The impact of breastfeeding on *FTO*-related BMI growth trajectories: An application to the RAINE Pregnancy cohort study

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SUMMARY

Introduction: For years, body mass index (BMI) has been used by scientists to track weight problems and obesity in children and adults. Recent studies have implicated the fat mass and obesity gene (FTO) in the increase of BMI in young adults. A longer duration of breastfeeding is known to reduce the risk of being overweight later in life but its ability to modify the effect due to FTO is not known. **Methods:** We studied 1,096 children from the Western Australian Pregnancy (Raine) cohort who were followed-up from birth to 14-years. Linear mixed effects models were used to investigate BMI growth trajectories in boys and girls separately. **Results:** An association was found between BMI growth and the duration of exclusive breastfeeding (EXBF) among carriers of the risk allele of the FTO SNP rs9939609. In girls, EXBF interacts with the SNP at baseline and can reverse the increase in BMI due to SNP risk allele by age 14 after three months of EXBF. In boys, EXBF reduces both BMI in carriers and non-carriers of the risk allele with an association found after 10 years of life. Six months of EXBF will put the boys' BMI growth curves back to the normal range. **Conclusions:** Our study could have major health implications by providing new perspectives for the prevention of growth problems in children carrying risk alleles in the FTO gene.

Key words: BMI; Obesity; Longitudinal study; Growth; FTO gene; Breastfeeding; Children; RAINE; Australia; Linear mixed effects model.

INTRODUCTION

BMI has been used by health professionals and scientists to track weight problems and obesity in adults for many years. Recent studies have supported the role of the (FTO) gene in increasing BMI in young adults (1-5). Genome-wide association studies on BMI and adiposity have successfully identified an association with genetic variants in the *FTO* locus in both adults and children (6,2,3,7,8). In meta-analyses, the addition of each minor (A) allele at the SNP rs9939609 within the first intron of *FTO* has been shown to be consistently associated with a higher BMI of up to 0.33 kg/m^2 or approximately 0.1 standard deviation (2,9). The biological mechanisms underlying this association are yet to be determined, however, evidence from population-based and functional studies suggest that this locus is likely involved in the hypothalamic regulation of appetite or energy expenditure and metabolic rate (4, 10-15).

What those studies have not yet reported is whether the long-term increase in BMI attributed to *FTO* risk alleles can be mitigated by environmental factors. Breastfeeding, particularly the duration of breastfeeding, is a good candidate as risk modifier. Some studies have shown that a longer duration of breastfeeding was associated with a decrease of the risk of being overweight later in life (16-19). A recent study from the RAINE cohort in Australia (20) showed that early infant feeding can affect BMI trajectories through a possible change in the timing of adiposity rebound (AR). These studies have also pointed out that the definition of breastfeeding can

affect the association results, in particular the distinction between exclusive and non-exclusive breastfeeding. It is still unknown in longitudinal studies whether a long duration of exclusive breastfeeding or mixed feeding can prevent the *FTO*-induced increase in BMI. Dedoussis et al. (21) showed that a short period of at least one month of breastfeeding was associated with reduced obesity in a childhood cohort from Greece, however the study was cross-sectional at different time points and there was no distinction between exclusive and non-exclusive breastfeeding. Also, the results were not replicated in the ALSPAC child cohort from the UK (22), even when a longer period of breastfeeding was considered. In our study, we investigate the long-term impact of the duration of breastfeeding, more specifically exclusive breastfeeding, on *FTO*-induced BMI growth curves in children from the Western Australian Pregnancy (Raine) cohort.

MATERIALS AND METHODS

The Western Australian Pregnancy Cohort (Raine)

Recruitment of the Western Australian pregnancy cohort (Raine) has been previously described in detail (23,24). In brief, between 1989 and 1991 2,900 pregnant women were recruited prior to 18-weeks gestation into a randomised controlled trial to evaluate the effects of repeated ultrasound in pregnancy. Recruitment predominantly took place at King Edward Memorial Hospital (Perth, Western Australia). Women were randomised to repeat ultrasound measurements at 18, 24, 28, 34 and 38 weeks gestation or to a regular ultra-

sound assessment at 18-weeks. Children have been comprehensively phenotyped from birth to 18 years of age by trained members of the Raine research team. Data up to 14 years only was available at the time of our study and these data were used in our analyses. DNA was collected at 14 years in addition to data on dietary intake and duration of exercise undertaken outside of school hours. Month and year of first menstrual period for girls was recorded. Weight was measured using a Wedderburn Digital Chair Scale to the nearest 100g with each child dressed in running shorts and a singlet top, height was measured to the nearest 0.1 cm with a Holtain Stadiometer and BMI was calculated as weight(kg) /height$(m)^2$. A description of the main characteristics of study population is given in table 1. The cohort was representative of the population presenting at an antenatal tertiary referral centre in Western Australia (25). The study was conducted with institutional ethics approval and a signed informed consent letter was obtained from all mothers.

Definition of breastfeeding-related variables

Information pertaining to early infant feeding was collected at 1, 2 and 3 years. Mothers recorded the age breastfeeding was stopped (in months) and the age at which milk other than breast milk was introduced (in months). This information was determined from the mother's diary of early feeding milestones, as well as from an interview with the study nurse at the early follow-ups and survey questions at later follow-ups. The duration of exclusive breastfeeding (EXBF) was defined for the subset of mothers who breastfed

their baby as the time from birth until they started feeding their baby with other milk (non-breast) or any solid. The duration of non-exclusive breastfeeding (BF) was derived as the time from birth until breastfeeding was stopped, where breastfeeding could be mixed with any other milk or solid. We also defined two instrumental variables (IVs). An IV is defined as a random variable that is correlated with the true variable but unobserved or is not an accurate variable, and uncorrelated with the model error term(s). In epidemiology, it is an observable variable that is predictive of an outcome, but has no direct association with the disease and is independent of the unobserved confounders (26). The first instrumental variable $IV1$ was defined as the time from birth to the mother stopped breastfeeding or introducing other milk, whichever came first. We also defined $IV2$ as the time from birth to the mother stopped breastfeeding or introducing solids, whichever came first. Both $IV1$ and $IV2$ were highly correlated with EXBF, $r = 0.79$ and $r = 0.89$, respectively.

Definition of other variables

Several known confounding variables were added to our longitudinal models including mother's eduction (college/university=1 vs. high-school/professional degree=0), gestational age (in months), and mother's pre-pregnancy BMI.

Statistical methods

For a typical child, the BMI curve increases from birth until it reaches a peak around 9 months (adiposity peak=AP) so that 1-year old children seem

chubby. Then the curve decreases until it reaches a minimum around 5.5/6 years (i.e the adiposity rebound =AR). After this, BMI typically increases again until adulthood. The proportion of fatness decreases after 1 year of age and varies across children so that the AR can occur between 4 and 8 years, with an earlier rebound often associated with greater adiposity in early adulthood (20). To our knowledge, there isn't a well-established parametric model that describes BMI growth throughout infancy and childhood. After careful investigation of the Raine data (Figures 1 & 2), it was decided to choose a break-point mixed effects model (27) for the following reasons:

1. BMI shows stages of "growth" and "decline" over this time period and a point separating these stages ("break-point");

2. BMI differs between individuals at the origin (intercept), so we need random intercept to explain this behavior;

3. Children exhibit variable rates of change in BMI with age, so we use a random slope to capture this feature.

This break-point model emphasizes that there are two distinct time windows: infancy (between birth and 1.5 years) and childhood until puberty (between 1.5 and 14 years) but does not require the data to be split. The break point was chosen such that it occurs between the times at adiposity peak and adiposity rebound and allows a more precise estimate of these two points (smaller standard deviation). To ensure our results were not dependent on the placement of the break point, we tested different break point

models with the point between 1 and 2 years; our results remained consistent. A random effect was not added to the model for the break point itself since it is quite challenging to estimate. A co-dominant genetic model was used to allow two genotype-associated parameters (TA vs. TT and AA vs. TT) to be estimated for the SNP of interest. Since the BMI growth pattern and the timing of pubertal growth differ between boys and girls, we fit a separate model for each gender. We also used models with no breastfeeding-related variables and other models adjusting for EXBF or BF.

Based on Scott et al.'s previous work (27) on break-point linear mixed effects for modeling changes in lung function in Duchenne's muscular dystrophy over time, we decided to use the same modelling framework for our longitudinal BMI. If we fix the break-point at age 1.5 years for all subjects, the model can be written as

$$Y = \begin{cases} \beta_0^{infant} + \beta_1^{infant} Age + \beta_2^{infant} Age^2 + X_r\beta_r + X_t\beta_t Age + \varepsilon & \text{if age} < 1.5 \\ \beta_0^{child} + \beta_1^{child} Age + \beta_2^{child} Age^2 + X_r\beta_r + X_t\beta_t Age + \varepsilon & \text{if age} \geq 1.5 \end{cases} \quad (1)$$

where Y is BMI at a given age, X_r represents time-independent covariates including the two *FTO* SNP genotypes, EXBF (BF), mother's education (MomEdu), gestational age (GA), mother's pre-pregnancy BMI (MomBMI), the interaction between the SNP and EXBF (or BF) and X_t represents time-dependent covariates including the interaction between age and BF (or

EXBF), SNP genotypes and mother's education. The coefficients β_1^{infant} and β_1^{child} represent the mean rate of change of BMI during infancy and childhood. The coefficients β_2^{infant} and β_2^{child} represent the mean acceleration/deceleration of BMI during infancy and childhood.

Physiologically, BMI is continuous across all ages. Thus models with enforced continuity at the breakpoint may fit the physiological process better. We modified model (1) to enforce continuity between the two time windows (27):

$$\begin{aligned} Y = \beta_0 + \beta_1(Age - 1.5) + I_{[Age<1.5]}\beta_2^{infant}(Age - 1.5)^2 + \\ I_{[Age\geq 1.5]}\beta_2^{child}(Age - 1.5)^2 + X_r\beta_r + X_t\beta_t Age + \varepsilon, \end{aligned} \tag{2}$$

where I is an indicator function. We have written model (2) in short form as

$$Y = X\beta + \varepsilon. \tag{3}$$

In this model, the vector of coefficients β_r measures the variation in BMI associated with a change in one unit of the time-independent covariates X_r at baseline (1.5 years), and the vector of coefficients β_t measures the variation in BMI associated with a change in one unit of the time-dependent covariates X_t, where time corresponds to a particular child's age.

Given BMI growth trajectories are not identical across individuals, we

can represent the model (3) in linear mixed effects model framework:

$$y_i = X_i\beta + Z_i b_i + \varepsilon_i, \qquad i = 1, ..., m, \tag{4}$$

where y_i is the response vector for the ith individual, β is the vector of fixed effects and $b_i \sim N(0, \Sigma)$ is the vector of random effects, X_i and Z_i are the fixed effect and random effect regressor matrices respectively and $\varepsilon_i \sim N(0, \sigma^2)$ is the within subject error vector. The linear mixed effects model was applied to our data with random intercept and random slope for age. The time dependency was accounted for by a continuous auto-regressive structure, which best fits our data.

The relation between EXBF and BMI growth curves at particular ages was estimated and tested using the General Linear Hypothesis approach (28) (See appendix). The times to AR and AP were obtained by maximizing the predicted BMI curves in the time intervals around the AR and AP using the Newton-Raphson maximization method. All the models were fitted with the function *lme* included in the *R* library *nlme* version 3.1-102.

RESULTS

Table 1 presents the characteristics of the 1,096 children in our analyses. Their distribution according to the SNP genotypes showed that 560 (44.9 %) were TT, 523 (42.0 %) AT and 163 (13.1 %) AA. The allele A is considered as the risk allele associated with increased BMI (1). The mean duration of EXBF was 3.1 months (SD=1.9 months) and did not vary with a child's

genotype. After removing children with missing covariate information at all time points, we had 959 children left for our analyses, 498 boys and 461 girls.

Cross-sectional analysis at age 14

Figure 3 represents the Box plot distribution of the sample-based estimate of BMI obtained from model 1 at age 14 with respect to the SNP genotypes and various durations of EXBF in boys and girls. The dashed horizontal line delineates the BMI categories "normal weight" and "overweight" as defined by the Centers for Disease Control and Prevention (CDC). This definition is based on expert committees' recommendations to classify BMI-for-age between the 85th and 95th percentile as at risk of overweight (29). It is clear from the left panel that EXBF impacts all genotype categories in boys with a substantial decrease in the median BMI as the duration of EXBF increases. For example, with less than 2 months of EXBF, the median BMI among boys who carry either the AT or AA genotype is in the overweight category. With 2 to 4 months of EXBF, less than 20% of boys are in the overweight category and no boys are overweight if they had 5 months or more EXBF. In girls (right panel), the parameter estimate for the duration of EXBF is smaller but we can still notice a decrease in the median BMI among the AA genotype carriers as the duration of EXBF increases. This decrease does not seem to be important as the confidence intervals overlap.

Longitudinal analysis of the SNP (rs9939609) genotypes associ-

ated with BMI growth curves

The estimated BMI growth curves obtained from model 1 from birth to 14 years by SNP genotype and gender are displayed in Figure 1. At baseline (i.e. 1.5 years), the parameter estimate for the AA genotype was associated with a higher BMI both in boys and girls but not the AT genotype (Table 2). Boys having the AA genotype have a higher BMI of 0.020 kg/m^2 (95%CI=[0.012-0.028], $P < 0.00001$) while girls have a higher BMI of 0.028 kg/m^2 (95%CI=[0.020-0.036], $P < 0.00001$). After 1.5 years, there is an interaction between the SNP genotypes (AT and AA) and age in boys only. Boys have an average linear increase of their BMI of 0.127 kg/m^2 per year (95%CI=[0.006-0.191], $P = 0.00001$) if they carry the AT genotype and 0.138 kg/m^2 per year (95%CI=[0.048-0.228], $P = 0.0022$) if they carry the AA genotype. There was no interaction with age for the girls.

Impact of the duration of breastfeeding on *FTO*-induced BMI growth curves

Table 3 gives the estimates of the fixed effects parameters from the linear mixed effects model (model 1), their standard errors and associated P values when EXBF was included in the model. There is a complex pattern of association between BMI growth curves, *FTO* SNP genotypes and EXBF, which differs between girls and boys (Figure 4). In girls only, an interaction between EXBF and the SNP genotypes was detected, resulting in a substantial

decrease in BMI of 0.119 kg/m^2 (95%CI=[0.001-0.237], $P = 0.043$) for each month of breastfeeding in the AT carriers and 0.180 kg/m^2 (95%CI=[0.002-0.358], $P = 0.045$) in the AA carriers. The association of the SNP genotypes and EXBF on BMI depends also largely on age. We used the general linear hypothesis tests (26) to compare the effect of no breastfeeding vs. 6 months of EXBF on BMI at different ages by gender and genotype (Table 4). This table shows clearly that in boys, EXBF impacts all SNP genotype categories after 10 years of life and leads to a decrease in BMI varying from 1 to 1.76 kg/m^2. In contrast, in girls, 6 months of EXBF reduces BMI only in the AA genotype category. The genotype-specific BMI growth curves estimated from the model fixed effects are depicted in Figure 4 for 0, 3 and 6 months of EXBF. In girls, the association with the *FTO* variant is reversed after 3 months of EXBF. In boys, 6 months of EXBF will put the BMI growth curves in the normal range. Carriers of the AT and AA genotypes breastfed exclusively for 6 months have a similar BMI growth as carriers of the TT genotype who have never been breastfed.

Assessing breastfeeding-related variables

To investigate whether our results are sensitive to the definition of breastfeeding, we refit the models using BF and the two instrumental variables (IV1 and IV2) instead of EXBF. No interaction was detected, in boys or girls, when looking at the duration of breastfeeding regardless of other milk/solids (BF). Neither IV had an interaction with *FTO* gene variant. If we consider

that EXBF is the true variable and BF, $IV1$ and $IV2$ are variables measuring breastfeeding but with measurement error, then our analysis emphasises the need for accurate information on the variables of interest for detecting important associations and gene environment interactions in longitudinal studies.

Assessing distribution assumptions

The distribution of the model residuals shows a slight departure from the normal distribution. Therefore, we also considered alternative models allowing the distribution of the random effects in model 1 to be either skew-normal or skew-t (30). Based on our diagnostic plots, the choice of the normal mixed effects model was justified. We have also investigated log and sqrt transformations of the response variable but the qq plots of the model residuals show that the best model fit was obtained when using the raw BMI values.

DISCUSSION

The aim of our study was to investigate the impact of EXBF on *FTO*-induced BMI growth trajectories from birth to age 14. The rationale being that while the *FTO* gene variant rs9939609 is associated with an increased BMI in children and adults, a longer period of exclusive breastfeeding could reverse the genetic association.

First, our study confirms the role of the *FTO* gene variant rs9939609 on BMI growth curves both at baseline and over time but also suggests an im-

portant gender- and age-specific relationship. At baseline (i.e. 1.5 years), the AA genotype was associated with a higher BMI both in boys and girls than the TT genotype but no association was detected with the AT genotype. After 1.5 years of age, a linear increase in BMI was only detected in boys among the AT and AA genotype carriers. A recent meta-analysis (1) also suggested an association with *FTO* gene variant on BMI growth trajectories in children but concluded that rs9939609 was associated with a decrease in BMI at the adiposity peak and an increase in BMI at the adiposity rebound. These two studies stress the complex time dependence of the *FTO* gene variant on BMI growth, however, Sovio et al (1) did not test the possibility of gender-specific associations.

The beneficial role of breastfeeding on many diseases (obesity, type 2 diabetes, cholesterol and insulin resistance) has been suggested in many publications (31-34) and there is a general consensus that breastfeeding should be recommended to all women (33). The protective association found with breast milk could come from its low protein and high fat content, which could lead to slower child growth and better nutritional balance between protein and fat during infancy (35). A study has also shown that infants who are bottle-fed in early infancy are more likely to empty the bottle or cup in late infancy than those who are breastfed. A possible reason for this is that parents may encourage an infant to finish the contents of the bottle whereas when breastfed, an infant naturally develops self-regulation of milk intake (36). Breastfeeding could therefore protect against later obesity by reducing

the occurrence of high weight gain in infancy (37). This is also consistent with previous findings from the Raine study where an increased duration of breastfeeding was found associated with reduced BMI in adolescence (38) and increased weight gain within the first year of life was associated with increased BMI in adolescence. Data from rat models (39) also suggest that lactation can mitigate some of the adverse effects of placental insufficiency on the later development of metabolic disease. Another possible role of breastfeeding is that breastfed and formula-fed infants have different hormonal responses to feeding, with formula feeding leading to a greater insulin response resulting in fat deposition and increased number of adipocytes (40). Finally, limited evidence suggests that breastfed infants adapt more readily to new foods such as vegetables, thus reducing the caloric density of their subsequent diets (41).

The additive genetic model has been widely used in genetic studies of BMI related to FTO gene variants. Most of these studies were cross-sectional in nature and considered adult BMI (42). In longitudinal studies of children BMI, the genetic model for FTO gene variants has been less studied. The meta-analysis from Sovio et al. (1) showed that although an additive model for the SNP rs99339609 could fit longitudinal BMI profiles in children well in different time periods, a more general genetic model was a more appropriate fit for alternative adiposity phenotypes such as age at adiposity peak and rebound. Besides, there was also a lot of variability across studies in terms of the best fitting genetic model. In our study, the estimation of FTO-related

BMI longitudinal profiles showed that boys with AA or AT genotypes have very similar longitudinal BMI profiles but differ greatly from boys with the TT genotype. On the other hand, girls who have the AT genotype have very different longitudinal BMI profiles than girls with a TT or AA genotype. These particular patterns would not have been detected under an additive model. Our study of the SNP by EXBF interaction and estimation of ages at AR and AP also show complex associations with the SNP genotypes, that could not be captured by an additive model. We therefore opted for the most general genetic model for the FTO SNP, i.e. the co-dominant model.

It has been previously noted that sex and age are associated with differences in obesity-related traits and body composition. For instance, women tend to store more fat subcutaneously rather than in visceral adipose tissue, so at the same BMI, women will tend to carry more body fat than men (43). This finding is of particular interest as it supports previous hypotheses that there are sex-specific genes contributing to variation of obesity-related traits and that genes account for more of the variability of fat distribution in women than in men (44, 45). Our study confirms the role of age- and gender-specific genetic effects and also adds to this observation that gene by environment interactions could also be gender-specific. The biological mechanisms underlying this observation require further investigation.

Despite the importance of breastfeeding in child growth development and prevention of metabolic diseases, its impact on carriers of *FTO* gene risk alleles has barely been studied. To our knowledge, it has only been investi-

gated by Dedoussis et al. (21), however their study was cross-sectional rather than longitudinal and there was no distinction between EXBF and BF. Their study concluded that a short period of at least one month of breastfeeding was associated with reduced obesity indices in a cohort of Greek children but this result was not replicated in the ALSPAC child cohort from the UK, even when a longer period of breastfeeding was considered (21). The major implication of our results is to support the possible role of exclusive breastfeeding in preventing the increase of BMI due to *FTO*. To better understand the role of *FTO* gene variant and EXBF on BMI growth, we also studied their association with the timing of AR. Indeed, it is hypothesized that an earlier age at AR is strongly associated with the risk of obesity later in life (1). We found that the *FTO* gene variant was associated with an earlier AR but only in boys. The mean age at AR was $5.16, 4.11, 4.17$ years in boys for the TT, AT, AA genotypes and was almost constant (around 4.60/4.70 years) in girls. EXBF had no impact on girls' AR however it delays the timing of AR in boys by about 4 months and 8 months after 3 and 6 months of EXBF respectively regardless of their genotype. The association between EXBF and BMI growth found in boys carriers of the SNP risk allele could therefore be partly explained by a change in the timing of AR. In girls, this association seems to be prior to AR occurrence and had little impact on growth after AR. The biological mechanisms explaining the role of breastfeeding on *FTO*-induced BMI growth curves are not known. A possible explanation is that breastfeeding could prevent *FTO*-induced fast weight gain during in-

fancy by providing better energy balance at critical periods of development during infancy, but this remains speculative. Further studies are required to determine the complex association of FTO and EXBF on BMI growth trajectories throughout childhood.

KEY MESSAGES

- Recent studies have implicated the fat mass and obesity gene (FTO) in the increase of BMI in young adults and children.
- The addition of each minor (A) risk allele at the SNP rs9939609 within the first intron of *FTO* has been shown to be consistently associated with a higher BMI of up to 0.33 kg/m^2 or approximately 0.1 standard deviation.
- We studied 1,096 children from the RAINE cohort that were followed-up from birth to 14-years and analyzed their longitudinal BMI growth trajectories.
- We showed that the duration of exclusive breastfeeding (EXBF) could attenuate the increase in BMI among carriers of the risk allele of the FTO SNP rs9939609 .
- In girls, EXBF can reverse the increase in BMI due to SNP risk allele by age 14 after three months of EXBF.
- In boys, EXBF reduces both BMI in carriers and non-carriers of the risk allele with an association found after 10 years of life. Six months of

EXBF will put the boys' BMI growth curves back to the normal range.

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LIST OF FIGURES

Figure 1. Population-based estimate of BMI versus age by SNP genotypes in children from birth to 14 years in the RAINE study

Figure 2. Body mass index versus age (for a sample of 12 individuals) in children from birth to 14 years in the RAINE study

Figure 3. Percentage of individuals in different categories of BMI and genotypes at age 14 years in the RAINE study

Figure 4. Fitted BMI versus Age for different EXBF categories in children from birth to 14 years in the RAINE study

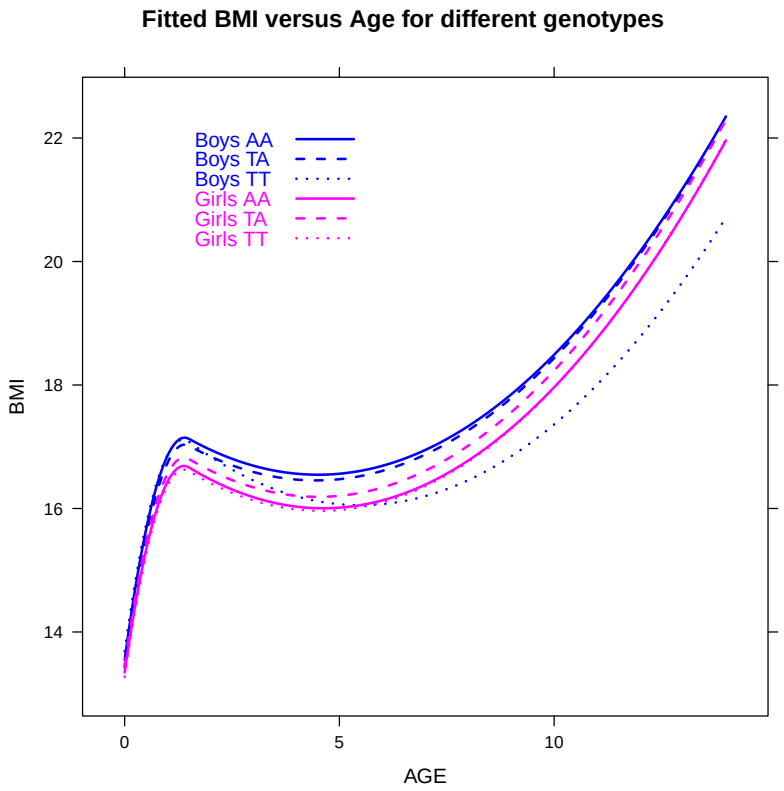
Fitted BMI versus Age for different genotypes
Boys AA
Boys TA
Boys TT
Girls AA
Girls TA
Girls TT
BMI
22
20
18
16
14
0
5
10
AGE

Figure 1:

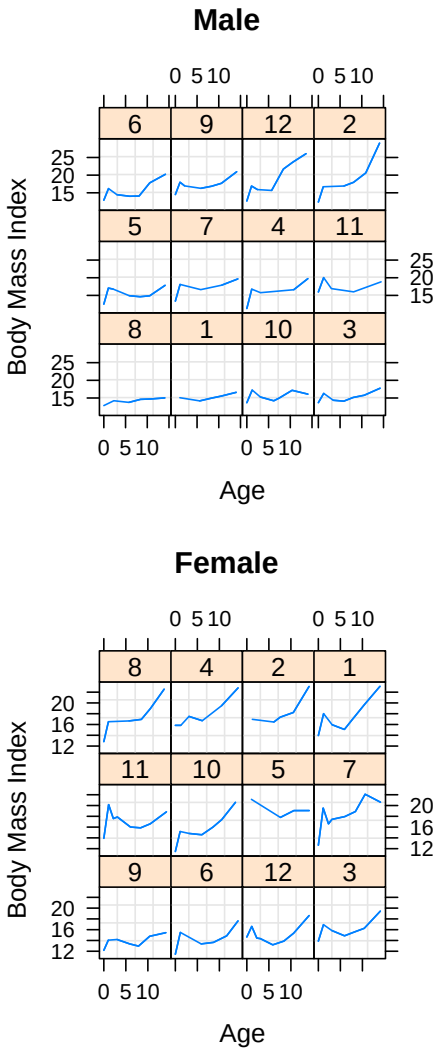
Male
0 5 10
0 5 10
6
9
12
2
5
7
4
11
8
1
10
3
25
20
15
Body Mass Index
Age
Female
8
4
2
1
11
10
5
7
9
6
12
3
20
16
12

Figure 2:

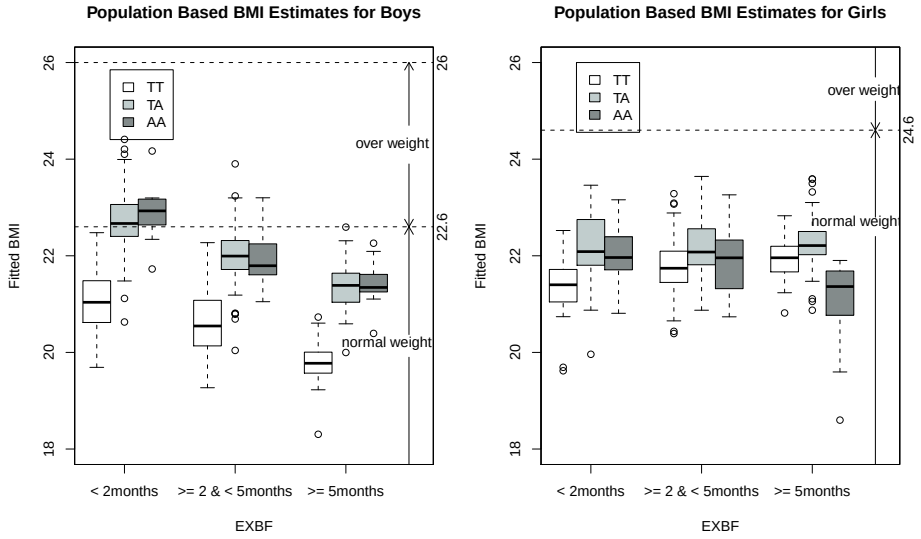
Population Based BMI Estimates for Boys
Population Based BMI Estimates for Girls
Fitted BMI
EXBF
TT
TA
AA
over weight
normal weight
22.6
24.6
< 2months
>= 2 & < 5months
>= 5months

Figure 3:

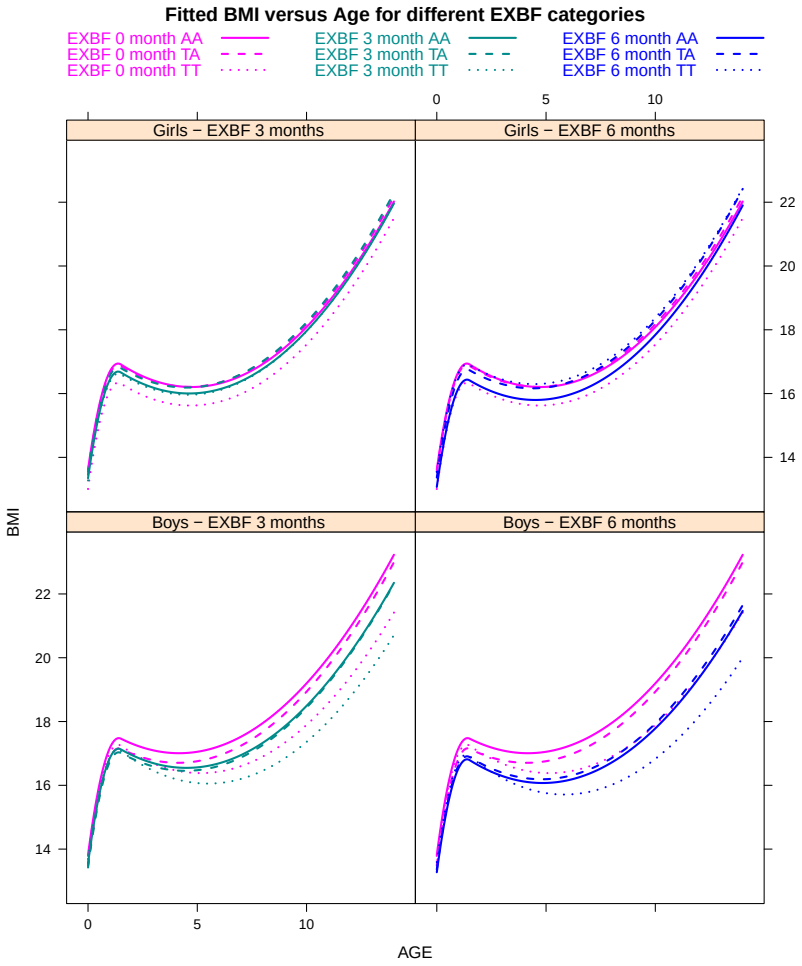
Fitted BMI versus Age for different EXBF categories
EXBF 0 month AA
EXBF 0 month TA
EXBF 0 month TT
EXBF 3 month AA
EXBF 3 month TA
EXBF 3 month TT
EXBF 6 month AA
EXBF 6 month TA
EXBF 6 month TT
Girls − EXBF 3 months
Girls − EXBF 6 months
Boys − EXBF 3 months
Boys − EXBF 6 months
BMI
AGE

Figure 4:

Table 1: Cohort Characteristics, Perth, Western Australia, 1989-2005

	Boys (n=417)		**Girls (n=389)**	
	mean	**sd**	**mean**	**sd**
EXBF (months)[†]	3.07	1.87	3.09	1.98
BF (months)[‡]	7.69	6.57	7.67	6.58
Gestational age (weeks)	39.31	2.06	39.30	2.09
Mother's BMI[#]	26.17	5.56	26.47	6.15
Mother's education	n		n	
high-school/professional degree=0	92		88	
college/university=1	325		301	
SNP rs9939609 genotypes				
TT	149		153	
TA	208		185	
AA	60		51	
Number of BMI measurements by AGE				
0	374		349	
1	404		377	
2	124		133	
3	288		279	
6	392		368	
7	13		10	
8	351		316	
9	58		63	
10	121		112	
11	270		246	
14	411		377	
Average number of measurements per child				
	6.76		6.8	

[†] EXBF= Duration of exclusive breastfeeding

[‡] BF= Duration of breastfeeding (non exclusive)

[#] Mother's pre-pregnancy BMI

Table 2: Estimates of SNP (rs9939609) and SNP by age interaction fixed effects, standard errors, and P values for a model with no breastfeeding variables, Perth, Western Australia, 1989-2005

	Boys			**Girls**		
	Estimate[†]	**SE**	P **value**	**Estimate**[†]	**SE**	P **value**
SNP genotype TA[‡]	0.14	0.13	0.29	0.042	0.12	0.73
SNP genotype AA	0.020	0.004	<0.0001	0.028	0.004	<0.0001
(AGE - 1.5):TA	0.127	0.032	0.0001	-0.009	0.033	0.78
(AGE - 1.5):AA	0.138	0.045	0.0022	-0.026	0.049	0.61

[†] Estimates were adjusted for age, quadratic age before and after the breakpoint, mother's education, gestational age, mother's BMI and the interaction age by mother's education. Age was centered at 1.5 years.

[†] The baseline genotype category is TT, i.e the low risk genotype

Table 3: Estimates of fixed effects parameters for the SNP (rs9939609) and EXBF main effects and the SNP by age and SNP by EXBF interactions, standard errors, and P values, Perth, Western Australia, 1989-2005

	Boys			**Girls**		
	Estimate	**SE**	P **value**	**Estimate**	**SE**	P **value**
EXBF	-0.054	0.050	0.28	0.097	0.045	0.033
SNP genotype TA[‡]	-0.098	0.22	0.66	0.56	0.22	0.011
SNP genotype AA	0.22	0.36	0.54	0.60	0.31	0.053
AGE:EXBF	-0.014	0.008	0.084	0.005	0.008	0.55
AGE :TA	0.13	0.034	<0.0001	0.010	0.035	0.78
AGE:AA	0.13	0.049	0.0088	-0.006	0.052	0.90
EXBF:TA	0.011	0.063	0.86	-0.119	0.059	0.043
EXBF:AA	-0.058	0.100	0.56	-0.180	0.089	0.045

[†] Estimates were adjusted for age, quadratic age before and after the breakpoint, mother's education, gestational age, mother's BMI and the interaction age by mother's education. Age was centered at 1.5 years.

[†] The baseline genotype category is TT, i.e the low risk genotype

Table 4: Linear estimates for 6 months vs. 0 month of EXBF at age 1.5, 5, 10, 14 by gender and SNP genotype, Perth, Western Australia, 1989-2005

		Boys			**Girls**		
FTO	**AGE**	**Estimate**	**SE**	P **value**	**Estimate**	**SE**	P **value**
TT	1.5	-0.32	0.30	0.28	0.56	0.27	**0.033**
	5	-0.63	0.35	0.073	0.69	0.33	**0.038**
	10	-1.06	0.52	**0.043**	0.84	0.52	0.11
	14	-1.41	0.70	**0.044**	0.96	0.70	0.17
TA	1.5	-0.26	0.23	0.26	-0.14	0.23	0.56
	5	-0.56	0.29	0.053	-0.030	0.30	0.92
	10	-1.00	0.49	**0.041**	0.12	0.50	0.81
	14	-1.35	0.67	**0.045**	0.24	0.68	0.72
AA	1.5	-0.67	0.52	0.20	-0.50	0.47	0.29
	5	-0.98	0.55	0.076	-0.39	0.50	0.43
	10	-1.41	0.67	**0.037**	-0.24	0.64	0.70
	14	-1.76	0.82	**0.032**	-0.12	0.79	0.88

APPENDIX

The relation between EXBF and BMI growth curves at particular ages was estimated and tested using the General Linear Hypothesis approach (26). The hypothesis can be specified through a constant matrix L to be matched with the fixed effects of the model such that $H_0 : L\beta = m$ where the m are the hypothesized values. The estimates of fixed effects β, follows asymptotically a multivariate normal distribution $\hat{\beta} \sim N\big(\beta, cov(\hat{\beta})\big)$ by Central Limit Theorem. Thus, the linear form $L\hat{\beta}$ also follows asymptotically a multivariate normal distribution

$$L\hat{\beta} \sim N\big(L\beta, Lcov(\hat{\beta})L'\big)$$

The P value and the 95% confidence interval for the hypothesized value can be obtained accordingly.

For example, in Table 4, we have estimated the linear effect for the comparison: 6 months of EXBF vs. 0 month on BMI by genotype and age. The matrix L corresponding to 6 months of EXBF for the genotype TA at age 5 is obtained by $L\hat{\beta} = L_2'\hat{\beta} - L_1'\hat{\beta}$ where $L_1'\hat{\beta}$ and $L_2'\hat{\beta}$ are the estimated BMI's at age 5 for TA with 0 and 6 months of EXBF respectively assuming MomEdu = 0, GA = 40 and MomBMI = 22 (See Table 5 in Appendix). And Ł$\hat{\beta}$ in this example is a vector following an approximate normal distribution $L\hat{\beta} \sim N\big(L\beta, Lcov(\hat{\beta})L'\big)$.

Table 5: An example of constructing the L matrix, Perth, Western Australia, 1989-2005

	$\hat{\beta}$	L_1'	L_2'	L'
Intercept	17.18	1	1	0
$(AGE - 1.5)$	-0.43	(5-1.5)	(5-1.5)	0
$(AGE - 1.5)^2 (< 1.5)$	-1.85	0	0	0
$(AGE - 1.5)^2 (\geq 1.5)$	0.064	$(5 - 1.5)^2$	$(5 - 1.5)^2$	0
EXBF	-0.054	0	6	6
TA	-0.098	1	1	0
AA	0.22	0	0	0
MomEdu	0.12	0	0	0
GA	0.019	40	40	0
MomBMI	0.036	22	22	0
(AGE - 1.5):EXBF	-0.014	0	$(5 - 1.5) * 6$	$(5 - 1.5) * 6$
(AGE - 1.5):TA	0.13	$(5 - 1.5) * 1$	$(5 - 1.5) * 1$	0
(AGE - 1.5):AA	0.13	0	0	0
(AGE - 1.5):MomEdu	-0.083	0	0	0
EXBF:TA	0.011	0	$6 * 1$	6
EXBF:AA	-0.058	0	0	0