FH did not have a cholesterol recorded >7.0 mmol/L in the database, although this was the case on reviewing their paper notes. It is also possible to use the electronic records from a GP practice to alert the GP when a patient is at risk of FH. Some very early research has indicated GPs are supportive of this concept, although further work is required to explore this area.\(^{(173)}\)

Very recently in 2015, a case ascertainment tool (FAMCAT) has been developed and validated for FH using centrally collated primary care data.\(^{(172)}\) This tool demonstrated nine variables were important and significantly increased case detection above cholesterol levels alone (area under receiver operator curve cholesterol alone =
0.56, combined = 0.86). However, these variables included components of family history, which is known to be suboptimal in primary and secondary care.\textsuperscript{92, 174} The predictive ability of FAMCAT is very encouraging, and the outcome of studies prospectively trialling this tool in primary care incorporating specialist assessment and genetic analysis are eagerly awaited.\textsuperscript{172}

1.3.5 Impact of detecting and treating adults with FH

Lowering LDL-cholesterol is recommended in all the international FH guidelines.\textsuperscript{55-58, 175} Diet and lifestyle modifications are central to lipid-lowering in these FH guidelines, although there is strong evidence that reducing LDL-cholesterol with statin therapy reduces the risk of cardiovascular events in individuals with FH.\textsuperscript{4, 12, 13, 104, 176} Ezetimibe lowers LDL-cholesterol in addition to statins and has been demonstrated to be safe.\textsuperscript{112, 177} However, the impact ezetimibe has on cardiovascular outcome in FH remains to be determined.

Early detection and treatment with statins has been shown to reduce the onset of CAD by 80\% in individuals with FH compared to untreated patients, such that the risk of myocardial infarction in statin-treated patients was not significantly greater than that in an age-matched sample from the general population.\textsuperscript{13} Other estimates suggest that cholesterol reduction could prevent 96-98\% of CAD deaths in patients aged <40 years.\textsuperscript{178}

Cascade screening provides an opportunity for primary CAD prevention, by identifying individuals at high risk of CAD who have not had a CAD event; studies from Norway and the Netherlands have demonstrated significant reductions in LDL-cholesterol after diagnosis and therapy.\textsuperscript{179, 180} Data from Ohio over the last three decades has demonstrated lipid therapy has reduced the average LDL-cholesterol in FH patients by 55\%, and suggested an increase in the interval between cardiovascular events by ~2 years.\textsuperscript{181} Cascade screening has also increased the proportion of FH
patients being treated with statins, which has subsequently decreased the LDL-cholesterol concentrations of these individuals.\(^{(182)}\) A Dutch study demonstrated that 92.5% of adults were on lipid-lowering therapy 1 year after cascade genetic screening compared with 39% before screening; which resulted in a 30% reduction in LDL-cholesterol levels in previously untreated patients, and a further 10% reduction in individuals already receiving treatment.\(^{(183)}\)

1.3.6 Benefit of detecting and treating children with FH

Atherosclerosis commences in childhood,\(^{(184)}\) and most FH guidelines recommend children with FH are treated with diet, lifestyle and lipid-lowering therapies.\(^{(11, 56, 139)}\) Statin treatment has been shown to reduce plasma LDL-cholesterol levels in children with FH, and to be generally safe and tolerable.\(^{(139, 185-188)}\) There is evidence that early statin therapy in children improves endothelial function,\(^{(103)}\) and carotid intima medial thickness (CIMT)\(^{(189, 190)}\) as markers of atherosclerotic disease processes. Statin therapy in children with FH has been associated with normalisation of carotid intima medial progression compared to unaffected siblings during a ten-year follow-up.\(^{(191)}\) Initiating statin therapy at a younger age was associated with a thinner CIMT.\(^{(191)}\) However, there is as yet no randomised control trial evidence of the long-term health benefits of statin treatment in children with FH, nor evidence of benefit compared to the detection and treatment of the disorder in adulthood, although early treatment is predicted to be beneficial.\(^{(11, 192-194)}\)
1.3.7 Cost-effectiveness of screening for and treating FH

The NICE guideline development group in the UK found that the most cost-effective testing strategy, at £2,700 (~$4,160AUD) per quality-adjusted life year (QALY), was DNA testing in relatives of patients with mutations identified and/or using cholesterol testing to cascade from mutation-negative patients with definite or possible FH as determined by Simon Broome criteria.\textsuperscript{(153)} This screening approach was supported by a subsequent economic evaluation showing that it was the most cost-effective strategy at £3,666/QALY (~$5,650AUD).\textsuperscript{(195)}

The Netherlands has been operating a successful FH screening program since 1994. Data showed that new cases diagnosed by the screening program gain an average of 3.3 years of life each.\textsuperscript{(196)} Twenty-six myocardial infarctions were avoided for every 100 persons treated with statins between the ages of 18 and 60 years. The average total lifetime incremental costs, over all age ranges and both sexes, including costs for screening and testing, lifetime drug treatment, and treatment of cardiovascular events, was US$7,500 (~$7,080AUD) per new case identified. Cost per life-year gained was US$8,700 (~$8,200AUD).\textsuperscript{(196)}

The feasibility and sustainability of any screening program is dependent on adequate funding, which is usually provided nationally. Thus if a government is going to sanction a screening program for FH, there will need to be evidence it is cost-effective in terms of cost of detection and cost per QALY gained. Health economic evaluations have been performed subsequently for FH programs form the UK, Netherlands and Spain.\textsuperscript{(197, 198)} These studies suggested the cost of detecting an individual with FH ranged from $955 for a lipid profile and microchip based DNA test,\textsuperscript{(199)} to $1237 via a lipid profile and $1829 for a DNA test.\textsuperscript{(195)} The cost per QALY ranged from $3,960\textsuperscript{(200)} to $36,856.\textsuperscript{(199)}
However, whether these figures are applicable in an Australian health care system in the mid 2010’s was uncertain, as outcome data and screening methods were often very different.\(^{(197)}\) Furthermore, the cost of statins has significantly reduced over the last decade due to the patents for some statins expiring, thus FH treatment strategies will become significantly cheaper, with a 42% reduction in cost predicted in the UK.\(^{(201)}\) Advances in DNA diagnostics will also improve the cost-effectiveness of screening.

A health economic evaluation of genetic cascade screening was undertaken to determine the cost-effectiveness of cascade screening in a contemporary Australian setting.\(^{(202)}\) This study utilised a Markov model\(^{(203)}\) with yearly cycles over a 10 year period to simulate the onset of CAD, and applied an annual discount rate of 5% to all costs and benefits. This study suggested that for every 100 individuals screened, there was a gain of ~25 life years and ~29 QALY. The costs were encouraging; $4,155 per life year saved and $3,565 per QALY.\(^{(202)}\)

### 1.3.8 Summary of FH screening

FH is a dominantly inherited disorder that causes marked elevation in LDL-cholesterol and premature CAD. However, the vast majority of individuals with FH remain undetected. Screening options for FH include population screening, targeted screening of patients with premature CAD, or opportunistic screening. These methods still require investigation to determine their effectiveness, acceptability and cost. Once an index case is identified cascade screening can be performed, which is very cost-effective. However, despite the above evidence in favour of FH screening, only a few countries have initiated screening programs, and the approaches to FH detection vary widely.\(^{(204)}\) Thus the current challenge in FH management is the systematic identification of index cases, without which cascade screening cannot occur.
1.4 CVD RISK FACTOR SCREENING IN THE GENERAL POPULATION

1.4.1 Overview of CVD in Australia

CVD aggregates in families and remains the leading cause of morbidity and mortality in Australia with nearly 50,000 deaths attributable to CVD in 2008 - 34% of the total mortality. Genetic, pathological and epidemiological studies have demonstrated that high plasma concentrations of LDL-cholesterol and low levels of high density lipoprotein cholesterol (HDL-cholesterol) are directly related to the incidence of coronary events and cardiovascular deaths. Increased concentrations of plasma LDL-cholesterol and decreased levels of HDL-cholesterol are associated with an increased risk of developing atherosclerotic CVD in a continuous and graded fashion with no clear threshold.

CVD mortality is associated with multiple risk factors, including dyslipidaemia, hypertension, cigarette smoking, diabetes, obesity, physical inactivity, a family history of premature CVD, age, male gender, diet and socioeconomic status. Based on 2006 estimates, 218,143 years of life lost and 263,487 disability adjusted life years were attributable to CAD in Australia. Approximately 6% of the burden of disease and injury in Australia in 2003 was attributed to high blood cholesterol, placing it fifth out of the 14 risk factors examined. At 35%, high blood cholesterol was the second highest contributor to the burden of CVD after hypertension.

Lipid disorders are common in Western societies. High cholesterol (and in particular LDL-cholesterol) is a major risk factor for CVD, with an estimated 51% of Australians aged ≥25 years (~6.4 million people) having a plasma cholesterol ≥5.5 mmol/L. A small percentage of individuals have a genetic basis to their dyslipidaemia. The considerable disease burden from CVD and strong epidemiological association with abnormalities of plasma lipids have directed efforts to identify
individuals with lipid disorders and to modify their cardiovascular risk with lifestyle changes and pharmacologic measures.

1.4.2 Lipid disorders

Lipid disorders, also known as dyslipidaemias, are abnormalities of lipoprotein metabolism and include elevations of total cholesterol, LDL-cholesterol, triglyceride, and reductions in HDL-cholesterol. They can be acquired (secondary) or familial (primary) in nature. Secondary dyslipidaemia should be excluded before a diagnosis of primary dyslipidaemia can be considered. Common secondary causes of dyslipidaemias include diet, hypothyroidism, obesity, alcohol excess, diabetes mellitus, chronic kidney disease (CKD), chronic liver disease, cholestasis, nephrotic syndrome, and drugs.

The more common dyslipidaemias include FH, familial dysbetalipoproteinaemia, familial combined hyperlipidaemia, and familial hypertriglyceridaemia. These familial dyslipidaemias may be associated with clinical examination findings which in some cases are considered pathognomonic, such as tendon xanthomata in FH\(^{(208)}\) and palmar xanthomata in familial dysbetalipoproteinaemia. Additional stigmata include other cutaneous xanthomata (eruptive, planar, tuberous, striated, xanthelasmata),\(^{(209)}\) and premature arcus cornealis (<45 years of age). Importantly, some people with a dyslipidaemia do not have an identifiable secondary or monogenic cause and these people are considered to have polygenic dyslipidaemia. The lipid profile in people with genetic dyslipidaemias may also be exacerbated by any coexisting condition that causes a secondary dyslipidaemia. Dyslipidaemia is defined by laboratory testing using statistically determined criteria. Age, gender, racial differences and temporal trends may alter these statistical cut-points.
1.4.3 Absolute cardiovascular risk

Total cardiovascular risk is a continuum. The prevention and treatment of dyslipidaemias should always be considered within the broader concept of CVD prevention. Patients screened for lipid disorders should be assessed for other cardiovascular risk factors including smoking, diabetes, hypertension, obesity, and personal and family history of premature CVD. However, for cost-effective strategies, it is necessary to identify those individuals at highest absolute risk of a cardiovascular event who have the most to benefit. Clinical decisions based on absolute risk can lead to improved health outcomes by identifying people most at risk and directing the appropriate treatments to them. The importance of measuring plasma lipids in determining the absolute CVD risk is well established.

The National Vascular Disease Prevention Alliance has published Australian guidelines for the assessment of absolute CVD risk.\(^{(210)}\) Absolute risk was defined as the numerical probability of a cardiovascular event occurring within a five-year period. It reflects a person’s individual risk of CVD. These guidelines make recommendations for assessing absolute CVD risk in all adults aged 45-74 years (from 35 years for Aboriginal or Torres Strait Islander adults and 45-60 years in adults with diabetes mellitus) without CVD or not known to be at increased risk of CVD using the Framingham Risk Equation. Of note, plasma LDL-cholesterol and triglyceride are not variables in the equation. An absolute CVD risk calculator is available online at http://www.cvdcheck.org.au/.
1.4.4 Indications for cardiovascular risk screening in adults

The Australian Lipid Management Guidelines recommend that a fasting lipid profile - total cholesterol, HDL-cholesterol, triglyceride concentrations should be measured, and LDL-cholesterol level estimated using the Friedewald calculation (unless the triglyceride is >4.5 mmol/L) - for all adults ≥45 years and for those <45 years who may be at higher risk due to other (non-lipid) risk factors. A fasting lipid profile in all adults age >20 every five years is recommended by the National Cholesterol Education Program Adult Treatment Panel (NCEP ATP-III) and is endorsed by the American Heart Association.

The US Preventive Services Task Force (USPSTF) strongly recommends screening men aged ≥35 for lipid disorders; screening is recommended for men aged 20 to 35 if they are at increased risk for CAD. The USPSTF strongly recommends screening for women aged ≥45 for lipid disorders if they are at increased risk for CAD; screening is also recommended for women aged 20 to 45 if they are at increased risk for CAD, although this was not a strong recommendation. The USPSTF makes no recommendation for or against routine screening for lipid disorders in men aged 20 to 35, or in women aged ≥20 who are not at increased risk for CVD.

The European Society of Cardiology and the European Atherosclerosis Society recommend risk factor screening, including the lipid profile, be considered in adult men >40 years, and in women >50 years or post-menopausal, particularly in the presence of other risk factors. It was recommended that a lipid profile be assessed in all subjects with evidence of atherosclerosis or with type 2 diabetes and those with a family history of CVD merited early screening. The Fifth Joint Task Force of the European Society of Cardiology reinforced this in 2012.
1.4.5 Indications for lipid screening in children and adolescents

Interest in screening children and adolescents for dyslipidaemia has heightened with an emphasis on primary prevention for CAD, due to the relationships between childhood and adult dyslipidaemia, and the increased prevalence of obesity and diabetes.\(^{(216)}\) Screening programs should identify children and adolescents with dyslipidaemia early, and should be sustainable in terms of cost versus benefit. The NCEP Expert Panel paediatric cut points are shown in Table 8.\(^{(217)}\) However, it should be noted that age, gender, racial differences and temporal trends might alter these cut points.

---

TABLE 8. PLASMA LIPID AND LIPOPROTEIN CONCENTRATIONS IN CHILDREN AND ADOLESCENTS AS CLASSIFIED BY THE NCEP

<table>
<thead>
<tr>
<th>Category</th>
<th>Total cholesterol (mmol/L)</th>
<th>HDL-cholesterol (mmol/L)</th>
<th>Triglyceride (mmol/L)</th>
<th>LDL-cholesterol (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acceptable</td>
<td>&lt;4.4</td>
<td>&gt;1.2</td>
<td>&lt;1.0</td>
<td>&lt;2.8</td>
</tr>
<tr>
<td>Borderline</td>
<td>4.4-5.1</td>
<td>0.9-1.2</td>
<td>1.0-1.4</td>
<td>2.8-3.3</td>
</tr>
<tr>
<td>Unacceptable</td>
<td>≥5.2</td>
<td>&lt;0.9</td>
<td>≥1.5</td>
<td>≥3.4</td>
</tr>
</tbody>
</table>
Different methods of guideline development for lipid screening in children have resulted in conflicting recommendations and clinical practice variations.\textsuperscript{(218)} In 2007, USPSTF concluded that the evidence was insufficient to recommend for or against routine screening for lipid disorders in infants, children, adolescents, or young adults (up to age 20).\textsuperscript{(193)} In contrast, one year later, the American Academy of Paediatrics released an updated policy statement recommending a targeted approach to detect dyslipidaemia in childhood using a fasting lipid profile in children with a positive family history of dyslipidaemia or premature CVD.\textsuperscript{(219)} It was also recommended that screening occur in children with an unknown family history or those with other non-lipid CVD risk factors such as overweight, obesity, hypertension, cigarette smoking and diabetes. These guidelines were further updated in 2011.\textsuperscript{(220)}

However, it should be noted that such a targeted strategy fails to identify up to 60\% of children and adolescents with hypercholesterolaemia, especially those with young parents who are free of CVD and unaware of their plasma lipid profiles.\textsuperscript{(221-223)} Moreover, the CARDIAC project, which used family history to determine the need for cholesterol screening in children would have missed many children with moderate dyslipidaemia and failed to detect a number who were likely to have a primary dyslipidaemia and require lipid-lowering therapy.\textsuperscript{(158)} It remains to be seen as to whether the inability to identify and treat those children results in increased CVD outcomes.

Disappointingly, screening strategies for dyslipidaemia in children and adolescents have low adherence by health practitioners and limited compliance by parents and children.\textsuperscript{(224)} Furthermore, no studies have addressed the optimal age and intervals for screening children and adolescents.
1.4.6 Lipid screening tests

Lipid measurements are affected by many biological, behavioural and clinical factors, and by variability in specimen collection and handling.\(^{(225)}\) Failure to control for these variables can lead to misclassification of patient cardiovascular risk. Preanalytical variation in individuals results from differences in lifestyle, altered lipid metabolism due to disease, the source of the specimen, and the conditions of sample collection.\(^{(225)}\) Standardising these preanalytical sources of variation improves the accuracy and utility of lipid testing in the assessment of cardiovascular risk.\(^{(226)}\)

1.4.6.1 Fasting versus non-fasting

If possible, measurements of plasma lipid parameters should be performed on blood drawn from patients after a 12 h fast.\(^{(227)}\) A fasting sample is essential for triglyceride analysis, as triglyceride concentrations increase postprandially (as early as 2 h and peaking at 4 to 6 h), and is required to estimate LDL-cholesterol using the Friedewald calculation.\(^{(228)}\) In contrast, total cholesterol, HDL-cholesterol, apoB and apoA-I concentrations vary little between fasting and non-fasting states.\(^{(229)}\)

1.4.6.2 Intra-individual variation

Biological variability is a major contributor to the inaccuracy of cardiovascular risk assessment based on the measurement of lipids, lipoproteins, or apolipoproteins. The biological coefficient of variation is ~6-7% for total cholesterol, HDL-cholesterol, apoA-I and apoB, ~9% for LDL-cholesterol, and ~28% for triglyceride.\(^{(230)}\)
1.4.7 Lipid, lipoprotein and apolipoprotein analyses

Minimally, plasma total cholesterol, HDL-cholesterol, triglyceride concentrations should be measured and the LDL-cholesterol level calculated in high-risk individuals after a 12 h fast. This testing should be performed in an accredited laboratory. However, it should be noted that cardiovascular risk equations are currently based on measures of total cholesterol and HDL-cholesterol and the clinical benefit from using other measures such as non-HDL-cholesterol, apoB, and various ratios have as yet not been proven. LDL-cholesterol remains the primary therapeutic target.

1.4.7.1 Total cholesterol

Total cholesterol is the best predictor of CAD events, with a continuous and graded risk, with most of this risk explained by the LDL-cholesterol concentration.

1.4.7.2 HDL-cholesterol

Epidemiological studies show that HDL-cholesterol concentrations are inversely correlated with the risk of cardiovascular events.\(^{(231)}\) Although measuring HDL-cholesterol provides information about the HDL pool size, it does not predict HDL composition or function. Robust assays to evaluate the function of HDL are needed to supplement the measurement of HDL-cholesterol in clinical practice.\(^{(232)}\)
1.4.7.3 Triglyceride

Increased plasma triglyceride concentrations are associated with increased cardiovascular risk, and often with reduced HDL-cholesterol levels and elevated small dense LDL particles: the atherogenic lipoprotein phenotype. Although guidelines recommend measuring fasting lipids for initial screening of adults without CVD, recent studies suggest that non-fasting triglyceride levels may be superior to fasting by providing information with respect to remnant lipoproteins and associated cardiovascular risk. Currently, triglyceride concentrations are not included in cardiovascular risk calculators.

1.4.7.4 LDL-cholesterol

The evidence linking LDL-cholesterol and CAD is derived from epidemiological, laboratory and clinical trial data. At this juncture, most guidance documents rely on LDL-cholesterol to assess cardiovascular risk and tailor therapy. Although the LDL-cholesterol used in routine practice is calculated using the Friedewald equation, rather than being measured. There has been recent debate over the accuracy of the Friedewald equation, especially when cholesterol <1.8 mmol/L, although it is recommended for routine use.

1.4.7.5 Non-HDL-cholesterol

Non-HDL-cholesterol, which is total cholesterol minus HDL-cholesterol, is a measure of the cholesterol in VLDL, IDL and LDL particles. Non-HDL-cholesterol can be easily calculated (total cholesterol – HDL-cholesterol) and is an indirect estimate of apoB, which is a measure the total atherogenic particle number. Non-HDL-cholesterol has been recommended as a secondary therapeutic target in individuals with high triglyceride concentration, such as the cardiometabolic syndrome, because it includes the cholesterol in VLDL particles.
1.4.7.6 Apolipoprotein B and A-I

It is important to note that each circulating particle of VLDL, IDL, LDL, and lipoprotein (a) [Lp(a)] contains only a single, nonexchangeable molecule of apoB-100. Similarly, chylomicrons and their remnants contain a single molecule of apoB-48. Hence, plasma apoB concentrations reflect the total number of atherogenic particles present in the circulation. Both apoB-100 and apoB-48 are recognised by conventional clinical immunoassays for apoB, and the assays are standardised. Like LDL-cholesterol, an increased plasma concentration of apoB has been shown to be a key risk factor for the development of atherosclerotic CVD. Because LDL particles differ in composition, LDL-cholesterol is not equivalent to LDL particle number. This discordance becomes critical when small dense LDL is the dominant LDL species, such as in the cardiometabolic syndrome. In a series of prospective epidemiological studies, plasma apoB has been shown to be superior to LDL-cholesterol as a marker of cardiovascular risk both on and off treatment, as well as in subclinical CVD risk prediction. ApoA-I, the major component of HDL, is largely responsible for reverse cholesterol transport via the macrophage adenosine triphosphate binding cassette transporter A1 (ABCA1). As is the case with HDL-cholesterol, higher concentrations of apoA-I, in general, are associated with a reduced cardiovascular risk.

1.4.7.7 Lipid and apolipoprotein ratios

Cardiovascular risk increases as plasma cholesterol increases and as HDL-cholesterol decreases. Based on these relationships, ratios such as total cholesterol to HDL-cholesterol are used by some laboratories in cardiovascular risk prediction. However, apoB and apoA-I also may serve as cardiovascular risk predictors. The apoB to apoA-I ratio integrates the risk due to proatherogenic and antiatherogenic lipoproteins. Thus, the apoB to apoA-I ratio may represent a surrogate marker not only
for predicting future cardiovascular risk, but also for evaluating the effects of lipid-lowering therapy.

While the proatherogenic/antiatherogenic ratio of apoB/apoA-I is a better risk discriminator than the single proatherogenic measurement (apoB), clinical trial data are lacking regarding the impact of increasing apoA-I and HDL on outcomes.

1.4.7.8 Lipoprotein particle size

Lipoproteins are heterogeneous in nature, varying in composition, density, and size. HDL and LDL particle subclasses have been associated with varying cardiovascular risk. It has been shown that patients who have or are at risk for CAD have increases in small, dense LDL.

1.4.7.9 Lipoprotein (a)

Lipoprotein a [Lp(a)] is a structurally complex particle which resembles LDL, but which also contains the distinctive glycoprotein apo(a). Although epidemiological studies indicate that elevated Lp(a) concentration and/or small apo(a) isoform sizes increase the risk of CAD, a causal role for Lp(a) in CAD has only recently been proven. Furthermore, while circulating Lp(a) is likely to be a causal risk factor in CAD and stroke, the magnitude of this association is modest. Differences in isoform specificity of the Lp(a) assays are a further potential complication, although whether these differences are clinically significant is unknown. Plasma Lp(a) is not recommended for general screening, however it should be considered in individuals at high cardiovascular risk or those with a family history of premature CAD and stroke. Elevated Lp(a) has recently been confirmed to be an independent CVD risk factor in individuals with FH, with higher rates of CVD seen in individuals with an Lp(a) >0.5 g/L.
1.4.8 Benefits of lipid screening in adults

There is good evidence that plasma lipid measurements can identify asymptomatic men and women who would benefit from primary preventive therapy. The absolute risk of CVD is lower in primary prevention (treating individuals who have not had a vascular event) than secondary prevention (where individuals have had a vascular event). However, the proportion of the population that could benefit from the reduction in absolute risk for a first CVD event is large. Thus, the net benefit from a small individual reduction in absolute risk is very significant at a population level for primary prevention. The absolute risk of CVD in an individual should be assessed, rather than focusing on an individual risk factor such as lipids.\(^\text{254}\) Absolute risk assessment identifies the individuals who would derive the largest absolute risk reduction from treatment, and enables this to be performed in a more cost-effective manner.\(^\text{254}\)

There is emerging evidence that clinical decisions based on absolute cardiovascular risk may lead to improved management of CVD risk. Access to absolute cardiovascular risk assessment tools has been shown to increase prescribing of lipid-modifying drugs for high-risk patients with diabetes and lead to both improvement in lipid profiles and significant reductions in CAD risk.\(^\text{255}\) Given that absolute cardiovascular risk assessment provides more accurate assessment of risk than individual risk factors, it is reasonable to expect that management decisions based on this assessment will improve outcomes.

The use of absolute risk in young people has caused some concern, as absolute CVD risk is also age dependent. Thus, the absolute risk of a younger person may still be below a population treatment threshold, but their risk relative to their peers may be significantly increased. The National Heart Foundation of New Zealand uses risk trajectory to address this issue, where the projected increase in an individual’s absolute
CVD risk with aging is compared to the projection of an individual with risk factors at optimal levels over the same ages.\(^\text{(254)}\)

1.4.9 Benefits of lipid screening in children and adolescents

The clinical benefits in identifying and treating dyslipidaemia have not been studied in children and adolescents, making the role of screening uncertain. Moreover, there is no evidence that such screening alters the progression of the dyslipidaemia or incidence of CAD in adulthood. However, in contrast to the general paediatric population, treatment with diet, lifestyle and lipid-lowering therapy is recommended for children with FH.\(^\text{(11, 56, 139)}\)

1.4.10 Effectiveness of primary CVD prevention in the general population

The West of Scotland Coronary Prevention Study (WOSCOPS) was the first trial to demonstrate that statin therapy for primary prevention of CVD in men with elevated cholesterol was beneficial. WOSCOPS enrolled 6,595 men aged 45-64 years with total cholesterol of 7.0 mmol/L to pravastatin 40 mg daily and followed them for an average of 4.9 years. The benefits this trial demonstrated were; a 32% reduction in CVD deaths, 31% reduction in nonfatal myocardial infarction and a 22% reduction in total mortality.\(^\text{(109)}\) The estimated absolute benefit of reduction in combined CVD death and nonfatal myocardial infarction was 3%. Multiple statin trials have shown similar benefits in primary prevention in both men and women; Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS) with lovastatin,\(^\text{(256)}\) Anglo-Scandinavian Cardiac Outcomes Trial - Lipid Lowering Arm (ASCOT-LLA) with atorvastatin,\(^\text{(257)}\) and the Justification for the Use of Statin in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) trial.\(^\text{(258)}\)
1.4.11 Lipid treatment targets for the general population

Statin therapy is generally recommended for the primary prevention of CVD for adults who are at increased risk of developing CVD, estimated using an appropriate risk calculator or by clinical assessment.\(^{(259)}\) However, regional differences in CVD risk calculators,\(^{(260)}\) treatment risk thresholds and LDL-cholesterol targets exist.\(^{(261)}\) Furthermore, the target values and even the need for lipid targets are frequently debated, which may lead to confusion and then to under treatment.\(^{(261)}\)

It is accepted that treatment should reflect the absolute cardiovascular risk of the individual. However, the current absolute CVD risk calculators for the general population are not appropriate for individuals with FH, as they can dramatically underestimate the absolute CVD risk, and thus should not be used.\(^{(11)}\) There are also many different recommendations for different population groups, for example; children,\(^{(220)}\) the elderly,\(^{(262)}\) women,\(^{(263)}\) individuals with diabetes or the metabolic syndrome\(^{(264)}\) and chronic kidney disease (CKD) and liver disease.\(^{(265)}\)

LDL-cholesterol targets for secondary prevention are generally lower than primary prevention treatment goals. Furthermore, there does not appear to be a lower LDL-cholesterol threshold below which there is no additional benefit of further LDL-cholesterol reduction.\(^{(100)}\) However, statins do not produce a linear reduction in LDL-cholesterol, with each doubling of dose only leading to a further 6-10\% decrease in LDL-cholesterol.\(^{(266)}\) The safety of statins has been re-affirmed in clinical trials, but the incidence of myopathy may be as high as 10\% in clinical practice.\(^{(267)}\)

The current Australian LDL-cholesterol targets were described in the 2005 Position Statement on Lipid Management, National Vascular Disease Prevention Alliance,\(^{(98)}\) and updated in 2012.\(^{(268)}\) High risk was defined as an absolute risk of CVD >15\% in the next 5 years, or people at 10-15\% absolute risk in the next 5 years if a family history of premature CVD (before age 60 years) or the metabolic syndrome.
People with diabetes, CKD and those of indigenous descent were classified as high risk. The LDL-cholesterol targets are described in Table 9.

The modifications proposed to the National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III) guidelines are outlined in Table 9, including the optional LDL-cholesterol goals for those deemed to be at high risk.\(^{(213)}\) The International Atherosclerosis Society issued their most recent guideline on the management of dyslipidaemia in 2013, which focused on the importance of lifestyle, non-HDL-cholesterol and determining the lifetime risk of CVD, their recommended LDL-cholesterol targets are described in Table 9.\(^{(269)}\)

The combined European Society of Cardiology and European Atherosclerosis Society dyslipidaemia guidelines were reported in 2011.\(^{(215)}\) They recommended the Systemic Coronary Risk Estimation (SCORE)\(^{(270)}\) system for calculation of absolute CAD risk and recommended targets are shown in Table 9.

The 2013 American College of Cardiology/American Heart Association (ACC/AHA) cholesterol treatment guideline altered the focus from LDL-cholesterol targets to the intensity of statin therapy, citing lack of evidence for or against LDL-cholesterol targets.\(^{(271)}\) This caused quite a degree of controversy and confusion after decades of focus on LDL-cholesterol targets.\(^{(261)}\) They also provided a novel risk calculator which focused on four groups of patients who were most likely to benefit from statin therapy: 1) individuals with CVD, 2) individuals with LDL \(\geq 4.9\) mmol/L, 3) individuals with diabetes aged 40-75 years with an LDL-cholesterol of 1.8-4.9 mmol/L, 4) individuals aged 40-75 years with an LDL-cholesterol of 1.8-4.9 mmol/L and an estimated 10 year risk of \(\geq 7.5\%\).\(^{(271)}\)

However, for comparison the 2008 American Diabetes Association and American College of Cardiology (ADA/ACC) consensus statement provided LDL-cholesterol goals; highest risk \(< 1.8\) mmol/L, or high risk \(< 2.6\) mmol/L.\(^{(264, 272)}\)
Treatment targets for primary prevention tend to be more conservative, as the absolute risk reduction of progressive LDL-cholesterol reductions decreases with decreasing LDL-cholesterol concentration.\(^{(254)}\) Thus, the number of people who require treatment to a progressively lower LDL-cholesterol target in order to prevent a CVD outcome increases. This leads to an increased cost for a relatively minimal benefit in primary prevention, and may actually lead to harm if higher doses of lipid-lowering therapy are required, which may be associated with a higher risk of side effects.

These LDL-cholesterol targets for general CVD risk reduction demonstrate regional differences, which are also reflected in the LDL-cholesterol targets for individuals with FH as discussed in Section 1.2.7.1, although the targets are different. The FH guidelines often have an emphasis on a \(\geq 50\%\) reduction in LDL-cholesterol from baseline, and then an absolute LDL-cholesterol target.

### 1.4.12 Role of the laboratory in general CVD screening

The laboratory performs a vital role in general CVD screening, by measuring lipid profiles and raising awareness of the regional absolute CVD guidelines.\(^{(268)}\) This usually occurs via automated interpretative commenting on the lipid results.\(^{(273)}\) The laboratory also screens for other conditions that increase CVD risk such as diabetes\(^{(274)}\) and CKD\(^{(275)}\) according to separate regional guidelines. However, FH is excluded from general CVD risk calculators, and is often only briefly described in the general CVD guidelines. This thesis explores the potential role a laboratory may have to opportunistically detect individuals with FH using the LDL-cholesterol results performed as part of general CVD screening.
TABLE 9. LDL-CHOLESTEROL TARGETS FOR CVD RISK REDUCTION IN THE GENERAL POPULATION

<table>
<thead>
<tr>
<th>Guideline</th>
<th>CAD risk category</th>
<th>LDL-cholesterol target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australian(^{(98, 268)})</td>
<td>High</td>
<td>&lt;2.0 mmol/L*</td>
</tr>
<tr>
<td>National Cholesterol Education Program Adult Treatment Panel III(^{(213)})</td>
<td>High risk</td>
<td>&lt;2.6 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Secondary prevention or 10 year risk &gt;20%</td>
<td>&lt;1.8 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Optional goal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderately high risk ≥2 risk factors 10 year risk 10-20%</td>
<td>&lt;3.4 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Optional goal</td>
<td>&lt;2.6 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Moderate risk ≥2 risk factors 10 year risk &lt;10%</td>
<td>&lt;3.4 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Low risk 0 to 1 risk factor</td>
<td>&lt;4.2 mmol/L</td>
</tr>
<tr>
<td>International Atherosclerosis Society(^{(269)})</td>
<td>Primary prevention</td>
<td>&lt;2.6 mmol/L</td>
</tr>
<tr>
<td></td>
<td>Secondary prevention</td>
<td>&lt;1.8 mmol/L</td>
</tr>
<tr>
<td>European Society of Cardiology &amp; European Atherosclerosis Society(^{(215)})</td>
<td>Very high risk CVD, diabetes CKD or SCORE ≥10%</td>
<td>&lt;1.8 mmol/L, or ≥50% reduction from baseline</td>
</tr>
<tr>
<td></td>
<td>High risk SCORE 5-9.9%</td>
<td>&lt;2.5 mmol/L</td>
</tr>
<tr>
<td>American College of Cardiology &amp; American Heart Association(^{(271)})</td>
<td>High(^{#})</td>
<td>High intensity statin (≥50% reduction from baseline)(^{\wedge})</td>
</tr>
<tr>
<td></td>
<td>Moderate(^{#})</td>
<td>Moderate intensity statin (30-49% reduction)(^{\wedge})</td>
</tr>
</tbody>
</table>

*Proposed target in 2005, with a caveat - awaiting trials in progress in 2005. \(^{\wedge}\)Refers to the average reduction in LDL-cholesterol achieved by these statins; these were not a goal and were not to be used to titrate statin therapy. \(^{\#}\)The risk assessment was based on age, comorbidity, additional CVD risk factors and lipid profile.
1.4.12 Overview of screening for lipid disorders in the general population

Dyslipidaemias are a group of conditions with abnormal lipid levels caused by disorders of lipoprotein metabolism, which can be acquired or familial in nature. Dyslipidaemia is a major risk factor for CVD, which is the leading cause of morbidity and mortality in Australia. There is compelling evidence based on multiple randomised controlled trials that decreasing LDL-cholesterol improves CVD outcomes, and subsequently LDL-cholesterol concentrations has become the target of lipid-lowering therapy.

Furthermore the benefits of screening for and treating lipid disorders in all men aged 35 years and older and women aged 45 years and older at increased risk for CVD substantially outweigh the potential harms.\textsuperscript{(214)} The benefits of screening for and treating lipid disorders in young adults at increased risk for CVD moderately outweigh the potential harms.\textsuperscript{(193)} However, the net benefits of screening for lipid disorders in young adults not at increased risk for CVD are insufficient to make a general recommendation.\textsuperscript{(193)}

The prevention and treatment of dyslipidaemias should always be considered within the broader concept of CVD prevention. Patients screened for lipid disorders should be assessed for other cardiovascular risk factors including smoking, diabetes, hypertension, obesity, and personal and family history of premature CAD. However, for cost-effective strategies, it is necessary to identify individuals at highest absolute CVD risk, as they are predicted to have the most to benefit.

Screening for absolute cardiovascular risk is advocated in the majority of western countries, although the age for initiating general CVD screening varies from 35 to 45 years. Absolute CVD screening includes measurement of lipids, usually including LDL-cholesterol. Thus, there may be potential to use the general CVD screening to opportunistically screen for FH.
1.5 IMPETUS TO OPTIMISE FH DETECTION

FH a most common inherited condition causing premature CAD. However, the vast majority of individuals with FH are undiagnosed and under treated\(^{(11)}\) despite four decades of detailed pathologic knowledge (described in Sections 1.2.2 – 1.2.3), and recent evidence current therapies are effective at reducing CAD and improving mortality in FH (Section 1.3.5). Most statins are now off patent reducing the costs of therapy, leading to improvements in the health economics of FH screening, which were already demonstrated to be cost-effective as described (Section 1.3.7). Furthermore, it is well recognised that attainment of the LDL-cholesterol targets for individuals with FH at high CAD risk is low.\(^{(10, 66)}\)

1.5.1 Emerging therapies for FH

Several new pharmacological strategies have been developed to lower LDL-cholesterol for patients with severe hypercholesterolaemia including FH. These therapies include; PCSK9 inhibitors, MTP inhibitors, cholesteryl ester transfer protein (CETP) inhibitors, as well as mipomersen an antisense oligonucleotide targeted to human apoB-100. These therapies have been reviewed in detail in multiple publications,\(^{(82, 276)}\) and a detailed review is beyond the scope of this thesis.

These agents are likely to be indicated for individuals with FH in the first instance, which highlights the urgency of identifying individuals with FH; as they are also likely to benefit the most from these therapies. These therapies are briefly described below, and the lipid-lowering effects are presented in Table 10.
### TABLE 10. EFFECTS OF EMERGING PHARMACOTHERAPIES ON LIPID, LIPOPROTEIN AND APOLIPOPROTEIN CONCENTRATIONS

<table>
<thead>
<tr>
<th>Class</th>
<th>Study</th>
<th>Patients</th>
<th>Duration</th>
<th>ApoB (%)</th>
<th>LDL-c (%)</th>
<th>TG (%)</th>
<th>HDL-c (%)</th>
<th>Lp(a) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ApoB antisense oligonucleotides</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mipomersen</td>
<td>McGowan et al(277)</td>
<td>HeFH (n=58)</td>
<td>26 w</td>
<td>-36</td>
<td>-36</td>
<td>-8.6</td>
<td>+5.8*</td>
<td>-31</td>
</tr>
<tr>
<td></td>
<td>Stein et al(278)</td>
<td>HeFH (n=124)</td>
<td>28 w</td>
<td>-26</td>
<td>-28</td>
<td>-14</td>
<td>+2.5*</td>
<td>-21</td>
</tr>
<tr>
<td></td>
<td>Raal et al(279)</td>
<td>HoFH (n=51)</td>
<td>26 w</td>
<td>-27</td>
<td>-25</td>
<td>-17.4</td>
<td>+15.1</td>
<td>-33</td>
</tr>
<tr>
<td><strong>PCSK9 inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evolocumab</td>
<td>Raal et al(280)</td>
<td>HeFH (n=168)</td>
<td>12 w</td>
<td>-43</td>
<td>-55</td>
<td>-10</td>
<td>+9.1</td>
<td>-27</td>
</tr>
<tr>
<td></td>
<td>Stein et al(281)</td>
<td>HoFH (n=8)†</td>
<td>≥24 w</td>
<td>-14.9</td>
<td>-16.5</td>
<td>NA</td>
<td>NA</td>
<td>-11.7</td>
</tr>
<tr>
<td></td>
<td>Blom et al(282)</td>
<td>Non-FH (n=901) LDL-cholesterol ≥1.9 mmol/L</td>
<td>52 w</td>
<td>-44</td>
<td>-57</td>
<td>-11.5</td>
<td>+5.4</td>
<td>-22.4</td>
</tr>
<tr>
<td></td>
<td>Sabatine et al(283)</td>
<td>FH and non-FH (n=4465)</td>
<td>48 w</td>
<td>-47.3</td>
<td>-70.5</td>
<td>-12.6</td>
<td>NA</td>
<td>-25.5</td>
</tr>
<tr>
<td>Alirocumab</td>
<td>Stein et al(284)</td>
<td>HeFH (n=77)</td>
<td>12 w</td>
<td>-50</td>
<td>-68</td>
<td>-17*</td>
<td>+12</td>
<td>-23*</td>
</tr>
<tr>
<td></td>
<td>Robinson et al(285)</td>
<td>Non-FH (n=2341) LDL-cholesterol &gt;1.8 mmol/L</td>
<td>24 w</td>
<td>-54.0</td>
<td>-61.9</td>
<td>-17.3</td>
<td>4.6</td>
<td>-25.6</td>
</tr>
<tr>
<td><strong>MTP inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lomitapide</td>
<td>Cuchel et al(286)</td>
<td>HoFH (n=29)†</td>
<td>78 w</td>
<td>-43</td>
<td>-38</td>
<td>-31</td>
<td>-5*</td>
<td>-1*</td>
</tr>
<tr>
<td><strong>CETP inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anacetrapib</td>
<td>Cannon et al(287)</td>
<td>Non-FH patients with or at high risk of CAD (n=1623)</td>
<td>76 w</td>
<td>-20</td>
<td>-40</td>
<td>-6.9*</td>
<td>+151</td>
<td>-17*</td>
</tr>
<tr>
<td></td>
<td>Kastelein et al(288)</td>
<td>HeFH (n=204 Anacetrapib, n=102 placebo)</td>
<td>52 w</td>
<td>-19.6</td>
<td>-36.4</td>
<td>-5.1</td>
<td>+105.8</td>
<td>-31.8</td>
</tr>
<tr>
<td>Evacetrapib</td>
<td>Nicholls et al(289)</td>
<td>Non-FH patients with elevated LDL-cholesterol or low HDL-cholesterol (n=398)</td>
<td>12 w</td>
<td>NA</td>
<td>-14</td>
<td>-8*</td>
<td>+88</td>
<td>NA</td>
</tr>
</tbody>
</table>

HeFH = heterozygous FH. HoFH = homozygous FH. TG = triglyceride. If more than one dose of investigative drug was used, the dose with the greatest effect on lipids and lipoproteins are shown in the table. In all of the studies, most patients continued on standard lipid-lowering therapy including statins. *Indicates \( P \geq 0.05 \) or not provided. For all other values, \( P < 0.05 \). NA, not available. †, single-arm trial.
1.5.1.1 ApoB antisense oligonucleotides

Mipomersen is a second-generation antisense oligonucleotide that concentrates in the liver and specifically binds to human apoB mRNA.\(^{(290)}\) Once bound, the mRNA duplex is degraded by RNase H, preventing the translation of apoB, and thus, the synthesis of apoB-containing lipoproteins. Mipomersen reduces the formation of apoB-containing lipoproteins thereby reducing LDL-cholesterol without the need for functional LDLR. Patients with heterozygous familial hypobetalipoproteinaemia, an autosomal dominant disorder caused by mutations in the \(APOB\) gene, have apoB levels ~25-30% of normal and provide a model for apoB antisense therapy. They are suggested to have decreased vascular disease, although they often have hepatic steatosis and increased transaminase levels.\(^{(291, 292)}\)

Mipomersen is administered subcutaneously and has an elimination half-life of 30 days. Mipomersen has Food and Drug Administration orphan drug approval for the treatment of homozygous FH. However, the European Medicines Agency recommended that mipomersen not be approved in the EU citing safety concerns.\(^{(293)}\)

Mipomersen lowers LDL-cholesterol by about 25-36% and Lp(a) by ~33% in FH and non FH patients (see Table 10). Injection site reactions were experienced by 76-93% of participants, and influenza-like symptoms by 46-49%.\(^{(277, 278, 294)}\) Discontinuation rates were relatively high (11-31%) and were predominantly due to side effects. Hepatotoxicity is a concern, with alanine transaminase (ALT) \(\geq 3\) times the upper reference limit observed in 12-33% of patients.\(^{(277, 278)}\) Mipomersen was associated with increases in hepatic fat content up to 47%, even in patients with a very low baseline hepatic fat.\(^{(278, 294)}\) It remains to be seen whether the mipomersen-induced hepatic steatosis progresses to fibrosis and further complications.

1.5.1.2 PCSK9 inhibition
PCSK9 is a secreted protease that promotes the degradation of the LDLR. People who have inherited a ‘loss-of-function’ mutation in PCSK9 usually have low LDL-cholesterol concentrations and a lower CVD risk, while ‘gain-of-function’ mutations cause FH. PCSK9 inhibitors cause a greater reduction in LDL-cholesterol than other agents, with 55-68% reductions seen in clinical trials (Table 10). In addition to lowering LDL-cholesterol, these agents also significantly lower Lp(a) by 22-27.

Injection site reactions have been described, but are much less frequent than with mipomersen. Furthermore these agents are generally very well tolerated and do not appear to be significantly hepatotoxic, although further monitoring is required to confirm this. Two individuals have been reported with naturally occurring homozygous PCSK9 ‘loss of function’ mutations, and they are both healthy and fertile, which is encouraging. However, PCSK9 inhibitors rely on residual LDL receptor function, and thus they are not as effective in individuals with receptor-negative FH.

In a large (n=901) 52-week trial of evolocumab, the most common side effects were nasopharyngitis, upper respiratory tract infections, influenza, back pain, myalgia and elevation of creatine kinase. However, only 5.5% were serious events compared with 4.3% of the placebo group, and only 2.2% dropped out of evolocumab compared with 1% ceasing placebo. PSCK9 inhibitors are the most encouraging of the emerging therapies, with ~50% reduction in major cardiac events with Alirocumab over 78 weeks, and Evolocumab over 1 year, although further research is required to confirm these findings.
1.5.1.3 Microsomal triglyceride transfer protein (MTP) inhibition

MTP is a critical chaperone for the assembly and secretion of hepatic and intestinal lipoproteins, interacting with apoB-100 to incorporate cholesterol ester and triglyceride into nascent VLDL (Figure 1).(298) Abetalipoproteinaemia, an autosomal recessive condition caused by mutations in MTP, is characterised by the absence of apoB containing lipoproteins, hepatic steatosis, ataxia, myopathy and retinitis pigmentosa.(299) Lomitapide is an oral small molecule MTP inhibitor that is licensed for use in individuals with homozygous FH in America and Europe.(82) Lomitapide was associated with a 38% reduction in LDL-cholesterol over 78 weeks in individuals with homozygous FH (Table 10).(286) However, 93% of participants had gastrointestinal side effects, which were severe enough for 10% to stop therapy. Hepatic fat increased from 0.1 to 8.6% on average, and one third of participants had significant elevations of transaminase levels.(286) However, lomitapide is very effective at reducing LDL-cholesterol, so much so, that over 25% of individuals with homozygous FH achieved an LDL-cholesterol of <2.6 mmol/L with combination therapy, and 17% could significantly reduce apheresis treatment.(286)

1.5.1.4 Cholesteryl ester transfer protein (CETP) inhibition

CETP transfers cholesteryl esters from HDL to the apoB containing lipoproteins in exchange for triglyceride. Naturally occurring CETP mutations are associated with hyperalphalipoproteinaemia and reduced coronary disease in some populations.(300) However, investigations with the CETP inhibitor torcetrapib were terminated when an increase in mortality was found,(301) and dalcetrapib did not demonstrate any benefit, although was safe.(302) Two newer CETP inhibitors, anacetrapib and evacetrapib, both ~ double the HDL and significantly lower LDL-cholesterol by one third. Although promising, trials are required to determine the long-term safety and efficacy of these agents.(287-289)
CHAPTER 2:

HYPOTHESES, SCOPE AND STRUCTURE OF THE THESIS
2.1 GENERAL HYPOTHESIS

The general hypothesis of this thesis was that the care of individuals with FH can be enhanced by employing laboratory, primary care and specialist lipid services.

2.1.1 Conception of the general hypothesis

The “care” of individuals with FH encompasses the detection, diagnosis, treatment and follow-up of individuals with FH and their family members. Hypothetico-deductive reasoning principles were used to conceptualise and test the hypothesis, through formulation and assessment of the specific hypotheses.

2.1.1.1 Perceived problem

The majority of individuals with FH are currently undiagnosed and those diagnosed are often undertreated.

2.1.1.2 Previous observations, beliefs and theories facilitating the genesis of the hypothesis

The pathophysiology, diagnosis and treatment of FH, as well as the principles and practices of general CVD risk screening are outlined and referenced in Chapter 1. The previous observations, beliefs and theories facilitating the genesis of the general hypothesis, and leading to the subsequent specific hypotheses and predictions are listed herein. These concepts are discussed more thoroughly in Chapter 1 and in the individual chapters assessing the validity of the specific hypothesis.
Previous observations

- FH causes elevations in LDL-cholesterol. However, there is an overlap between the LDL-cholesterol concentrations of people with and without FH.\(^{303}\)
- Despite the overlap in LDL-cholesterol concentrations, it is possible to identify an LDL-cholesterol concentration that selects individuals at high risk of FH.\(^{51}\)
- Most Western countries advocate measuring LDL-cholesterol as part of general CVD risk assessment (Chapter 1, Section 1.4)
- Laboratories performing LDL-cholesterol estimations could highlight individuals at high risk of FH to the requesting clinician.\(^{304}\)
- GPs are well placed to detect individuals with FH in the community.\(^{142}\)
- Genetic testing for FH can identify mutations in an acceptable proportion of people to allow effective genetic cascade screening to occur.\(^{305}\)
- Specialist lipid services can optimise the detection and management of FH.\(^{12}\)

Beliefs

- GPs may not recognise or optimally manage individuals and families with FH owing to the relatively low prevalence and current diagnosis rates.
- GPs are well placed and willing to have a role in FH management.

Theories

- GPs’ potential awareness and knowledge gaps can be overcome with support from lipid specialists and the community laboratory.
- The community laboratory can augment FH detection by alerting GPs when one of their patients is at high-risk of FH based on the LDL-cholesterol concentration.
- Identifying individuals with undiagnosed FH will lead to optimisation of LDL-cholesterol despite the increased use of statins in the community.
2.2 SCOPE OF THE THESIS

This thesis provides insight into the potential to opportunistically detect individuals with FH in the community using the lipid profiles currently performed for general cardiovascular risk assessment. This concept has not been previously described. It involves using the results of tests performed for general CVD risk screening to identify a specific condition, which while related, was not the reason for performing the test. Furthermore, FH is actually excluded from general CVD risk calculators, as these underestimate the absolute CVD risk associated with FH.

The role of the general practitioner was explored, to establish their knowledge and management practices regarding FH, and to investigate if GPs were willing and able to assist with FH detection. The FH mutation spectrum and detection rates in Western Australia were described to determine if genetic testing was a viable option. Then the effectiveness of genetic cascade screening in terms of detecting and treating individuals with FH was investigated; specifically, seeking to identify if detecting individuals with FH remains efficacious with the increased use of statins in the community.

2.2.1 General structure of the thesis

Chapter 1 encompasses a literature review of FH and general CVD screening, in order to conceptualise how opportunistic FH screening may be conducted using lipid tests performed in the community. The remainder of the thesis comprises a series of studies to assess the predictions and provide observations to test a specific hypothesis, each of which are described in an individual chapter. There are three specific hypotheses derived from the general hypothesis; which focus on the role of the laboratory, primary care and specialist lipid services, as outlined in Figure 4.

Chapters 3-10 start with an introduction to the specific topic of the chapter, which synthesises and augments information presented in Chapter 1. The chapters are
presented as an introduction, method, results, discussion and conclusion. The chapters cross-reference each other in addition to the literature. The limitations and areas for future research, some of which form the topics of subsequent chapters are also presented. The overview in Chapter 11 briefly summarises the findings of the studies and draws an overall conclusion on the validity of the specific hypotheses and general hypothesis. The clinical implications, limitations, areas for future research and overall conclusions of the complete thesis are then presented.

2.2.2 Overview of the studies performed to test the specific hypotheses

The structure of the thesis is outlined in Figure 4, which relates the three specific hypotheses and predictions to the general hypothesis. The first three studies focused on the ability to detect FH using a community laboratory. The first study determined the potential to screen for FH using the LDL-cholesterol measurements performed at a community laboratory, and selected an appropriate LDL-cholesterol threshold to conduct the further investigations to determine the yield and effectiveness of FH detection (Chapter 3). The second study ascertained the impact interpretative commenting on lipid profiles of individuals found to be at high risk of FH on the detection and treatment of FH (Chapter 4). The third study demonstrated the diagnostic yield of a telephone call between the chemical pathologist and requesting general practitioner of an individual found to be at high risk of FH (Chapter 5).

The third and fourth studies investigated the role primary care can have in the care of individuals with FH. Study four sought to identify GPs’ current knowledge and management practices regarding FH (Chapter 6). The fifth study investigated whether, after education, GPs could use the DLCNC in the community to identify individuals at high and low risk of FH (Chapter 7).

The final series of investigations focused on specialist lipid services. Insight into genetic testing for FH in Australia was provided by describing the spectrum of
mutations causing FH in Western Australia and the mutation detection rates (Chapter 8). The effectiveness of cascade genetic screening was demonstrated in terms of the diagnostic yield and whether CVD risk factors were optimised after diagnosis and specialist review, noting the increased use of statins the community (Chapter 9). Chapter 10 incorporated the observations from the investigations in the thesis with the published literature to formulate a practical approach to FH detection involving collaboration between the community laboratory, primary care and specialist lipid services.
General hypothesis:
The care of individuals with FH can be enhanced by employing laboratory, primary care and specialist lipid services

Specific hypothesis 1:
The community laboratory can optimise the detection of FH

Prediction 1: Community laboratories can opportunistically screen for FH

Prediction 2: Interpretative comments increase the detection of FH and optimise treatment

Prediction 3: A phone call and advice from the pathologist to the GP will optimise the detection of FH

Specific hypothesis 2:
General practitioners can effectively detect individuals with FH

Prediction 4: GP's knowledge of FH is suboptimal, although they will be willing to detect and manage individuals with FH

Prediction 5: GPs can use the DLCNC to identify individuals at high risk of FH in primary care

Specific hypothesis 3:
Specialist lipid services can effectively diagnose and treat individuals and families with FH

Prediction 6: A genetic testing strategy for FH can be developed and applied to an Australian clinic population to identify mutations causing FH

Prediction 7: Cascade screening will effectively detect family members with FH, and diagnosis and specialist review will result in additional reductions in CVD risk factors

FIGURE 4: OUTLINE OF GENERAL AND SPECIFIC HYPOTHESES, AND PREDICTIONS
CHAPTER 3:
OPPORTUNISTIC SCREENING FOR FAMILIAL HYPERCHOLESTEROLAEMIA VIA A COMMUNITY LABORATORY

A version of this chapter has been published:

3.1 INTRODUCTION

FH is a common monogenic condition that causes elevated LDL-cholesterol and premature atherosclerotic CVD.\(^{(3-6)}\) Statin therapy significantly reduces CAD and mortality in FH, with evidence that early treatment is more efficacious.\(^{(12,13)}\) Although FH fulfils the WHO criteria for systematic screening, few countries have implemented screening programs to date.\(^{(2,131)}\) As such, the vast majority of people with FH are currently undiagnosed worldwide, and those diagnosed are often inadequately treated.\(^{(7,8,11,55,56)}\)

3.1.1 Screening for FH

The screening approaches for FH are described in Section 1.3. Cascade screening - testing relatives of FH patients - is the most cost-effective approach to identify people with FH, and is discussed thoroughly in Chapter 9.\(^{(2,146)}\) However, cascade screening can only be performed in families where an individual has been diagnosed with FH. Identifying the initial individual (index case) in families with FH is one of the major challenges in FH management.\(^{(55)}\)

Thus despite the advantages of cascade screening for an autosomal co-dominant condition, it is not the sole solution for detecting FH. The diagnostic yield (number of new cases with FH) described with cascade screening programs is very variable worldwide, ranging from 0.4\(^{(307)}\) to 8.6\(^{(180)}\) new cases per index case. It has been estimated that to achieve FH detection rates >80% in the population, between 17-47% of FH cases need to be identified independent of cascade screening.\(^{(305)}\)
3.1.2 Diagnosis of FH: LDL-cholesterol concentration thresholds

There are currently no internationally agreed diagnostic criteria for FH. Three main criteria exist: DLCNC, the Simon Broome Registry criteria and the MED-PED criteria.\(^{(87, 88, 95)}\) All of these criteria use cholesterol concentrations (LDL-cholesterol and/or total cholesterol), although cholesterol alone is usually not adequate to appropriately diagnose FH in any of them.

However, considerable overlap exists between LDL-cholesterol concentrations in FH patients and the general population (Figure 5).\(^{(91, 303)}\) Thus the sensitivity and specificity of FH diagnosis depends on the actual LDL-cholesterol concentration, with specificity only increasing with very elevated LDL-cholesterol concentrations at the expense of marked decreases in sensitivity. Confirming the diagnosis of FH relies on either demonstrating a pathogenic mutation, or a combination of clinical features such as tendon xanthomata and a family history consistent with autosomal dominant inheritance of elevated LDL-cholesterol and/or premature vascular disease.
FIGURE 5. DISTRIBUTION OF LDL-CHOLESTEROL CONCENTRATIONS AMONG SUBJECTS GENETICALLY SCREENED FOR FH IN THE NETHERLANDS

LDL-c = LDL-cholesterol. A – 25,406 subjects tested for LDLR or APOB gene mutations, 20,520 subjects were not on lipid therapy. B – 20,595 subjects were tested for pathogenic LDLR or APOB mutations, 15,901 subjects were not on lipid therapy. C – 17,003 subjects were tested for pathogenic LDLR mutations, 13,067 were not on lipid therapy. D – 5,933 subjects were tested for LDLR mutations where no protein can be synthesised from the affected allele, 4,436 were not on lipid therapy. Figure reproduced from Huijgen et al Discriminative ability of LDL-cholesterol to identify patients with familial hypercholesterolemia: a cross-sectional study in 26,406 individuals tested for genetic FH, with permission. [303]
3.1.3 Prediction

Community laboratories are well placed to perform opportunistic screening for FH.

3.1.4 Aims

The primary aim was to determine the ability of a community laboratory to opportunistically screen for individuals at high risk of FH. The prevalence and appropriateness of the LDL-cholesterol thresholds employed in the varying FH diagnostic criteria and the proportion of LDL-cholesterol requested by GPs were also sought.

3.2 METHODS

3.2.1 Population selection

Serum LDL-cholesterol concentrations were reviewed over a one-year period (1 May 2010 – 30 April 2011) from St John of God Pathology (SJGP), a private laboratory in Western Australia providing services for ~500,000 people from the community and private hospitals. All serum LDL-cholesterol requests were included, with no exclusion criteria. This study protocol was approved by the Royal Perth Hospital Human Research Ethics Committee, and used anonymous data.

3.2.2 Specific LDL-cholesterol concentrations to be investigated

We sought to determine the prevalence of possible FH based on LDL-cholesterol thresholds employed by the three commonly used diagnostic criteria: the DLCNC, Simon Broome Registry and the MED-PED criteria (Tables 1-3 respectively). (87, 88, 95)
3.2.3 Presence of conditions that can cause elevations in LDL-cholesterol

Potential secondary causes of hypercholesterolaemia were sought within ± 30 days of the LDL-cholesterol result. However, hypercholesterolaemia is not uniformly present in patients with these conditions, and the actual severity that causes elevations in cholesterol is not well described for these conditions. Thus, as a conservative approach, we sought the presence of these conditions using the most conservative definition for each condition as described below.

- Hypothyroidism \[\text{TSH} > 4.0 \text{ mU/L}\]
- Nephrotic syndrome \[\text{proteinuria} > 3 \text{ g/L and serum albumin} < 30 \text{ g/L}\]
- Cholestasis alkaline phosphatase (ALP) > 135 U/L and γ glutamyltransferase (GGT) > 55 U/L in males or > 38 U/L in females

Cholesterol may be elevated along with elevated triglycerides representing a mixed hyperlipidaemia, which was defined as a triglyceride > 4.0 mmol/L. The aetiology of mixed hyperlipidaemia is complex, but includes: genetic conditions such as familial combined hyperlipidaemia and dysbetalipoproteinaemia, hypertriglyceridaemia overlaying a polygenic hypercholesterolaemia, and hypertriglyceridaemia overlaying FH.

Altered glucose metabolism occurs in the metabolic syndrome and type 2 diabetes is often associated with elevations in triglycerides.\(^{(308)}\) To estimate the prevalence of altered glucose homeostasis, fasting serum glucose and HbA1c were also sought within ± 30 days of the serum LDL-cholesterol result. Diabetes was defined as a serum glucose ≥ 7.0 mmol/L or a haemoglobin A1c (HbA1c) ≥ 6.5% (48 mmol/mol).

3.2.4 Analytical methods
Total cholesterol, triglyceride, HDL-cholesterol analyses were performed with enzymatic, colorimetric assays using Siemens reagents on a Siemens Dimension RXL chemistry analyser (Siemens Healthcare Diagnostics, Tarrytown NY, USA). LDL-cholesterol was calculated according to the Friedewald equation. For lipid assays, the inter- and intra-assay CVs were <5%. TSH was performed on the Siemens Advia Centaur XP (Siemens Healthcare Diagnostics, Tarrytown NY, USA). ALP, albumin and GGT were measured on the Siemens Dimension RXL. HbA1c was measured on the Primus Ultra, using boronate affinity high-performance liquid chromatography (Trinity Biotech, Kansas City, USA).

3.2.5 Change in LDL-cholesterol

To establish the change in LDL-cholesterol concentration from the LDL-cholesterol measured prior to the selection period LDL-cholesterol, the laboratory database was searched back until 1 January 2008. LDL-cholesterol changes were classified according to the selection period LDL-cholesterol concentration. LDL-cholesterol concentrations <6.5 mmol/L refer to the most recent LDL-cholesterol being an exact value; i.e. 3.5, 4.0, 4.5, 5.0, 5.5, or 6.0 mmol/L respectively. LDL-cholesterols greater than 6.5mmol/L were pooled into groups (i.e. 6.5-6.9 mmol/L) to provide a sufficient sample size.
3.2.6 Data handling

The laboratory database was searched with Business Objects Software, Crystal Reports version 11.0.0.1282, SAP AG, Business Objects, (Walldorf, Germany) and Microsoft Access 2003. If more than one serum LDL-cholesterol was performed in this period, prevalence data were calculated based on the highest concentration, and the most recent LDL-cholesterol was selected as the selection period LDL-cholesterol for the change in LDL-cholesterol analysis. Statistical analysis was performed using Microsoft Excel 2003 and Simple Interactive Statistical Analysis.(309)

3.3 RESULTS

3.3.1 Population description

Over the one-year period, 99,467 serum LDL-cholesterol results were reported on 84,823 people. GPs requested 91.8%, cardiologists requested 3.2%, with the remaining 5.0% requested by other specialists. There were 43,455 (51.2%) LDL-cholesterol measurements performed in females (mean age 56 ± 15 years; range 3-101 years) and 41,386 (48.8%) in males (mean age 56 ± 15 years; range 3-99 years).

In the 213 individuals with an LDL-cholesterol ≥6.5 mmol/L, there was a female predominance, 144 females (mean age 55 ± 12 years; range 17-86 years) vs. 69 males (mean age 51 ± 13 years, range 19-80 years).
3.3.2 Distribution of LDL-cholesterol concentrations

The serum LDL-cholesterol distribution is demonstrated in Figure 6. The median and mean serum LDL-cholesterol concentrations were 3.0 mmol/L and 3.1 mmol/L, respectively. The 95\textsuperscript{th}, 99\textsuperscript{th}, and 99.75\textsuperscript{th} percentiles for serum LDL-cholesterol were 4.8, 5.7, and 6.5 mmol/L, respectively.

There were 3,124 people aged $\geq$16 years who had an LDL-cholesterol $>$4.9 mmol/L, which equated to a prevalence of 1:27 in this sample population. An LDL-cholesterol $>$4.9 mmol/L is part of the Simon Broome Registry criteria, with clinical and history components forming the other aspects see Table 2. The number of individuals and prevalence using the MED-PED criteria, DLCNC and the non-age adjusted LDL-cholesterol cut points are shown in Tables 11, 12 and 13, respectively.
FIGURE 6. DISTRIBUTION OF LDL-CHOLESTEROL CONCENTRATIONS

Distribution of LDL-cholesterol concentrations from 84,823 community dwelling individuals measured by St John of God Pathology over a 12 month period. The insert demonstrates the frequencies of LDL-cholesterol concentrations above 6.5 mmol/L.
### TABLE 11. POTENTIAL FH PREVALENCE BASED ON THE MED-PED CRITERIA

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Number of people meeting the criteria</th>
<th>Number of people in the age category</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt;20 years LDL-cholesterol ≥5.1 mmol/L</td>
<td>6</td>
<td>748</td>
<td>1:124</td>
</tr>
<tr>
<td>Age 20-29 years LDL-cholesterol ≥5.6 mmol/L</td>
<td>19</td>
<td>2980</td>
<td>1:157</td>
</tr>
<tr>
<td>Age 30-39 years LDL-cholesterol ≥6.5 mmol/L</td>
<td>33</td>
<td>7169</td>
<td>1:217</td>
</tr>
<tr>
<td>Age ≥40 years LDL-cholesterol ≥6.7 mmol/L</td>
<td>118</td>
<td>73926</td>
<td>1:626</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>176</strong></td>
<td><strong>84823</strong></td>
<td><strong>1:482</strong></td>
</tr>
</tbody>
</table>

### TABLE 12. PREVALENCE OF THE LDL-CHOLESTEROL THRESHOLDS EMPLOYED BY THE DLCNC

<table>
<thead>
<tr>
<th>Categories</th>
<th>Number of people</th>
<th>Prevalence in this population</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-cholesterol 4.0 – 4.9 mmol/L</td>
<td>11030</td>
<td>1:7.7</td>
</tr>
<tr>
<td>LDL-cholesterol 5.0 – 6.4 mmol/L</td>
<td>2911</td>
<td>1:29</td>
</tr>
<tr>
<td>LDL-cholesterol 6.5 – 8.4 mmol/L</td>
<td>198</td>
<td>1:428</td>
</tr>
<tr>
<td>LDL-cholesterol ≥8.5 mmol/L</td>
<td>15</td>
<td>1:5655</td>
</tr>
</tbody>
</table>
### TABLE 13. PREVALENCE OF POTENTIAL LDL-CHOLESTEROL CUT POINTS

<table>
<thead>
<tr>
<th>LDL-cholesterol (mmol/L)</th>
<th>Number of people</th>
<th>Percentile</th>
<th>Prevalence in this population</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥8.5</td>
<td>15</td>
<td>0.02%</td>
<td>1:5655</td>
</tr>
<tr>
<td>≥8.0</td>
<td>23</td>
<td>0.03%</td>
<td>1:3688</td>
</tr>
<tr>
<td>≥7.5</td>
<td>48</td>
<td>0.06%</td>
<td>1:1767</td>
</tr>
<tr>
<td>≥7.0</td>
<td>90</td>
<td>0.11%</td>
<td>1:942</td>
</tr>
<tr>
<td>≥6.5</td>
<td>213</td>
<td>0.25%</td>
<td>1:398</td>
</tr>
<tr>
<td>≥6.0</td>
<td>472</td>
<td>0.56%</td>
<td>1:180</td>
</tr>
<tr>
<td>≥5.5</td>
<td>1227</td>
<td>1.45%</td>
<td>1:69</td>
</tr>
<tr>
<td>≥5.0</td>
<td>3124</td>
<td>3.68%</td>
<td>1:27</td>
</tr>
<tr>
<td>≥4.5</td>
<td>6879</td>
<td>8.11%</td>
<td>1:12</td>
</tr>
<tr>
<td>≥4.0</td>
<td>14154</td>
<td>16.69%</td>
<td>1:6</td>
</tr>
</tbody>
</table>

#### 3.3.3 Potential secondary causes of hypercholesterolaemia

A potential secondary cause of hypercholesterolaemia was identified in 260 people (8.3%) with an LDL-cholesterol ≥5.0 mmol/L and in 25 people (11.7%) with an LDL-cholesterol ≥6.5 mmol/L. Thyroid dysfunction was the most common potential secondary cause, subclinical (TSH 4-10 mU/L) was found in 7.25%, and overt (TSH > 10mU/L) in 3.99% of individuals. A detailed description of the potential secondary causes is presented in Table 14. A fasting glucose or HbA1c was available on 74,391 (74.8%) requests within ± 30 days of the serum LDL-cholesterol result. In individuals with a LDL-cholesterol ≥6.5 mmol/L, a fasting glucose ≥7.0 mmol/L was present in 6.6% and an HbA1c ≥6.5% (48 mmol/mol) in 5.6% of individuals, with one or both of these conditions present in 8.5%.
### TABLE 14. PREVALENCE OF POTENTIAL CAUSES OF SECONDARY HYPERCHOLESTEROLAEMIA

<table>
<thead>
<tr>
<th></th>
<th><strong>LDL-cholesterol</strong></th>
<th><strong>MEDPED</strong></th>
<th><strong>Aged &lt;20 LDL-cholesterol ≥5.1</strong></th>
<th><strong>Aged 20-29 LDL-cholesterol ≥5.6</strong></th>
<th><strong>Aged 30-39 LDL-cholesterol ≥6.5</strong></th>
<th><strong>Aged ≥40 LDL-cholesterol ≥6.7</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.0-6.4 mmol/L</td>
<td>≥6.5 mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG &gt;4.0</td>
<td>23 (0.79%)</td>
<td>3 (1.41%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (1.69%)</td>
</tr>
<tr>
<td>TSH 4.0 - 10</td>
<td>129 (4.43%)</td>
<td>6 (2.82%)</td>
<td>0</td>
<td>0</td>
<td>2 (6.06%)</td>
<td>3 (2.54%)</td>
</tr>
<tr>
<td>TSH &gt;10</td>
<td>34 (1.17%)</td>
<td>6 (2.82%)</td>
<td>0</td>
<td>1 (5.26%)</td>
<td></td>
<td>5 (4.24%)</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>0 (0.47%)</td>
<td>1 (0.47%)</td>
<td>0</td>
<td>0</td>
<td>1 (3.03%)</td>
<td>0</td>
</tr>
<tr>
<td>Cholestasis</td>
<td>44 (1.51%)</td>
<td>5 (2.35%)</td>
<td>0</td>
<td>1 (5.26%)</td>
<td>1 (3.03%)</td>
<td>3 (2.54%)</td>
</tr>
<tr>
<td>TG &gt;4.0 and TSH 4.0-10.0</td>
<td>2 (0.07%)</td>
<td>2 (0.94%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TSH &gt;4.0 and TSH &gt;10.0</td>
<td>0</td>
<td>2 (0.94%)</td>
<td>0</td>
<td>0</td>
<td>1 (3.03%)</td>
<td>1 (0.85%)</td>
</tr>
<tr>
<td>TG &gt;4.0 and TSH &gt;4.0 and cholestasis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TG &gt;4.0 and cholestasis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TSH 4.0-10.0 and cholestasis</td>
<td>3 (0.10%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TSH &gt;10.0 and cholestasis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nephrotic syndrome and cholestasis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Individuals with secondary cause(s)</td>
<td>235 (8.07%)</td>
<td>25 (11.74%)</td>
<td>0</td>
<td>2 (10.52%)</td>
<td>5 (15.15%)</td>
<td>14 (11.86%)</td>
</tr>
<tr>
<td>Individuals without secondary cause</td>
<td>2676 (91.93%)</td>
<td>188 (88.26%)</td>
<td>6 (100%)</td>
<td>17 (89.48%)</td>
<td>28 (84.85)</td>
<td>104 (88.14%)</td>
</tr>
</tbody>
</table>

TG = triglycerides, measured in mmol/L. TSH = thyroid stimulating hormone, measured in mU/L. TSH was separated in to subclinical 4.0-10.0 mU/L and clinical hypothyroidism >10.0mU/L. Nephrotic syndrome = proteinuria >3g/L and serum albumin <30 g/L. Cholestasis = alkaline phosphatase (ALP) >135 U/L and \( \gamma \) glutamyltransferase (GGT) >55 U/L in males or >38 U/L in females.
3.3.4 Change in LDL-cholesterol concentration

Overall 14,865 individuals meet the specific LDL-cholesterol concentration criteria to search for the change in LDL-cholesterol from their most recent LDL-cholesterol to the selection period LDL-cholesterol. A previous LDL-cholesterol was found in 5,842 (39.5%) individuals allowing an absolute change in LDL-cholesterol to be calculated. An increase from the most recent to the selection LDL-cholesterol was found in 3,588 (61.4%) and a decrease in 2,254 (38.6%) individuals (Table 15, and Figure 7). An absolute change in LDL-cholesterol between 0.1 and 1.0 mmol/L was found in 4,940 (84.6%) individuals; 2,929 (81.6%) of those with increases and 2,011 (89.2%) of those with decreases. An absolute change in LDL-cholesterol between 1.1 and 1.9 mmol/L was found in 668 (11.4%) individuals; 463 (12.9%) of those with increases and 205 (9.1%) of those with decreases. An absolute change in LDL-cholesterol of ≥2.0 mmol/L was found in 234 individuals; 196 (5.5%) of those with increases, and 34 (1.6%) of those with decreases.
TABLE 15. CHANGE IN LDL-CHOLESTEROL BEFORE THE SELECTION PERIOD

<table>
<thead>
<tr>
<th>Selection period LDL-cholesterol concentration (mmol/L)</th>
<th>3.5</th>
<th>4.0</th>
<th>4.5</th>
<th>5.0</th>
<th>5.5</th>
<th>6.0</th>
<th>6.5-6.9</th>
<th>7.0-7.4</th>
<th>7.5-7.9</th>
<th>8.0-8.5</th>
<th>≥8.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>6,698</td>
<td>4,354</td>
<td>2,183</td>
<td>937</td>
<td>332</td>
<td>113</td>
<td>148</td>
<td>47</td>
<td>26</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>Deltas</td>
<td>2,572</td>
<td>1,689</td>
<td>917</td>
<td>355</td>
<td>159</td>
<td>45</td>
<td>61</td>
<td>17</td>
<td>12</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>(%Delta)</td>
<td>38.4%</td>
<td>38.8%</td>
<td>42.0%</td>
<td>37.9%</td>
<td>47.9%</td>
<td>39.8%</td>
<td>41.2%</td>
<td>36.2%</td>
<td>46.2%</td>
<td>66.7%</td>
<td>52.4%</td>
</tr>
<tr>
<td>Increasing (%)</td>
<td>1,314</td>
<td>1,048</td>
<td>668</td>
<td>285</td>
<td>141</td>
<td>39</td>
<td>54</td>
<td>16</td>
<td>10</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>(%)</td>
<td>51.1%</td>
<td>62.0%</td>
<td>72.8%</td>
<td>80.3%</td>
<td>88.7%</td>
<td>86.7%</td>
<td>88.5%</td>
<td>94.1%</td>
<td>83.3%</td>
<td>75.0%</td>
<td>90.9%</td>
</tr>
<tr>
<td>0.1 -1.9 mmol/L</td>
<td>99.0%</td>
<td>97.5%</td>
<td>94.8%</td>
<td>90.9%</td>
<td>73.0%</td>
<td>61.4%</td>
<td>59.3%</td>
<td>43.8%</td>
<td>70.0%</td>
<td>33.3%</td>
<td>20.0%</td>
</tr>
<tr>
<td>≥2.0 mmol/L</td>
<td>1.0%</td>
<td>2.5%</td>
<td>5.2%</td>
<td>9.1%</td>
<td>27.0%</td>
<td>35.9%</td>
<td>40.7%</td>
<td>56.3%</td>
<td>30.0%</td>
<td>66.7%</td>
<td>80.0%</td>
</tr>
<tr>
<td>Decreasing (%)</td>
<td>1,258</td>
<td>641</td>
<td>249</td>
<td>70</td>
<td>18</td>
<td>6</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>(%)</td>
<td>48.9%</td>
<td>38.0%</td>
<td>27.2%</td>
<td>19.7%</td>
<td>11.3%</td>
<td>13.3%</td>
<td>11.5%</td>
<td>5.9%</td>
<td>16.7%</td>
<td>25.0%</td>
<td>9.1%</td>
</tr>
<tr>
<td>0.1 -1.9 mmol/L</td>
<td>98.3%</td>
<td>98.4%</td>
<td>99.2%</td>
<td>98.6%</td>
<td>88.9%</td>
<td>100.0%</td>
<td>85.7%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>≥2.0 mmol/L</td>
<td>1.7%</td>
<td>1.6%</td>
<td>0.8%</td>
<td>1.4%</td>
<td>11.1%</td>
<td>0.0%</td>
<td>14.3%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

Delta = individuals with a previously measured LDL-cholesterol, where an absolute change could be calculated. The database was searched back until 1 January 2008. Deltas are listed under the selection period LDL-cholesterol. LDL-cholesterol concentrations greater than 6.5 mmol/L were pooled into groups (i.e. 6.5-6.9 mmol/L) to provide a sufficient sample size. LDL-cholesterol concentrations <6.5 mmol/L refer to the selection period LDL-cholesterol being an exact value; i.e. 3.5 mmol/L. Increasing = the selection LDL-cholesterol is higher than the previous LDL-cholesterol. Decreasing = the selection LDL-cholesterol is lower than the previous LDL-cholesterol.
The change in LDL-cholesterol concentrations were sought from the selection period to the LDL-cholesterol measured prior to this, searching back to 1 January 2008. LDL-cholesterol changes were classified according to the selection period LDL-cholesterol concentration. LDL-cholesterol concentrations <6.5 mmol/L refer to the selection period LDL-cholesterol being an exact value; i.e. 3.5, 4.0, 4.5, 5.0, 5.5, or 6.0 mmol/L respectively. LDL-cholesterol concentrations greater than 6.5 mmol/L were pooled into groups (i.e. 6.5-6.9 mmol/L) to provide a sufficient sample size. An increase from the most recent to the selection LDL-cholesterol was found in 3,588 (61.4%) and a decrease in 2,254 (38.6%) individuals.
3.4 DISCUSSION

These findings demonstrate that given the large number of serum LDL-cholesterol measurements requested in the community, primarily by GPs, the community laboratory is well placed to opportunistically screen for individuals with potential FH. Previous studies have suggested that individuals with a serum LDL-cholesterol $\geq 6.5$ mmol/L have a high likelihood of FH.$^{(51,90)}$ Over a one-year period, we found 213 individuals with an LDL-cholesterol $\geq 6.5$ mmol/L, equating to a prevalence of 1:398 in this sample population. FH is conventionally considered to have a population prevalence of 1:500 in Australia.$^{(55)}$ Thus, a serum LDL-cholesterol cut point $\geq 6.5$ mmol/L, irrespective of the patient’s age, gives a result close to the estimated prevalence of FH. However, exactly how many of these individuals will be confirmed to have FH after specialist review had yet to be determined, but is discussed in detail in Chapters 4 and 5.

3.4.1 LDL-cholesterol concentrations employed in FH diagnostic criteria

The Simon Broome criteria rely on clinical features and a serum LDL-cholesterol of $>4.9$ mmol/L to diagnose FH in adults. However, our data suggest that this LDL-cholesterol threshold, if used in isolation of history and clinical features, lacks specificity for FH since an LDL-cholesterol $>4.9$ mmol/L had a prevalence of 1:27 in this sample population, almost 20 times the predicted FH prevalence in an unselected community sample.

The age-adjusted serum LDL-cholesterol criteria employed by MED-PED identified 176 individuals with possible FH, equating to a prevalence of 1:482 in this sample population, very similar to the predicted population prevalence. Hence, the MED-PED criteria could be applied in a community laboratory to detect FH, although this would require specialised laboratory software to identify these individuals. A benefit of applying the MED-PED criteria is their ability to ascertain individuals at a
younger age. The MED-PED criteria identified 25 individuals aged <30 years at high risk of FH; 18 of whom would not have been detected using an LDL-cholesterol threshold of ≥6.5 mmol/L.

The multiple LDL-cholesterol categories employed by the DLCNC could be used to raise the likelihood of FH in a graded manner. However, the lower LDL-cholesterol categories would lack specificity. The LDL-cholesterol category of 6.5–8.4 mmol/L had a prevalence of 1:428 in this sample population, although it would also be important to include individuals with LDL-cholesterol of ≥8.5 mmol/L. Selecting an LDL-cholesterol cut point ≥6.5 mmol/L was more efficient and gave a prevalence of 1:398 in this sample population.

3.4.2 Relevance of these results

This community laboratory provides diagnostic services for ~500,000 people. Thus, ~17% of this population had a serum LDL-cholesterol measured during the one-year period of study. Based on a population prevalence for FH of 1:500, ~1,000 people would be predicted to have FH in this sample population. A serum LDL-cholesterol cut point of ≥6.5 mmol/L identified 213 individuals who were likely to have FH; ~20% of the predicted FH in this sample population. These findings emphasise the huge potential of a community laboratory has in the opportunistic detection of FH.

The mean age of this sample population was 56 years, with 12.8% aged less than 40 years, 4.4% aged less than 30 years and 0.9% aged less than 20 years, suggesting that routine CVD screening is occurring, as discussed in Sections: 1.4.4, 1.4.5 and 1.4.12. Although younger adults and children are underrepresented in this population, these individuals could be identified during the cascade screening process. The detection of index cases is one of the major challenges in FH management.10

The findings of this study should be generally applicable given the large community population, the lack of exclusion criteria and the fact that 92% of requests
were from GPs. Furthermore, this community laboratory sample would appear representative of the general Australian population in that the 95th percentile for LDL-cholesterol of 4.8 mmol/L was very similar to the age- and gender-specific 95th percentiles from the AusDiab study (males 4.43 and females 4.74 mmol/L), and that diabetes was not over-represented (GF Watts personal communication). The average LDL-cholesterol (3.1 mmol/L) was also similar to that reported in a randomly sampled rural South Australian population from 2004-2006 with an average of 3.23 mmol/L.\(^{(310)}\)

Potential secondary causes of hypercholesterolaemia were identified in a minority of individuals (8%), indicating that an LDL-cholesterol cut-point alone could be applicable for screening for FH.

### 3.4.3 Trends in LDL-cholesterol

The relatively low number of repeat LDL-cholesterol measurements within this ~2.5 year period is of concern. It is even more concerning that the majority of individuals demonstrated an increase in their LDL-cholesterol prior to the selection period. The majority of individuals with very elevated LDL-cholesterol concentrations (≥6.5 mmol/L) had increases of ≥2.0 mmol/L in their LDL-cholesterol, which may suggest they are coming off, or are poorly compliant with their lipid-lowering therapy – noting that statins have been proven to improve mortality and morbidity in FH.\(^{(12,13)}\)
3.4.4 **Strengths and limitations**

The large number of individuals having LDL-cholesterol measurements and the unselected community population are strengths of this research. The research was novel, and sought to ascertain if a community laboratory could opportunistically screen for FH. However, there are some limitations of using a community laboratory to screening for FH, as this represents a form of opportunistic screening of individuals referred for LDL-cholesterol testing, rather than the systematic screening of a population. Due to the large number of LDL-cholesterol measurements performed by this community laboratory, LDL-cholesterol is calculated rather than measured directly, which would exclude some, but not all, patients with familial combined hyperlipidaemia. However, it could also exclude some individuals with FH and elevated triglyceride concentrations for another reason.\(^{19}\)

Given the considerable overlap in LDL-cholesterol concentrations of people with FH and the normal population, any LDL-cholesterol threshold suggesting high risk for FH is likely to have reduced sensitivity.\(^{91, 303}\) FH individuals with lower LDL-cholesterol concentrations may be detected via cascade screening.

This study used LDL-cholesterol results from only one community laboratory, so it is possible that more individuals had repeat lipid profiles performed at a different laboratory. However, this study was performed in a period where individuals had to present to the laboratory the requesting doctor chose, and the requesting doctors tended to remain with one pathology provider.
3.4.5 Areas for future research

The practicalities and logistics of how the community laboratory can play an active role in highlighting potential index cases with FH remain to be elucidated. Identifying patients with an LDL-cholesterol of $\geq 6.5$ mmol/L would be achievable with most community laboratory information systems. The prevalence of LDL-cholesterol of $\geq 6.5$ mmol/L (the 99.75th percentile) allows each case to be reviewed by a chemical pathologist, enabling individualised interpretation and advice on further testing and referral pathways.

The interaction between the chemical pathologist and the requesting GP will be a central aspect of this process, although this also requires further study to ensure it is performed in an efficient and effective manner. Moreover, the best method of ensuring these people are referred to a specialist lipid clinic remains to be determined. It is essential that a proposed system can be implemented in a high volume, rapid turnaround laboratory, without necessitating large amounts of staff time. Further investigation into LDL-cholesterol trends and reasons why the lipid profiles do not appear to be repeated as often as recommended in the national CVD guidelines for high-risk individuals are required.

3.5 CONCLUSIONS

This study has shown that the community laboratory has the ability to opportunistically screen for individuals with potential FH. However, the proportion of these people who are subsequently confirmed to have FH after specialist review remains to be determined. Secondary causes were only present in a minority of cases, suggesting that an LDL-cholesterol cut point alone would be applicable for detecting FH. Further investigation is required to determine the most effective method of identifying these people and ensuring they are referred for specialist review.
CHAPTER 4:
IMPACT OF INTERPRETATIVE COMMENTING ON
THE DETECTION OF FAMILIAL
HYPERCHOLESTEROLAEMIA

A version of this chapter has been published:

4.1 INTRODUCTION

Community laboratories perform a large number of lipid profiles, and are well placed to opportunistically detect FH as described in Chapter 3.\(^{(306)}\) However, the best method to highlight individuals found to be at high risk of FH based on their LDL-cholesterol concentration to the requesting GP remains to be determined. This chapter describes an investigation to determine if interpretative comments on the lipid report of individuals found to be at high risk of FH improves FH detection and management.

4.1.1 Interpretative commenting

Interpretative commenting involves applying additional information to a report to assist the clinician interpreting the results and is typically performed by the duty biochemist, a pathologist or, less frequently in Australia, a professionally qualified scientist.\(^{(312)}\) Interpretative commenting is employed in the vast majority laboratories in the UK,\(^{(273)}\) despite debate over the influence this has on patient management and outcomes.\(^{(313, 314)}\) There was previously only one published manuscript describing the impact of interpretative commenting.\(^{(315)}\) This was a historically controlled observational study of community patients on thyroxine conducted over a three-year period. Interpretative comments were associated with a 22% reduction in the number of under replaced patients.\(^{(315)}\) Further work is required to establish the impact interpretative comments have on management outcomes.

However, interpretative comments are well received, especially by GPs, with 78% stating that they influenced patient management.\(^{(316, 317)}\) Interpretative commenting on large volume tests such as lipid profiles often necessitates the use of sophisticated computer software.\(^{(273)}\) Given the multifactorial nature of absolute cardiovascular risk, interpretation of lipid profiles should occur within the broader concept of CVD prevention.\(^{(1, 210, 318, 319)}\)
4.1.2 Detecting FH in Australia

Australia does not currently have a systematic FH screening program, but recommends a combination of opportunistic, targeted and cascade screening for FH.\(^{(55)}\)

It has been previously demonstrated that community laboratories have the potential to detect FH, as they perform large numbers of LDL-cholesterol measurements with the vast majority requested by GPs (Chapter 3).\(^{(306)}\) The DLCNC are the preferred diagnostic criteria for FH in Australasia.\(^{(55)}\) People with an LDL-cholesterol \(\geq 6.5\) mmol/L have a high risk of FH.\(^{(51, 90)}\)

4.1.3 Prediction

Appending an interpretative comment highlighting the risk of FH to lipid reports of individuals with an LDL-cholesterol \(\geq 6.5\) mmol/L will increase the detection of FH and optimise treatment.

4.1.4 Aims

To determine the impact of adding interpretative comments highlighting the possibility of FH to lipid profiles of individuals with an LDL-cholesterol \(\geq 6.5\) mmol/L. The impact of interpretative commenting on specialist referral for FH assessment and rates of LDL-cholesterol re-measurement were established, and the subsequent LDL-cholesterol concentrations were assessed to determine if lipid-lowering therapy was added.
4.2 METHODS

4.2.1 Population selection

This case-historical control study used serum lipid profiles measured at SJGP between the 1st of January 2008 and the 19th of October 2011. SJGP is private not-for-profit organisation providing clinical laboratory services to patients in primary care and private hospitals. In Western Australia, SJGP performs ~100,000 LDL-cholesterol measurements per year, 92% of which are requested by GPs.\(^{(306)}\)

Individuals in the intervention group were selected on the basis of an LDL-cholesterol concentration ≥6.5 mmol/L on a lipid profile requested by a GP between the 23rd of June and the 19th of October 2010. The control group, consisting of the first 100 individuals requested by a GP, was selected retrospectively by reviewing the LDL-cholesterol results from the 1st of May 2009. Individuals in both groups were excluded if there was an identifiable potential secondary cause for the hypercholesterolaemia [hypothyroidism (TSH >4.0 mU/L), mixed hyperlipidaemia (triglyceride >4.0 mmol/L), nephrotic syndrome (proteinuria >3 g/L and serum albumin <30 g/L), and cholestasis (ALP >135 U/L and GGT >55 U/L in males or >38 U/L in females) within ± 30 days of the LDL-cholesterol result].

4.2.2 Interpretative comments

Interpretative comments were added to the lipid results with the assistance of an expert system, LabWizard® (Pacific Knowledge Systems, Sydney, Australia), with all comments reviewed by a chemical pathologist.\(^{(320)}\) Four different comments were used, all raising FH as a consideration, but with increasing content and specificity.
The comments were as follows:

1. Familial hypercholesterolaemia (FH) is an important consideration when LDL-cholesterol ≥6.5 mmol/L.

2. FH Familial hypercholesterolaemia (FH) is an important consideration when LDL-cholesterol ≥6.5 mmol/L. Suggest review of clinical stigmata of FH (tendon xanthomata, corneal arcus), review family history and repeat full lipid profile.

3. Familial hypercholesterolaemia (FH) is an important consideration when LDL-cholesterol ≥6.5 mmol/L. Suggest review of clinical stigmata of FH (tendon xanthomata, corneal arcus) and consider specialist referral.

4. Familial hypercholesterolaemia (FH) is very likely when LDL-cholesterol >8.4 mmol/L.

The expert system assisted in the selection of these comments; comment 3 could only be applied if there was a previous LDL-cholesterol of ≥6.5 mmol/L after reviewing LDL-cholesterol results back to the 1st January 2008, whereas comment 4 could only be applied when the LDL-cholesterol was >8.4 mmol/L.

4.2.3 Analytical methods

Total cholesterol, triglyceride, and HDL-cholesterol analyses were performed with enzymatic, colorimetric assays using Siemens reagents on a Siemens Dimension RXL chemistry analyser (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). LDL-cholesterol was calculated according to the Friedewald equation. The coefficient of variation for total cholesterol was 1.8%.
4.2.4 Data handling

The community laboratory database was searched with Crystal Reports software version 11.0.0.1282, SAP AG, Business Objects (Walldorf, Germany), and Microsoft Access 2003. Referral to and the outcome of review by the regional lipid disorders clinic were determined by manual comparison of the case and the clinic databases. In order to capture patients who may have been reviewed by a private specialist, the regional Cardiovascular Genetics Laboratory database was manually reviewed to determine if genetic testing had been ordered on any of these individuals.

This study was approved by the Royal Perth Hospital Human Research Ethics Committee (EC 2011/069).

4.2.5 Statistical analysis

Statistical analysis was performed using Microsoft Excel 2003, STATA, StataCorp. 2011, Stata Statistical Software: release 12. A repeated measures, random effects, linear regression model was used to investigate the changes in LDL-cholesterol over time between the interpretative comment and control groups via an interaction term. Baseline characteristics were included as covariates to investigate any possible effect on the interaction term. Bootstrapped standard errors were employed in this analysis. Chi-squared or Fisher’s exact tests were used to compare the specialist referral rates.
4.3 RESULTS

4.3.1 Description of study population

During the case selection period, 30,336 LDL-cholesterol results were issued, with 109 individuals identified with an LDL-cholesterol \( \geq 6.5 \) mmol/L. Two individuals were excluded due to a potential secondary cause of hypercholesterolaemia (TSH >4.0 mU/L). Eleven individuals did not have FH raised in the interpretative comments after review by the chemical pathologist: seven were aged \( \geq 75 \) years, one the result was copied to a cardiologist, and one had recent hypothyroidism. The reason for exclusion by the chemical pathologist was not stated in the remaining two individuals. The subject characteristics for the 96 individuals remaining in the intervention group are described in Table 16 with a mean LDL-cholesterol of 7.1 ± 0.8 mmol/L (range, 6.5–11.2). There were 83 different GP requestors for the 96 individuals.

The first one hundred individuals who met the same inclusion and exclusion criteria were selected from the 153 individuals in the control selection period with an LDL-cholesterol \( \geq 6.5 \) mmol/L; their mean LDL-cholesterol was also 7.1 ± 0.8 mmol/L (range, 6.5–9.9) (Table 16) with 88 different GP requestors. There were no statistically significant differences in the demographics between the cases and controls.
TABLE 16. SUBJECT CHARACTERISTICS FOR THE INTERPRETATIVE
COMMENT INVESTIGATION

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>96</td>
<td>100</td>
</tr>
<tr>
<td>Females</td>
<td>68</td>
<td>68</td>
</tr>
<tr>
<td>Age (years) mean ± SD, [range]</td>
<td>53.7 ± 10.7 [26–74]</td>
<td>53.8 ± 14.8 [16–92]</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L) mean ± SD, [range]</td>
<td>7.1 ± 0.8 [6.5–11.2]</td>
<td>7.1 ± 0.8 [6.5–9.9]</td>
</tr>
<tr>
<td>No history provided (%)</td>
<td>36 (37.5)</td>
<td>31 (31)</td>
</tr>
<tr>
<td>Clinical history provided:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>8 (8.3)</td>
<td>9 (9)</td>
</tr>
<tr>
<td>Ischaemic heart disease (%)</td>
<td>2 (2.1)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Statin (%)</td>
<td>5 (5.2)</td>
<td>7 (7)</td>
</tr>
</tbody>
</table>
4.3.2 Subsequent change in LDL-cholesterol concentration

A subsequent LDL-cholesterol was recorded in 63 individuals in the intervention group in the 12 months following their selection LDL-cholesterol. If more than one LDL-cholesterol was performed in the follow up period, the first was selected. Fifty nine (89%) individuals from the intervention group demonstrated a reduction in their LDL-cholesterol, whereas four showed an increase. The mean follow up LDL-cholesterol was 4.1 ± 1.6 mmol/L, demonstrating a significant 3.0 ± 1.7 mmol/L reduction (p<0.0001; Figure 8).

A subsequent LDL-cholesterol was recorded in 70 controls in the 12 months following their selection LDL-cholesterol. Sixty one (87%) controls demonstrated a reduction in their LDL-cholesterol, one remained the same and eight showed an increase. The mean follow up LDL-cholesterol was 4.7 ± 1.7 mmol/L demonstrating a mean reduction of 2.3 ± 1.8 mmol/L (p<0.0001; Figure 9). The additional 0.7 mmol/L reduction LDL-cholesterol in the intervention group when compared with controls was statistically significant (p<0.005).

Adjusting for the patient demographics and characteristics including, presence of a clinical history provided on the request form, statin use, history of CAD and diabetes had no impact on the difference between the LDL-cholesterol reduction between the interpretative comment group and controls. Although fewer males had a repeat LDL-cholesterol when compared with females (p=0.02), there was no effect seen for age, statin use, history of CAD, or diabetes. Interestingly, there was a trend towards a higher LDL-cholesterol repeat rate in individuals without any clinical history provided (36%) compared with individuals with a clinical history provided (19%), although this failed to reach statistical significance.
FIGURE 8. CHANGE IN LDL-CHOLESTEROL CONCENTRATION AFTER INTERPRETATIVE COMMENTING

Change in LDL-cholesterol concentrations from the selection period to the LDL-cholesterol measured after the interpretative comment was appended to the selection period lipid report, presented as a waterfall plot. The follow up LDL-cholesterol concentrations were sought within 365 days of the selection period LDL-cholesterol being measured.
FIGURE 9. CHANGE IN LDL-CHOLESTEROL CONCENTRATION IN THE CONTROL GROUP

Change in LDL-cholesterol concentrations from the selection period to the LDL-cholesterol measured within 365 days of the selection period LDL-cholesterol being measured in the control group, presented as a waterfall plot.
4.3.3 Specialist referral

Four individuals from the intervention group were referred to the regional lipid disorders clinic compared with one control, although this was not statistically significant (p=0.20). However, when specialist referral was stated in the interpretative comment, three (11.5%) of the 26 individuals were referred to a specialist, which was significantly greater than the one (1%) referred from the controls (p<0.05). The mean time to referral was 229 days, (range, 169-311).

The specific interpretative comment added to the individual cases and their outcomes are shown in Table 17. There were no statistically significant differences in the LDL-cholesterol reduction between the comment groups.

All four individuals were diagnosed with FH clinically after specialist review using the DLCNC; two had ‘definite’ FH, two had ‘probable’ FH. Both individuals with ‘definite’ FH had an identifiable LDLR gene mutation. The control individual was not clinically diagnosed with FH and did not have genetic testing after specialist review.
<table>
<thead>
<tr>
<th>Interpretative comment</th>
<th>Individuals receiving the comment</th>
<th>Repeat lipid profile (%)</th>
<th>Reduction in LDL-cholesterol (mmol/L) mean ± SD</th>
<th>Referral to the Lipid Disorders Clinic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 FH is an important consideration when LDL-cholesterol ≥6.5 mmol/L.</td>
<td>18</td>
<td>12 (66.7%)</td>
<td>2.6 ± 2.4</td>
<td>0</td>
</tr>
<tr>
<td>2 FH is an important consideration when LDL-cholesterol ≥6.5 mmol/L. Suggest review of clinical stigmata of FH (tendon xanthomata, corneal arcus), review family history and repeat full lipid profile.</td>
<td>50</td>
<td>33 (66.0%)</td>
<td>3.1 ± 1.6</td>
<td>1</td>
</tr>
<tr>
<td>3 FH is an important consideration when LDL-cholesterol ≥6.5 mmol/L. Suggest review of clinical stigmata of FH (tendon xanthomata, corneal arcus) and consider specialist referral.</td>
<td>26</td>
<td>18 (69.2%)</td>
<td>3.0 ± 1.6</td>
<td>3</td>
</tr>
<tr>
<td>4 FH is very likely when LDL-cholesterol &gt;8.4 mmol/L.</td>
<td>2</td>
<td>0 (0.0%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
4.4 DISCUSSION

This is the first study to investigate the impact adding an interpretative comment to the lipid profile of individuals at high risk for FH has on their subsequent management. Interpretative commenting was associated with a significant additional reduction in LDL-cholesterol compared with controls without an interpretative comment. The significant reductions in LDL-cholesterol seen in both the interpretative comment and control groups would be consistent with the initiation of statin therapy.\(^\text{(266)}\) However, the additional LDL-cholesterol reduction demonstrated in the interpretative comment group would be consistent with approximately double the dose of statins used in the control group.\(^\text{(266)}\) Statin therapy has been demonstrated to significantly reduce CAD and mortality in FH.\(^\text{(12, 13)}\) The additional LDL-cholesterol reduction seen in the interpretative comment group would be predicted to provide a clinically significant further reduction in cardiovascular risk. High intensity statin therapy has also been predicted to be cost-effective in FH.\(^\text{(111)}\)

4.4.1 Specialist referral

Although specifically suggesting specialist referral in the interpretative comment was associated with a 10-fold increase in referrals, only the minority (11.5\%) of these individuals were referred for specialist assessment. A possible explanation for the relatively low specialist referral rates is the observation by some GPs that FH was just synonymous with a family history of raised cholesterol, and thus, the benefit of formal diagnosis of FH was not felt to be important.\(^\text{(141)}\)

Alternatively, it may be that requestors did not recognise that FH is a dominantly inherited genetic condition with the resulting ramifications for family members. A further possibility may be the perception that individuals with FH just require statin therapy, which GPs were very comfortable providing, thus negating a
need for specialist review. Investigation of GPs knowledge and attitudes towards FH is required to guide further measures to increase the identification and treatment of individuals with FH.

4.4.2 Strengths and limitations

The LDL-cholesterol selection criteria designed to identify individuals at very high risk for FH are one of the strengths of this study, as reflected by four of the five individuals referred for specialist assessment being clinically diagnosed with FH. The large sample size, relative to the specificity of the selection criteria, and the well-matched cases and controls are other strengths.

However, there were some limitations of this study. Our study used historical controls, although both the control and case periods occurred during a time of stable statin availability and FH case detection, as the FH Western Australia program had been active since 2007. Furthermore, the majority of the study occurred when government regulations stated that a patient had to attend the laboratory named on the request form, and an individual’s GP tended to remain with a single pathology provider during this time. Although we may have missed individuals who had subsequent LDL-cholesterol measurements performed at a different laboratory, this was a constant for both the interpretative comment group and controls.

The improvement in clinical outcome with interpretative commenting is encouraging and highlights the role clinical biochemists have in the detection and management of FH. However, due to the observational nature of this study, we were unable to elucidate if the greater LDL-cholesterol reductions in the interpretative comment group were due to increased dose, compliance, or use of more potent statin therapy. The clinical biochemist’s role in FH detection could be further advanced by investigating the reasons specialist referral rates were low compared with lipid-lowering treatment rates.
4.4.3 Areas for future research

Interpretative commenting was associated with a significant increase in specialist referrals, although these remained relatively low which requires further investigation. To try to improve this referral rate, we investigated the impact of a phone call between the chemical pathologist and requesting GP in addition to the interpretative comments (Chapter 5).

There seemed to be a discrepancy between referral and optimising LDL-cholesterol, which may suggest GPs are comfortable using medications but may not have recognised the relevance of diagnosing FH for the patient and their family. GP knowledge and practices of FH has been investigated and is described in Chapter 6.

4.5 CONCLUSION

Interpretative commenting was associated with a significantly greater reduction in LDL-cholesterol concentrations and an increase in specialist referrals compared with controls. However, interpretative commenting did not increase the LDL-cholesterol re-measurement rates, and even specifically suggesting specialist referral resulted in only a minority of individuals being referred. Hence while the potential role interpretative commenting may have in individuals at risk for FH is encouraging, further investigation is required to optimise FH detection.
CHAPTER 5:

IMPACT OF A TELEPHONE CALL FROM THE CHEMICAL PATHOLOGIST TO THE REQUESTING GENERAL PRACTITIONER ON THE DETECTION OF FAMILIAL HYPERCHOLESTEROLAEMIA

A version of this chapter has been published:

5.1 INTRODUCTION

Most countries do not have a systematic FH screening program despite FH fulfilling the WHO criteria for disease screening,\(^{(2,11,55)}\) and the majority of individuals with FH are currently undiagnosed, and often undertreated.\(^{(11,55)}\) Detecting individuals with FH early is important, as statin therapy significantly reduces CAD and mortality in FH.\(^{(12,13)}\) Chapter 3 demonstrated that community laboratories are well placed to opportunistically screen for FH, as they measure large numbers of lipid profiles.\(^{(304,306)}\) Interpretative comments on lipid results of individuals at high risk of FH was associated with increased FH detection, although only a minority of individuals were actually referred to a lipid specialist (Chapter 4).\(^{(311)}\) The most effective method of highlighting individuals at high risk of FH to their requesting GP remains to be elucidated.

5.1.1 Prediction

A phone call and advice from the chemical pathologist to the requesting GP of individuals identified at high risk of FH from a community laboratory will optimise the detection of FH.

5.1.2 Aim

To determine the impact of a telephone call from the chemical pathologist to the requesting GP of individuals at high risk of FH (LDL-cholesterol $\geq6.5$ mmol/L) on FH detection and specialist referral rates.
5.2 METHODS

5.2.1 Population Selection

This case-historical control study consisted of individuals selected on the basis of an LDL-cholesterol ≥6.5 mmol/L measured by SJGP, a private community laboratory in Western Australia, if the lipid profile was requested by a GP. This LDL-cholesterol threshold has previously been shown to identify individuals at high risk of FH.\(^{(51, 90)}\) Individuals were excluded if they had a potential secondary cause of hypercholesterolaemia [hypothyroidism (TSH >4.0 mU/L), mixed hyperlipidaemia (triglyceride >4.0 mmol/L), nephrotic syndrome (proteinuria >3 g/L and serum albumin <30 g/L), or cholestasis ((ALP >135 U/L and GGT >55 U/L in males or >38 U/L in females)] identifiable within 30 days of the LDL-cholesterol result.

5.2.2 Intervention: Phone call and advice

The intervention group, identified between the 1\(^{st}\) of November 2010 and the 6\(^{th}\) of October 2011, consisted of the first 100 individuals meeting the above selection criteria whose GP answered the telephone call from one of the three chemical pathologists at SJGP. During this call the chemical pathologist informed the GP that the patient was at high risk of FH, provided information on the mode of inheritance and increased premature CVD risk associated with FH, and suggested referral to the regional lipid disorders clinic. A handout on FH specifically designed for health professionals was also offered. GPs were asked if the phone call was useful, and the chemical pathologist made notes on the discussion and comments the GPs made with regard to the utility of laboratory alerts.
The control group, selected between the 23rd of June and the 19th of October 2010, consisted of 96 individuals with the same inclusion and exclusion criteria as above, except their GP did not receive a telephone call from the chemical pathologist. The control group has previously been described in Chapter 4. Individuals in both the intervention and control groups received interpretative comments with the lipid results highlighting the patient is at risk of FH, as previously described (Section 4.2.2).

5.2.3 Data handling

To determine the impact the telephone call had on FH detection, the number of referrals to the regional lipid disorders clinic was compared over the 12 months following the LDL-cholesterol being reported for each individual in the intervention and control groups. This was performed by manually comparing the study and lipid disorders clinic databases. In order to capture data on individuals who may have been reviewed by a private specialist, the regional Cardiovascular Genetics Laboratory database was screened to determine if genetic testing had been performed on any of these individuals.

The impact on FH detection was ascertained by reviewing the outcome of the specialist consultation for individuals referred to the lipid disorders clinic by manually searching the lipid disorders clinic database, and by reviewing the cardiovascular genetics database for the individuals not referred to the clinic. Genetic testing was performed as part of routine care and is thoroughly described in Chapter 8. In brief, for index cases all 18 exons of the \textit{LDLR}, part of exon 26 and 29 of \textit{APOB} and exon 7 of \textit{PCSK9} were Sanger sequenced, and MLPA of the \textit{LDLR} was performed to assess for deletions or duplications.
This study was approved by the Royal Perth Hospital Human Research Ethics Committee (EC 2011/069). The investigation and management of all individuals referred to the lipid disorders clinic was performed as part of routine clinical service.

5.2.4 Statistical analysis

Statistical analysis was performed using Microsoft Excel 2003, STATA, StataCorp. 2011, Stata Statistical Software, release 13. Two-tailed Chi-squared or Fisher’s exact tests were used for categorical data, and unpaired two-tailed t-tests for continuous data.

5.3 RESULTS

5.3.1 Description of the study population

During the case selection period, 94,799 LDL-cholesterol results were issued by SJGP, with 164 LDL-cholesterol results ≥6.5 mmol/L. In order to contact the requesting GP for the 100 individuals in the intervention group, a chemical pathologist made 158 telephone calls about 113 individuals; 13 GPs could not be contacted. There were 82 different GPs for the 100 individuals in the intervention group; their mean LDL-cholesterol was 7.1 ± 0.7 mmol/L (Table 18). There were 83 different GP requestors for the control group; their mean was 7.1 ± 0.8 mmol/L. The characteristics of the control group have been previously described in Section 4.3.1 and Table 16.(311) The cases (49.3 years) were younger than controls (53.7 years), but there were no other significant differences in demographics.

5.3.2 Specialist referral

Twenty seven (27%) individuals in the intervention group were referred to the lipid disorders clinic during the 12 months follow up after the telephone call. One was already known to the clinic (LDLR mutation-positive FH) and two failed to attend their
appointments, thus 25 individuals underwent specialist review (Table 19). No patients were referred for FH genetic testing from private specialists over this time.
### TABLE 18. SUBJECT CHARACTERISTICS AND FH DETECTION RATES

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls</th>
<th>Cases</th>
<th>Significance (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>96</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Females, n</td>
<td>68</td>
<td>57</td>
<td>0.05</td>
</tr>
<tr>
<td>Age (years), mean ± SD, [range]</td>
<td>53.7 ± 10.7 [26–74]</td>
<td>49.3 ± 12.4 [15–76]</td>
<td>0.009</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L), mean ± SD, [range]</td>
<td>7.1 ± 0.8 [6.5–11.2]</td>
<td>7.1 ± 0.7 [6.5–9.5]</td>
<td>1.00</td>
</tr>
<tr>
<td>Referred to specialist, n (%)</td>
<td>4 (4%)</td>
<td>27 (27%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Clinical FH (probable or definite) n (%)</td>
<td>4 (4%)</td>
<td>18 (18%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Probable FH, n (% of clinically assessed)</td>
<td>2 (50%)</td>
<td>6 (24%)</td>
<td>0.28</td>
</tr>
<tr>
<td>Definite FH, n (% of clinically assessed)</td>
<td>2 (50%)</td>
<td>12 (48%)</td>
<td>0.01&quot;</td>
</tr>
<tr>
<td>Mutation identified, n (% of genetically tested)</td>
<td>2 (50%)</td>
<td>7 (30%)</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Continuous variables were compared with two-tailed unpaired t-tests. Categorical data were compared with either two-tailed Fisher’s exact or χ² tests. # p refers to the absolute numbers referred.
TABLE 19. LIKELIHOOD OF FH AFTER SPECIALIST REVIEW AND GENETIC TESTING IN THE TELEPHONE GROUP

<table>
<thead>
<tr>
<th>ID</th>
<th>Gender</th>
<th>Age</th>
<th>Pre Rx LDL-cholesterol mmol/L</th>
<th>Clinical DLCNC score</th>
<th>FH causing mutation</th>
<th>Complete DLCNC score</th>
<th>FH likelihood (DLCNC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F38</td>
<td>38</td>
<td>6.8</td>
<td>5</td>
<td>No</td>
<td>5</td>
<td>Possible</td>
</tr>
<tr>
<td>2</td>
<td>M40</td>
<td>40</td>
<td>6.5</td>
<td>6</td>
<td>No</td>
<td>6</td>
<td>Probable</td>
</tr>
<tr>
<td>3</td>
<td>F62</td>
<td>62</td>
<td>8.5</td>
<td>13</td>
<td>Yes</td>
<td>22</td>
<td>Definite</td>
</tr>
<tr>
<td>4</td>
<td>F63</td>
<td>63</td>
<td>6.5</td>
<td>10</td>
<td>No</td>
<td>10</td>
<td>Definite</td>
</tr>
<tr>
<td>5</td>
<td>F69</td>
<td>69</td>
<td>9.5</td>
<td>8</td>
<td>Yes</td>
<td>16</td>
<td>Definite</td>
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<td>6</td>
<td>F44</td>
<td>44</td>
<td>6.5</td>
<td>6</td>
<td>No</td>
<td>6</td>
<td>Probable</td>
</tr>
<tr>
<td>7</td>
<td>M19</td>
<td>19</td>
<td>7.5</td>
<td>6</td>
<td>Yes</td>
<td>14</td>
<td>Definite</td>
</tr>
<tr>
<td>8</td>
<td>F60</td>
<td>60</td>
<td>6.5</td>
<td>5</td>
<td>No</td>
<td>5</td>
<td>Possible</td>
</tr>
<tr>
<td>9</td>
<td>M59</td>
<td>59</td>
<td>6.6</td>
<td></td>
<td>Did not attend</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>M38</td>
<td>38</td>
<td>6.8</td>
<td>9</td>
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<td>9</td>
<td>Definite</td>
</tr>
<tr>
<td>11</td>
<td>F52</td>
<td>52</td>
<td>6.9</td>
<td>10</td>
<td>No</td>
<td>10</td>
<td>Definite</td>
</tr>
<tr>
<td>12</td>
<td>F36</td>
<td>36</td>
<td>7.7</td>
<td>9</td>
<td>Yes</td>
<td>17</td>
<td>Definite</td>
</tr>
<tr>
<td>13</td>
<td>F54</td>
<td>54</td>
<td>6.9</td>
<td>6</td>
<td>Yes</td>
<td>14</td>
<td>Definite</td>
</tr>
<tr>
<td>14</td>
<td>F48</td>
<td>48</td>
<td>7.4</td>
<td>10</td>
<td>No</td>
<td>10</td>
<td>Definite</td>
</tr>
<tr>
<td>15</td>
<td>F63</td>
<td>63</td>
<td>9.2</td>
<td></td>
<td>Did not attend</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>M50</td>
<td>50</td>
<td>6.8</td>
<td>5</td>
<td>No</td>
<td>5</td>
<td>Possible</td>
</tr>
<tr>
<td>17</td>
<td>F48</td>
<td>48</td>
<td>7.2</td>
<td>5</td>
<td>Yes</td>
<td>13</td>
<td>Definite</td>
</tr>
<tr>
<td>18</td>
<td>F51</td>
<td>51</td>
<td>6.7</td>
<td>5</td>
<td>No</td>
<td>5</td>
<td>Possible</td>
</tr>
<tr>
<td>19</td>
<td>M51</td>
<td>51</td>
<td>6.6</td>
<td>5</td>
<td>Not done</td>
<td>5</td>
<td>Possible</td>
</tr>
<tr>
<td>20</td>
<td>F59</td>
<td>59</td>
<td>7.4</td>
<td>8</td>
<td>No</td>
<td>8</td>
<td>Probable</td>
</tr>
<tr>
<td>21</td>
<td>F62</td>
<td>62</td>
<td>7.2</td>
<td>6</td>
<td>No</td>
<td>6</td>
<td>Probable</td>
</tr>
<tr>
<td>22</td>
<td>M60</td>
<td>60</td>
<td>7.2</td>
<td>6</td>
<td>No</td>
<td>6</td>
<td>Probable</td>
</tr>
<tr>
<td>23</td>
<td>M71</td>
<td>71</td>
<td>7.5</td>
<td>5</td>
<td>Not done</td>
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<td>Possible</td>
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<td>24</td>
<td>M40</td>
<td>40</td>
<td>6.7</td>
<td>9</td>
<td>No</td>
<td>9</td>
<td>Definite</td>
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<td>25</td>
<td>F31</td>
<td>31</td>
<td>6.5</td>
<td>5</td>
<td>No</td>
<td>5</td>
<td>Possible</td>
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<tr>
<td>26</td>
<td>F32</td>
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<td>9</td>
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<td>17</td>
<td>Definite</td>
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<tr>
<td>27</td>
<td>M49</td>
<td>49</td>
<td>6.5</td>
<td>6</td>
<td>No</td>
<td>6</td>
<td>Probable</td>
</tr>
</tbody>
</table>

Clinical DLCNC score excluded the genetic testing. The complete DLCNC score included scoring for genetic testing.
Using the DLCNC to assess the clinical likelihood of FH in the 25 individuals, 18 (72%) were clinically diagnosed with FH, with 6 probable (24%) and 12 definite (48%) FH. Genetic testing was performed in 23 individuals, seven (30%) of whom had identifiable FH-causing mutations. Four individuals from the control group were referred to the lipid disorders clinic in the 12 months following selection. All four controls were clinically diagnosed with FH: two probable and two definite FH. Genetic testing was performed on all four, the two clinically definite FH individuals had identifiable FH-causing mutations, and the two clinically probable individuals did not.

The specialist referral rate was significantly greater in the intervention group than in the control group (27% vs. 4%; p<0.0001). Genotypic cascade screening has been performed in 12 family members from four mutation-positive FH individuals in the intervention group to date; seven were confirmed carry the FH mutation and five did not.

In general, the phone calls made by the chemical pathologists were well received and deemed to be useful by the GPs; 82% were positive with those GPs engaging in discussion with the chemical pathologists with respect to the clinical aspects of the case, 12% were neutral, and 4% negative, citing clinic time pressures.

5.4 DISCUSSION

This case-historical control study demonstrates that a telephone call from a chemical pathologist to the requesting GP of a patient at high risk of FH significantly improves FH detection and specialist referral rates in addition to interpretative comments. These findings also confirm the important role that a community laboratory can play in FH detection, and that an LDL-cholesterol threshold of ≥6.5 mmol/L has a high specificity for FH in this Australian community population.
5.4.1 Mutation detection

The mutation detection rate for individuals with clinical FH in this study was lower than has previously been demonstrated in Western Australia (Chapter 8).\(^3\) However, it compares favourably to FH mutation detection rates from other populations.\(^9, 16\) Identifying the mutation causing FH in an index case then allows cascade screening to occur using genotype, which has previously been demonstrated to be very cost-effective.\(^55, 57, 146, 160, 195\) To date, seven additional family members with FH have been detected in four families with identifiable FH-causing mutations, which suggests two additional family members may be identified with FH for each FH index case in Australia. Although this requires confirmation, it supports the need to combine FH index case detection with cascade screening to optimise FH detection.

5.4.2 Advantages of the phone call and advice

Consultation between the chemical pathologist and the requesting GP for individuals identified at high risk of FH when their LDL-cholesterol was measured has advantages for opportunistic FH detection. Firstly, it removes the need to search the primary care database and overcomes any potential cholesterol concentration transcription inaccuracies.\(^141\) Secondly, there is the potential to perform additional blood tests to exclude secondary causes of hypercholesterolaemia, if these tests have not been performed recently. Thirdly, it provides an opportunity to discuss FH inheritance and the ramifications this has for the patient’s family and to address any gaps in FH knowledge,\(^322, 323\) which will hopefully empower the GP to pursue the diagnosis and appropriate treatment.

Given that there are approximately 45,000 individuals predicted to have FH in Australia\(^2\), a co-ordinated multidisciplinary team is needed to detect and manage individuals with FH. This study confirms the important role the community laboratory
has in opportunistic FH detection, and demonstrates that direct interaction between the chemical pathologist and requesting GP improves FH detection.

### 5.4.3 Specialist referral rate

Despite the statistically significant increase in specialist referrals and the detection of FH associated with a telephone call, only the minority (27%) of these high-risk individuals were referred to the regional lipid disorders clinic. GPs have knowledge deficiencies with respect to the mode of inheritance, prevalence, clinical features and CVD risk of FH, which are discussed in detail in Chapter 6.\(^{322}\) While the majority of GPs believed they were the best health professional to detect FH, most were unaware of national FH guidelines, and only one half would screen family members of known FH patients.\(^{322}\) The reduced GP awareness of FH may, in part, explain the relatively low referral rates for these individuals at high risk of FH. Moreover, the awareness and knowledge of FH in cardiologists was comparable to that of GPs, suggesting that an education campaign is required for all medical practitioners to optimise FH detection and reduce the burden of premature CVD.\(^{323, 324}\) Further investigation is required to formulate strategies to ensure appropriate specialist referral for these very high-risk individuals.

### 5.4.4 Strengths and limitations

This is the first study to demonstrate that a telephone call from a chemical pathologist to the requesting GP of a patient at high risk of FH significantly improves FH detection and specialist referral rates in addition to interpretative comments. However, the relatively small sample size and use of historical controls represent limitations of this study, although both the case and control periods occurred during a time of stable statin availability and FH case detection, as the FH Western Australia program had been active since 2007. Index case detection remains a fundamental aspect
of FH detection. It has been estimated that index case detection rates between 17-47% are required to achieve FH detection rates of 80%.\(^{(305)}\) Even when very proactive cascade screening is performed, such as occurs in the Netherlands, Spain and Wales,\(^{(325)}\) it is estimated that 17% of cases need to be unrelated index cases.\(^{(305)}\)

5.4.5 **Areas for future research**

These findings should be confirmed, preferably with a large multicentre prospective randomised control trial incorporating written and telephone alerts from the community laboratory. It would also be useful to combine this trial with a campaign to increased medical practitioners’ knowledge and awareness of FH, as this would clarify the role a community laboratory has in FH detection, as detection yields may be improved by addressing potential knowledge and awareness deficiencies.

5.5 **CONCLUSION**

This study has shown that a telephone call from a chemical pathologist to the requesting GPs of individuals at high risk of FH was associated with significantly higher specialist referral and FH detection rates. The study has also confirmed the LDL-cholesterol threshold of \(\geq 6.5 \text{ mmol/L} \) selects individuals at very high risk of FH in an Australian community population. This suggests that a community laboratory may be an integral part of a multidisciplinary approach to opportunistic FH detection. However, the reason for the relatively low specialist referral rates remains to be elucidated.
CHAPTER 6:
GENERAL PRACTITIONERS’ KNOWLEDGE AND
PRACTICES REGARDING FAMILIAL
HYPERCHOLESTEROLAEMIA IN WESTERN
AUSTRALIA

A version of this chapter has been published:

6.1 INTRODUCTION

FH fulfils the WHO’s criteria for screening (Section 1.3.1 and 1.3.2), however, Australia like most countries, currently does not have a formal FH screening program, and the majority of the people with FH in Australia remain undiagnosed. (2) GPs request 92% of the lipid profiles in the community as demonstrated and reviewed in Chapter 3, and are well placed to opportunistically detect individuals with FH. (306)

6.1.1 GPs’ potential role in detecting FH

The GPs’ potential role in FH detection has been reviewed in Section 1.3.4. Opportunistic screening for FH by GPs could address the deficit in FH detection. (55, 142, 170, 326) However, little is known of current GP knowledge and management practices regarding FH in Australia. However, the relatively low specialist referral rates for individuals at high risk of FH despite interpretative comments and a telephone call highlighting FH described in Chapters 4 and 5 heighten concern that GP knowledge of FH may be suboptimal. (311, 321)

6.1.2 Prediction

GPs’ FH knowledge and awareness of FH is suboptimal, although they will be willing to manage individuals with FH.

6.1.3 Aim

To determine the awareness, knowledge and management practices regarding FH among GPs in Western Australia.
6.2 METHODS

6.2.1 Subjects
A formal questionnaire was offered to all GPs attending education sessions on the assessment and management of cardiovascular risk, before the session commenced. The surveys were voluntary, kept anonymous and were completed without discussion with either the specialist leading the education session, or other GPs attending the session. There were six sessions in metropolitan Perth, and two in rural Western Australia. Approval was obtained from the Royal Perth Hospital Clinical Safety and Quality Unit (Reference number 120928-2).

6.2.2 Questionnaire
Participants were asked about their familiarity with FH, awareness of the National Heart Foundation / Australian Atherosclerosis Society FH Network guidelines, description of FH, identification of the typical lipid profile, prevalence and inheritance of FH, risk of CVD, definitions of premature CVD, clinical features of FH, whether the diagnosis requires genetic confirmation, methods for alerting the possibility of FH, which health professional is best placed to detect FH, number of patients with FH they currently care for, whether they perform family screening, their treatment and referral practices regarding patients with severely elevated cholesterol. GPs were asked to select one or more answers from a list; there were no open questions. The questionnaire is provided in Appendix 1.

De-identified demographic data were sought from the participants including, gender, Fellowship of the Royal Australasian College of General Practitioners (FRACGP) status, years of practice, number of patients seen per month and if their practice was metropolitan or rural.
6.2.3 Analysis

Data were collated and analyses performed using Microsoft Excel 2003 and R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria). Chi squared or Fisher’s exact tests were used to determine if there were differences in FH awareness, knowledge, practices and opinions on FH detection using FRACGP status and metropolitan or rural practice as categorical variables.

6.3 RESULTS

6.3.1 Demographics and practice details

The questionnaire was completed by 191 of 200 GPs (95.5%) attending the education sessions. Of the 191 GPs, 134 (70%) were males and 47 (25%) females, and 10 (5%) did not, or preferred not to respond. Seventy four percent of GPs were from the metropolitan area, 20% were rural, and 6% did not respond. FRACGP was held by 44%, 49% were non-Fellows, and 7% did not respond. The median time in practice post Fellowship was 10 years (range 1-39 years) for the 42% of GPs who responded to this question. The mean number of patient seen per month was 492, median of 500 (range 15-1000) for the 160 GPs who responded.

6.3.2 Knowledge and awareness of FH

One hundred and nineteen (62%) GPs rated their familiarity with FH as average or above. Sixty three (33%) were aware of the National Heart Foundation/Australian Atherosclerosis Society FH Network Guidelines for the detection and management of FH. A summary of the results is presented in Table 20. The mean age given for premature CVD was 43.6 years (SD 10.9) in males and 49.0 years (SD 11.1) in females.
<table>
<thead>
<tr>
<th></th>
<th>Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Awareness</strong></td>
<td></td>
</tr>
<tr>
<td>Familiarity with FH rated as average or above</td>
<td>62</td>
</tr>
<tr>
<td>Aware of the NHF/AAS FH guidelines</td>
<td>33</td>
</tr>
<tr>
<td>Aware of lipid specialists</td>
<td>62</td>
</tr>
<tr>
<td><strong>Knowledge</strong></td>
<td></td>
</tr>
<tr>
<td>Correctly described FH</td>
<td>80</td>
</tr>
<tr>
<td>Correctly identified the lipid profile</td>
<td>68</td>
</tr>
<tr>
<td>Correctly identified the prevalence of FH in the community</td>
<td>27</td>
</tr>
<tr>
<td>Correctly identified the transmission rate to first degree relatives</td>
<td>45</td>
</tr>
<tr>
<td>Correctly identified the CVD risk in untreated FH</td>
<td>29</td>
</tr>
<tr>
<td>Correctly identified the age threshold for premature CVD</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>30</td>
</tr>
<tr>
<td>Females</td>
<td>22</td>
</tr>
<tr>
<td>Correctly identified that genetic testing was not required to accurately diagnosis FH</td>
<td>50</td>
</tr>
<tr>
<td>Selected statins to treat hypercholesterolaemia</td>
<td>95</td>
</tr>
<tr>
<td>Selected a combination of statin and ezetimibe to treat severe hypercholesterolaemia</td>
<td>74</td>
</tr>
<tr>
<td><strong>Practice</strong></td>
<td></td>
</tr>
<tr>
<td>Screened patients with premature CVD for FH, including screening family members</td>
<td>56</td>
</tr>
<tr>
<td>Unaware or unsure whether they had FH patients under their care</td>
<td>65</td>
</tr>
<tr>
<td>Performed routine family screening of patients with FH</td>
<td>53</td>
</tr>
<tr>
<td>The most prevalent age for screening young people in a kindred with FH was 13-18 years; which was selected by</td>
<td>52</td>
</tr>
<tr>
<td>Referred patients to lipid specialists</td>
<td>27</td>
</tr>
<tr>
<td><strong>Opinions on detection</strong></td>
<td></td>
</tr>
<tr>
<td>Selected GPs as the most effective health care provider for the early detection of FH</td>
<td>84</td>
</tr>
<tr>
<td>Selected interpretative commenting on lipid profiles to highlight patients at risk of FH</td>
<td>90</td>
</tr>
</tbody>
</table>
6.3.3 Detecting FH

The health care providers that GPs considered had a role in the early detection of FH are demonstrated in Figure 10. Interpretative commenting on the laboratory report was the preferred method to alert GPs to FH, selected by 59% as a single method, and by 90% as a single or combined approach. An alert generated by the practice clinical software system was selected as the single method by 2% of GPs, with 2% preferring a direct phone call from the laboratory, and 31% preferred all three of the above approaches.

6.3.4 Management practices

In patients with premature CVD, 56% of GPs indicated they would examine for corneal arcus, tendon xanthomata and take a detailed family history and screen close relatives. Data on screening practices was provided by 88 GPs, 15% stated they would screen the patient’s children only, 57% would screen the patient’s children and close relatives, 9% would not screen any relatives and 19% stated the question was not applicable. The most frequent age GPs would theoretically screen children of patients with premature CVD was 13-18 years old, selected by 52% of GPs; 25% would test children aged under 13 years. Most GPs (62%) were aware of the regional specialist lipid service, although only 43% had referred patients to this service.
The proportion of GPs who selected these health care providers had a role in the detection of individuals with FH.

One hundred and ten GPs provided information on the number of people with FH in their practice; 55 stated they did not have any patients with FH, while the remaining 55 GPs reported 1-10 patients with FH, 36 stating they had 1-3 patients. The medications GPs use to treat hypercholesterolaemia are shown in Figure 11. The combination of statins plus ezetimibe was selected by 74% of GPs to treat severe hypercholesterolaemia.
FIGURE 11. MEDICATIONS SELECTED BY GPS FOR THE TREATMENT OF FH

Proportion of GPs who selected these medication(s) were useful to treat severe hypercholesterolaemia.

The term severe hypercholesterolaemia was used instead of FH, as the other aspects of the questionnaire assess knowledge of the diagnosis of FH, thus the level of knowledge on FH could not be assumed. More than one medication could be selected.

6.3.5 Comparison of GP knowledge

There was no significant difference in awareness, knowledge, practices or opinions on detection between Fellows and non-Fellows of the RACGP. Rural GPs were less likely to rate their knowledge of FH as average or above compared to metropolitan GPs (p = 0.03). However, no actual FH knowledge differences were demonstrated.
6.4 DISCUSSION

This is the first formal survey of GP knowledge and management practices regarding FH. While most GPs perceived their knowledge of FH as average or above, we found some important areas of knowledge deficit. GPs were generally able to clinically define FH, although only one third were aware of the national guidelines and a third of GPs failed to correctly identify the typical lipid profile. Knowledge of the prevalence and heritability of FH were also suboptimal, which are important factors when formulating the pre-test probability an individual may have FH.

6.4.1 Screening related knowledge

GPs also considered premature CVD to occur over ten years earlier than defined in national guidelines. Thus, they would miss an opportunity to screen for FH in a significant proportion of individuals at high risk of FH. Even if premature CVD was recognised, only half the GPs would screen close relatives appropriately, further reducing the potential to detect FH in a kindred. This is important, as testing first-degree relatives of individuals with FH, termed cascade screening, has previously been demonstrated to be the most cost-effective FH screening method.\(^{(146)}\)

A minority of GPs considered they currently had patients with FH in their practice, reflecting that FH is under diagnosed in the community.\(^{(2, 55)}\) The per capita rate for GPs in Australia is 112 GPs per 100,000 people (~892 patients per GP).\(^{(327)}\) On average, 80% of Australians visited a GP in the preceding 12 months, suggesting a GP would review 713 different people per year, although the actual number will vary widely.\(^{(328)}\) This suggests that a GP is only likely to see one or two people per year with FH. However, a recent large Danish population study suggested the prevalence of ‘probable’ or ‘definite’ FH may be as high as 1:137, suggesting 17,500 individuals in Western Australia may have FH.\(^{(16)}\) However, this prevalence was amended to 1:223,\(^{(17)}\)
although even this prevalence reinforces that FH will predominantly need to be detected and managed in primary care.\(^{(142)}\)

### 6.4.2 Opinions on FH screening

The majority of GPs stated they were the most effective healthcare provider to detect FH, which is encouraging as GPs are central to both systematic and opportunistic FH detection.\(^{(55, 142, 170, 326)}\) However, this survey has highlighted that GP awareness and knowledge of some important aspects of FH were suboptimal. Professional interventions to improve GP knowledge and awareness need to be formulated in collaboration with primary care and specialists in lipidology and preventative cardiology. It will also be important to investigate the impact these interventions have on FH detection and management.

Most GPs preferred interpretative comments on the laboratory forms to alert possible FH. Interpretative comments were associated with greater LDL cholesterol reductions and increased specialist referral in individuals at high risk of FH (Chapter 4).\(^{(311)}\) One third of GPs preferred an informatics approach applied to the practice database to highlight individuals with potential FH, although predominantly combined with interpretative commenting. An informatics approach using the GP practice database to identifying individuals with FH has been described.\(^{(141)}\) Grey \textit{et al.} identified 402 individuals at high risk of FH; 20 individuals were confirmed to have definite or probable FH after review of the notes.\(^{(141)}\) However, only six individuals were unknown to the specialist lipid clinic, and they uncovered multiple significant database transcription errors.\(^{(141)}\)

In primary care the diagnosis of FH will be made clinically with the DLCNC.\(^{(55)}\) However, after specialist review and clinical confirmation, these individuals may have genetic testing for FH mutations.
6.4.3 Knowledge of lipid-lowering therapies

Most GPs identified the more effective cholesterol-lowering treatments, which is encouraging as CVD was the 3rd most prevalent presentation to a GP. Lipid-lowering medications were prescribed in 3.7% of consultations. GP knowledge and familiarity with lipid-lowering medication is consistent with the previously marked LDL-cholesterol reductions demonstrated in individuals at high risk of FH in primary care (Chapter 4). Despite this, the specialist referral rates were low, even after highlighting the potential for FH on the lipid report, which may reflect the deficits in FH knowledge and awareness.

The relatively low long-term statin adherence rates in Australia are a further concern, as low statin adherence would decrease the cardiovascular protection. This is especially important in FH, with a high CVD incidence. GPs are central to improving adherence to lipid-lowering therapies. Statin compliance has been demonstrated to be higher in individuals formally diagnosed with FH, reinforcing the benefit of formal FH diagnosis.

6.4.4 Family history

An accurate family history is integral to both CVD risk assessment and the diagnosis of FH. However, this is often neglected or poorly recorded in clinical practice, both in primary and tertiary care. Collecting family history systematically using a self-administered questionnaire has been shown to significantly increase the number of individuals identified with high CVD risk, without increasing patient anxiety. If family history was available to the laboratory, either provided on the request form or via a self-administered questionnaire, an expert computer system could use this to identify individuals at high risk of FH, and then assist in selecting an age- and gender-appropriate interpretative comment.
6.4.5 Strengths and limitations

This research was novel, and has provided some very useful information to help guide future research in this field. However, the opportunistic selection of GPs represents a significant limitation. Although, the demographics (gender, RACGP Fellowship and the metropolitan/regional practice distribution) of our sample are reasonably representative of the GP population in Australia.\(^{327, 329}\) The questionnaire was also handed out at the start of a talk on CVD and lipids, which may have altered the GPs’ perceptions, although this was completed prior to the education session commencing.

6.4.6 Areas for future research

A larger survey of randomly selected GPs conducted by an independent body should be performed to confirm these findings, which should be conducted on a national if not international scale. We have demonstrated that the more specific the interpretative comment, the greater the associated clinical impact (Chapter 4).\(^{311}\) Given that GPs preferred interpretative comments to alert them to the possibility of FH, appending a comment outlining the additional clinical information required to diagnose FH may improve FH detection, although this requires investigation.

Furthermore, it has recently been demonstrated that knowledge of FH among American cardiologists\(^{323}\) and physicians in the Asia-Pacific region\(^{334}\) was also suboptimal, and very comparable to these GPs and Australian pharmacists.\(^{324, 335}\) Taken together these findings suggest the awareness of FH among medical practitioners is suboptimal, and a general education and awareness campaign for all medical practitioners is likely to be required. Formal investigation to confirm the effect an education and awareness campaign has on FH detection is warranted.
6.5 CONCLUSION

The majority of GPs considered they were the most effective health practitioners to manage FH, although GP awareness of national guidelines and knowledge of hereditability, prevalence and diagnostic features of FH were suboptimal. This may explain the relatively low specialist referral rate – even when alerted an individual was at high risk of FH. Implementing a community model of care for FH requires more extensive GP education combined with interventions to highlight at risk individuals which should augment FH detection and help close this important gap in CVD prevention. Further work is required to formulate professional interventions to improve FH awareness and knowledge, and then to determine the effect these interventions have on actual FH detection and management.
CHAPTER 7:

CAN PATIENTS BE ACCURATELY ASSESSED FOR FAMILIAL HYPERCHOLESTEROLAEMIA IN PRIMARY CARE?

A version of this chapter has been published:

7.1 INTRODUCTION

7.1.1 Defining GPs role in FH detection

Chapter 6 highlighted that there are areas where GPs’ knowledge of FH require improvement, although GPs remain well-placed and are willing to assist with FH detection and management in the community.\textsuperscript{170, 322} Chapter 3 described the distribution of LDL-cholesterol concentrations in the community, and highlighted the magnitude of patients who may be at risk of FH, depending on any particular LDL-cholesterol cut point. If a lower LDL-cholesterol cut point is selected in order to improve sensitivity, the specificity tends to reduce.\textsuperscript{303} For example, if an LDL-cholesterol cut point of $\geq 5.0$ mmol/L was selected, 3.7% of the population having their LDL-cholesterol measured would meet this criteria (Table 13).

However, FH is not diagnosed by LDL-cholesterol alone and clinical features remain central for diagnosis, as they improve the likelihood of detecting an individual with FH in addition to LDL-cholesterol.\textsuperscript{172} A case ascertainment tool (FAMCAT) has recently been developed in the UK for use in primary care, which incorporated family history and other features in addition to LDL-cholesterol to augment FH case detection.\textsuperscript{172} However, the DLCNC are the preferred diagnostic criteria for FH in Australia.\textsuperscript{55, 87} If GPs are able to use these clinical features in primary care to improve the likelihood their patient has FH, this would optimise specialist referrals and ensure these patients could access appropriate assessment and treatment in a more timely manner. However, the agreement in DLCNC score (DLCNCS) between specialists and GPs remains to be established.
7.1.2 Prediction

GPs will be able to use the DLCNC to select individuals at high risk of FH in primary care.

7.1.3 Aim

To determine whether GPs can accurately establish the likelihood of FH with the DLCNC.

7.2 METHODS

7.2.1 Population selection and primary care assessment

Assessment in primary care was carried out by one of 3 nurses and one of 2 GPs in a rural primary care setting in Western Australia. The nurses were provided with education on calculating the DLCNCS at the regional specialist lipid clinic. Individuals at risk of FH were identified by either the laboratory highlighting individuals with elevated LDL-cholesterol, or by using an informatics tool to search general practice databases. This occurred as part of a research project that sought to determine the best methods of detecting FH in the community. A nurse would interview and examine all individuals at risk enrolled in the research project and collate the data required for calculating the DLCNCS. One of the GPs would review the data and calculate the DLCNCS.
7.2.2 Specialist assessment

The lipid specialist was then provided with the same de-identified data and calculated their own DLCNCS. The lipid specialist remained blind to the GPs’ DLCNCS. The DLCNCS were preferably calculated using a documented LDL-cholesterol off lipid-lowering treatment. If an off-treatment LDL-cholesterol was unavailable, the pre-treatment LDL-cholesterol was estimated by adding 30% to the LDL-cholesterol obtained while on lipid-lowering therapy.\(^{(16)}\)

The lipid specialist subsequently reviewed thirty individuals (DLCNCS $\geq 4$ as assessed by the specialist with primary care data) in a telehealth clinic and determined their likelihood of FH using information obtained during this consultation. The specialist was blind to all previous DLCNCS and the results of FH genetic testing for these individuals, until they had calculated the DLCNCS for the telehealth consultation. Clinical FH was defined as probable or definite using the DLCNC categories.

7.2.3 Analytical methods

Total cholesterol, triglyceride and HDL-cholesterol analyses were performed with enzymatic, colorimetric assays using either Abbott, Siemen’s or Roche analysers and reagents, depending on the community laboratory the patient presented to. All laboratories were nationally certified. LDL-cholesterol was calculated according to the Friedewald equation.\(^{(228)}\) Genetic testing was performed as part of routine care, as described in Chapter 8.\(^{(35)}\)
7.2.4 Statistical methods

Statistical analysis was performed with STATA, StataCorp. 2011, Stata Statistical Software. Agreement on the DLCNCS was assessed using Lin’s concordance correlation coefficient, presented with 95% confidence intervals. The agreement between the GPs and specialist’s FH likelihood categories based on the DLCNC was assessed using Cohen’s kappa statistic, presented with 95% confidence intervals. Demographic data are presented as mean and standard deviation (SD). The sensitivity and specificity of GP diagnosis of FH was calculated for the 30 individuals who had undergone specialist reviewed, using the specialist’s diagnosis of FH including knowledge of the genetic testing as the standard.

7.3 RESULTS

7.3.1 Study population

There were 153 individuals identified at risk of FH, their mean age was 54±9 years, 80 (53%) were male, and 93 (72%) were on a statin. Their mean total cholesterol was 7.5±1.7 mmol/L, mean LDL-cholesterol 5.1±1.1 mmol/L and mean triglyceride 1.7±1.3 mmol/L (Table 21). The mean age for the 30 individuals assessed by the specialist in the telehealth clinic was also 54±9.5 years, 19 (63%) were male and 29 (97%) were on a statin. Their mean total cholesterol was 8.4±1.7 mmol/L and LDL-cholesterol 6.0±1.1 mmol/L and triglycerides 1.9±1.1 mmol/L (Table 21).
### TABLE 21. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE INDIVIDUALS IDENTIFIED IN PRIMARY CARE, AND THOSE WHO UNDERWENT SPECIALIST REVIEW

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Individuals identified at risk of FH from the community</th>
<th>n</th>
<th>Individuals with DLCNC ≥4 who underwent specialist review</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age years</td>
<td>153</td>
<td>54 (9)</td>
<td>30</td>
<td>54 (9.5)</td>
</tr>
<tr>
<td>Male</td>
<td>153</td>
<td>80 (53%)</td>
<td>30</td>
<td>19 (63%)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>109</td>
<td>7.5 (1.7)</td>
<td>23</td>
<td>8.4 (1.7)</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>95</td>
<td>5.1 (1.1)</td>
<td>16</td>
<td>6.0 (1.1)</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>97</td>
<td>1.7 (1.3)</td>
<td>16</td>
<td>1.9 (1.0)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>95</td>
<td>1.4 (0.3)</td>
<td>16</td>
<td>1.2 (0.4)</td>
</tr>
<tr>
<td>Premature CVD</td>
<td>153</td>
<td>21 (14%)</td>
<td>30</td>
<td>8</td>
</tr>
<tr>
<td>Premature other vascular disease</td>
<td>153</td>
<td>9 (6%)</td>
<td>30</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>FHx premature CVD</td>
<td>153</td>
<td>72 (47%)</td>
<td>30</td>
<td>19 (63%)</td>
</tr>
<tr>
<td>FHx elevated cholesterol</td>
<td>153</td>
<td>93 (61%)</td>
<td>30</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>Tendon xanthoma</td>
<td>153</td>
<td>3 (2%)</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Corneal arcus</td>
<td>153</td>
<td>11 (7%)</td>
<td>30</td>
<td>5 (17%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>130</td>
<td>15 (12%)</td>
<td>30</td>
<td>5 (17%)</td>
</tr>
<tr>
<td>Statin</td>
<td>130</td>
<td>93 (72%)</td>
<td>30</td>
<td>29 (97%)</td>
</tr>
<tr>
<td>DLCNC score range (primary care data)</td>
<td>153</td>
<td>0-12</td>
<td>30</td>
<td>4-11</td>
</tr>
</tbody>
</table>

Continuous variables expressed as mean and standard deviation. Categorical variables expressed as absolute number and percentage. N for lipid results is for the number of individuals with documented pre-treatment results. Data for the 153 individuals was from primary care; data for the 30 high-risk (DLCNCs ≥4) individuals was from the specialist review.
7.3.2 Correlation between primary care and specialist assessment

Lin’s concordance correlation coefficient between the DLCNCS calculated by GPs and specialists for the 153 individuals was 0.832 (0.783-0.881) p<0.001. There was an 83.6% agreement between DLCNCS, kappa 0.744 (0.642-0.831). The specialist DLCNCS was on average 0.196 (-3.23, 2.84) higher than the GPs. The DLCNCS and DLCNC based FH likelihood categories are compared in Table 22 and Figure 12.

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TABLE 22. COMPARISON OF DLCNC FH CATEGORIES AS ASSESS BY GPS AND SPECIALISTS

<table>
<thead>
<tr>
<th>Likelihood of FH as assessed by GPs</th>
<th>Likelyhod of FH as assessed by the specialist</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unlikely (0-2)</td>
</tr>
<tr>
<td>Unlikely (0-2)</td>
<td>32</td>
</tr>
<tr>
<td>Possible (3-5)</td>
<td>1</td>
</tr>
<tr>
<td>Probable (6-8)</td>
<td>1</td>
</tr>
<tr>
<td>Definite (&gt;8)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
</tr>
</tbody>
</table>
FIGURE 12. COMPARISON OF THE DLCNCS ASSESSED BY GPS AND SPECIALISTS, WITH PRIMARY CARE DATA

This figure is a comparison of DLCNCS calculated by GPs and lipid specialists using data that had been gathered in primary care. This comparison was performed to assess the ability of GPs to apply the DLCNCS, using the specialist score as a reference standard. DLCNCS – Dutch lipid clinic network criteria score, GP – general practitioner.
The specialist classified 45 (29.6%) individuals with clinical FH (probable or definite DLCNCS categories). Of these individuals with clinical FH, the GPs correctly classified 39 (86.7%). The GPs classified 16 (10.5%) individuals with a lower likelihood of FH than the specialists, and nine (5.9%) with a higher likelihood. GPs correctly classified 32 (94%) individuals with unlikely FH (DLCNC <3).

A primary care DLCNCS <4 selected 58 (37.9%) individuals at low risk of FH, only one of whom was determined to have clinical FH by the specialists using the same information. A DLCNCS <5 selected 100 (65.4%) individuals, however 10 were categorised with clinical FH by the specialist.

### 7.3.3 Diagnosis of FH in the individuals assessed in the telehealth clinic

The specialist diagnosed 15 individuals with clinical FH after telehealth review, 12 (80%) of whom were correctly classified with probable or definite FH by GPs. GPs correctly classified 9 (60%) of the individuals classified with possible FH by the specialist after telehealth review. GPs had a diagnostic sensitivity of 80% and specificity of 60% using the DLCNCS calculated with data they had obtained in primary care, relative to the specialist’s diagnosis of FH after telehealth review.

Genetic testing was performed in all thirty individuals reviewed by the specialist; four individuals had FH causing mutations. The specialist DLCNCS classified all four with clinical FH (two probable and two definite FH), whereas the GPs classified three with clinical FH (two probable and one definite FH) and one with possible FH. The mutation detection rate in individuals classified with clinical FH was 26.7% for the specialists and 16.7% for primary care, although this was not significantly different (p=0.67).
7.4 DISCUSSION

This is the first study to demonstrate that GPs can accurately assess the likelihood of FH in a primary care setting using the DLCNC. There was good agreement between GPs and specialists overall, although primary care slightly over-estimated FH in individuals at high risk. However, GPs very accurately identified individuals at low risk of FH, suggesting the DLCNC could be used to optimise specialist referrals, by selecting individuals at high risk of FH for specialist confirmation.

7.4.1 Detecting FH in primary care

Identifying FH index cases is one of the current major challenges in FH management.\textsuperscript{(55)} GPs are central to both systematic and opportunistic FH screening strategies.\textsuperscript{(57, 142, 145, 170, 326)} However, the most efficient method to identify individuals at high risk of FH in primary care remains to be determined. Enabling GPs to identify individuals at high risk of FH is important, as it may facilitate appropriate specialist referral without overloading specialist lipid clinics.\textsuperscript{(172)} Once an FH index case is identified, family cascade screening may occur, which has previously been demonstrated to be clinically and financially effective, and is recommended in FH guidelines worldwide.\textsuperscript{(11, 55-57, 139, 146)}
Identifying the mutation causing FH allows cascade screening to occur by genotype, although it is well recognised that 10-40% of individuals with clinically diagnosed FH will not have identifiable mutations.\(^9,11\) The mutation detection rate for individuals with clinical FH was lower than previously described in Western Australia (Chapter 8),\(^{35}\) which may reflect the small sample size, but compares favourably with other community studies.\(^9,16\) However, if mutation testing was restricted to clinical FH calculated in primary care, one individual with genetically proven FH would have been missed.

### 7.4.2 Strengths and limitations

The primary care DLCNCS were only generated by five primary care staff: two GPs and three nurses; and the nurses had received some training in the specialist clinic. This represents a major limitation of this study, as the actual agreements in routine clinical practice may not be high. However, the inclusion of nurses is also a strength of the study, as calculating the DLCNC could potentially be incorporated into nursing practice. Also of note, the specialist review was conducted in a telehealth clinic, so mild xanthomata may have been missed, although individuals with suspected Achilles tendon thickening were referred for ultrasound assessment. There is no internationally accepted gold standard for the diagnosis of FH, although the DLCNC are the recommended diagnostic criteria for Australasia,\(^{55}\) thus it is possible the specialist may have misdiagnosed some of the individuals.
7.4.3 Areas of future investigation

While the GPs FH detection rates were not as high as the specialists, the general agreement in DLCNCS were encouraging and support further research using the DLCNC in a larger cohort primary care practitioners. Furthermore, interventions to improve the relatively low FH knowledge and awareness among primary care, specialist (322) and allied health practitioners (335) would be expected to improve this agreement. One possible intervention to improve both knowledge and agreement involves an interactive website to assist practitioners with calculating the DLCNCS. The website could assist by accurately defining premature CAD, demonstrating and defining arcus cornealis and could estimate the pre-treatment LDL-cholesterol concentration for individuals currently on lipid-lowering treatment, this approach could also be combined with the FAMCAT tool. (172) Furthermore, the community laboratory may further assist GPs with FH detection by alerting them when one of their patients is found to be at high risk of FH through knowledge of clinical factors such as premature CVD if this is recorded on the request form, or previously very elevated LDL-cholesterol.

7.5 CONCLUSIONS

GPs can accurately identify individuals at high and low risk of FH using the DLCNCS, which may augment efficient opportunistic detection of FH in the community. Increased FH awareness and education in primary care may also improve the sensitivity and specificity of diagnosing FH in primary care. Further investigations are required to confirm these findings in a larger and more diverse population of GPs. Furthermore, the impact of enhanced GP knowledge and awareness of FH combined with interventions that alert them when individuals are at high risk of FH remains to be elucidated.
CHAPTER 8:

GENETIC ANALYSIS OF FAMILIAL
HYPERCHOLESTEROLAEMIA IN WESTERN
AUSTRALIA

A version of this article has been published:

8.1 INTRODUCTION

It is well established that elevated plasma concentrations of LDL-cholesterol are associated with increased risk of CVD. FH with a prevalence of approximately one in 300 to 500 is one of the most common heritable human diseases, affecting at least 10 million people worldwide.\(^{(19)}\) The high levels of LDL-cholesterol in FH are caused by reduced clearance of LDL from blood by the LDLR pathway, leading to premature CAD with a 50% cumulative risk of myocardial infarction in males before the age of 50 years.\(^{(19)}\) The majority of the estimated 45,000 individuals with FH in Australia remain undiagnosed and inadequately treated.\(^{(96)}\)

Lifetime exposure to high blood cholesterol concentrations also causes the formation of cholesterol deposits in and around the eye as corneal arcus and xanthelasma, and in tendons, particularly the Achilles, as xanthomas as described in Section 1.2.4.\(^{(19, 23, 338)}\) These clinical characteristics together with raised LDL-cholesterol levels and a positive family history can be used in diagnostic algorithms such as DLCNC.\(^{(87)}\)

DNA testing facilitates cascade screening for FH from index cases and previous studies have shown that genetic testing is superior to phenotypic measurement of LDL-cholesterol levels as a cascade screening tool.\(^{(182)}\) The FH Western Australia (FHWA) pilot program was established to identify and treat individuals with FH in Western Australia, by cascade screening of families from index cases.\(^{(55)}\) Recent guidelines for the screening, diagnosis and management of FH all focus on family or “cascade” DNA testing as the most cost-effective way for new case detection.\(^{(55)}\)
8.1.1 Spectrum of mutations causing FH

As outlined in Chapter 1 FH is mainly caused by mutations in the *LDLR* gene; over 1000 have been described.\(^{(36,339)}\) In some populations, a large proportion of FH is accounted for by relatively few *LDLR* mutations.\(^{(20)}\) Large deletions or insertions in the *LDLR* are estimated to occur in 10% of FH patients and are not readily detected by conventional DNA sequence analysis, although they are detected with using MLPA. Combining MLPA with exon-by-exon sequencing of the 18 exons of the *LDLR* gene (plus part of *APOB* containing the FDB mutation p.Arg3527Gln and exon 7 of *PCSK9* containing p.Asp374Tyr) is a comprehensive means of mutation detection for FH.

8.1.2 Predicted genetic ancestry of Australians

Owing to significant European migration in the 1950s and Asian migration in the 1970s and 1980s, Australia has a genetically heterogeneous population, which poses challenges for a screening program. Western Australians are mainly of Northern European ancestry (80%) with a significant proportion of the remainder from South-East Asia and Southern Africa.

8.1.3 Prediction

A genetic testing strategy for FH can be developed and applied to an Australian clinic population to identify mutations causing FH.

8.1.4 Aims

To describe the spectrum of mutations causing FH in Australia and yield of mutation detection in FH patients referred to a dedicated lipid disorders clinic in Perth, Western Australia.
8.2 METHODS

8.2.1 Study population and clinical assessment

Subjects were consecutive patients (n=343) considered to have phenotypic FH who were referred for DNA testing from the lipid disorders clinic at Royal Perth Hospital; this clinic takes referrals from the community via GPs and from medical specialists, particularly cardiologists. A physician specialising in lipidology then reviewed each subject, obtained written consent for genetic testing, and then elicited a full history and examination, including waist circumference, body mass index (BMI) and blood pressure. Biochemical laboratory testing included: fasting lipid profile including apolipoprotein B (apoB), lipoprotein(a) [Lp(a)] and glucose; TSH, free thyroxine, hepatic aminotransferases, albumin, urea, creatinine and electrolytes along with a urinary albumin:creatinine ratio. This information was used to exclude secondary causes of elevated cholesterol.

DLCNC scores were calculated using phenotypic criteria alone, by the physician requesting the genetic test,\(^{(55)}\) when estimating this score pre-treatment fasting plasma LDL-cholesterol concentrations were used for patients who were already on statins. A phenotypic diagnosis of FH was made in all cases after secondary causes of hypercholesterolaemia were excluded.\(^{(55)}\)

8.2.2 Genetic analysis

Genomic DNA was isolated from peripheral blood leukocytes using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). The MLPA-LDLR kit P062 (MRC-Holland, The Netherlands) was used according to the manufacturer’s instructions. Deletions/duplications were confirmed by a second MLPA reaction. M13-tagged primers for the amplification of the 18 exons of the \(LDLR\) plus part of \(APOB\) exon 26 and 29 were designed de novo using CLC Main Workbench (CLC Bio, Denmark) with reference to the NCBI and the UCL databases. \(PCSK9\) exon
7 primers have been reported previously.\textsuperscript{(340)} Sequencing was performed using Big Dye Terminator chemistry (Applied Biosystems) in the forward and reverse directions, and aligned using CLC Main Workbench. Mutations were described using Human Genome Variation Society nomenclature \url{www.hgvs.org} for the coding DNA (c.) and the protein (p.) with reference sequence NM\textunderscore 000384.2 \textit{(APOB)}, AY114155.1 \textit{(LDLR)} or NM\textunderscore 174936.3 \textit{(PCSK9)}. Mutations were confirmed by sequencing of a second PCR product. Pathogenicity was assessed by reference to published data and for novel variants by \textit{in-silico} methods using the online tools PolyPhen-2,\textsuperscript{(341)} SIFT,\textsuperscript{(342)} and Mutation Taster,\textsuperscript{(343)} and investigating whether different mutations at the same position had been reported.

8.2.3 Biochemical analysis

All laboratory measurements were performed using routine assays in an accredited laboratory. Cholesterol, triglyceride, HDL-cholesterol and glucose analyses were performed with enzymatic assays using automated analysers (Abbott, Siemens or Roche). LDL-cholesterol was calculated using the Friedewald equation\textsuperscript{(228)} with direct LDL-cholesterol measured when triglyceride exceeded 4.5 mmol/L. ApoB and Lp(a) were measured by immunonephelometry or immunoturbidimetry. Other biochemical tests were carried out using routine laboratory methods in accredited laboratories.

8.2.4 Statistical analysis

Data were collated and analyses performed using Microsoft Excel 2003. T-tests were used to compare age, plasma LDL-cholesterol, triglyceride and HDL-cholesterol concentrations between patients found to carry a mutation and those in whom a mutation could not be detected, with statistical significance defined as <0.05.
8.3 **RESULTS**

Overall, 86 different pathogenic mutations were identified in 129 of the 343 patients referred for DNA testing on the basis of a clinical suspicion of FH.

8.3.1 **Compound heterozygous patients**

Four of these patients were compound heterozygotes, including one patient heterozygous for a mutation in each of the *APOB* and *LDLR* genes, described in Table 23. The mean plasma LDL-cholesterol concentration in these patients was 12.9 mmol/L, ranging from 7.9 mmol/L (the individual carrying both the *APOB* p.Arg3527Gln and an *LDLR* mutation) to 16.8 mmol/L.

8.3.2 **Mutation detection rate according to clinical phenotype**

Of the 337 FH genetic testing requests where a DLCNC score was available, 38% were classified ‘definite’ (DLCNC score >8), 26% ‘probable’ (DLCNC score 6-8), 33% ‘possible’ (score 3-5) and 3% ‘unlikely’ FH. While 70% of ‘definite’ FH patients were found to carry a mutation, only 29% of ‘probable’ and 11% of ‘possible’ FH patients were mutation-positive, as shown in Figure 13.
### TABLE 23. MUTATIONS FOUND IN THE FOUR COMPOUND HETEROZYGOUS PATIENTS WITH FH

<table>
<thead>
<tr>
<th>Compound heterozygote</th>
<th>Mutation 1</th>
<th>Mutation 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LDLR c.326G&gt;A</td>
<td>LDLR c.660delC</td>
</tr>
<tr>
<td></td>
<td>p.Cys109Tyr</td>
<td>p.Asp221Thrfs*44</td>
</tr>
<tr>
<td>2</td>
<td>LDLR c.417C&gt;G</td>
<td>LDLR c.693C&gt;A</td>
</tr>
<tr>
<td></td>
<td>p.Asp139Glu†</td>
<td>p.Cys231*</td>
</tr>
<tr>
<td>3</td>
<td>LDLR c.661G&gt;A</td>
<td>LDLR c.681C&gt;G</td>
</tr>
<tr>
<td></td>
<td>p.Asp221Asn</td>
<td>p.Asp227Glu</td>
</tr>
<tr>
<td>4</td>
<td>APOB c.10580G&gt;A</td>
<td>LDLR c.681C&gt;G</td>
</tr>
<tr>
<td></td>
<td>p.Arg3527Gln</td>
<td>p.Asp227Glu</td>
</tr>
</tbody>
</table>

†Novel mutation, further described in the text and table 25.

#### FIGURE 13. MUTATION DETECTION IN PATIENTS STRATIFIED BY DLCNC SCORE

![Mutation Detection Bar Chart](image-url)
8.3.3 Mutation spectrum in the heterozygous patients

Of the 125 heterozygous mutation-positive FH patients, the most common mutations were the FDB mutation \( APOB \) p.Arg3527Gln (also known as R3500Q) and the \( LDLR \) intron 3 splice site mutation c.313+1G>A, found in 9 (7.2%) and 6 (4.8%) heterozygous mutation-positive patients, respectively, as demonstrated in Table 24. Twenty-two patients (18%) carried a mutation in exon 4, the largest exon. Twelve \( LDLR \) gene deletions or duplications (9.6% of mutation-positive patients) were detected by MLPA analysis. MLPA results suggesting reduced copy number in a single \( LDLR \) exon were sequenced for that exon; c.912C>G (p.Asp304Glu), c.1880C>A (p.Ala627Asp), and c.1885T>G (p.Phe629Val) were identified in this manner.

8.3.4 Novel mutations

In total, fourteen novel mutations were detected. Of these, there was one \( APOB \) variant of unknown pathogenicity, c.10477G>A (p.Glu3493Lys), found in an individual with a DLCNC score of 8. Four small \( LDLR \) deletions were found; three of these were frameshifts and one affected a single amino acid, p.Gly593del, as demonstrated in Table 25. An \( LDLR \) promoter variant c.-121C>T was found and predicted to be pathogenic; a mutation affecting the adjacent nucleotide was shown to affect LDLR expression \textit{in vitro} \cite{344}. Five novel missense mutations (p.Pro84Leu, p.Asp139Glu, p.Asp178Val, p.Ala627Asp, and p.Tyr828Ser) were likely to be pathogenic and three (p.Gln125Lys, p.Phe629Val and p.Tyr828Ser) were likely to be benign (Table 25). In addition p.Gly516Ser was detected, which has been previously considered to be benign.\cite{345}
### TABLE 24. KNOWN PATHOGENIC MUTATIONS IDENTIFIED IN WESTERN AUSTRALIAN HETEROZYGOUS FH PATIENTS REFERRED TO A LIPID DISORDERS CLINIC

<table>
<thead>
<tr>
<th>cDNA</th>
<th>Predicted effect on protein</th>
<th>Exon</th>
<th>Number of patients</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.10580G&gt;A</td>
<td>p.Arg3527Gln</td>
<td>26</td>
<td>9</td>
<td>(37)</td>
</tr>
<tr>
<td>LDLR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.6delG</td>
<td>p.Trp4Glyfs*202</td>
<td>1</td>
<td>1</td>
<td>LDLR_00248</td>
</tr>
<tr>
<td>c.58G&gt;A</td>
<td>p.Gly20Arg</td>
<td>1</td>
<td>1</td>
<td>LDLR_00868</td>
</tr>
<tr>
<td>c.81C&gt;G</td>
<td>p.Cys27Trp</td>
<td>2</td>
<td>1</td>
<td>LDLR_00004</td>
</tr>
<tr>
<td>c.131G&gt;A</td>
<td>p.Trp44*</td>
<td>2</td>
<td>1</td>
<td>LDLR_00007</td>
</tr>
<tr>
<td>c.190+4A&gt;T</td>
<td></td>
<td>(intron 2)</td>
<td>1</td>
<td>LDLR_01031</td>
</tr>
<tr>
<td>c.232C&gt;T</td>
<td>p.Arg78Cys</td>
<td>3</td>
<td>1</td>
<td>LDLR_00251</td>
</tr>
<tr>
<td>c.246C&gt;A</td>
<td>p.Cys82*</td>
<td>3</td>
<td>1</td>
<td>LDLR_00983</td>
</tr>
<tr>
<td>c.259T&gt;G</td>
<td>p.Trp87Gly</td>
<td>3</td>
<td>1</td>
<td>LDLR_00011</td>
</tr>
<tr>
<td>c.269A&gt;G</td>
<td>p.Asp90Gly</td>
<td>3</td>
<td>3</td>
<td>LDLR_00012</td>
</tr>
<tr>
<td>c.301G&gt;A</td>
<td>p.Glu101Lys</td>
<td>3</td>
<td>3</td>
<td>LDLR_00013</td>
</tr>
<tr>
<td>c.311G&gt;A</td>
<td>p.Cys104Tyr</td>
<td>3</td>
<td>1</td>
<td>LDLR_01117</td>
</tr>
<tr>
<td>c.313_313+1delCG</td>
<td></td>
<td>3</td>
<td>1</td>
<td>LDLR_00015</td>
</tr>
<tr>
<td>c.313+1G&gt;A</td>
<td></td>
<td>(intron 3)</td>
<td>6</td>
<td>LDLR_00163</td>
</tr>
<tr>
<td>c.326G&gt;A</td>
<td>p.Cys109Tyr</td>
<td>4</td>
<td>2</td>
<td>LDLR_00646</td>
</tr>
<tr>
<td>c.427T&gt;G</td>
<td>p.Cys143Gly</td>
<td>4</td>
<td>2</td>
<td>(345)</td>
</tr>
<tr>
<td>c.501C&gt;A</td>
<td>p.Cys167*</td>
<td>4</td>
<td>2</td>
<td>LDLR_00250</td>
</tr>
<tr>
<td>c.551G&gt;A</td>
<td>p.Cys184Tyr</td>
<td>4</td>
<td>4</td>
<td>LDLR_00236</td>
</tr>
<tr>
<td>c.589T&gt;C</td>
<td>p.Cys197Arg</td>
<td>4</td>
<td>1</td>
<td>LDLR_00400</td>
</tr>
<tr>
<td>c.654_656delTGG</td>
<td></td>
<td>4</td>
<td>1</td>
<td>LDLR_00030</td>
</tr>
<tr>
<td>c.660delC</td>
<td>p.Asp221Thrfs*44</td>
<td>4</td>
<td>2</td>
<td>LDLR_00382</td>
</tr>
<tr>
<td>c.661G&gt;A</td>
<td>p.Asp221Asn</td>
<td>4</td>
<td>1</td>
<td>LDLR_00490</td>
</tr>
<tr>
<td>c.662A&gt;G</td>
<td>p.Asp221Gly</td>
<td>4</td>
<td>1</td>
<td>LDLR_00031</td>
</tr>
<tr>
<td>c.664T&gt;C</td>
<td>p.Cys222Arg</td>
<td>4</td>
<td>1</td>
<td>LDLR_00707</td>
</tr>
<tr>
<td>c.680_681delAC</td>
<td></td>
<td>4</td>
<td>2</td>
<td>LDLR_00206</td>
</tr>
<tr>
<td>c.681C&gt;G</td>
<td>p.Asp227Glu</td>
<td>4</td>
<td>2</td>
<td>LDLR_00036</td>
</tr>
<tr>
<td>c.798T&gt;A</td>
<td>p.Asp266Glu</td>
<td>5</td>
<td>1</td>
<td>LDLR_00044</td>
</tr>
<tr>
<td>c.912C&gt;G</td>
<td>p.Asp304Glu</td>
<td>6</td>
<td>1</td>
<td>LDLR_00048</td>
</tr>
<tr>
<td>c.938G&gt;A</td>
<td>p.Cys313Tyr</td>
<td>6</td>
<td>1</td>
<td>LDLR_00210</td>
</tr>
<tr>
<td>c.986G&gt;A</td>
<td>p.Cys329Tyr</td>
<td>7</td>
<td>1</td>
<td>LDLR_00380</td>
</tr>
<tr>
<td>c.1027G&gt;A</td>
<td>p.Gly343Ser</td>
<td>7</td>
<td>1</td>
<td>LDLR_00056</td>
</tr>
<tr>
<td>c.1033C&gt;T</td>
<td>p.Gln345*</td>
<td>7</td>
<td>2</td>
<td>LDLR_00213</td>
</tr>
<tr>
<td>c.1048C&gt;T</td>
<td>p.Arg350*</td>
<td>7</td>
<td>4</td>
<td>LDLR_00215</td>
</tr>
<tr>
<td>c.1049G&gt;C</td>
<td>p.Arg350Pro</td>
<td>7</td>
<td>2</td>
<td>LDLR_00214</td>
</tr>
<tr>
<td>c.1066G&gt;T</td>
<td>Asp356Tyr</td>
<td>8</td>
<td>1</td>
<td>LDLR_00523</td>
</tr>
<tr>
<td>c.1121_1122insGT</td>
<td></td>
<td>8</td>
<td>1</td>
<td>LDLR_00923</td>
</tr>
<tr>
<td>c.1187-10G&gt;A</td>
<td></td>
<td>(intron 8)</td>
<td>2</td>
<td>LDLR_00882</td>
</tr>
<tr>
<td>c.1196C&gt;A</td>
<td>p.Ala399Asp</td>
<td>9</td>
<td>1</td>
<td>LDLR_00217</td>
</tr>
<tr>
<td>c.1222G&gt;A</td>
<td>p.Glu408Lys</td>
<td>9</td>
<td>1</td>
<td>LDLR_00065</td>
</tr>
<tr>
<td>c.1238C&gt;T</td>
<td>p.Thr413Met</td>
<td>9</td>
<td>2</td>
<td>LDLR_00967</td>
</tr>
<tr>
<td>c.1285G&gt;A</td>
<td>p.Val429Met</td>
<td>9</td>
<td>2</td>
<td>LDLR_00066</td>
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<tr>
<td>c.</td>
<td>Change</td>
<td>Description</td>
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<td>Reference</td>
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<tr>
<td>c.1330T&gt;C</td>
<td>p.Ser444Pro</td>
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<td>2</td>
<td>LDLR_01052</td>
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<tr>
<td>c.1358+2T&gt;A</td>
<td>(intron 9)</td>
<td>1</td>
<td>LDLR_00544</td>
<td></td>
</tr>
<tr>
<td>c.1444G&gt;A</td>
<td>p.Asp482Asn</td>
<td>10</td>
<td>2</td>
<td>LDLR_00237</td>
</tr>
<tr>
<td>c.1618G&gt;A</td>
<td>p.Ala540Thr</td>
<td>11</td>
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<td>LDLR_00243</td>
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<td>p.Gly546Asp</td>
<td>11</td>
<td>1</td>
<td>LDLR_00078</td>
</tr>
<tr>
<td>c.1705+1G&gt;A</td>
<td>(intron 10)</td>
<td>1</td>
<td>LDLR_00303</td>
<td></td>
</tr>
<tr>
<td>c.1715_1719delGTGGCinsA</td>
<td>p.Ser572Asnfs*92</td>
<td>12</td>
<td>1</td>
<td>LDLR_00494</td>
</tr>
<tr>
<td>c.1747C&gt;T</td>
<td>p.His583Tyr</td>
<td>12</td>
<td>2</td>
<td>LDLR_00263</td>
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**PCSK9**

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<td>p.Asp374Tyr</td>
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Large **LDLR** deletions and duplications

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<td>Deletion of exons 2 to 6</td>
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<td>Duplication of exons 3 to 8</td>
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<tr>
<td>c.314-?_694+?del</td>
<td>Deletion of exon 4</td>
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<tr>
<td>c.314-?_1060+?del</td>
<td>Deletions of exons 4 to 7</td>
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<tr>
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Human Genome Variation Society nomenclature is used, (> substitution, (del) deletion, (dup) duplication, _ insertion. ^ LDLR and PCSK9 numbers refer to UCL LDLR Mutation Database ID(www.ucl.ac.uk/ldlr)
<table>
<thead>
<tr>
<th>Variant</th>
<th>Effect on protein</th>
<th>Mutation Taster</th>
<th>Polyphen2</th>
<th>SIFT</th>
<th>Comments</th>
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<td>p.Gly593del</td>
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<td><strong>Novel mutations likely to be pathogenic</strong></td>
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<td>c.-121T&gt;C</td>
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<td>n/a</td>
<td>n/a</td>
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<td>Probably damaging</td>
<td>Protein function affected</td>
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<td>p.Asp139Glu</td>
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<td>Probably damaging</td>
<td>Protein function affected</td>
<td>(Compound heterozygote with known mutation)</td>
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<td>p.Asp178Val</td>
<td>Disease-causing</td>
<td>Probably damaging</td>
<td>Protein function affected</td>
<td>Other mutations reported at same position (Asp178Asn, Asp178Tyr, Asp178Gly, Asp178Gly)</td>
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<td>Possibly damaging</td>
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<td>Disease-causing</td>
<td>Probably damaging</td>
<td>Protein function affected</td>
<td>Other mutation reported at same position (Tyr828Cys)</td>
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<tr>
<td><strong>Mutations of unknown pathogenicity</strong></td>
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<td>c.373C&gt;A</td>
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<td>Poly morphism</td>
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<td>Benign</td>
<td>Novel, probably benign</td>
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<td>p.Gly516Ser</td>
<td>Poly morphism</td>
<td>Probably damaging</td>
<td>Protein function affected</td>
<td>Reported as ‘benign’ in (345)</td>
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<tr>
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<td>Disease-causing</td>
<td>Probably damaging</td>
<td>Benign</td>
<td>Novel</td>
</tr>
<tr>
<td>c.2289G&gt;T</td>
<td>p.Glu763Asp</td>
<td>Poly morphism</td>
<td>Possibly damaging</td>
<td>Benign</td>
<td>Novel</td>
</tr>
</tbody>
</table>

The mean highest LDL-cholesterol in heterozygous mutation-positive patients was significantly higher than in patients without a detectable mutation (6.6 ±2.2 mmol/L vs. 4.9 ±1.5 mmol/L, p=3x10^{-15}). Of interest, 96% of patients with highest recorded LDL-cholesterol above 8.5 mmol/L (n=24) were found to carry a mutation. Patients with heterozygous mutations were also younger at age of referral than those where no mutation could be detected (47 vs. 51 years, p=0.02). In addition, triglyceride values at the time of highest recorded LDL-cholesterol, were higher in mutation-negative patients (2.1±1.2 mmol/L vs. 1.6±0.9 mmol/L in mutation-positive patients; p=0.002).

8.4 DISCUSSION

8.4.1 Overview of findings

There were 86 different pathogenic mutations identified in 129 (37.6%) patients in this study. These included four compound heterozygotes manifesting a much higher mean LDL-cholesterol level than heterozygotes (12.9 vs. 6.6 mmol/L), suggesting a “trans” rather than “cis” mode of inheritance of the mutations in each of the compound heterozygotes. The most common mutations were APOB p.Arg3527Gln (7.2% of mutation-positive patients) and LDLR c.313+1G>A (4.8%). Overall, 70% of ‘definite’ FH patients were found to carry a mutation, only 29% of ‘probable’ and 11% of ‘possible’ FH patients were mutation-positive. These results are similar to those obtained in northern European screening centres using comprehensive sequence-based strategies. Taylor et al. had an overall mutation detection rate of 36.5% (compared to our overall detection rate of 37.6%), with a detection rate of 56% in definite FH and 28% in possible FH (by Simon Broome FH criteria).^46^
8.4.2 PCSK9 mutation

Only one patient heterozygous for PCSK9 p.Asp374Tyr was identified (0.8% of mutation-positive FH patients), which is similar to the 1.7% observed in the UK.\(^{(46)}\) Although PCSK9 mutations are rare, it is important to identify these patients, as they generally have a more severe phenotype than FH patients carrying LDLR mutations.\(^{(346)}\) It has been shown that PCSK9 p.Asp374Tyr mutation carriers are younger at presentation, have higher pre-treatment serum cholesterol levels, and are affected by premature CAD more than 10 years earlier than the LDLR patients.\(^{(346)}\) Of interest, the highest recorded LDL-cholesterol in our study (excluding compound heterozygotes) was seen in a patient heterozygous for PCSK9 p.Asp374Tyr, at 12.4 mmol/L. Full exon-by-exon sequencing of PCSK9 is underway in patients classed as ‘definite’ FH in whom we could not detect a causative mutation.

8.4.3 Lipid concentration and mutations

Patients who carry a mutation have higher LDL-cholesterol levels and CAD risk than patients in whom a mutation is not detected, and null-allele carriers have higher LDL-cholesterol levels compared with receptor-defective mutations.\(^{(347, 348)}\) Our study also showed that heterozygous mutation-positive patients were younger and had a significantly higher LDL-cholesterol than in patients without a detectable mutation.

The triglyceride concentrations at the time of highest recorded LDL-cholesterol, were higher in mutation-negative than mutation-positive patients, suggesting possible inclusion of patients with familial combined hyperlipidaemia in this group.\(^{(349)}\)
8.4.4 Mutation detection rates

The mutation detection rate was comparable to that reported in other countries, and the distribution of LDLR mutation types (57% missense, 9.7% nonsense, 9.7% frameshift, 1.6% small in-frame deletions, 11.3% splice, 0.8% 5’UTR, 9.7% major rearrangements) similar to that reported in France.\(^{(330)}\) The proportion of large LDLR deletions and duplications that we observed was double that observed in the UK (~5%),\(^{(46)}\) reflecting a possible ascertainment bias in Western Australia due to the introduction of genetic testing for FH only in the last few years. Possible reasons for not finding a mutation include the presence of polygenic rather than monogenic hypercholesterolaemia, intronic mutations occurring outside of amplified regions or within primer binding sites, a balanced rearrangement of the LDLR (no net gain or loss of LDLR exons and hence unable to be detected by MLPA), or the involvement of other, as yet unidentified, genes influencing cholesterol metabolism.

8.4.5 Strengths and Limitations

This is the first description of the spectrum of mutations and mutation detection rates in Australia, and used comprehensive genetic analysis. The mutation detection rates in this study are very comparable to the international literature, although they highlight a very significant proportion (~40%) of individuals clinically labelled with FH who do not have identifiable mutations.\(^{(11)}\) Furthermore, approximately 15% of the LDLR variants suggested to cause FH, are not associated with marked elevations in LDL-cholesterol,\(^{(303)}\) nor with increased risk of CAD.\(^{(351)}\) The lower mutation detection rates are likely related to both the presence of individuals with polygenic hypercholesterolaemia and undiscovered mutations causing FH. However, the mutation rates may have been increased due to possible case ascertainment bias, as some of the
individuals tested in the early stages of the FH Western Australia program were already known to the lipid clinic.

8.4.6 Areas for future research

It would be important to confirm these finding, removing the possibility of ascertainment bias by reviewing mutation detection rates in the next 300 cases referred for genetic testing. Targeted or whole exome sequencing using next generation sequencing may augment FH detection, by increasing the number of mutations identified. However, the reports to-date have demonstrated relatively poor detection yields. In the case of novel mutations (particularly missense mutations) and those of questionable pathogenicity by in-silico methods will need to be interpreted with caution, requiring further in-vitro or pedigree studies to confirm their pathogenic role.

8.5 CONCLUSIONS

The spectrum of mutations causing FH, and mutation detection rates from the FH Western Australia program are presented. It should be noted that the clinic spectrum of patients initially enrolled in the program may not accurately reflect the community spectrum of disease and mutations causative of FH in Western Australia, and that further studies are required in unselected patients and samples. These findings suggest genetic testing should be prioritised to those with high DLCNC scores and that it offers a cost-effective family screening method from FH index cases. Family mutation cascade screening from affected individuals is ongoing and should identify further family members at a much younger age than these probands thereby providing the additional benefit of initiation of preventative measures at an earlier age and optimising cardio-protective intervention.
CHAPTER 9:

DETECTION OF FAMILIAL HYPERCHOLESTEROLAEMIA IN WESTERN AUSTRALIA: EFFECTIVENESS OF GENETIC CASCADE SCREENING IN A CLINICAL SERVICE SETTING

A version of this chapter has been published:

9.1 INTRODUCTION

Individuals with untreated FH have a ~13 fold higher CAD risk than non-FH individuals.\textsuperscript{(11)} On average, males with untreated FH have a 50% risk of CAD by age 50 years and females a 30% risk by age 60 years.\textsuperscript{(4)} Individuals with FH generally have markedly higher LDL-cholesterol concentrations than people without FH, although there is overlap in the distribution of values.\textsuperscript{(303)} However, individuals with FH have elevated LDL-cholesterol concentrations from birth and consequently their coronary arteries have had a higher cumulative exposure to cholesterol.\textsuperscript{(11)}

Cascade screening, introduced in Section 1.3.3.2 is potentially clinically and economically effective, and is recommended in adult and paediatric FH management guidelines.\textsuperscript{(11, 55, 56, 139, 160, 202)} The effectiveness of cascade screening was originally based on early reports of national and regional cascade screening programs from the late 1990s and early 2000s.\textsuperscript{(180, 353)} However, since the late 1990s the availability and use of HMG-CoA reductase inhibitor (statin) therapy has increased markedly. Statin therapy leads to a significant reduction in LDL-cholesterol and reduces CAD mortality in individuals with and without FH.\textsuperscript{(13, 100)} However, only 47% of individuals with probable FH were reported to be taking statin therapy in a recent survey of a large community population.\textsuperscript{(16)}

Cascade screening can occur based on genotype and/or phenotype. The molecular pathology and genetic testing for FH were discussed in Section 1.2.3, the spectrum of mutations causing FH in Western Australia and mutation detection yields were described in Chapter 8.\textsuperscript{(35)}
9.1.1 Australasian model of care for FH

9.1.1.1 Diagnosis of FH in Australia and the role of genetic testing

A comprehensive FH model of care for Australia was published in 2011.\(^{(55)}\) The DLCNC\(^{(87)}\) constitute the preferred phenotypic diagnostic tool for FH in Australia.\(^{(55)}\) There are other criteria used to diagnose FH, but at present there is no international consensus on the best method of diagnosis, other than the detection of a pathogenic gene variant.\(^{(87, 88, 95, 354)}\) These diagnostic criteria are discussed thoroughly in Section 1.2.5.

9.1.1.2 Role of FH genetic testing in Australia

There are currently over 1200 \textit{LDLR} mutations which account for >90\% of FH mutations.\(^{(3, 6)}\) \textit{APOB} mutations are responsible for a further 5-10\% and \textit{PCSK9} approximately 1\% of FH mutations.\(^{(6, 11)}\) The genetic aspects of FH are discussed in Chapter 1, Section 1.2.3. Genetic testing is recommended to confirm FH and if a mutation is found, to conduct cascade screening using genotype.\(^{(55)}\)

9.1.3 Prediction

Cascade screening will effectively detect family members with FH, and diagnosis and specialist review will result in additional reductions in CVD risk factors despite the increased use of statins in the community.

9.1.4 Aim

To investigate the effectiveness of cascade screening family members of the first 100 index cases with genetically confirmed FH in a clinical service setting in Western Australia. The effectiveness will be determined by reviewing the diagnostic yield and improvement in LDL-cholesterol and non-cholesterol CVD risk factors.
9.2 METHODS

9.2.1 Assessment of subjects

The first 100 index cases (probands) from kindreds with genetically confirmed FH where at least one family member had been genetically screened were studied. The index cases were reviewed at the lipid disorders clinic at Royal Perth Hospital between March 2007 and August 2013. The index cases were assessed and treated according to national guidelines,\(^{(55)}\) with the phenotypic diagnosis of FH made according to the DLCNC and details of all CAD risk factors and treatments were recorded.

9.2.2 Cascade screening process

Family cascade screening was performed after obtaining written consent from the index case according to national guidelines.\(^{(55)}\) A trained nurse contacted the family members and obtained verbal consent to contact family members, after providing counselling as indicated; eight relatives declined testing (Figure 14).
FIGURE 14. PROTOCOL FOR GENETIC CASCADE SCREENING IN WESTERN AUSTRALIA
9.2.3 Medical assessment

A physician specialising in lipidology then reviewed each subject, obtained written consent for genetic testing for the family mutation, and then elicited a full history and examination, including waist circumference, BMI and blood pressure. Biochemical laboratory testing included: fasting lipid profile, apoB, Lp(a), fasting glucose, TSH, free thyroxine, hepatic aminotransferases, albumin, urea, creatinine and electrolytes along with a urinary albumin:creatinine ratio. This information was collated to determine the spectrum of cardiovascular risk factors and to exclude secondary causes of elevated cholesterol. Family members with newly diagnosed FH were followed-up in the clinic to receive advice on lifestyle modification and cholesterol-lowering therapy, according to national guidelines.\(^{(55)}\)

Diabetes was defined as a fasting glucose of $\geq 7.0$ mmol/L, a random glucose of $\geq 11.1$ mmol/L, a known history of diabetes or use of hypoglycaemic medications. Hypertension was defined as a known history of hypertension, a systolic blood pressure $\geq 140$ mmHg and/or a diastolic blood pressure $\geq 90$ mmHg, or the use of antihypertensive medication.

9.2.4 Participant satisfaction

A random sample of 146 individuals completed a questionnaire (using a discrete scale of 5 grades) that assessed their perceptions of the service. The questionnaire enquired about satisfaction with the program as a whole, the care provided by medical and nursing staff, the waiting times in clinic and educational material provided.
9.2.5 Ethical approval

Ethics approval for de-identified data analysis and reporting was obtained from the Human Research Ethics Office of the University of Western Australia (RA/4/1/6188), the Human Research Ethics Committee of Royal Perth Hospital (EC 2012/040) and the Clinical Audit and Safety Unit at Royal Perth Hospital (QI Registration No: 120928-3).

9.2.6 Biochemical and genetic analyses

All laboratory measurements were performed using routine assays in an accredited laboratory. Cholesterol, triglyceride and HDL-cholesterol analyses were performed with enzymatic assays using automated analysers (Abbott, Siemens or Roche, depending on the laboratory). LDL-cholesterol was calculated using the Friedewald equation\textsuperscript{(228)} with direct measurement if triglyceride exceeded 4.5 mmol/L; non-HDL-cholesterol was calculated as total cholesterol minus HDL-cholesterol. ApoB and Lp(a) were measured by immunonephelometry or immunoturbidimetry. Other biochemical tests were performed using routine laboratory methods. Genetic testing was performed as previously described in Chapter 8, Section 8.2.2.\textsuperscript{(35)} Cascade testing was dependent on the mutation present in the family, either sequencing the exon containing the mutation, or performing MLPA.

9.2.7 Data handling

We assessed the integrated effectiveness of cascade screening as the number of new cases of genetically confirmed FH per index case and as the magnitude of reduction in plasma LDL-cholesterol concentration in the new cases after review in the clinic. Data were collated using Microsoft Access and Excel 2010 and analysed using STATA, StataCorp. 2011, Stata Statistical Software: release 13.
9.2.8 Statistical analyses

Continuous variables were described as mean ± standard deviation unless otherwise stated; log normal data were presented as geometric mean ± standard deviation, or as median and interquartile range (IQR). Discontinuous or categorical variables were described as numbers and percentages. Skewed data were logarithmically transformed for regression analyses. The maximal cumulative LDL-cholesterol exposure per individual was estimated by multiplying age in years by the highest documented plasma LDL-cholesterol concentration. The lowest and the most recent lipid values were employed to assess the effectiveness of therapy. The proportion of cases achieving therapeutic targets for LDL-cholesterol was also calculated.\(^{(11, 56)}\)

Differences between index cases and relatives, sub-divided into gene mutation positive (M+) and negative (M-), as well as differences in patient characteristics between mutation positive relatives on and not on therapy, were assessed using linear regression for continuous variables and logistic regression for dichotomous variables; exact logistic regression was employed if cell numbers were small. Inter-group comparisons were Bonferroni adjusted, so that in these analyses, a p<0.016 was considered statistically significant. Gender effects were investigated using a two-way interaction of group and gender. To account for correlation among family members, a variance adjustment (Stata’s vce option) was included on all regression analyses. Changes in lipid, lipoprotein and apolipoprotein concentrations at follow-up were tested in a hierarchical linear mixed model for individuals with baseline and follow-up data (which accounted for repeated measures within a subject, nested within the family cluster). This analysis used maximum likelihood estimation to avoid the bias and imprecision from complete case analysis. Differences in changes over time between groups were investigated using a group and time interaction.
9.3 RESULTS

9.3.1 Study population

Genetic cascade screening was undertaken in 366 family members from the 100 index cases. The majority of the index cases were adults, although six (6%) were aged <18 years. The majority of index cases were referred by primary care (62%), with the remainder by specialists (38%), predominately cardiologists. An FH-causing mutation was found in 188 (51.4%) individuals; 178 (48.6%) individuals did not have a mutation. Kindred size was skewed, with a median of nine family members (IQR 11). A median of four (IQR 2) relatives were genetically tested per index case. On average two (IQR 2) new relatives with FH detected per index case. There were 84 kindreds in whom three or more relatives were genetic tested, with a median of three (IQR 3) new FH cases detected per index case.

9.3.2 Demographic and clinical characteristics of the study population

The baseline demographic and clinical characteristics of the index cases and M+ and M- relatives are shown in Table 26. There were 219 males and 247 females, males were younger (p<0.0001) with no other gender differences. Individuals with FH (index and M+) had higher total cholesterol, LDL-cholesterol, non HDL-cholesterol and apoB concentrations, and were more likely to have xanthomata and corneal arcus than M- relatives. Xanthomata and corneal arcus were more frequent in index cases than M+ relatives; M+ exhibited these clinical signs more frequently than M- relatives. The maximum LDL-cholesterol exposure was greater in index cases than M+ relatives (p<0.001), and greater in M+ than M- relatives (p<0.001). Statins and ezetimibe were used more frequently (p<0.001) by index cases than relatives, and more frequently (p<0.001) by M+ than M- relatives. Antihypertensives were used by 73 (15.6%) of individuals; use in index cases (26.0%) was higher (p<0.01) than M+ (12.8%) and M- (12.9%) relatives.
<table>
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<tr>
<th>Characteristics</th>
<th>Mean (SD)</th>
<th>p-value for difference</th>
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<tbody>
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<td>Index cases with FH (n=100)</td>
<td>Mutation positive relatives, M+ (n=188)</td>
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<td>Male (%)</td>
<td>41 (41.0)</td>
<td>91 (48.4)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>47.0 ± 15.8</td>
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<td>Body mass index (kg/m²)</td>
<td>26.9 ± 4.6</td>
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<td>Systolic BP (mmHg)</td>
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<td>121.6 ± 17.3</td>
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<td>Diastolic BP (mmHg)</td>
<td>74.5 ± 15.7</td>
<td>71.2 ± 11.1</td>
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<td>Corneal arcus (%)</td>
<td>42 (42.0)</td>
<td>49 (26.1)</td>
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<td>Tendon xanthomata (%)</td>
<td>39 (39.0)</td>
<td>35 (18.6)</td>
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<td>178 (94.7)</td>
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<td>1.21 ± 1.38</td>
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<td>Lipoprotein (a) (g/L)</td>
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<td>0.22 ± 0.64</td>
</tr>
<tr>
<td>Highest LDL-cholesterol (mmol/L)</td>
<td>7.21 ± 1.32</td>
<td>6.02 ± 1.45</td>
</tr>
<tr>
<td>Maximum Cumulative LDL-cholesterol exposure (mmol/years)</td>
<td>278.4 ± 1.7</td>
<td>163.2 ± 2.1</td>
</tr>
<tr>
<td>Statin therapy (%)</td>
<td>74 (74.0)</td>
<td>91 (48.4)</td>
</tr>
<tr>
<td>Ezetimibe therapy (%)</td>
<td>44 (44.0)</td>
<td>36 (19.1)</td>
</tr>
</tbody>
</table>

Continuous variables are presented as arithmetic mean and standard deviation (SD), except the lipid data, which are geometric mean and SD after log transformation.
9.3.3 Cardiovascular risk factors, events and interventions

The frequencies of non-cholesterol CVD risk factors and CVD events in the FH index cases and relatives are shown in Table 27. Overall 14.6% were smokers, with no significant difference between groups. The overall frequency of hypertension was 15.4%, with index cases (26.0%) having a greater frequency than M+ (12.8%) and M- (12.9%) relatives (p<0.01). The overall frequency of diabetes was 3.6%, with no significant group differences. The prevalence of premature CAD and of myocardial infarction and coronary revascularisation (angioplasty; coronary artery bypass grafting) was significantly higher (p<0.005) in index cases than either group of family members, and higher (p<0.02) in M+ than M- family members, but became non-significant after adjustment for inter-group comparisons. Premature non-coronary vascular disease was more frequent (p<0.05) in index cases than either M+ or M- relatives, but became non-significant after adjustment for inter-group comparisons.
TABLE 27. NUMBER AND FREQUENCY OF NON-CHOLESTEROL CARDIOVASCULAR RISK FACTORS AND EVENTS IN THE INDEX CASES WITH FH AND THEIR AFFECTED AND UNAFFECTED RELATIVES

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Index cases with FH (n=100)</th>
<th>Mutation positive relatives, M+ (n=188)</th>
<th>Mutation negative relatives, M- (n=178)</th>
<th>p-value for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Index vs. M+</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>26 (26.0)</td>
<td>24 (12.8)</td>
<td>23 (12.9)</td>
<td>0.007</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>5 (5.0)</td>
<td>5 (2.7)</td>
<td>7 (3.9)</td>
<td>0.349</td>
</tr>
<tr>
<td>Current Smoker (%)</td>
<td>12 (12.0)</td>
<td>30 (16.0)</td>
<td>26 (14.6)</td>
<td>0.278</td>
</tr>
<tr>
<td>Former Smoker (%)</td>
<td>31 (33.0)</td>
<td>38 (20.2)</td>
<td>41 (23.0)</td>
<td>0.099</td>
</tr>
<tr>
<td>Premature coronary artery disease (%)</td>
<td>28 (28.0)</td>
<td>18 (9.6)</td>
<td>5 (2.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Myocardial infarction (%)</td>
<td>10 (10.0)</td>
<td>5 (2.7)</td>
<td>0 (0)</td>
<td>0.002</td>
</tr>
<tr>
<td>Coronary revascularisation (%)</td>
<td>18 (18.0)</td>
<td>12 (6.4)</td>
<td>1 (0.6)</td>
<td>0.002</td>
</tr>
<tr>
<td>Premature non-coronary vascular disease (%)</td>
<td>7 (7.0)</td>
<td>1 (0.5)</td>
<td>3 (1.7)</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Premature coronary disease was classified as myocardial infarction, angioplasty or coronary bypass <age 55 for males and <60 years in females. Myocardial infarction and coronary revascularisation (angioplasty and bypass graft) includes premature and non-premature events.
9.3.4 Comparison of family members on and off lipid therapy at diagnosis

The demographic and clinical characteristics of the newly diagnosed family members with FH, in relation to whether they were receiving cholesterol-lowering therapy at diagnosis are shown in Table 28. Cholesterol-lowering medications, mainly statins, were used by 91 of the 188 mutation positive family members. Individuals on therapy were significantly older, had a higher pre-treatment cholesterol level and BMI, a greater frequency of CAD and hypertension, and were more likely to be female and a former smoker.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>M+ relatives on therapy (n=91)</th>
<th>M+ relatives not on therapy (n=97)</th>
<th>p-value for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>34 (37.4)</td>
<td>57 (58.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48 ± 17</td>
<td>29 ± 17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.7 ± 5.5</td>
<td>24.8 ± 5.6</td>
<td>0.049</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>21 (23.1)</td>
<td>3 (3.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>5 (5.5)</td>
<td>0 (0)</td>
<td>0.727</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>14 (15.4)</td>
<td>16 (16.5)</td>
<td>0.701</td>
</tr>
<tr>
<td>Former smoker (%)</td>
<td>26 (28.6)</td>
<td>12 (12.4)</td>
<td>0.017</td>
</tr>
<tr>
<td>Highest total cholesterol (mmol/L)</td>
<td>9.86 ± 2.16</td>
<td>8.12 ± 2.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Coronary artery disease (%)</td>
<td>24 (26.4)</td>
<td>4 (4.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Coronary revascularisation (%)</td>
<td>12 (13.2)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Coronary artery disease was classified as myocardial infarction, angina or revascularisation.
9.3.5 Changes to lipid therapy and LDL-cholesterol after specialist review

Follow-up LDL-cholesterol data were available on 146 (77.7%) of the M+ family members; their average duration of follow-up was 25.0 (18.7) months with no significant differences between those previously on and not on therapy. There were no significant differences between relatives followed-up and not followed-up in the clinic, other than a greater proportion of smokers in the former (21% vs. 6%, p<0.05). Of the 91 family members with FH already on therapy, eighteen relatives had moved interstate or overseas, or declined followed-up in the clinic. In the remaining 73 family members, statin dose was increased in 22 (30.1%) and 16 (21.9%) had ezetimibe therapy added; the types and mean daily dose of statins in this group were: atorvastatin (n=27) 56mg; rosvastatin (n=22) 32mg; simvastatin (n=21) 76mg; pravastatin (n=1) 80mg; 42 individuals received 10 mg of ezetimibe, with two individuals on ezetimibe 10mg alone. Of the 97 new cases not on therapy 24 could not be followed up, 23 had moved interstate or overseas, or declined followed-up in the clinic, and one elderly patient had died from malignancy. In the remaining 73 family members, statins were started in 46 (63.0%) and ezetimibe in 11 (15.0%), of which two received ezetimibe alone; the types and mean daily dose of statins in this group were: atorvastatin (n=12) 36mg; rosvastatin (n=16) 17mg; simvastatin (n=9) 40mg; pravastatin (n=8) dose 37mg; fluvastatin (n=1) 80mg. Twenty-five (34.2%) relatives were not treated with medication for the following reasons: LDL-cholesterol <4.0 mmol/L without CVD or other risk factors in (n=14); reluctance to take a statin or initial intolerance to a statin (n=7); pregnancy or breast feeding (n=4); all received dietary and lifestyle advice. Of 15 people who reported myalgia on statins, 9 were able to continue on a low-dose regimen.

The changes in the lipid lipoprotein and apolipoprotein concentrations are shown in Table 29. There were significant (p<0.001) overall reductions in plasma total cholesterol (-17.7%), LDL-cholesterol (-24.3%), non-HDL-cholesterol (-22.0%) and
apoB (-19.5%) in M+ relatives after review (Table 29A). Significant reductions in total cholesterol, LDL-cholesterol, non-HDL-cholesterol and apoB were seen in M+ relatives previously on treatment in whom drug therapy was revised or unaltered (Table 29B), as well as in those not on treatment in whom drug therapy was initiated, in whom LDL-cholesterol fell by an average of 42% (Table 29C). In relatives in whom drug therapy was not initiated, there were no significant changes in the plasma lipids and lipoproteins (Table 29D). HDL-cholesterol did not change in any of the groups, and triglyceride only in relatives initiated on drug therapy (-19.7%, p<0.001). The significant reductions in LDL-cholesterol, non-HDL-cholesterol and apoB shown in Table 4 were similar when the most recent plasma lipid results were employed in analyses.

The proportional reductions in cholesterol, LDL-cholesterol, non-HDL-cholesterol and apoB were significantly greater (all interaction p-values<0.001) in relatives initiated on drug therapy than those previously on medication. Overall, 75% of the treated cases achieved an LDL-cholesterol <4.0 mmol/L and 19% an LDL-cholesterol <2.5 mmol/L. The proportion of cases achieving an LDL-cholesterol target <4.0 mmol/L was greater among those previously on therapy than those in whom drug therapy was initiated, but this was not statistically significant (81% vs. 64%, p=0.057), there were no significant group difference for an LDL-cholesterol target <2.5 mmol/L.

At follow-up in the clinic, 82.6% of the new cases of FH attained a blood pressure <140/90 mmHg on antihypertensive medication. Of 30 smokers at diagnosis, 12 (40%) discontinued (p<0.01) smoking at follow-up.
TABLE 29. PLASMA LIPID, LIPOPROTEIN AND APOLIPOPROTEIN CONCENTRATIONS OF FH FAMILY MEMBERS AT DIAGNOSIS AND AFTER SPECIALIST REVIEW

<table>
<thead>
<tr>
<th>Lipid, lipoprotein and apolipoprotein concentrations</th>
<th>Period</th>
<th>(A) All mutation positive relatives</th>
<th>(B) Relatives on cholesterol-lowering therapy; drug therapy revised or unaltered</th>
<th>(C) Relatives not on cholesterol-lowering therapy; drug therapy initiated</th>
<th>(D) Relatives not on cholesterol-lowering therapy; drug therapy not initiated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>At diagnosis</td>
<td>6.56 ± 1.03  p&lt;0.001</td>
<td>5.63 ± 1.03  p&lt;0.001</td>
<td>8.14 ± 1.04  p&lt;0.001</td>
<td>6.89 ± 1.04  p=0.34</td>
</tr>
<tr>
<td></td>
<td>Post-review</td>
<td>5.40 ± 1.02</td>
<td>5.01 ± 1.03</td>
<td>5.39 ± 1.04</td>
<td>6.62 ± 1.05</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>At diagnosis</td>
<td>4.52 ± 1.04  p&lt;0.001</td>
<td>3.66 ± 1.04  p&lt;0.001</td>
<td>6.05 ± 1.05  p&lt;0.001</td>
<td>4.89 ± 1.05  p=0.13</td>
</tr>
<tr>
<td></td>
<td>Post-review</td>
<td>3.42 ± 1.03</td>
<td>3.06 ± 1.04</td>
<td>3.48 ± 1.05</td>
<td>4.49 ± 1.07</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>At diagnosis</td>
<td>1.29 ± 1.02  p=0.61</td>
<td>1.29 ± 1.03  p=0.60</td>
<td>1.30 ± 1.04  p=0.64</td>
<td>1.28 ± 1.04  p=0.79</td>
</tr>
<tr>
<td></td>
<td>Post-review</td>
<td>1.28 ± 1.01</td>
<td>1.28 ± 1.03</td>
<td>1.28 ± 1.04</td>
<td>1.29 ± 1.05</td>
</tr>
<tr>
<td>Non-HDL-cholesterol (mmol/L)</td>
<td>At diagnosis</td>
<td>5.17 ± 1.03  p=0.001</td>
<td>4.27 ± 1.04  p&lt;0.001</td>
<td>6.75 ± 1.05  p&lt;0.001</td>
<td>5.53 ± 1.05  p=0.32</td>
</tr>
<tr>
<td></td>
<td>Post-review</td>
<td>4.03 ± 1.03</td>
<td>3.65 ± 1.04</td>
<td>4.02 ± 1.05</td>
<td>5.25 ± 1.06</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>At diagnosis</td>
<td>1.20 ± 1.05  p=0.01</td>
<td>1.18 ± 1.06  p=0.48</td>
<td>1.27 ± 1.09  p&lt;0.001</td>
<td>1.17 ± 1.09  p=0.66</td>
</tr>
<tr>
<td></td>
<td>Post-review</td>
<td>1.09 ± 1.04</td>
<td>1.13 ± 1.07</td>
<td>1.02 ± 1.09</td>
<td>1.13 ± 1.10</td>
</tr>
<tr>
<td>Apolipoprotein B (g/L)</td>
<td>At diagnosis</td>
<td>1.23 ± 1.03  p&lt;0.001</td>
<td>1.08 ± 1.04  p&lt;0.001</td>
<td>1.55 ± 1.05  p&lt;0.001</td>
<td>1.22 ± 1.05  p=0.32</td>
</tr>
<tr>
<td></td>
<td>Post-review</td>
<td>0.99 ± 1.03</td>
<td>0.95 ± 1.04</td>
<td>0.98 ± 1.05</td>
<td>1.16 ± 1.06</td>
</tr>
</tbody>
</table>

Geometric mean ± standard error shown. (A) all relatives, (B) relatives who were on cholesterol-lowering therapy in whom drug therapy was revised or unaltered, (C) relatives not on cholesterol-lowering therapy at diagnosis in whom therapy was initiated, (D) relatives not on cholesterol-lowering therapy in whom drug therapy was not initiated.
9.4 DISCUSSION

This study establishes the feasibility and effectiveness of cascade screening employing genetic testing within an Australian context. It also demonstrated that significant reductions in LDL-cholesterol are achievable after diagnosis and clinic review. If sustained this would translate into a proportional reduction in coronary events in newly diagnosed patients with FH.(12)

9.4.1 Yield of cascade screening

The success of this genetic screening program was underscored by an average of two new cases of FH detected per index case, with three per index case if three or more family members were tested. This yield compares favourably with other studies of the detection rate of FH employing genetic testing.(9, 180, 305, 355, 356) Cascade screening is an internationally acknowledged approach to the care of families with FH,\(^{(11, 55-57, 139, 160)}\) and has been confirmed to be highly cost-effective.(146, 197, 198, 202)

Predictably, the diagnostic yield of cascade screening is directly proportional to the kindred size tested\(^{305}\) and, consistent with expert guidelines, is likely to be most successful if, as in the present study, the process is centrally co-ordinated\(^{55, 56}\). In contrast to the Netherlands,\(^{180}\) identifying large kindreds suitable for cascade screening is a major challenge for a country with the migration history and geography of Australia. The Australasian FH guidelines advocate a proactive, but ethically grounded, approach to cascade screening that was consistent with legislation within our jurisdiction.\(^{55}\) After informed consent (or assent) was obtained from the index case, genetic testing was initially extended to first-degree relatives and then to second- and third-degree relatives. Genetic testing was generally performed in adults before minors, consistent with the Australasian FH guidelines.\(^{55}\) However, testing children as index
cases is advocated in rare clinical or family circumstances, and when combined reverse cascade screening is recommended by international guidelines. 

The success and acceptability of this approach to cascade screening is confirmed by the yield of new cases, as well as the favourable response to testing by screenees. Cascade screening may also be undertaken using cholesterol testing alone, but the accuracy and yield of this approach is less than that of genetic testing. Nevertheless, in accordance with national and international guidelines, both a genetic and cholesterol testing strategy was adopted in the present study.

9.4.2 Mutation detection rate

The mutation spectrum of our population has been previously described (Chapter 8), being comparable to reports from the UK and France. As in other studies, mutations were detected in approximately half of the family members who underwent genetic testing, consistent with the autosomal co-dominant transmission of FH. Lower detection rates were described from the Netherlands between 1994 and 1999, which may be explained by premature mortality among untreated family members; modern day use of statins can improve survival rate of FH. As recommended elsewhere, genetic testing was restricted to known pathogenic mutations causative of FH.
9.4.3 **Coronary artery disease**

The prevalence of CAD was higher in family members who were on cholesterol-lowering therapy prior to the diagnosis of FH, probably reflecting appropriate use of statins in secondary prevention\(^{100, 215, 271}\) in individuals with significant hypercholesterolaemia that has not been attributed to FH. The data also reflects a lack of awareness of FH amongst both GPs and specialists\(^{322, 323}\) and points to the need to enhance education and training concerning the diagnosis of FH and the value of cascade screening\(^{324}\).

The prevalence of CAD in the index cases was similar to that reported from Spain in 2008\(^{360}\) but lower than reported from the UK in 2000\(^{353}\). Notwithstanding selection bias, the difference may reflect the impact of more potent statins and combination therapy in lowering LDL-cholesterol on the incidence of coronary artery disease over the last decade. That the screening program identified family members with FH who had less CAD and non-cholesterol cardiovascular risk factors than their index relatives is consistent with the younger age of the former. This also underscores the fact that cascade screening is a potentially highly effective method for primary prevention of coronary disease in families with FH\(^{11, 55, 56}\).

9.4.4 **Reductions in LDL-cholesterol**

Overall, significant reductions in LDL-cholesterol, non-HDL-cholesterol and apoB were achieved in family members diagnosed with FH. However, these reductions were greater in those who were not on statins prior to the diagnosis, consistent with other studies\(^{10, 66, 303}\). Importantly, 90% of eligible and accessible newly diagnosed individuals with FH were initiated on statin therapy according to national guidelines\(^{55}\).

LDL-cholesterol targets were only achieved in a relatively low number of newly diagnosed cases, which is consistent with other reports\(^{10, 66}\). However, this also reflects that higher doses of high potency statins nor combination therapy with ezetimibe were
not initially employed. Other studies have shown that only one in five individuals with FH can attain a plasma LDL-cholesterol of <2.5 mmol/L with best current therapy.\(^\text{(66)}\) The greater proportion of new cases who were already on statins at diagnosis who then attained LDL-cholesterol targets compared to those not on statins at diagnosis reflects the effectiveness of up-titrating the dose of statins and/or the addition of ezetimibe at review in the clinic.

Another potential factor limiting efficacy relates to side effects, principally myalgia, with high dose statins,\(^\text{(361, 362)}\) although the prevalence of this was approximately 8% in this sample population. Nevertheless, the overall mean reduction in LDL-cholesterol of 25% could potentially translate into a commensurate reduction in the incidence cardiovascular events\(^\text{(12)}\) and therefore significant costs averted.\(^\text{(363)}\) The attainment of ideal therapeutic LDL-cholesterol goals in FH awaits the clinical use of anti-PCSK9 monoclonal antibody therapies.\(^\text{(364)}\)

Non-HDL-cholesterol concentration fell significantly in all newly diagnosed groups of FH, which suggests that reduction in post-prandial remnant lipoproteins may contribute to the impact of therapy on future cardiovascular events,\(^\text{(365)}\) although this remains to be established in FH.\(^\text{(122)}\)

### 9.4.5 Non-cholesterol cardiovascular risk factors

This study confirms that non-cholesterol cardiovascular risk factors are present in a significant proportion of people with FH,\(^\text{(121, 366)}\) particularly those that are older and have established CAD. The data emphasize the need to engage in the general cardiovascular prevention and treatment of non-cholesterol risk factors, including smoking, diabetes and hypertension.\(^\text{(11, 55, 56, 175)}\) The effectiveness of an absolute vascular risk approach to new cases of FH in the clinic is supported by the finding that 80% of those with hypertension attained therapeutic blood pressure targets and 40% discontinued cigarette smoking.
The Lp(a) concentration was not significantly different between family members with or without FH, which is consistent with the fact that this quantitative trait is under separate genetic control.\(^{(252)}\) Furthermore, the higher Lp(a) concentration in index cases than family members with FH could reflect selection bias, possibly related to Lp(a) being an independent risk factor for CAD.\(^{(253)}\)

### 9.4.6 Strengths and limitations

A particular strength of this study is that it tested the principle of cascade screening using a rigorous genetic strategy in a ‘real world’ clinical setting using a relatively large sample of index cases and family members. Our results validate the recommendations of expert bodies\(^{(11, 55, 56)}\) and set a precedent for implementation of a national cascade screening program in Australia. The study emphasises that a centralised service may be more effective than *ad hoc* screening in healthcare systems where the care of family members may be fragmented across several providers; cascade screening may also be embedded into telehealth services.\(^{(55, 56)}\) Cascade screening is highly cost-effective,\(^{(198, 202)}\) with further reduction in expenditure anticipated owing to a fall in the costs of statins and genetic testing, as well as the integration of clinical management with primary care.

This study also employed rigorous statistical analyses that adjusted for correlations within family clusters. Failure to adjust for this may lead to incorrect estimates of statistical significance and inappropriate inferences. To assess treatment effects hierarchical linear mixed modelling with maximum likelihood estimation were applied, allowing all data to be utilised. Although the maximal reduction in LDL-cholesterol to assess the effectiveness of therapy in the analyses was employed, the statistical conclusions were similar when we used the most recent lipid values recorded in the clinic.
Genetic cascade screening is inherently limited to individuals with identifiable mutations and is not applicable to all cases with FH, as up to 40% of individuals with clinically overt FH do not have identifiable mutations.\textsuperscript{(11, 56)} Furthermore, approximately 15% of mutations suggested to cause FH are not associated with marked elevations in LDL-cholesterol,\textsuperscript{(303)} or with increased risk of CAD.\textsuperscript{(351)} Cholesterol testing is a critical component of cascade screening and should be employed in parallel with genetic testing and as the sole method of screening when genetic testing is not available.\textsuperscript{(11, 55-57, 354)}

There were only a limited number of children screened. The clinical characteristics of the newly diagnosed cases of FH who were followed up in clinic did not differ significantly from those not followed up, which mitigates against potential bias and supports the generalisability of the effectiveness of this management strategy. The present study was also restricted to the detection of heterozygous FH.

Finally, cascade screening has limitations, as it is recognised that to detect >80% individuals with FH in a population also requires a combination of universal, targeted and opportunistic screening\textsuperscript{(56, 305)} to identify index cases, that can then trigger the cascade testing process. It is estimated that 20 to 50% of FH cases in the community need to be identified by these alternative methods to cascade screening.\textsuperscript{(305)}

\textbf{9.4.7 Areas for future research}

This study has focused on genetic cascade screening; however, a mutation cannot be identified in up to 40% of people with a clinical diagnosis of FH, and further work is required to clarify the yield and effectiveness of cascade screening in these families based on clinical phenotype. Further research is required into the detection and management of children with FH, including genetic and phenotypic cascade screening. There have been some early reports on the use of next generation sequencing for the detection of FH, which may assist in identifying individuals with mutations. However, further work is required to classify mutations as pathogenic or unlikely to be
pathogenic, as currently ~15% of \textit{LDLR} variants suggested to cause FH are not associated with a marked elevation in LDL-cholesterol, nor with increased vascular disease.\textsuperscript{(303, 351)}

While the role of genetic cascade screening for FH has been confirmed in a non-European population and remains effective despite increased statin use in the community; further research is required to optimise its application in a forever-changing health care environment. Furthermore, future studies should explore the coordination of cascade screening with universal and selective screening methods, and also explore their integration within primary care services. The lessons learnt from cascade screening for FH may also apply to the detection and care of other inherited cardiovascular conditions.

A national registry to augment the detection and management of FH in Australia is another area of future research, to clarify the practical, ethical and governance issues.\textsuperscript{(56, 367)}

\textbf{9.5 CONCLUSION}

This study demonstrated the feasibility, acceptability and effectiveness of cascade screening for FH in Australasia based on genetic testing using a centralised service with specialist follow-up. This information, supported by health economic evaluations,\textsuperscript{(198, 202)} provides an impetus for health policy makers and commissioners to fund a national program for the early detection of FH. The success of such a program is dependent on enhancing the awareness of FH and genetic testing amongst primary care physicians, specialists and the community at large,\textsuperscript{(322-324)} as well as on the establishment of a registry and family support group,\textsuperscript{(56, 367)} both of which have recently been initiated in Australia.
CHAPTER 10:

OPTIMISING THE DETECTION OF FAMILIAL HYPERCHOLESTEROLAEMIA IN THE COMMUNITY: COLLABORATION BETWEEN THE COMMUNITY LABORATORY, PRIMARY CARE AND SPECIALIST LIPID SERVICES

A version of this chapter has been published:

Vickery AW, Bell D, Garton-Smith J, Kirke AB, Pang J, Watts GF. Optimising the detection and management of familial hypercholesterolaemia: central role of primary care and its integration with specialist services. *Heart Lung Circ* 2014; 23(12): 1158-64. (368)
10.1 INTRODUCTION

FH a common monogenic lipid disorder associated with elevated LDL-cholesterol\(^3, 4, 6\) and premature CAD.\(^{55}\) However, currently, the majority of people with FH are undiagnosed, and those who are diagnosed are often undertreated\(^{10, 11}\). Untreated, 50% of men with FH will have CAD by age 50 years and 30% of women by 60 years.\(^4\) There are 50,000 people estimated to have FH in Australasia.\(^2\)

10.1.1 Benefits of early detection and treatment of individuals with FH

Most patients with FH can be simply and effectively treated with life-long cholesterol-lowering treatment, especially with statins, which have been demonstrated to reduce mortality.\(^{12, 13}\) However, people with FH under 30 years of age are rarely identified or treated, although they are predicted to benefit most in terms of life years gained.\(^{11}\) Early treatment before the development of atherosclerosis may well prove to be crucial,\(^{191}\) as individuals with genetically low LDL-cholesterol levels have lower risk of CAD.\(^{295, 369, 370}\) Mendelian randomisation studies have shown that exposure to lower LDL-cholesterol in early life is associated with a substantially greater reduction in CAD.\(^{371}\)

10.1.2 Laboratory and primary care collaboration to detect FH

FH occurs in approximately 1 in 300-500 people and GPs are well placed to detect people with FH, as the average GP in Australia may encounter up to 25 patients with FH every year.\(^{170}\) Once an individual with FH is identified cascade screening of first-degree relatives (i.e. parents, siblings and children) is essential, as 50% of them are predicted to have the condition. Cascade screening of relatives either clinically or genetically, is the most cost-effective FH screening method.\(^{161, 202}\)

However, cascade screening requires effective and accurate mechanisms for identifying FH index cases, which was the focus of Chapters 3-7 of this thesis. These
studies have demonstrated that the community laboratory has the potential to augment opportunistic FH detection (Chapter 3), and that an interpretative comment specifically suggesting specialist referral increased the referral rates, although only minimally (Chapter 4). A phone call from the community laboratory chemical pathologist to the requesting GP of an individual found to be at high-risk of FH significantly increased the referral rate. Furthermore, over 70% of these patients were confirmed to have FH, although only a quarter of those identified at high risk of FH were actually referred (Chapter 5).

GPs request over 90% of LDL cholesterol measurements in the community, and are thus critical to detecting FH index cases. However, GPs and cardiologists demonstrated significant knowledge gaps and a relatively low awareness of FH (Chapter 6). Thus a broad FH awareness and education program will be an integral aspect of a multidisciplinary approach to FH detection in the community.

10.1.3 Aim

To provide an algorithm that highlights the integration of care between community laboratories, GPs and specialist services to try to improve the detection and management of individuals with FH.
10.2 COLLABORATIVE APPROACH TO FH DETECTION: AN ALGORITHM

An algorithm with high specificity for FH incorporating the knowledge gained from the studies in this thesis with the published literature is shown in Figure 15. The algorithm can be used to visualise the interaction and flow of patients suspected of having FH, between primary and specialist care. The algorithm is designed for use by GPs and nurse practitioners in primary care and specialists with an interest in lipid metabolism.

The algorithm starts when the laboratory alerts the clinician responsible for a patient found to have an untreated LDL-cholesterol concentration >5.0 mmol/L. The clinician is then prompted to use an online tool to calculate the DLCNC score to assess the overall likelihood of the patient having FH. Patients already taking lipid-lowering medication can have their pre-treatment LDL-cholesterol level estimated by multiplying their current LDL-cholesterol by 1.3, (16) or a more accurate estimation can be performed using a medication and dose specific calculation. (372) Secondary causes for hypercholesterolaemia (hypothyroidism, cholestasis, nephrotic syndrome, corticosteroids) should be excluded before considering FH, and the LDL-cholesterol should be repeated and confirmed.

Both specialists and primary care clinicians have a role in identifying index cases and referring for cascade screening. GPs may also refer patients to cardiologists for non-invasive screening of subclinical CAD or symptomatic patients for invasive angiography and/or revascularisation procedures. Patients with well controlled FH and stable CAD may also be referred back from the specialist to primary care.
FIGURE 15. ALGORITHM DESCRIBING A COLLABORATIVE APPROACH TO FH DETECTION AND MANAGEMENT

The algorithm is initiated when an LDL-cholesterol >5.0 mmol/L is measured by the community laboratory, which is communicated to the GP via interpretative commenting and/or by phone. The GP then uses the DLCNC to determine the overall likelihood the individual has FH, individuals with probable or definite FH (DLCNC scores >5) are referred to the specialist centre for formal review. The specialists confirm the diagnosis (possibly with genetic testing if available), assess the complexity in order to determine the best management and coordinate the cascade screening process. Low risk FH patients are cared for by the GP, intermediate complexity patients are cared for by both the GP and specialist, and high complexity patients are predominantly cared for by the specialist. LDL - low density lipoprotein cholesterol. GP - general practitioner. FH - familial hypercholesterolaemia.
10.2.1 FH diagnosis and CAD risk assessment

The DLCNC score provides a categorical likelihood of FH: Unlikely, Possible, Probable or Definite. Patients with a DLCNC score greater than 5 are considered to have probable or definite FH (Table 1). Patients should then be assessed for complexity to determine the best clinical management (Table 30). Note that global CVD risk assessment using published CVD risk algorithms should not be employed for individuals with FH, as these underestimate the CVD risk in patients with FH.\textsuperscript{11, 56} Risk of premature CAD in FH is exceptionally high as the LDL-cholesterol is elevated from birth, although other CVD risk factors such as smoking, diabetes and hypertension should be identified and treated.\textsuperscript{352}

Low complexity patients require cholesterol-lowering medication and are best managed in primary care. Medium complexity may require specialist input and shared-care management with the primary care physician. High complexity patients may require tertiary hospital care including procedural interventions and potentially lipoprotein apheresis. Unpublished data from the FH Western Australia Program suggests that ~30% of patients with a DLCNC score >5 are of high complexity and should remain under specialist care, for example, unstable CAD, uncontrolled multiple risk factors and need for apheresis. The remaining 70% of patients who appear to be stable after, diagnosis, treatment and family screening, can be referred back to primary care or to a shared-care option (Figure 15).

Having identified a probable FH index case, it is essential to commence cascade screening. Cascade screening of relatives should be coordinated at a centralised specialist service to efficiently identify affected family members (Figure 16). Referral to specialist services should be considered in two situations: index cases for cascade screening family members for FH, and high-complexity patients with multiple risk factors, unstable CVD and other special problems (Table 30).\textsuperscript{55}
# TABLE 30. ASSESSMENT OF COMPLEXITY AND RECOMMENDATION FOR CARE LEVEL

<table>
<thead>
<tr>
<th>Complexity</th>
<th>Characteristics</th>
<th>Care Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>• No CVD&lt;br&gt;• No CVD risk factors&lt;br&gt;• Attained their LDL-cholesterol target on statin treatment</td>
<td>Primary Care</td>
</tr>
<tr>
<td>Medium</td>
<td>• Stable CVD&lt;br&gt;• Stable CVD risk factors&lt;br&gt;• Nearly reached their LDL-cholesterol target on statin treatment&lt;br&gt;• Minor statin intolerance&lt;br&gt;• Heterozygous FH and are under 18</td>
<td>Shared care</td>
</tr>
<tr>
<td>High</td>
<td>• Multiple uncontrolled risk factors&lt;br&gt;• Symptomatic CVD&lt;br&gt;• Recent myocardial infarction or revascularisation&lt;br&gt;• Not attained their LDL-cholesterol target despite dual therapy&lt;br&gt;• Severe statin intolerance&lt;br&gt;• Special situations e.g. pregnancy, LDL-apheresis, issues with cascade screening</td>
<td>Tertiary care</td>
</tr>
</tbody>
</table>
• A family history is taken from the index case and consent is obtained to contact family members
• These family members are then invited to be assessed by telephone or at the FH Specialist centre
• Lipid profile and blood tests to exclude secondary causes of elevated LDL-cholesterol are performed and results are reviewed by the FH specialist
• Results are given to the patient and their GP by letter
• The letter includes an estimation of the likelihood the individual has FH, and recommendations for formal referral if required
  o If the family member is confirmed to have FH but is of low complexity, management will be undertaken by the GP
  o If the family member is an intermediate or high complexity FH, a referral is required from the GP to the FH Specialist centre for ongoing management.

The coordination of cascade screening including contacting and managing of these relatives, is best performed at the centralised FH specialist service. Confirmation of FH may include genetic testing if the index case has genetically confirmed FH.

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FIGURE 16. ESSENTIAL ASPECTS OF A CASCADE SCREENING PROGRAM FOR FH

Cascade screening is a systematic method to detect FH in close relatives of patients identified with FH (index cases). This figure outlines the essential aspects of a centralised FH cascade screening process.
10.3 INTEGRATED CARE: COLLABORATORS’ ROLES

10.3.1 Role of the clinical biochemist

A combination of index case detection and cascade screening are required to identify over 80% of people with FH in the community, with estimates of 17-47% of individuals required to be identified as unrelated index cases. Community laboratories are well placed to assist with opportunistic FH detection. This may be achieved by the clinical biochemist appending an interpretative comment highlighting FH or calling the requesting GP. The majority of GPs like detailed interpretative comments and value the opinions outside the remit of imaging or pathology when suggesting further patient management. The clinical biochemist is ideally placed to assist with FH detection and management in general practice by adding an effective recommendation to lipid reports that alerts the requestor to the possibility of FH and may direct them to online diagnostic tools and education.

The experiments in the thesis suggest a staged approach, with an interpretative comment appended alone for the lower risk individuals who have LDL-cholesterol between 5-6.4 mmol/L, and a phone call for very high-risk individuals (LDL-cholesterol ≥6.5 mmol/L) in addition to the interpretative comment. The interpretative comment should be brief but informative, provide a simple description of FH, the potential complications and mode of inheritance, as these were identified as areas of knowledge deficiency. Furthermore they should direct the requestor to a tool to assist with FH diagnosis and management (an online DLCNC calculator is in the final stages of development in Australia), or a tool such as FAMCAT could also be made available online. An additional advantage of a website is that an active link could be inserted by the laboratory and sent to the GP practice, depending on the laboratory and practice software.

The interpretative comments may read:
**LDL-cholesterol 5.0-6.4 mmol/L**

“Familial hypercholesterolaemia (an autosomal dominant condition causing premature cardiovascular disease) is likely (~20%). Recommend the FH Network website to assist with diagnosis and management.”

**LDL-cholesterol ≥6.5 mmol/L**

“Familial hypercholesterolaemia (an autosomal dominant condition causing premature cardiovascular disease) is very likely (~70%). Recommend the FH Network website to assist with diagnosis and management.”

Further research to determine the yield and effectiveness of the comments and website is planned.

### 10.3.2 Role of the lipid specialist

Lipid specialists include any specialist with an interest and expertise in lipid metabolism and CVD prevention, which includes general physicians, cardiologists, endocrinologists and chemical pathologists, although this is likely to depend on the country and individual centre. Cardiologists can play a significant role in FH across the continuum of health care from prevention to management of acute coronary syndrome.\(^{(374)}\) As many index cases present for the first time with acute coronary syndrome, cardiologists may be one of the first clinicians to encounter patients with FH and their families. However, while 10-15% of patients with premature CAD will have FH, nearly one third will not have cholesterol measured at the time.\(^{(7,375)}\) Coronary care and rehabilitation units provide an ideal setting to identify FH and to lead the secondary prevention of CAD in FH. However, cardiologists need to recognise that FH is a preventable cause of CAD, and that it is an inherited disorder, which requires cascade screening of family members. To perform this important role, awareness and knowledge of FH amongst cardiologists needs to be improved.\(^{(323)}\)
Cardiologists also have an essential role communicating the importance of primary and secondary CAD prevention in individuals with FH to the family’s GP. Preventive cardiology and cardiovascular genetics have been recommended as a requirement for cardiology training. (376) Additional specialist clinics dealing with lipid disorders and FH are required, either delivered by cardiologists specialising in preventative cardiology or dedicated lipidologists. (55, 374) There should be one clinic specialising in lipid disorders and FH per 250,000 people. (147)

10.3.3 Role of the general practitioner

The role of the GP in screening for FH has been described in Section 1.3.4. (170) The GP has also been central to the opportunistic detection of FH described in Chapters 3-5 of the thesis, and has a critical role in the collaborative algorithm. The GP’s role encompasses both detection and treatment of FH. Although, in order to ensure this occurs, an education and awareness campaign is crucial to correct the knowledge deficiencies uncovered in Chapter 6. (322) The laboratory can alert the GP when one of their patients is found to be at high risk of FH, as GPs preferred interpretative comments as an alert. (322) The GP is then able to include clinical information and diagnostic tools such as the DLCNC, (336) or perhaps FAMCAT (172) to determine the overall likelihood of FH, and if specialist referral is warranted. GPs also have a role in managing the lower complexity FH patients after diagnosis and initial treatment at a specialist centre, as per the algorithm (Figure 15).
It is possible for the GP to increase identification of FH index cases by interrogating the general practice electronic database.\textsuperscript{(141)} In Australia, general practice quickly adopted electronic records with more than 90\% of Australian GPs using a computer software package by 2006.\textsuperscript{(377)} The general practice database can be searched with existing practice software or an extraction tool. This identifies patients at risk of FH who may then require clinical follow-up. The GP can then follow-up either by: formal case note review, opportunistic review at a normal appointment, patient recall to their normal GP or to a specific review clinic held in primary care run by GPs, practice nurses, or other clinical staff, but they may require specific DLCNCS training by the GP or a specialised lipid nurse.

10.3.4 Role of the geneticist

FH can effectively be diagnosed clinically, although it is recommended individuals at high risk of FH are referred to a specialist to confirm the diagnosis.\textsuperscript{(57)} Genetic testing may be offered in specialist centres to confirm the diagnosis and assist with cascade screening, although it is not formally required to diagnose FH.\textsuperscript{(55)} For individuals with a DLCNCS $>5$ and those with xanthoma plus a family history of premature CAD, the Australian Heart Foundation strongly recommends molecular genetic testing. The geneticist is central to this process, ensuring appropriate counselling and consent are provided and that genetic testing is appropriate and performed according to legislative standards. When a causative mutation is found, genetic testing should be offered to all first-degree relatives.\textsuperscript{(55)}

Genetic cascade screening enables accurate diagnosis and becomes much cheaper once the genetic mutation in the index case is known. Without a genetic diagnosis, cascade screening is less efficient,\textsuperscript{(305)} as it is complicated by including families with polygenic hypercholesterolaemia.\textsuperscript{(53)} Genetic testing is also particularly
useful for children and adolescents, where an LDL-cholesterol may not be easily
discriminative of FH.\textsuperscript{(56, 139, 378)}

10.4 CONCLUSION

A collaborative approach between primary and specialist care is required to
increase the detection and management of FH, and to reduce the burden of premature
CVD associated with this condition. A multidisciplinary approach incorporating
education and awareness campaigns with novel mechanisms to alert health
professionals to the possibility of FH is required to close this important gap in
cardiovascular prevention. The algorithm presented in this paper outlines an approach to
augment FH detection and management. Ongoing monitoring of care pathways to
identify persisting gaps remains warranted.
CHAPTER 11:

OVERVIEW: CLINICAL IMPLICATIONS, LIMITATIONS
AND PERSPECTIVES FOR FUTURE RESEARCH
11.1 OVERVIEW

This thesis tested the hypothesis that the care of individuals with FH can be enhanced by employing laboratory, primary care and specialist lipid services. The thesis comprises a review of the relevant literature followed by a series of studies designed to test the hypothesis. This chapter provides a brief summary of FH and then focuses on the studies in the thesis, highlighting their main findings, inter-relationships, clinical relevance and limitations; perspectives for future research are also provided. The validity of the general and specific hypotheses in relation to the observations and conclusions from the predictions are summarised in Figures 17-20.

FH is the most important monogenic cause of elevated LDL-cholesterol and premature CAD. Cholesterol-lowering therapy reduces cardiovascular events and improves mortality in individuals with FH, with evidence that early therapy may abrogate the excess cardiovascular risk. However, only a minority of individuals with FH are currently diagnosed worldwide, and these individuals are often under-treated. In spite of the evidence that screening for and treating FH is cost-effective, most countries have not implemented systematic FH screening. Nevertheless, most developed countries perform general cardiovascular risk assessments, which include measurement of LDL-cholesterol.

11.1.1 Optimising FH detection using the community laboratory

Most developed countries advocate general cardiovascular screening to reduce the overall cardiovascular risk at a population level, as reviewed in Chapter 1. FH is characterised by an elevated LDL-cholesterol concentration, although marked overlap exists in the LDL-cholesterol concentrations between individuals with and without FH. This heterogeneous clinical phenotype is primarily due to the spectrum of residual hepatic LDL-cholesterol clearance capacity, which varies among the ~ 1200 mutations.
A smaller variance in phenotype is also seen among carriers of the same mutation, which is likely to be secondary to other gene variants, as well as environmental and lifestyle factors.

Chapter 3 describes the study that demonstrated that the community laboratory could play an important role in FH detection. The data showed a large number of individuals identified at high risk of FH based on their LDL-cholesterol concentrations, with secondary causes of elevated LDL-cholesterol only present in a minority (8.3%) of individuals. GPs requested the vast majority of lipid profiles, consistent with the notion that general screening for CVD occurs in primary care. An LDL-cholesterol of ≥6.5 mmol/L had a frequency of 1:398 people and was proposed as the threshold to investigate methods to optimise FH detection.

Chapter 4 demonstrates that interpretative comments highlighting the possibility of FH on lipid profiles of individuals with an LDL-cholesterol ≥6.5 mmol/L were associated with a significantly greater reduction in LDL-cholesterol concentration than controls, who did not receive interpretative comments. There was also a trend to increased specialist referral associated with interpretative comments. However, the specialist referral rate was only significantly higher when referral was specifically suggested on the interpretative comment, but even then only a minority (11.5%) of these individuals were referred to the regional lipid disorders clinic. While the impact of interpretative comments on LDL-cholesterol concentrations was encouraging, further investigations were required to optimise FH detection.

A telephone call and advice from the chemical pathologist to the requesting GP was associated with significant increases in the rate of referral to a lipid specialist as shown in Chapter 5. This study also ascertained that phenotypically defined FH was present in 72% of individuals with an LDL-cholesterol ≥6.5 mmol/L in an Australian community population, 30% of whom had identifiable mutations.
11.1.2 Primary care aspects of the detection of FH

GP awareness of national guidelines and knowledge of hereditability, prevalence and diagnostic features of FH were found to be suboptimal in Chapter 6. These deficiencies in awareness and knowledge were postulated to be the reason for the relatively low (27%) rate of referral to a specialist. The majority of GPs considered they were the most effective health practitioners for managing FH. These findings may explain the differences between LDL-cholesterol reduction and referral rates, since GPs were knowledgeable and proficient at lipid-lowering, but were relatively unaware of FH and the ramifications this has for the patient and their family. This survey also demonstrated that the majority of GPs (90%) preferred interpretative comments on laboratory reports to alert them to the possibility of FH.

Furthermore, Chapter 7 demonstrated that there was a good agreement (83.6%) in the DLCNCS calculated by trained GPs and specialists. GPs also accurately categorised individuals at high (86.7%) and low (94.0%) risk of FH with the DLCNCS, suggesting that the DLCNC can be used in primary care to augment FH detection by ensuring specialist referrals are appropriate.
11.1.3 Yield and effectiveness of genetic cascade screening for FH

Chapter 8 describes the spectrum of mutations and the mutation detection rates for individuals with phenotypic FH in Australia. A mutation known to be pathogenic or likely causative of FH was identified in 70% of individuals with clinically definite FH, which reduced to 29% of probable and 11% of possible FH categorised using the DLCNC. There were 86 pathogenic mutations identified in 129 individuals, 14 of which were novel. The spectrum of mutations was similar to that described in the UK and France.

The diagnostic yield and effectiveness of cascade screening family members of the first 100 index cases with genetically confirmed FH in Western Australia are shown in Chapter 9. Cascade screening detected on average two new FH cases per index case, with a ~50% mutation detection rate. Cholesterol-lowering therapy (almost exclusively statins) was initiated in 90% of the individuals found to have FH who were eligible for treatment, and not already receiving therapy. Of note, cholesterol-lowering therapy had already been started in ~50% the family members in the community before they were diagnosed with FH, although only a minority were reaching the recommended targets. Significant improvements were seen in non-lipid CVD risk factors; 80% of individuals with hypertension attained the recommended blood pressure targets and 40% of smoker’s ceased smoking. This study confirmed that genetic cascade screening is effective in a centralised service, and that significant additional reductions in LDL-cholesterol were achieved despite the high use of cholesterol-lowering therapy in the community.
11.1.4 Summary of findings and their relationship to the general hypothesis

The studies support the specific and the general hypothesis of this thesis (Figures 17-20). In summary, an LDL-cholesterol of ≥6.5 mmol/L from a community laboratory selected individuals at high risk of FH. LDL-cholesterol concentrations were improved by interpretative comments, and a telephone call significantly improved referral and FH detection rates. Deficiencies in GPs knowledge of FH were uncovered, which may explain the relatively low referral rates to a specialist. Although, once trained GPs can accurately use the DLCNC to identify individuals who are at high and low risk of FH.

Genetic testing identified a mutation in an acceptable proportion of individuals with phenotypic FH to allow cost-effective genetic cascade screening to occur. Detecting individuals with FH remains clinically beneficial and significant reductions in LDL-cholesterol are still achieved despite the increase use of statins in the community.
Specific hypothesis 1: The community laboratory can optimise the detection of FH

Prediction 1: Community laboratories can opportunistically screening for FH

Results 1:
- GPs requested 92% of Lipids
- 8.3% of elevated LDL-cholesterols had a potential secondary cause identified
- 1:398 individuals had an LDL-cholesterol ≥5.5mmol/L

Conclusion 1: Laboratories are well placed to opportunistically detect FH Consistent with prediction 1. Supports hypothesis 1.

Prediction 2: Interpretative comments increase the detection of FH and optimise treatment

Results 2:
- Interpretative comments were associated with:
  - Significant additional reductions in LDL-cholesterol
  - A significant increase in referrals to a specialist was achieve when referral was specified

Conclusion 2: Interpretative comments improve FH management and increase, but do not optimise FH detection Partly consistent with prediction 2. Supports hypothesis 1.

Prediction 3: A phone call and advice from the pathologist to the requesting GP will optimise the detection of FH

Results 3:
- The telephone call significantly increased referral to a specialist
- 72% of referred patients had FH
- 30% had an identifiable mutation
  - However, only 27% were referred to a specialist

Conclusion 3: A telephone call from the chemical pathologist to the GP increases the detection of FH Consistent with prediction 3. Supports hypothesis 1.

FIGURE 17. SPECIFIC HYPOTHESIS 1 - THE COMMUNITY LABORATORY CAN OPTIMISE THE DETECTION OF FH
Specific hypothesis 2:
GPs can effectively detect individuals with FH

Prediction 4:
GPs knowledge of FH is suboptimal, although they will be willing to detect and manage individuals with FH

Results 4:
- GPs awareness of national guidelines and knowledge of hereditability, prevalence and diagnostic features of FH were suboptimal
- The majority of GPs considered they were best placed to manage patients with FH
- 90% of GPs preferred interpretative comments to alert them when a patient is at high risk of FH

Conclusion 4:
GPs awareness and knowledge were suboptimal although they were willing to manage individuals with FH

Consistent with prediction 4. Supports hypothesis 2

Prediction 5:
GPs can use the DLCNC to identify individuals at high risk of FH in primary care

Results 5:
- 83.6% agreement between the Dutch Lipid Clinic Network Criteria score calculated by GPs and lipid specialists
- GPs accurately categorised 86.7% of high risk and 94.0% of low risk individuals

Conclusion 5:
The DLCNC can be used in primary care to augment the detection of FH by ensuring specialist referrals are appropriate

Consistent with prediction 5. Supports hypothesis 2

FIGURE 18. SPECIFIC HYPOTHESIS 2 - GPS CAN EFFECTIVELY DETECT INDIVIDUALS WITH FH
FIGURE 19. SPECIFIC HYPOTHESIS 3 - SPECIALIST LIPID SERVICES CAN EFFECTIVELY DIAGNOSE AND TREAT INDIVIDUALS WITH FH, AND PERFORM FAMILY SCREENING
**General Hypothesis**

The care of individuals with FH can be enhanced by employing laboratory, primary care and specialist lipid services

**Specific hypothesis 1:**
The community laboratory can optimise the detection of FH

Supported by the observations

**Specific hypothesis 2:**
GPs can effectively detect individuals with FH

Supported by the observations

**Specific hypothesis 3:**
Specialist lipid services can effectively diagnose and treat individuals with FH, and perform family screening

Supported by the observations

---

**FIGURE 20. ASSESSMENT OF THE GENERAL HYPOTHESIS**
11.2 CLINICAL IMPLICATIONS

The studies in this thesis have shown that opportunistic screening for FH can be successfully conducted using the LDL-cholesterol results from a community laboratory. A potential collaborative approach to FH detection is presented in Chapter 10. Interpretative commenting was associated with a significant reduction in LDL-cholesterol, and was the mode GPs’ preferred to alert them of patients at high risk of FH. This suggests that interpretative comments highlighting FH should be instituted on all lipid profiles of patients considered to be at risk of FH. This study is one of the first in the literature to demonstrate the impact of interpretative commenting on clinical outcome, and denotes a potentially wider role of the chemical pathologist in the detection and management of metabolic disorders.

While GPs stated they were the best-placed health professionals to detect FH, some concerning deficiencies in knowledge were identified. This suggests that an education campaign is required to augment the detection of FH in primary care. This is perhaps best coordinated at a national level. There are implications for training and accreditation in FH management for medical schools, medical colleges and state health services. A telephone call and advice from the chemical pathologist to the requesting GP was shown to increase FH detection rates. These findings suggest that a pragmatic approach incorporating a tiered method of alerting GPs when a patient is at risk of FH should be employed; interpretative commenting for moderate risk (LDL-cholesterol 5.0 – 6.4 mmol/L), and an additional phone call for those at very high risk (LDL-cholesterol ≥6.5 mmol/L). This proposition requires testing.

The implications of these studies for tertiary care include integrating and coordinating the care of FH across primary care and tertiary care. Tertiary care is responsible for establishing a centralised service, and coordinating cascade screening and optimising treatment for individuals with FH. These centralised services should be
multidisciplinary, as they will also have an important role in training and accreditation and as such should have representation from lipid specialists, geneticists, chemical pathologists and primary care physicians. The studies in this thesis may be used to support national and international guidelines on the detection of FH using the LDL-cholesterol results already being generated by community laboratories.

11.3 LIMITATIONS

This section will initially summarise the limitations of the studies in the thesis, as the specific limitations are discussed in the individual chapters, and then consider the general limitations related to this area of FH research. The non-randomised case-historical control methodology used in the interpretative comment and phone call studies represent methodological limitations, as while they were controlled, a historical control is not as robust as a prospective randomised control. However, there are currently no randomised controlled trials of the outcome of interpretative comments in the literature. All of the studies in the thesis were of a relatively small size, although the findings were statistically significant. All studies were conducted in Western Australia, and while the inclusion and exclusion criteria were not overly restrictive, a larger multicentre, preferably international, randomised controlled trial should be conducted to confirm the benefit of detecting individuals with FH from the community laboratory, and ensure these finding can be applied to other regions.

The studies investigating the ability of a community laboratory to opportunistically identify individuals with FH were novel, and as such were conducted to test the principle, rather than to provide definitive proof of the hypothesis or prediction. The individuals conducting this research were also interested in FH, and thus may have introduced bias into these investigations – although comments and the information provided in the telephone calls was standardised to try to reduced this possibility. The assessment of both GP knowledge and their ability to use the DLCNC
to evaluate an individual likelihood of FH were performed on opportunistic samples, which significantly limits their generalisability. The study assessing the ability to use the DLCNC in primary care was also severely limited by the very low number of primary care staff, and is perhaps best viewed as providing data to generate a specific hypothesis rather than providing definitive evidence.

The study describing the yield and effectiveness of cascade screening in Western Australia reported the first 100 index cases with identifiable FH causing mutations who had at least one family member who had under gone genetic testing, and as such may be affected by ascertainment bias, and thus not accurately reflect the yield and effectiveness generally. Individuals were also lost to follow-up during this report. However, this limitation may also be viewed as a strength as it reports an outcome of a clinical service rather than a formally controlled trial.

A major limitation of this thesis is that it predominantly focused on opportunistic screening, which is inherently limited in that it can only detect individuals who have had LDL-cholesterol testing performed. The lack of internationally agreed diagnostic criteria for FH remains a limitation of FH research, in that the findings are not necessarily directly transferable to countries using alternate diagnostic criteria. This thesis used the DLCNC and comprehensive genetic testing to diagnose FH, which are well-recognised diagnostic methods. However, even genetic testing for FH has weaknesses, as a mutation is not found in 10-40% of individuals with a clinical diagnosis of FH, and 10-15% of identifiable mutations are only associated with a mild phenotype, and may not be associated with premature CVD.

11.4 PERSPECTIVES FOR FUTURE RESEARCH

This section will discuss future research related to the thesis as a whole, as areas of future research relating to each individual study were discussed in the respective chapters.
11.4.1 Optimising the detection of FH

Detecting FH index cases remains one of the major challenges in FH research. This thesis has described the potential of the community laboratory to opportunistically detect FH in collaboration with primary care and specialist lipid services. However, while the detection of FH was significantly improved, it was still not ideal and further research to optimise the detection of FH is required. Low awareness and significant FH knowledge deficits among health professionals represent major barriers to the detection of FH. A national FH awareness and education campaign aimed at medical professionals is required, although the best method of providing this education remains to be determined. Furthermore, the optimal governing body to oversee the training and accreditation of health professionals in the care of individuals with FH remains to be determined. The medical colleges are likely to be the best placed organisations to perform this role. However, the current level of teaching and FH knowledge of fellows of the college of physicians, general practice and pathology remains to be elucidated.

11.4.1.1 Verifying the role of the community laboratory

It would also be important to confirm that the community laboratory can play an important role in opportunistic FH detection. This could be achieved by performing a multicentre randomised control trial comparing a telephone call from the chemical pathologist to requesting GP of individuals found to be at high risk of FH, with current practice, which is likely to be a generic comment. Using the data in Chapter 5, a trial would require a minimum of 58 individuals in each group to have 95% power to detect a 23% difference (4 vs. 27%) with 95% confidence. However, if the effect size was not as large as shown in Chapter 5, a trial with 207 individuals in each arm would have 95% power to detect a 10% difference (4 vs. 14%) with 95% confidence. A 10% difference would also remain clinically significant.
Furthermore, it would also be important to conduct a randomised control trial comparing a specific interpretative comment suggesting referral for specialist assessment with no interpretative comment. Using the information in Chapter 4, this trial would require a minimum of 141 individuals in each group to have 95% power to detect a 10% difference (1 vs. 11%) with 95% confidence. Of note, there are currently no randomised controlled trials investigating the outcome of interpretative comments, so this trial could have wider implications than FH detection, and may be a pivotal study the field of laboratory medicine.

11.4.1.2 Augmenting the detection on FH using expert computer systems

There is also a potential to augment the detection of FH through the use of the Internet and expert computer systems. For example, GPs can effectively use the DLCNC score to determine whether an individual is at high or low risk of FH, thus linking an interpretative comment with an online DLCNC calculator may streamline this process. In addition, laboratory comment alerts may be optimised by an expert computer system that could highlight individuals at risk of FH using clinical information and previous cholesterol results irrespective of the LDL-cholesterol concentration on the current sample. This would be important for detecting the ~50% of individuals with undiagnosed FH currently on cholesterol-lowering therapy, and for younger individuals with a family history of premature CAD who may not have an LDL-cholesterol above a threshold selected to screen for FH.

11.4.2 Diagnostic criteria for FH

The diagnostic criteria for FH are a crucial area for further research, and more importantly international consensus, which would optimise further research by providing an internationally accepted diagnostic standard and ensure research findings were applicable worldwide. Furthermore, the laboratory could play a more central role
in FH detection if there was consensus surrounding the LDL-cholesterol criteria for diagnosis of FH. Laboratory computer systems could also incorporate LDL-cholesterol into FH risk algorithms as a continuous variable, rather than relying on thresholds. This may improve both the sensitivity and specificity of LDL-cholesterol for screening, noting the large overlap in LDL-cholesterol concentrations between individuals with and without FH.

11.4.2.1 Employing diagnostic criteria as continuous variables

LDL-cholesterol could be employed as a continuous variable in a computer or web-based diagnostic tool, which could generate a linear quantitative risk of FH rather than the current threshold based diagnostic criteria. A computer model could also adjust the LDL-cholesterol to use an age, gender and county specific LDL-cholesterol percentile or multiple of the median, rather than using LDL-cholesterol as an absolute number and categorical variable. The computer model could also adjust the LDL-cholesterol to estimate a pre-treatment LDL-cholesterol concentration for individuals on cholesterol-lowering treatment, in whom a pre-treatment LDL-cholesterol is unknown.

A computer model could also use the clinical features of FH as continuous variables if appropriate. Tendon xanthomata are pathognomonic for FH, and hence may be a categorical variable. However, the pathogenesis of xanthomata is not completely known, and the incidence may be decreased by the increase use of statins in individuals with undiagnosed FH. It would be possible to use the Achilles tendon width as a continuous variable, although research is required to determine if this has any value in predicting FH.

Premature CVD is currently a dichotomous variable based on age and gender, but the current age threshold may no longer be appropriate given the increased use of statins in the community and an aging population. Research should also be performed to determine if CVD or just CAD should be included. Moreover, the age of onset of
vascular disease for the patient and their family members could be adjusted to provide an age and/or generation adjusted risk, as the ages of premature CVD may not be the same among different generations. This may explain the finding that a personal history of CVD was not an independent predictor of FH in FAMCAT, although this requires investigation. The adjustment could be made either using a percentile or a multiple of the median, in order to provide a more accurate and continuous estimate of premature vascular disease risk. Other features could also be incorporated, such imaging results, CIMT or computerised tomography coronary angiographic findings, after adjusting for age. The algorithm could then combine both the LDL-cholesterol clinical and imaging features of FH as continuous variables to estimate the overall likelihood of FH.

While this concept is complicated and is only suited to computer based estimates, the vast majority of clinicians have access to computers in their clinic rooms. Furthermore, the data inputs are relatively simple, consisting of LDL-cholesterol, type and dose of cholesterol-lowering medication, date of birth, age and imaging result if performed, age at first CVD event for the patient and their family members. This information would be known to the clinician, or could be obtained quickly during a consultation.
11.4.3 Implication for the detection of FH in tertiary care

The studies in this thesis have demonstrated that laboratories can play an important role in alerting GPs that their patient is at high risk of FH. However, further research is required to determine whether this can be modified and applied in a tertiary care setting. Given the high prevalence of premature vascular disease associated with FH, a higher frequency of individuals with FH would be anticipated in coronary care unit and cardiothoracic and vascular surgery wards compared with the community. While specialists are caring for these patients, there is evidence that the knowledge and awareness of FH among some specialists is not optimal, with missed opportunities for identifying index cases with FH and their affected family members. Investigations would be required to determine the prevalence of FH on these wards and the number of individuals currently diagnosed, and then to formulate and test strategies to optimise the detection of FH in these high-risk clinical settings.

11.4.4 Detecting children with FH

Identifying paediatric FH is another priority for future research. There is observational evidence that detecting and treating adolescents with FH is associated with reduced mortality and morbidity. However, again there is no international consensus on the best methods to identify children with FH; universal screening of 9-11 year old children is recommended in the United States (US), whereas testing children is only recommended as part of cascade screening in Belgium. While not the focus of this thesis, children could potentially benefit from community laboratory based opportunistic screening. LDL-cholesterol was measured on 444 individuals aged <18 years in the population described in Chapter 3. An LDL-cholesterol of $\geq 3.5$ mmol/L, which is an LDL-cholesterol concentration where further assessment for FH has been advised, was present in 43 of these children. These preliminary data suggest that investigating this potential method for detecting children with FH is warranted. It would
be important to perform reverse cascade screening from children with FH, conducted within regional legal and ethical guidelines.

One of the barriers to screening children for FH is the requirement for a blood sample, although there are alternative methods of obtaining an estimate of the LDL-cholesterol, including finger prick testing. However, children often have blood tests for other reasons, and it may be possible to detect FH by offering to add a lipid profile to samples of any child undergoing blood testing, if the parents consent and the child assents. While this may be logistically more challenging, it is worthy of consideration. Studies have investigated universal screening of neonates, but to date these have not been very successful, and this requires further investigation.

This thesis demonstrates that it is feasible to use LDL-cholesterol measurements from a community laboratory to opportunistically screen for FH. However, the cost-effectiveness of this method remains to be elucidated. Health economic evaluations of cascade screening incorporating investigation and treatment have been conducted and demonstrate this is cost-effective. Further investigations need to be conducted into the cost-effectiveness of all methods to detect index cases – as 17-45% of individuals need to be detected outside of cascade screening to detect more than 80% of individuals with FH.

11.4.5 Genetic testing for FH

The role of genetic testing in the diagnosis of FH is also an area of controversy, with some opinion leaders stating it is fundamental and others believing it has no role. A randomised controlled trial investigating the detection yield of genetic and phenotypic screening is currently planned in the US, which addresses this question. Genetic testing is currently expensive, and limited by the lack of agreement with the clinical phenotype in some cases. Accurately classifying the ~1200 mutations currently identified and discovering the remaining mutations that cause the FH phenotype are
other areas of research that are entering an exciting phase. Next generation sequencing may assist in detecting these mutations, but it will also necessitate strict pathogenicity classification of these variants, since multiple variants of uncertain significance are likely to be uncovered.

11.4.6 Implications for health policy

The development and implementation of effective health policy for the detection and management of FH remain paramount. The studies in this thesis have explored the possibility of augmenting the detection of FH using the laboratory interacting with primary and specialist care. However, involving consumers, and ascertaining their perspectives and the acceptability of these proposals is required. Furthermore, it is essential to optimise these processes, and then to assess them against health performance indicators such as; access, efficiency, efficacy, acceptability, integration, continuity of care and sustainability.

11.5 CONCLUSIONS

The studies in this thesis collectively demonstrate that laboratory, primary care and specialist lipid services can be employed to enhance the care of individuals with FH, by augmenting the detection and treatment of the condition. Interpretative comments were associated with significant LDL-cholesterol reductions, and a telephone call to the requesting GP of high-risk individuals significantly improved the detection of FH. Identifying individuals with FH remains effective and leads to additional reductions in LDL-cholesterol and improvements in non-lipid cardiovascular risk factors after specialist review. Additional research is required to further investigate methods for optimising the detection of FH and the translation of the findings into effective health policy.


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308. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation;


339. UCL LDLR Database. [http://www.ucl.ac.uk/ldlr/LOVDv.1.1.0/](http://www.ucl.ac.uk/ldlr/LOVDv.1.1.0/). [Accessed 20.04.12].


APPENDIX 1

GP Familial Hypercholesterolaemia knowledge and awareness questionnaire

Q1. On a scale of 1 to 7; how familiar are you with familial hypercholesterolaemia?

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

Please circle:

Q2. Are you aware of the National Heart Foundation/Cardiac Society of Australian and New Zealand guidelines on the detection and management of familial hypercholesterolaemia?

✓ Yes

No

Q3. Which one description below best describes familial hypercholesterolaemia?

Please tick one

- The presence of family members with diagnosed high cholesterol
- A genetic disorder that is characterized by very high cholesterol and a family history of premature heart disease
- The presence of multiple lipid abnormalities that may be genetic in nature
- An ultra-rare, potentially fatal condition caused by cholesterol levels that can be up to six times the normal level
- Other (please specify)
- Don’t know

Q4. Which one of the following lipid profiles is most consistent with the diagnosis of familial hypercholesterolaemia?

<table>
<thead>
<tr>
<th>Reference intervals</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>&lt; 5.5mmol/L</td>
<td>6.0</td>
<td>6.3</td>
<td>8.0</td>
<td>5.4</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>&lt; 1.7mmol/L</td>
<td>3.4</td>
<td>12.2</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td>HDL – cholesterol</td>
<td>&gt; 1.0mmol/L</td>
<td>0.8</td>
<td>1.0</td>
<td>1.0</td>
<td>1.7</td>
</tr>
<tr>
<td>LDL – Cholesterol</td>
<td>&lt; 3.5mmol/L</td>
<td>3.8</td>
<td>-</td>
<td>6.5</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Please tick one
**Q5.** Which of the following options could usefully assist you in detection of familial hypercholesterolaemia in your practice?

<table>
<thead>
<tr>
<th>✓ Please tick one.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory report on a lipid profile alerting possible familial hypercholesterolaemia</td>
</tr>
<tr>
<td>Alert by the clinical software system in your practice</td>
</tr>
<tr>
<td>Direct telephone call from the laboratory</td>
</tr>
<tr>
<td>All of the above</td>
</tr>
<tr>
<td>None of the above</td>
</tr>
<tr>
<td>Other (please specify)</td>
</tr>
<tr>
<td>Don’t know</td>
</tr>
</tbody>
</table>

**Q6.** What is the prevalence of familial hypercholesterolaemia in Australia?

<table>
<thead>
<tr>
<th>✓ Please tick one</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 in 100 persons</td>
</tr>
<tr>
<td>1 in 500 persons</td>
</tr>
<tr>
<td>1 in 1,000 persons</td>
</tr>
<tr>
<td>1 in 2,000 persons</td>
</tr>
<tr>
<td>1 in 5,000 persons</td>
</tr>
<tr>
<td>Don’t know</td>
</tr>
</tbody>
</table>

**Q7.** What is the likelihood that first-degree relatives (i.e. parents, siblings and children) of someone who has familial hypercholesterolaemia will also have the condition themselves?

<table>
<thead>
<tr>
<th>✓ Please tick one</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
</tr>
<tr>
<td>25%</td>
</tr>
<tr>
<td>50%</td>
</tr>
<tr>
<td>75%</td>
</tr>
<tr>
<td>100%</td>
</tr>
<tr>
<td>Don’t know</td>
</tr>
</tbody>
</table>
Q8. How much greater is the risk of premature coronary heart disease (CHD) in untreated familial hypercholesterolaemia patients compared to the general population?

- Please tick one
- 2 times greater
- 5 times greater
- 10 times greater
- 20 times greater
- 50 times greater
- Don’t know

Q9. When you are assessing a patient’s family history, at what age for males and females do you consider heart disease to be “premature”?

- Premature heart disease in males: # years of age or younger
- Premature heart disease in females: # years of age or younger

- Don’t know

Q10. In patients with documented premature coronary artery disease which of the following do you routinely carry out?

- Please tick all that apply.
- Look for arcus cornealis
- Look for tendon xanthomata
- Take a detailed family history of coronary artery disease
- Screen close relatives for hypercholesterolaemia
- All of the above
- None of the above

Q11. Is the following statement true or false?

- An accurate diagnosis of familial hypercholesterolaemia can only be made via genetic test.
- True
- False
- Don’t know
Q12. How many patients currently under your care, if any, have been formally diagnosed with familial hypercholesterolaemia?

Enter "0" if appropriate

patients

Don’t know

Q13. If you have patients with familial hypercholesterolaemia under your care do you routinely screen close relatives for this condition with a lipid profile?

Please tick one

Yes, patient’s children only

Yes, patient’s children and other close relatives

No

Not applicable

Q14. In your view, which healthcare providers would be most effective at early detection of familial hypercholesterolaemia and screening first-degree relatives?

Please tick up to two.

Lipid specialists

General practitioners

Cardiologists

Nurses with experience in cardiac risk prevention

Pediatricians

Obstetricians/Gynecologists

Endocrinologists

Other (please specify)

Q15. At what age would you test young individuals for hypercholesterolaemia in a family with premature coronary heart disease?

Please tick one.

0 – 6 years

7 – 12 years

13 – 18 years

None of the above

Don’t know
Q16. Are you aware of any specialist-clinical services for lipid disorders to whom you can refer patients?

✓

Yes

No (Go to question 18)

Q17. If yes to question 16, have you referred patients with familial hypercholesterolaemia to this service?

✓

Yes

No

Don’t know

Q18. Which drugs do you use to treat hypercholesterolaemia?

✓ Please select all that apply

- Exchange resins / bile acid sequestants
- Ezetimibe
- Statins
- Fibrates
- Nicotinic acid
- Other (please specify)

None of the above

Q19. Which drug combinations do you use to treat severe hypercholesterolaemia?

✓ Please select all that apply

- Statin + Exchange resins / bile acid sequestants
- Statin + nicotinic acid
- Statin + ezetimibe
- Statin + ezetimibe + nicotinic acid
- Statin + ezetimibe + Exchange resins / bile acid sequestants
- Other (please specify)

None of the above
GP Demographics and Practice details

The following questions are for classification purposes only. Your responses will only be reported in aggregate.

1. What is your gender?

☒ Please tick one.

| Male | Female | Prefer not to say |

2. How would you describe the area of your primary practice?

☒ Please tick one.

| Metropolitan | Outer metropolitan | Rural |

3. Are you a fellow of the RACGP?

☒ Please tick one.

| Yes | No | Prefer not to say |

4. If you are FRACGP, how many years have you been in practice since completing your fellowship?


5. Approximately how many patients do you see for any condition in an average month?


6. Have you previously completed this questionnaire?

☒ Please tick one.

| Yes | No | Not sure |

Thank you for completing this questionnaire