Claire Poppelaars - Monnereau

GENETICS — AND — EPIGENETICS — OF — CHILDHOOD ADIPOSITY

The Generation R Study

GENETICS
— AND —
EPIGENETICS
— OF —
CHILDHOOD ADIPOSITY

Claire Poppelaars - Monnereau

Colofon

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Genetics and Epigenetics of Childhood Adiposity
The Generation R Study

Genetica en epigenetica van adipositas bij kinderen
Het Generation R Onderzoek

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Chapter 3.1

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Chapter 4.2

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Chapter 1

General Introduction

General introduction

Childhood overweight and obesity

Overweight and obesity represent excessive or abnormal accumulation of fat in the body.[1] Worldwide, over 1.9 billion adults (39%) are overweight and 600 million adults (13%) are considered obese.[1] In the Netherlands, the prevalences of overweight and obesity in adults are 32% and 12%, respectively.[2] Since the 1980s the prevalence of obesity in the Netherlands has more than doubled, reflecting the increase in obesity worldwide.[1,3] A variety of environmental and lifestyle related factors may contribute to the development of obesity, such as a poor diet, physical inactivity, alcohol intake, smoking, and stress.[1,4] In children comparable risk factors apply, including poor diet and physical inactivity, but also maternal obesity during pregnancy.[5] These factors all contribute to a disturbed energy balance in which energy intake is larger than energy expenditure. Body mass index (BMI) is the most commonly used measure of overweight and obesity. It reflects a person's weight corrected for height and is calculated by dividing weight by height squared (kg/m^2). In adults values of 18.5-24.9 are considered normal, whereas values of ≥25 and ≥30 represent overweight and obesity, respectively.[1,6] In children the definition of overweight and obesity is more complex. Whereas in adults, weight is made independent of height by dividing it by height-squared in the formula for BMI, in children, exponentiation of height to a different power than two may more adequately remove the effect of height. This power may differ across ages. This indicates that BMI calculated as kg/m^2 may not be the best representation of weight corrected for height in early life and illustrates the complexity of BMI as a phenotype in childhood.[7] In addition, what is considered a normal BMI in early life varies with age, which is why age-related reference curves are generally used.[8] During the first year of life BMI rapidly increases, showing a peak, the adiposity peak, between 6 and 12 months of age (Figure 1).[8,9] Thereafter, BMI gradually decreases until a dip, the adiposity rebound, is reached at approximately 5.5 to 6 years of age (Figure 1).[8,10] In general, children that have a higher BMI in early life tend to be at a higher risk of becoming an obese adult.[11,12] More specifically, a high and/or late adiposity peak as well as an early adiposity rebound have been shown to be associated with a higher BMI later in life.[9,13-15]

Childhood body fat distribution

The use of a general measure like BMI implies that adipose tissue is evenly distributed throughout the body which in general is not the case. In addition to BMI as an overall measure of adiposity, the distribution and accumulation of fat in specific areas in the body is also important.[17] Health consequences of fat accumulation may differ depending on

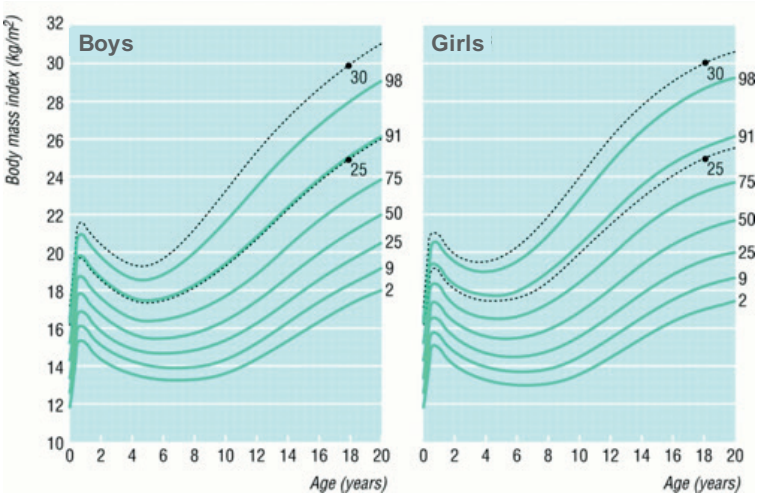

Figure 1. Centile curves showing the development of BMI with age for boys (left panel) and girls (right panel). The numbers at the right end of each green curve refer to the percentile of BMI that curve represents. The dashed black curves marked with values 25 and 30 represent extrapolated curves for BMI values of 25 kg/m^2 (cutoff for overweight) and 30 kg/m^2 (cutoff for obesity) at age 18 years. The peak in BMI around the age of 1 year is the adiposity peak, whereas the dip in BMI following the adiposity peak represents the adiposity rebound. Reproduced from Cole TJ, *et al*.[16], copyright notice 2018, with permission from BMJ Publishing Group Ltd.

the location in the body. Specific locations of fat storage include abdominal fat, which can be subdivided into subcutaneous (under de skin (SAT)) and visceral (around organs (VAT)) adipose tissue, and fat accumulated in the liver and around the heart (pericardial). Abdominal adipose tissue, especially VAT, is associated with an increased risk of type 2 diabetes, liver fat was shown to be associated with dyslipidemia and dysglycemia, and fat around the heart was shown to be associated with coronary artery disease.[18-20] Children with high levels of BMI and adipose tissue may thus already be at risk of various metabolic health complications in later life.[1,11,21] In addition, body fat distribution differs between boys and girls. This becomes even more apparent in puberty under the influence of sex hormones.[22] Prepuberty, girls generally already have less fat stored at the waist area, but more at the hip area than boys.[23] Postpuberty, females have a larger amount of SAT, whereas males have more VAT.[22]

Genetics of body fat measures

Next to environmental factors genetic susceptibility is also known to play a role. Heritability estimates of up to 80% were reported for BMI in twin studies.[24,25] Genetic studies in animals have identified several loci within gene coding regions, for example in the melanocortin 4

receptor (*MC4R*) and pro-opiomelanocortin (*POMC*) genes, which nowadays are well-known for their role in human obesity.[26] Unfortunately, extrapolation of the results from animal studies to humans is not always straightforward. Early human genetic studies included family studies and candidate gene studies, which highly rely on previous knowledge of the underlying pathways of the trait of interest and may be less suitable for diseases in which not a single genetic variant, but a combination of multiple genetic variants and environmental factors together contribute to the development of a disease, a so-called complex disease. A more recent approach to investigate the genetic background of complex diseases are genome-wide association studies (GWAS) in which to date over 40 million genetic variants (single nucleotide polymorphisms (SNPs)) in the genome can be tested for association with a phenotype of interest in one analysis. Large sample sizes are necessary for this type of association studies to obtain reproducible and reliable findings.[27] To unravel the genetic factors underlying BMI and many other complex phenotypes large consortia have been formed. Within these consortia GWAS are performed in order to find common variants associated with BMI, but also with more specific adiposity measures such as waist-hip ratio (WHR), SAT and VAT, liver and pericardial fat.[28-32] The largest number of genetic variants has been identified for BMI. To date, 97 loci were found to be genome-wide significantly associated (p-value $<5*10^{-8}$) with adult BMI, accounting for only 2.7% of the phenotypic variation.[33] In total, up to 21% of the variation in BMI is estimated to be explained by common genetic variants. This indicates that additional research is still needed in even larger sample sizes to examine potential effects of rare variants as well as gene-environment and gene-gene interactions. Although a substantial amount of knowledge has been gained on adult BMI so far, the genetics and underlying pathways of BMI during childhood are less extensively studied. Further examination of the genetic background of childhood BMI will be extremely valuable in terms of expending our knowledge on overlap and differences in genetic variants and pathways underlying BMI in early life as compared to adulthood.

Epigenetics of body fat measures

Next to genetic variants, epigenetic processes may influence gene expression without changing the actual deoxyribonucleic acid (DNA) sequence. Epigenetic processes include DNA methylation, histone modifications, and the silencing of genes by noncoding ribonucleic acids (RNAs).[34,35] DNA methylation is the most extensively studied epigenetic process, comprising the addition or removal of a methyl group to specific positions in the DNA, mainly those where a cytosine is adjacent to a guanine, linked by a phosphate bond (cytosine phosphate guanine (CpG) sites) (Figure 2).[36] This process may be influenced by both genetic and environmental factors and changes in DNA methylation may subsequently contribute to health and disease phenotypes by influencing gene expression.[37,38] In

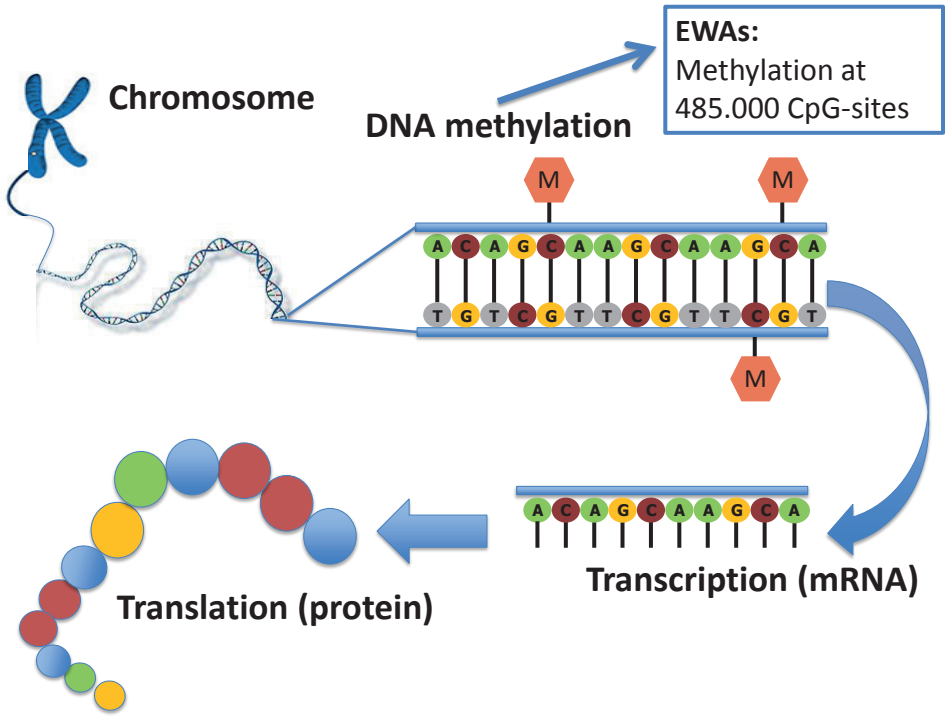

Figure 2. Schematic presentation of the process of DNA methylation. Adapted from Felix JF, *et al.*[51] CpG: cytosine-phosphate-guanine; EWAS: epigenome-wide association study; M: methyl group; mRNA: messenger ribonucleic acid; adenine (A); cytosine (C); guanine (G); thymine (T).

contrast to the static DNA sequence a person's epigenetic profile may change over time through methylation and demethylation.[39] Early life, and especially the *in utero* period, is a particularly sensitive period for DNA methylation changes.[36] Similar to GWAS, one of the most commonly used methods to examine DNA methylation in population studies is using a genome-wide approach (epigenome-wide association study (EWAS)), in which hundreds of thousands of CpG sites are tested for association with a phenotype of interest in one analysis. Interestingly, and in contrast to GWAS, due to its dynamic nature, methylation may be both an exposure and an outcome of phenotypes such as adiposity. It has been shown previously that maternal overweight or obesity is associated with offspring DNA methylation at 28 CpG sites.[40,41] At all 28 CpG's, there was evidence supporting a direct causal *in utero* association.[40,41] Maternal underweight is also suggested to be associated with offspring DNA methylation.[41] DNA methylation may also be associated with a variety of offspring health outcomes. Birthweight is the result of fetal growth and has been used as a proxy for intrauterine exposures. Both high and low birthweight have been

associated with a predisposition for overweight in later life.[42,43] To date, no large EWAS has been performed on DNA methylation and either birthweight or childhood adiposity. Smaller epigenetic studies have identified several CpG sites associated with birthweight or childhood adiposity.[44-50] Nevertheless, these studies either had limited power, were performed in a single cohort without replication, or only examined previously defined candidate regions of the genome for associated CpG sites. More extensive knowledge on the background of how the epigenome and phenotype are linked could be very valuable for future risk prediction, prevention and treatment of overweight and obesity.

Objectives

The general aims of this thesis are:

1) To assess associations of maternal adiposity with offspring adiposity in childhood.
2) To identify and examine the role of genetic variants in childhood adiposity.
3) To identify DNA methylation variants associated with maternal adiposity and birthweight.

General design

The studies described in this thesis were embedded in the Generation R Study, a population-based prospective cohort study, and in international consortia.

Generation R Study

The Generation R Study is a population-based, prospective cohort study from fetal life onwards in Rotterdam, the Netherlands.[52] The aim of the Generation R Study is to identify early environmental as well as (epi)genetic determinants and underlying pathways of growth, development and health. All pregnant women with an expected delivery date between April 2002 and January 2006 and living in Rotterdam were asked to participate. The study was approved by the local Medical Ethical Committee and written consent was obtained for each participating child. Enrolment was preferred during the first trimester of pregnancy, but was allowed until the date of delivery. At baseline 9,778 women were enrolled in the study (Figure 3). The Generation R Study is a multi-ethnic cohort. Participants of European origin constitute the largest ethnic group (58%), followed by Surinamese (9%), Turkish (7%) and Moroccan (6%).[52] Extensive data collection was performed in mothers, fathers and their children. Measurements were planned in early-, mid- and late pregnancy.

The fathers were assessed once during their partners' pregnancy. In the preschool period data collection was performed by a home-visit, questionnaires and routine child health center visits. At school-age, at the ages of 6 and 10 years, hands-on measurements, advanced imaging studies, behavioural observations and biological sample collection were performed in both children and parents at a dedicated research center in the Erasmus MC-Sophia Children's Hospital. Furthermore, the parents received 6 questionnaires during this period. Children received their first own questionnaire around the age of 10 year.[52] Data collection at age 13 years is currently ongoing.

Data used in this thesis include anthropometric measures, parent report questionnaires on eating behavior at age 4 years, detailed general and abdominal adiposity measures, using ultrasound, Dual-energy X-ray absorptiometry (DXA), and Magnetic Resonance Imaging (MRI) at ages 6 and 9 years, non-fasting blood samples for serum alanine aminotransferase (ALT) concentration at age 6 years, cord blood for genetic and epigenetic analyses, and blood samples at 6 and 9 years for epigenetic analyses. For a small subgroup without available cord blood samples, blood samples were taken at the age 6 years. Genotyping of the DNA samples was performed using Illumina 610 and 660 Quad arrays. For DNA methylation analyses, DNA was bisulfite-converted using the EZ-96 DNA Methylation kit (Zymo Research Corporation, Irvine, USA) and methylation was measured at 485,577 CpG sites using the Illumina Infinium HumanMethylation450 BeadChip (Illumina Inc., San Diego, USA). Both genetic and epigenetic samples underwent quality control based on standardized criteria.

EGG Consortium

As part of this thesis, we conducted a GWAS on childhood BMI within the Early Growth Genetics (EGG) Consortium. The EGG Consortium is an international collaboration that aims to identify genetic variants in the human genome involved in a variety of traits regarding early life. Our study meta-analysed GWAS data on childhood BMI of 35,668 children from 20 different cohorts.[53]

PACE Consortium

Two Epigenome-Wide Association Studies (EWAS) were performed within the Pregnancy And Childhood Epigenetics (PACE) Consortium. The PACE Consortium is an international collaboration of pregnancy, birth and childhood studies, which aims to facilitate the joint analysis of DNA methylation data to identify differences in DNA methylation associated with early life exposures and outcomes. To date, the study comprises 39 studies with a total sample size of 29,000 subjects with information on DNA methylation in pregnant

women, newborns, and children.[54,55] Within the PACE Consortium we first assessed the associations of pre-pregnancy maternal BMI with offspring DNA-methylation. In total, we meta-analysed 9,340 mother-child pairs originating from 19 cohorts. Our second study within the PACE Consortium comprised the assessment of associations between cord blood DNA methylation and birthweight, in 8,809 newborns originating from 24 birth cohorts.

Outline of this thesis

In this thesis, we address the objectives as follows: **Chapter 2** focusses on the associations of maternal adiposity and offspring outcomes. We examine whether maternal body mass index and gestational weight gain are associated with several childhood general abdominal and organ fat measures assessed by Magnetic Resonance Imaging. **Chapter 3** presents multiple studies on the genetics of childhood adiposity. In ***Chapter 3.1*** we present the results of a genome-wide association study which identified three new susceptibility loci for body mass index in children. In ***Chapter 3.2*** we examine whether genetic risk scores based on adult adiposity pathways are associated with early growth and general and abdominal fat measures in childhood. In ***Chapter 3.3*** we examine whether genetic variants associated with various adiposity measures in adulthood and childhood influence childhood body fat measures assessed by Magnetic Resonance Imaging. ***Chapter 3.4*** presents the associations of genetic risk scores based on known genetic variants for body mass index with eating behavior in childhood. In ***Chapter 3.5*** we focus on the influence of liver enzyme and fatty liver associated genetic variants on alanine transferase levels in childhood. **Chapter 4** focuses on epigenetic aspects of maternal and childhood adiposity. In ***Chapter 4.1*** we present an epigenome-wide association study on the association of maternal body mass index before pregnancy with DNA methylation in offspring. In ***Chapter 4.2*** we discuss the results of an epigenome-wide association study on the association of DNA methylation measured in cord blood with birthweight. All findings are discussed in **Chapter 5** where we will place our results in a broader context. An overall summary of this thesis in both English and Dutch can be found in **Chapter 6**.

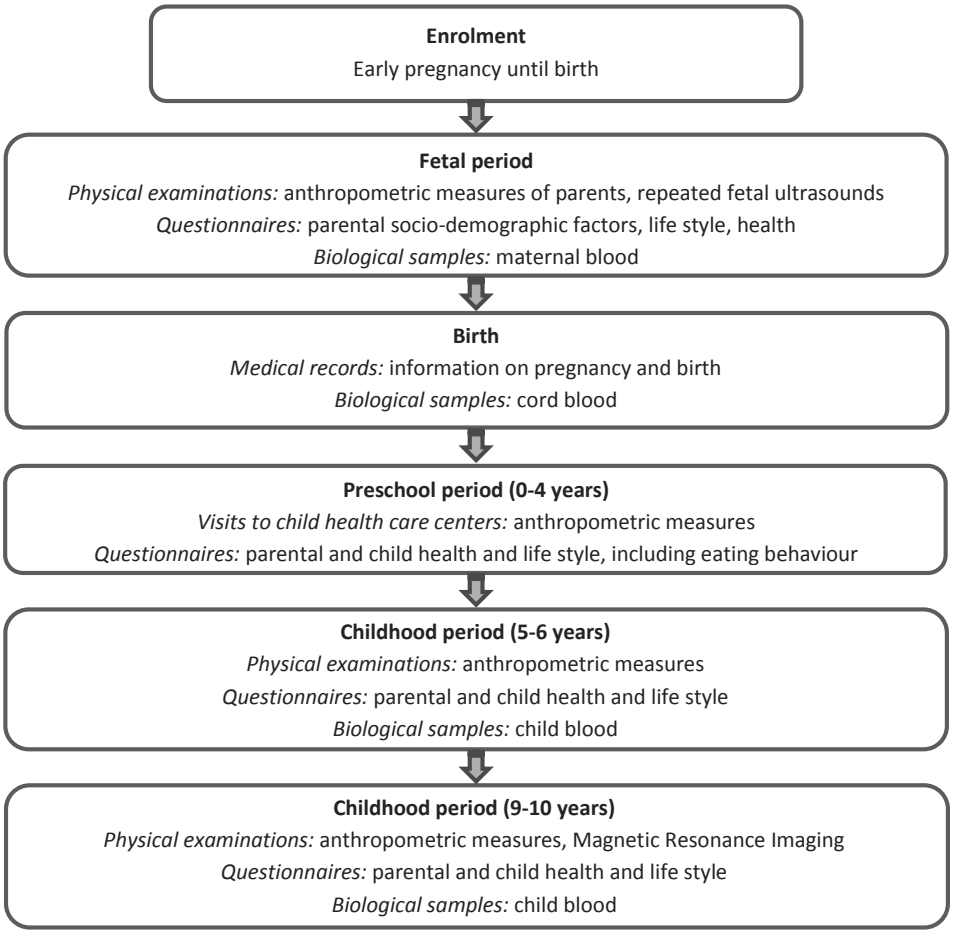

Figure 3. Design and data collection of interest for this thesis in the Generation R Study.

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Chapter 2

Maternal body mass index, gestational weight gain and childhood abdominal, pericardial and liver fat assessed by Magnetic Resonance Imaging

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Abstract

Background: Maternal obesity and excessive gestational weight gain are associated with an increased risk of obesity in offspring. It remains unclear whether maternal adiposity also affects organ fat, which has important adverse cardiometabolic health consequences and whether the associations reflect intrauterine causal mechanisms. We examined the associations of parental pre-pregnancy body mass index (BMI) and gestational weight gain with general, abdominal, pericardial and liver fat in 10-year-old children.

Methods: In a population-based prospective cohort study among 2,354 parents and their children, we obtained pre-pregnancy maternal and paternal BMI and gestational weight gain and offspring BMI, fat mass index (total fat/height^4) by dual-energy X-ray absorptiometry and subcutaneous fat index (subcutaneous fat/height^4), visceral fat index (visceral fat/height^3), pericardial fat index (pericardial fat/height^3) and liver fat fraction by Magnetic Resonance Imaging (MRI) at 10 years.

Results: A 1-standard deviation score (SDS) higher maternal pre-pregnancy BMI was associated with higher childhood BMI (difference 0.32 (95% Confidence Interval (CI) 0.28, 0.36) SDS), fat mass index (difference 0.28 (95% CI 0.24, 0.31) SDS), subcutaneous fat index (difference 0.26 (95% CI 0.22, 0.30) SDS), visceral fat index (difference 0.24 (95% CI 0.20, 0.28) SDS), pericardial fat index (difference 0.12 (95% CI 0.08, 0.16) SDS) and liver fat fraction (difference 0.15 (95% CI 0.11, 0.19) SDS). After conditioning each MRI adiposity measure on BMI at 10 years, higher maternal pre-pregnancy BMI remained associated with higher childhood subcutaneous and visceral fat indices. Smaller but not statistically different effect estimates were observed for paternal BMI. Gestational weight gain was not consistently associated with organ fat.

Conclusions: Higher maternal pre-pregnancy BMI, but not gestational weight gain, was associated with higher general and organ fat. Similar associations of pre-pregnancy maternal and paternal BMI with offspring adiposity suggest a role of family shared lifestyle factors and genetics.

Background

Maternal obesity is associated with several short- and long-term adverse health effects, including an increased risk of obesity in the offspring.[1,2] It has been hypothesized that maternal obesity is related to an increased placental transfer of nutrients to the fetus, which might affect the development of adipocytes, the appetite control system, and the energy metabolism.[3] However, the associations of maternal obesity with offspring outcomes might also be explained by shared family-based lifestyle or genetic factors. To help disentangling the underlying mechanisms, previous studies have compared the strength of associations of maternal and paternal body mass index (BMI) with offspring BMI and total fat mass and have shown conflicting results.[4-6] Stronger associations for maternal BMI with offspring outcomes suggest that intrauterine programming effects might be part of the underlying mechanisms, whereas similar or stronger associations for paternal BMI suggest a role for lifestyle or genetic factors.

Although many studies reported the associations between maternal and offspring obesity, it remains unclear whether maternal obesity also affects body fat distribution in the offspring. Information about body fat distribution is important since, as compared to BMI, body fat distribution, and more specifically excess visceral, heart and liver fat, may be better indicators of adverse cardiometabolic health.[7-10] Previous studies have reported that higher maternal BMI is associated with higher abdominal and liver fat in newborns.[11,12] Maternal pre-pregnancy obesity was also associated with higher visceral fat mass in Greek schoolchildren.[13] Whether these findings reflect an effect on specific fat accumulation or are just explained by general adiposity remains unknown. Next to maternal pre-pregnancy BMI, gestational weight gain may also affect childhood body fat distribution, but evidence remains scarce and not consistent.[14-17] Thus, a better understanding of the influence of maternal adiposity on body fat distribution in offspring, and the underlying mechanisms is important for development of preventive strategies.

We examined, in a population-based prospective cohort study among 2,354 mothers, fathers and their children, the associations of parental pre-pregnancy BMI and gestational weight gain with offspring BMI, fat mass index measured by dual-energy X-ray absorptiometry (DXA) and subcutaneous fat index, visceral fat index, pericardial fat index and liver fat fraction measured by Magnetic Resonance Imaging (MRI) at 10 years. We explored whether any association with organ specific fat measures reflects specific accumulation, or just reflects general adiposity.

Subjects and Methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands.[18] The study was approved by the Medical Ethical Committee of the Erasmus MC, Rotterdam (MEC 198.782/2001/31). Written informed consent was obtained from parents.[18] Pregnant women were enrolled between 2001 and 2005. Of all the eligible children in the study area, 61% participated at birth in the study. In total, 5,706 mothers and their singleton children attended the study visit at 10 years, of whom information about pre-pregnancy BMI was available in 4,298 subjects. Further, we excluded children without any organ specific fat measures assessed by MRI (N=1,944). Thus, the population for analysis was 2,354 mothers and their children (Supplemental Figure 1).

Parental anthropometrics

Maternal pre-pregnancy BMI was calculated from pre-pregnancy weight obtained by questionnaire and height measured at enrolment. Paternal height and weight were measured at enrolment and BMI was calculated. Maternal pre-pregnancy BMI was categorized into underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), and obesity (≥30.0 kg/m^2). For the parental BMI comparison analyses, pre-pregnancy maternal and paternal BMI were categorized into normal weight (18.5-24.9 kg/m^2) and overweight/obesity (≥ 25.0 kg/m^2) and combined in 4 groups: maternal and paternal normal weight; only maternal overweight/obesity; only paternal overweight/obesity; and maternal and paternal overweight/obesity. As previously described, we measured maternal weight at early, mid and late pregnancy (median 13.2 weeks of gestation (95% range 9.8, 18.9), median 30.1 weeks of gestation (95% range 20.5, 31.4) and median 39.0 weeks of gestation (95% range 32.8, 42.0), respectively).[16] Information about maximum weight during pregnancy was assessed by questionnaire 2 months after delivery. We calculated maximum weight gain during pregnancy as the difference between maximum weight and pre-pregnancy weight. Further, we divided maximum weight gain by gestational age at birth to obtain the maximum weight gain per week. Maximum gestational weight gain was also classified as insufficient, sufficient and excessive weight gain in relation to maternal pre-pregnancy BMI according to the Institute of Medicine guidelines.[19]

Measures of adiposity at 10 years

We measured child's height and weight without shoes and heavy clothing and calculated BMI (kg/m^2). We calculated sex- and age- adjusted standard deviation scores (SDS) of childhood BMI based on Dutch reference growth charts (Growth Analyzer 4.0, Dutch Growth Research Foundation).[20] We measured total body fat mass using a DXA scanner (iDXA, GE-Lunar, 2008, Madison, WI, USA, enCORE software v.12.6), according to standard procedures.[21] Previous studies have validated DXA against computed tomography for body fat assessment.[22]

Measures of organ fat at 10 years were obtained from MRI scans.[18] MRI has been described as an accurate and reproducible technique and considered the gold standard for the measurement of intra-abdominal and organ fat deposition.[23] All children were scanned using a 3.0 Tesla MRI (Discovery MR 750w, GE Healthcare, Milwaukee, WI, USA) for body fat imaging using standard imaging and positioning protocols, while performing expiration breath-hold maneuvers of maximum 11 seconds duration. They wore light clothing without metal objects while undergoing the body scan.[24] Pericardial fat imaging in short axis orientation was performed using an ECG triggered black-blood prepared thin slice single shot fast spin echo acquisition (BB SSFSE) with multi-breath-hold approach. An axial 3-point Dixon acquisition for fat and water separation (IDEAL IQ) was used for liver fat imaging. This technique also enables the generation of liver fat fraction images.[25] An axial abdominal scan from lower liver to pelvis and a coronal scan centered at the head of the femurs were performed with a 2-point DIXON acquisition (LavaFlex).

The obtained fat scans were subsequently analyzed by the Precision Image Analysis company (PIA, Kirkland, Washington, United States), using the sliceOmatic (TomoVision, Magog, Canada) software package. All extraneous structures and any image artifacts were removed manually.[23] Pericardial fat included both epicardial- and paracardial fat directly attached to the pericardium, ranging from the apex to the left ventricular outflow tract. Total subcutaneous and visceral fat volumes were generated by summing the volumes of the liver, abdominal and if necessary the femoral fat-only scans, encompassing the fat volume ranging from the dome of the liver to the superior part of the femoral head. Fat masses were obtained by multiplying the total volumes by the specific gravity of adipose tissue, 0.9 g/ml. Liver fat fraction was determined by taking four samples of at least 4 cm^2 from the central portion of the hepatic volume. Subsequently, the mean signal intensities were averaged to generate an overall mean liver fat fraction estimation.

To create measures of general and organ fat independent of height at 10 years, we estimated the optimal adjustment by log-log regression analyses and subsequently we

divided total and subcutaneous fat mass by height^4 (fat mass index and subcutaneous fat index) and visceral and pericardial fat mass by height^3 (visceral and pericardial fat indices) (More details given in the Supplemental Methods).[26,27]

Covariates

Information on maternal and paternal age, educational level, and ethnicity, and maternal parity and smoking habits was obtained by questionnaires during pregnancy. Information on child's sex was obtained from medical records. Information on breastfeeding duration and timing of introduction of solid foods was obtained by questionnaires in infancy, and information on the average television watching time was obtained by questionnaires at the age of 10 years.

Statistical analysis

First, we used linear regression models to examine the associations of maternal and paternal pre-pregnancy BMI and maximum gestational weight gain, continuously and using clinical categories, with measures of adiposity (BMI, fat mass index, subcutaneous, visceral and pericardial fat indices and liver fat fraction) at 10 years. Second, we examined the independent associations of maternal pre-, early, mid, and late pregnancy weight with the childhood outcomes using conditional linear regression analyses to account for the correlations between the weight measurements.[28] For these models, we obtained standardized residuals for each weight from the regression of a maternal weight at a specific time point on prior maternal weights. These variables correspond to the difference between the actual weight and the expected weight based on prior weights and thus are statistically independent from each other and can be included simultaneously in the regression models.[28] Third, we used conditional regression analyses to assess whether the associations of maternal and paternal pre-pregnancy BMI and gestational weight gain with measures of organ fat at 10 years were independent of BMI at 10 years. We used as outcomes the standardized residuals for each measure of organ fat at 10 years obtained from the regression of those outcomes on BMI.[28] For all analyses, we used a basic model including child´s sex and age at outcome measurements, and a confounder model, which additionally included covariates. We included covariates in the models if they were strongly associated with parental anthropometrics and childhood adiposity in our study, or if they changed the effect estimates substantially (>10%). We log-transformed the non-normally distributed childhood DXA and MRI adiposity measures. We constructed SDS [(observed value - mean)/SD] of the sample distribution for all continuous exposures and DXA and MRI outcomes to enable comparisons of effect sizes. No statistical interactions between maternal pre-pregnancy BMI and gestational weight gain, and between maternal pre-

pregnancy BMI and paternal BMI were observed in these associations. We also tested for statistical interaction between maternal pre-pregnancy BMI and gestational weight gain with child´s sex since body fat development and body fat distribution pattern during childhood is known to differ between boys and girls, but no significant interaction was observed. Since the maximum gestational weight gain was self-reported, sensitivity analyses using weight gain measured until late pregnancy were performed. Missing values in covariates (ranging from 0 to 28%) were multiple-imputed by using Markov chain Monte Carlo approach. Five imputed datasets were created and analyzed together. All statistical analyses were performed using the Statistical Package of Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Table 1 shows the subject characteristics. In our sample, 26.3% of mothers and 49.2% of fathers had overweight/obesity and 45.0% of mothers gained excessive weight during pregnancy. Non-response analyses showed that parents of children with MRI follow-up data available were slightly older and had a higher educational level, and mothers were more likely to be non-smokers (p-values <0.05). No differences were observed for maternal pre-pregnancy BMI and gestational weight gain and paternal BMI (Supplemental Table 1). Supplemental Table 2 shows that the correlation coefficients of BMI and fat mass index with subcutaneous and visceral fat indices are moderate to strong and higher than the correlation coefficients with pericardial fat index and liver fat fraction.

Maternal and paternal BMI and childhood organ fat measures

Table 2 shows that a 1-SDS higher maternal pre-pregnancy BMI was associated with higher childhood BMI (difference 0.32 (95% Confidence Interval (CI) 0.28, 0.36) SDS), fat mass index (difference 0.28 (95% CI 0.24, 0.31) SDS), subcutaneous fat index (difference 0.26 (95% CI 0.22, 0.30) SDS), visceral fat index (difference 0.24 (95% CI 0.20, 0.28) SDS), pericardial fat index (difference 0.12 (95% CI 0.08, 0.16) SDS) and liver fat fraction (difference 0.15 (95% CI 0.11, 0.19) SDS). As compared to maternal pre-pregnancy normal weight, maternal pre-pregnancy underweight was associated with lower fat measures whereas maternal pre-pregnancy overweight and obesity were associated with higher fat measures in childhood (p-values <0.05). After conditioning each MRI measure of adiposity on BMI at 10 years, higher maternal pre-pregnancy BMI remained associated with higher childhood subcutaneous and visceral fat indices (p-values <0.05).

Table 1. Characteristics of mothers, fathers and their children.[a]

Characteristics	Total group (N=2,354)	Maternal underweight (N=97)	Maternal normal weight (N=1,639)	Maternal overweight (N=451)	Maternal obesity (N=167)	p-value[b]
Maternal characteristics						
Age, mean (SD), years	31.0 (4.8)	29.0 (5.3)	31.1 (4.7)	31.0 (4.7)	30.3 (4.8)	<0.001
Education, N (%)						
Low	160 (6.9)	7 (7.3)	87 (5.4)	53 (12.1)	13 (8.2)	<0.001
Medium	935 (40.5)	41 (42.7)	594 (36.8)	200 (45.8)	100 (63.3)	
High	1,211 (52.5)	48 (50.0)	934 (57.8)	184 (42.1)	45 (28.5)	
Ethnicity, N (%)						
European	1,532 (65.3)	60 (61.9)	1,135 (69.4)	253 (56.6)	84 (50.3)	<0.001
Non-European	815 (34.7)	37 (38.1)	501 (30.6)	194 (43.4)	83 (49.7)	
Parity, N (%)						
Nulliparous	1,419 (60.3)	55 (56.7)	1,036 (63.2)	237 (52.5)	91 (54.5)	<0.001
Multiparous	934 (39.7)	42 (43.3)	602 (36.8)	214 (47.5)	76 (45.5)	
Pre-pregnancy BMI, median (95% range), kg/m^2	22.5 (18.0, 34.9)	17.9 (15.8, 18.5)	21.7 (18.8, 24.8)	26.7 (25.1, 29.8)	33.0 (30.1, 44.7)	<0.001
Maximum gestational weight gain, mean (SD), kg	14.8 (5.8)	15.2 (5.5)	15.3 (5.2)	14.3 (6.3)	11.0 (9.0)	<0.001
Gestational weight gain clinical categories (IOM criteria), N (%)						
Insufficient gestational weight gain	299 (20.5)	16 (31.4)	236 (22.2)	25 (9.6)	22 (25.3)	<0.001
Sufficient gestational weight gain	505 (34.5)	25 (49.0)	407 (38.3)	56 (21.5)	17 (19.5)	
Excessive gestational weight gain	658 (45.0)	10 (19.6)	421 (39.6)	179 (68.8)	48 (55.2)	
Weight early pregnancy, mean (SD), kg	69.0 (12.9)	53.6 (9.2)	64.3 (7.2)	78.4 (8.7)	97.0 (14.2)	<0.001
Weight mid pregnancy, mean (SD), kg	76.0 (12.6)	61.7 (8.8)	72.0 (8.4)	84.8 (9.5)	100.4 (13.2)	<0.001
Weight late pregnancy, mean (SD), kg	81.6 (12.5)	66.5 (6.2)	78.0 (9.0)	91.4 (10.5)	105.6 (13.2)	<0.001
Smoking during pregnancy, N (%)						

Table 1. *(Continued)*

Characteristics	Total group (N=2,354)	Maternal underweight (N=97)	Maternal normal weight (N=1,639)	Maternal overweight (N=451)	Maternal obesity (N=167)	p-value[b]
Yes	504 (22.3)	27 (28.4)	336 (21.3)	104 (24.5)	37 (23.0)	0.238
No	1,757 (77.7)	68 (71.6)	1,244 (78.7)	321 (75.5)	124 (77.0)	
Paternal characteristics						
Age, mean (SD), years	33.6 (5.3)	31.9 (4.9)	33.7 (5.3)	33.6 (5.3)	33.7 (6.5)	0.052
Education, N (%)						
Low	83 (4.9)	4 (6.3)	50 (4.1)	20 (6.6)	9 (9.6)	<0.001
Medium	652 (38.6)	20 (31.7)	428 (34.9)	150 (49.3)	54 (57.4)	
High	953 (56.5)	39 (61.9)	749 (61.0)	134 (44.1)	31 (33.0)	
Ethnicity, N (%)						
European	1,383 (74.4)	52 (70.3)	1,020 (76.5)	233 (69.1)	78 (67.8)	0.011
Non-European	477 (25.6)	22 (29.7)	314 (23.5)	104 (30.9)	37 (32.2)	
BMI, mean (SD), kg/m^2	25.3 (3.3)	23.4 (2.8)	25.0 (3.1)	26.2 (3.3)	27.5 (4.5)	<0.001
BMI clinical categories, N (%)						
Underweight	9 (0.5)	1 (1.4)	6 (0.4)	1 (0.3)	1 (0.8)	<0.001
Normal weight	945 (50.3)	53 (71.6)	726 (54.0)	128 (37.8)	38 (31.7)	
Overweight	771 (41.1)	19 (25.7)	521 (38.8)	180 (53.1)	51 (42.5)	
Obesity	152 (8.1)	1 (1.4)	91 (6.8)	30 (8.8)	30 (25.0)	
Birth and infant characteristics						
Child's sex, N (%)						
Boys	1,151 (48.9)	52 (53.6)	812 (49.5)	206 (45.7)	81 (48.5)	0.389
Girls	1,203 (51.1)	45 (46.4)	827 (50.5)	245 (54.3)	86 (51.5)	
Breastfeeding duration, median (95% range), months	3.5 (0.0, 12.0)	2.5 (0.0, 12.0)	3.5 (0.0, 12.0)	3.5 (0.0, 12.0)	1.5 (0.0, 12.0)	<0.001

Table 1. *(Continued)*

Characteristics	Total group (N=2,354)	Maternal underweight (N=97)	Maternal normal weight (N=1,639)	Maternal overweight (N=451)	Maternal obesity (N=167)	p-value[b]
Introduction of solid foods, N (%)						
< 3 months	123 (7.0)	6 (10.0)	68 (5.4)	33 (10.4)	16 (13.6)	0.002
3-6 months	1,435 (81.7)	47 (78.3)	1,044 (82.7)	251 (79.2)	93 (78.8)	
> 6 months	199 (11.3)	7 (11.7)	150 (11.9)	33 (10.4)	9 (7.6)	
Childhood characteristics						
Age, mean (SD), years	9.8 (0.3)	9.9 (0.4)	9.8 (0.3)	9.8 (0.4)	9.9 (0.4)	0.012
Television watching time, N (%)						
< 2 hours/day	1,342 (70.0)	60 (82.2)	996 (72.5)	221 (64.4)	65 (50.8)	<0.001
≥ 2 hours/day	575 (30.0)	13 (17.8)	377 (27.5)	122 (35.6)	63 (49.2)	
BMI, mean (SD), kg/m^2	17.5 (2.6)	16.2 (2.1)	17.1 (2.3)	18.5 (2.9)	20.0 (3.5)	<0.001
Total fat mass, median (95% range), g	8,451 (4,549, 21,235)	7,268 (3,782, 17,498)	8,003 (4,549, 19,478)	9,749 (4,829, 23,547)	13,014 (4,791, 31,236)	<0.001
Subcutaneous fat mass, median (95% range), g	1,297 (603, 5,226)	1,063 (539, 4,516)	1,210 (601, 4,632)	1,638 (656, 5,994)	2,335 (738, 6,032)	<0.001
Visceral fat mass, median (95% range), g	365 (163, 1,004)	285 (128, 800)	350 (159, 905)	416 (176, 1,119)	494 (233, 1,305)	<0.001
Pericardial fat mass, median (95% range), g	10.6 (4.6, 22.6)	9.4 (3.5, 18.2)	10.4 (4.4, 21.9)	11.1 (5.2, 23.5)	13.3 (5.5, 25.1)	<0.001
Liver fat fraction, median (95% range), %	2.0 (1.2, 5.2)	1.9 (1.1, 5.1)	2.0 (1.2, 4.7)	2.1 (1.3, 6.0)	2.3 (1.4, 9.3)	<0.001

[a] Values are observed data and represent means (SD), medians (95% range) or numbers of subjects (valid %). IOM, Institute of Medicine. [b] Differences in subject characteristics between groups were evaluated using one-way-ANOVA-tests for continuous variables and Chi-square tests for proportions. BMI; body mass index, SD; standard deviation.

Figure 1A shows that, as compared to normal weight parents, those in which only mothers or only fathers were overweight/obese had children with higher levels of all adiposity measures at the age of 10 years (p-values< 0.05). The associations tended to be stronger when only mothers rather than only fathers were overweight/obese but had overlapping CI and thus seem not to be statistically different. The strongest associations were observed for children in which both parents were overweight/obese. After conditioning each MRI measure of child's adiposity on BMI at 10 years (Figure 1B), no significant associations were observed for couples in which only mothers or only fathers were overweight/obese. Those couples in which both parents were overweight/obese had children with higher subcutaneous, visceral and pericardial fat indices (p-values <0.05).

Maternal gestational weight gain and childhood organ fat measures

Table 3 shows that a 1-SDS higher maximum weight gain per week was only associated with higher childhood BMI (difference 0.08 (95% CI 0.03, 0.13) SDS). Excessive weight gain, as compared to sufficient weight gain, was associated with higher childhood BMI, fat mass index and subcutaneous and visceral fat indices (p-values <0.05). After conditioning each MRI measure of adiposity on BMI at 10 years, a 1-SDS higher maximum weight gain per week was associated with lower childhood subcutaneous fat index (p-value <0.05). Similar results were observed when using maternal weight gain measured until late pregnancy (Supplemental Table 5). Figure 2A shows that independent from weights in other periods, higher pre-pregnancy weight was associated with higher levels of all adiposity measures (p-values <0.05). Higher early pregnancy weight was associated with higher BMI and fat mass index, but not with organ fat measures at 10 years. No associations were observed for mid and late pregnancy weight. After conditioning each MRI measure of adiposity on BMI at 10 years (Figure 2B), higher pre-pregnancy weight remained associated with higher subcutaneous and visceral fat indices. No associations were observed for early, mid and late pregnancy weight.

Table 2. Maternal body mass index and childhood general and organ fat measures.[a]

	Measures of adiposity at 10 years in SDS[b]					
	Body mass index (N=2,354)	**Fat mass index (N=2,339)**	**Subcutaneous fat index (N=2,049)**	**Visceral fat index (N=2,052)**	**Pericardial fat index (N=2,123)**	**Liver fat fraction (N=2,319)**
BMI (kg/m^2 in SDS)	0.32 (0.28, 0.36)*	0.28 (0.24, 0.31)*	0.26 (0.22, 0.30)*	0.24 (0.20, 0.28)*	0.12 (0.08, 0.16)**	0.15 (0.11, 0.19)*
Underweight (<18.5 kg/m^2)	-0.49 (-0.69, -0.29)*	-0.32 (-0.50, -0.14)*	-0.31 (-0.50, -0.12)*	-0.37 (-0.58, -0.17)*	-0.26 (-0.47, -0.05)**	-0.17 (-0.37, 0.04)
Normal weight (18.5 – 24.9 kg/m^2)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Overweight (25.0 – 29.9 kg/m^2)	0.46 (0.36, 0.56)*	0.39 (0.30, 0.48)*	0.40 (0.30, 0.50)*	0.35 (0.24, 0.45)*	0.15 (0.04, 0.26)*	0.19 (0.09, 0.30)*
Obesity (≥30.0 kg/m^2)	0.88 (0.73, 1.04)*	0.81 (0.66, 0.95)*	0.76 (0.61, 0.92)*	0.69 (0.52, 0.86)*	0.42 (0.24, 0.59)*	0.45 (0.28, 0.61)*
			MRI measures of adiposity at 10 years in SDS conditional on body mass index[c]			
			Subcutaneous fat index (N=2,049)	**Visceral fat index (N=2,052)**	**Pericardial fat index (N=2,123)**	**Liver fat fraction (N=2,319)**
BMI (kg/m^2 in SDS)			0.05 (0.01, 0.09)**	0.07 (0.03, 0.11)*	0.02 (-0.02, 0.07)	0.03 (-0.01, 0.07)
Underweight (<18.5 kg/m^2)			0.09 (-0.09, 0.28)	-0.12 (-0.33, 0.10)	-0.10 (-0.31, 0.11)	0.02 (-0.19, 0.23)
Normal weight (18.5 – 24.9 kg/m^2)			*Reference*	*Reference*	*Reference*	*Reference*
Overweight (25.0 – 29.9 kg/m^2)			0.12 (0.03, 0.22)**	0.13 (0.02, 0.24)**	0.02 (-0.09, 0.13)	0.02 (-0.09, 0.13)
Obesity (≥30.0 kg/m^2)			0.26 (0.10, 0.41)*	0.24 (0.07, 0.42)*	0.16 (-0.01, 0.34)	0.12 (-0.04, 0.29)

[a] Estimates are based on multiple imputed data. Model includes child´s sex and age at outcome measurements (except for sex- and age-adjusted body mass index SDS), maternal age, educational level, ethnicity, parity, and smoking habits during pregnancy, and child's breastfeeding duration, timing of introduction of solid foods and television watching time. Results from the basic model are given in Supplemental Table 3. **p-value <0.05, *p-value <0.01. [b]Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal pre-pregnancy body mass index or for body mass index clinical groups as compared to the reference group (normal weight). [c]Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in the standardized residuals of the childhood outcomes (obtained by conditional regression analyses on body mass index at 10 years) per SDS change in maternal pre-pregnancy body mass index or for body mass index clinical groups as compared to the reference group (normal weight). BMI; body mass index, SDS; standard deviation scores.

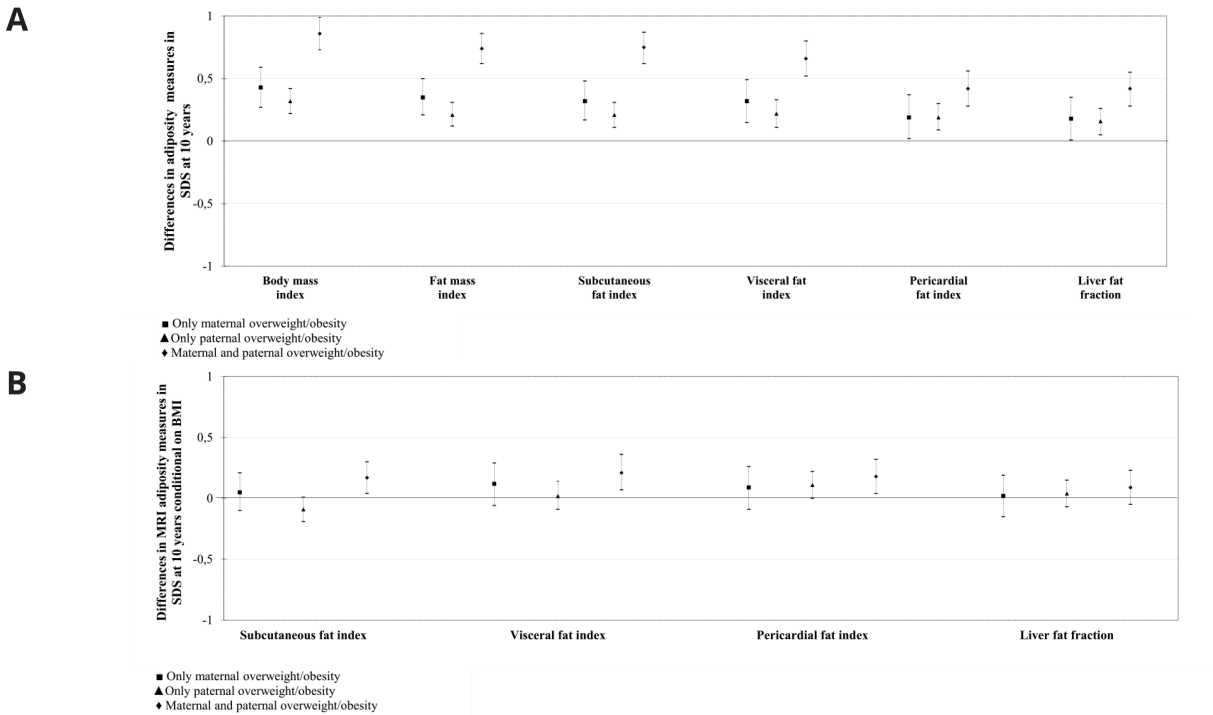

Figure 1. Parental body mass index and childhood general and organ fat measures 204 (N=1,795). Estimates are based on multiple imputed data. Model includes child´s sex and age at outcome measurements (except for sex- and age-adjusted body mass index SDS), parental age, educational level, and ethnicity, parity, maternal smoking habits during pregnancy, and child's breastfeeding duration, timing of introduction of solid foods and television watching time. Results from the basic model are given in Supplemental Figure 2. Values in A are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood outcomes in SDS for parental body mass index clinical groups as compared to the reference group (maternal and paternal normal weight). Values in B are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in the standardized residuals of the childhood outcomes (obtained by conditional regression analyses on body mass index at 10 years) for parental body mass index clinical groups as compared to the reference group (maternal and paternal normal weight). SDS, standard deviation scores.

Table 3. Maternal gestational weight gain and childhood general and organ fat measures.[a]

	Measures of adiposity at 10 years in SDS[b]					
	Body mass index (N=1,462)	**Fat mass index (N=1,451)**	**Subcutaneous fat index (N=1,287)**	**Visceral fat index (N=1,288)**	**Pericardial fat index (N=1,336)**	**Liver fat fraction (N=1,444)**
Maximum weight gain per week (kg in SDS)	0.08 (0.03, 0.13)*	0.02 (-0.03, 0.06)	0.01 (-0.04, 0.05)	0.03 (-0.02, 0.08)	0.02 (-0.03, 0.08)	0.00 (-0.05, 0.05)
Insufficient weight gain	-0.09 (-0.23, 0.05)	0.01 (-0.12, 0.14)	-0.01 (-0.14, 0.13)	-0.03 (-0.17, 0.12)	0.04 (-0.11, 0.20)	0.05 (-0.09, 0.19)
Sufficient weight gain	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Excessive weight gain	0.19 (0.07, 0.30)*	0.14 (0.04, 0.25)*	0.12 (0.01, 0.23)**	0.16 (0.04, 0.28)**	0.09 (-0.03, 0.22)	0.06 (-0.05, 0.18)
	MRI measures of adiposity at 10 years in SDS conditional on body mass index[c]					
			Subcutaneous fat index (N=1,287)	**Visceral fat index (N=1,288)**	**Pericardial fat index (N=1,336)**	**Liver fat fraction (N=1,444)**
Maximum weight gain per week (kg in SDS)			-0.09 (-0.13, -0.04)*	-0.02 (-0.07, 0.04)	0.00 (-0.06, 0.05)	-0.04 (-0.09, 0.02)
Insufficient weight gain			0.11 (-0.02, 0.23)	0.04 (-0.10, 0.19)	0.08 (-0.07, 0.23)	0.10 (-0.04, 0.24)
Sufficient weight gain			*Reference*	*Reference*	*Reference*	*Reference*
Excessive weight gain			-0.03 (-0.13, 0.08)	0.07 (-0.05, 0.19)	0.04 (-0.09, 0.16)	-0.01 (-0.12, 0.11)

[a]Estimates are based on multiple imputed data. Model includes child´s sex and age at outcome measurements (except for sex- and age- adjusted body mass index SDS), maternal age, educational level, ethnicity, parity, smoking habits during pregnancy, and child's breastfeeding duration, timing of introduction of solid foods and television watching time. Models for maximum weight gain per week were additionally adjusted for pre-pregnancy body mass index. Results from the basic model are given in Supplemental Table 4. **p-value <0.05, *p-value <0.01. [b]Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal maximum weight gain per week or for IOM weight gain clinical groups as compared to the reference group (sufficient weight gain). [c]Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in the standardized residuals of the childhood outcomes (obtained by conditional regression analyses on body mass index at 10 years) per SDS change in maternal maximum weight gain per week or for IOM weight gain clinical groups as compared to the reference group (sufficient weight gain). SDS, standard deviation scores.

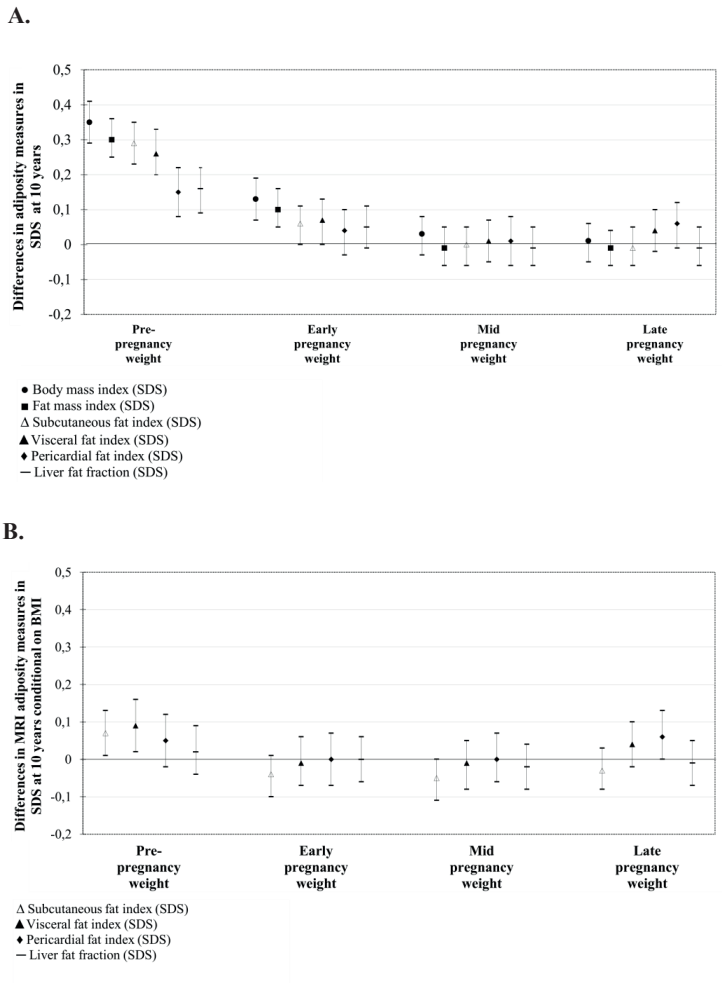

Figure 2. Maternal pre-, early, mid, and late pregnancy weight with childhood general and organ fat measures (N=1,121). Estimates are based on multiple imputed data. Model includes child´s sex and age at outcome measurements (except for sex- and age-adjusted body mass index SDS), maternal age, educational level, and ethnicity, parity, height at intake, smoking habits during pregnancy, and child's breastfeeding duration, timing of introduction of solid foods and television watching time. Results from the basic model are given in Supplemental Figure 3. Values in A are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal pre-pregnancy weight and per change in standardized residuals of maternal early, mid, and late pregnancy weight obtained from conditional regression analyses. Values in B are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in the standardized residuals of the childhood outcomes (obtained by conditional regression analyses on body mass index at 10 years) per SDS change in maternal pre-pregnancy weight and per change in standardized residuals of maternal early, mid, and late pregnancy weight obtained from conditional regression analyses. SDS, standard deviation scores.

Discussion

We observed, in this population-based prospective cohort study, that higher maternal pre-pregnancy BMI was associated with higher BMI, fat mass index, subcutaneous, visceral and pericardial fat indices and liver fat fraction at 10 years. The associations of maternal pre-pregnancy BMI with offspring subcutaneous and visceral fat indices seemed to be independent of offspring BMI. Total and period-specific gestational weight gain were not consistently associated with organ fat measures.

Interpretation of main findings

Maternal obesity is a major public health concern.[29] A meta-analysis of published studies showed an increased risk of overweight in offspring of mothers with overweight and obesity, as compared to offspring of mothers with normal weight.[1] In the same cohort as the current study, we have previously reported that maternal overweight and obesity were strongly associated with increased risks of overweight and obesity in the offspring aged 4 and 6 years.[6,30] In the present study, maternal pre-pregnancy overweight and obesity were associated with higher BMI and fat mass index at 10 years.

Large cohort studies such as the Framingham Heart Study and the Jackson Heart Study have reported that excess visceral, pericardial and liver fat depositions are related to various cardiometabolic abnormalities in adults.[7-10,31] As compared to visceral abdominal fat, excess subcutaneous abdominal fat was less strongly associated with an adverse cardiometabolic risk profile in adults.[8,31] In 105 healthy mother-newborn pairs, higher maternal BMI was associated with higher infant abdominal fat, independently of weight, and higher intrahepatocellular lipid content.[11] In another study among 25 newborns, infants born to obese mothers with gestational diabetes had higher intrahepatocellular fat compared with infants born to normal weight mothers.[12] Maternal pre-pregnancy obesity was also associated with higher visceral fat mass levels in 1,228 Greek children aged 9-13 years.[13] In the present study, higher maternal pre-pregnancy BMI was associated with higher subcutaneous, visceral and pericardial fat indices and liver fat fraction at 10 years. The associations of maternal pre-pregnancy BMI with offspring subcutaneous and visceral fat indices seemed to be independent of offspring BMI. This means that higher maternal pre-pregnancy BMI is associated with a specific accumulation of fat in abdominal depots that is not a result of general adiposity. We did not observe differences in the results when we conditioned on fat mass index instead of BMI. These results are not in line with those of our previous study in 6-year-old children suggesting that higher maternal pre-pregnancy

BMI was not associated with subcutaneous and preperitoneal abdominal fat measured by ultrasound, independently of child´s BMI.[6] The differences in results may be due to different ages or different imaging methods.

Previously, we reported that higher paternal BMI was associated with higher BMI but was not associated with subcutaneous and preperitoneal abdominal fat at the age of 6 years, independently of child's BMI.[6] In the present study, paternal overweight was associated with higher BMI, fat mass index and organ fat measures in children aged 10 years. The associations observed with MRI adiposity measures were not independent of BMI at 10 years. Our results suggest that both maternal and paternal BMI before pregnancy may be risk factors for offspring cardiometabolic health by influencing general and organ fat accumulation in later life. Previous studies comparing the associations of maternal and paternal BMI with childhood BMI and total fat mass have shown conflicting results.[4-6] A recent study using genetic variants in a Mendelian randomization approach found little evidence to support strong causal intrauterine effects of maternal BMI on offspring adiposity.[32] Although we observed a tendency for stronger associations of maternal pre-pregnancy BMI, as compared to paternal BMI, with general and abdominal fat measures, the differences between the maternal and paternal effect estimates were not statistically significant. These findings may suggest that the associations of maternal pre-pregnancy BMI with offspring adiposity might be explained by shared family-based lifestyle and genetic characteristics rather than by intrauterine programming.[33]

Next to maternal pre-pregnancy obesity, excessive gestational weight gain also seems to be associated with an increased risk of childhood overweight.[34] In our study, excessive weight gain was associated with higher BMI and fat mass index. A previous study among 313 mother-child pairs reported that higher maternal BMI was associated with higher childhood subcutaneous and visceral fat, particularly among mothers with excessive gestational weight gain.[15] However, in the same cohort as the current study, maternal weight gain in early, mid and late pregnancy was not associated with childhood subcutaneous and preperitoneal abdominal fat mass levels at 6 years, independently of BMI.[16] In our study, total and period-specific weight gain was not consistently associated with any MRI adiposity measures. Thus, gestational weight gain, contrary to BMI before pregnancy, seems to have a limited influence on offspring organ fat in later life.

The mechanisms by which maternal adiposity during pregnancy affects offspring organ fat accumulation are not fully known yet. Maternal over-nutrition may affect the development of adipocytes and their capacity to expand or contract, the appetite control system and the energy metabolism in later life,[3] which might lead to increased body fat in the offspring. Maternal over-nutrition might also lead to accumulation of fat in the liver and

other developing organs of the fetus, especially during early and mid-pregnancy due to the absence of adipose tissue.[35] The postnatal persistence of increased fat in these depots might be related to reduced fatty acid oxidation, changes in lipogenesis and lipoprotein export.[35]

Our study shows that higher maternal pre-pregnancy BMI, as opposed to gestational weight gain, is related to higher organ fat measures, which have important adverse cardiometabolic health consequences. Future preventive strategies focused on promoting a healthy weight in women of reproductive age before pregnancy are needed to improve cardiometabolic health of the offspring. Considering the uncertainty about the causality of the associations, strategies directed at both parents might be more effective. Future studies may also analyse body fat distribution patterns based on various measures of general and organ fat by cluster analyses and relate these to early life exposures and later life outcomes.

Methodological considerations

Strengths of this study were the large sample size, prospective design and data available on multiple maternal weight measurements throughout pregnancy and detailed childhood adiposity measures. Of the 4,298 mothers and their singleton children with information on pre-pregnancy BMI available, 2,354 had information on MRI adiposity measures at 10 years. The non-response could lead to biased effect estimates if the associations of maternal pre-pregnancy BMI and gestational weigh gain with childhood adiposity measures differ between mothers and children included and not included in the present analyses. However, this seems unlikely since participants and non-participants did not differ regarding maternal pre-pregnancy BMI and weight gain during pregnancy. We relied on self-reported pre-pregnancy weight and maximum weight during pregnancy. Women tend to underestimate their weight on self-report,[36] which might have led to an underestimation of observed effects for maternal pre-pregnancy BMI and weight gain. However, bias seems unlikely since strong correlations were observed between self-reported pre-pregnancy weight and weight measured at enrolment ($r=0.96$, p-value <0.01) as well as between self-reported maximum weight and weight measured at late pregnancy ($r=0.99$, p-value <0.01). Also, since pre-pregnancy weight and maximum gestational weight are both self-reported and probably underestimated, the influence on maximum weight gain is likely to be minimal, which is confirmed by the fact that similar results were observed for weight gain measured until late pregnancy. Finally, although we adjusted for a large number of potential confounders, residual confounding due to lifestyle-related characteristics such

as parental and child nutritional intake and physical activity might still be present in the observed associations. Thus, from the current observational data, no conclusions can be drawn on the causality and mechanisms underlying the observed associations.

Conclusions

Our study suggests that higher maternal pre-pregnancy BMI, but not gestational weight gain, is associated with organ fat accumulation, especially abdominal fat, in the offspring. Our findings emphasize the importance of promoting a healthy BMI in women who are planning to become pregnant rather that influencing weight gain during pregnancy. Similar associations were observed in our study for pre-pregnancy maternal and paternal BMI with offspring adiposity, suggesting a role of family shared lifestyle factors and genetics.

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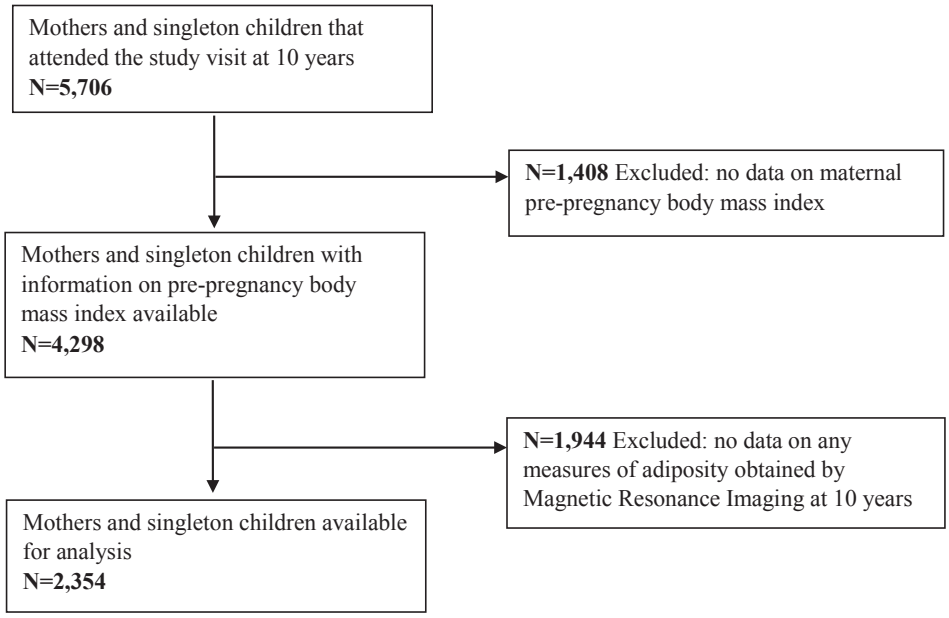
Supplement



Supplemental Figure S1. Selection of study participants.

Supplemental Methods

Log-log regression analyses

To create measures of adiposity independent of height at 10 years, we estimated the optimal adjustment by log-log regression analyses.[1] Total fat mass, subcutaneous fat mass, visceral fat mass and pericardial fat mass and height were log-transformed, using natural logs. Log-adiposity measures were regressed on log-height. The regression slope corresponds to the power by which height should be raised in order to calculate an index uncorrelated with height. Thus, we divided total fat mass by height^4, subcutaneous fat mass by height^4, visceral fat mass by height^3, and pericardial fat mass by height^3.

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Supplemental Table S1. Comparison of subject characteristics between participants and non-participants[a]

Characteristics	Participants (N=2,354)	Non-participants (N=1,944)	p-value
Maternal characteristics			
Age, mean (SD), years	31.0 (4.8)	30.6 (5.1)	0.01
Education (higher education), N (%)	1211 (52.5)	887 (46.9)	<0.01
Ethnicity (European), N (%)	1532 (65.3)	1256 (64.7)	0.72
Parity (nulliparous), N (%)	1419 (60.3)	1123 (57.9)	0.10
Pre-pregnancy body mass index, median (95% range), kg/m^2	22.5 (18.0, 34.9)	22.6 (18.2, 34.2)	0.90
Obesity, N (%)	167 (7.1)	164 (8.4)	0.22
Maximum gestational weight gain, mean (SD), kg	14.8 (5.8)	14.9 (5.5)	0.62
Excessive gestational weight gain (IOM criteria), N (%)	658 (45.0)	416 (42.9)	0.16
Weight in early pregnancy, mean (SD), kg	69.0 (12.9)	68.7 (12.0)	0.47
Weight in mid pregnancy, mean (SD), kg	76.0 (12.6)	75.5 (12.5)	0.17
Weight in late pregnancy, mean (SD), kg	81.6 (12.5)	80.1 (11.8)	0.01
Smoking during pregnancy (yes), N (%)	504 (22.3)	473 (25.5)	0.02
Paternal characteristics			
Age, mean (SD), years	33.6 (5.3)	33.1 (5.7)	0.02
Education (higher education), N (%)	953 (56.5)	713 (52.5)	0.02
Ethnicity (European), N (%)	1383 (74.4)	1089 (72.9)	0.36
Body mass index, mean (SD), kg/m^2	25.3 (3.3)	25.2 (3.4)	0.33
Birth and infant characteristics			
Boys, N (%)	1151 (48.9)	986 (50.7)	0.23
Breastfeeding duration, median (95% range), months	3.5 (0.0, 12.0)	3.5 (0.0, 12.0)	0.58
Introduction of solid foods (>6 months), N (%)	199 (11.3)	99 (9.8)	0.44
Childhood characteristics			
Age, mean (SD), years	9.8 (0.3)	9.8 (0.4)	0.02
Television watching time (≥2 hours/day), N (%)	575 (30.0)	477 (32.8)	0.09
Body mass index, mean (SD), kg/m^2	17.5 (2.6)	17.7 (2.9)	0.04
Total fat mass, median (95% range), g	8,451 (4,549, 21,235)	8,557 (4,505, 23,454)	0.26

[a] Values are observed data and represent means (SD), medians (95% range) or numbers of subjects (valid %). Differences were tested using Student's t-tests and Mann-Whitney tests for normally and non-normally distributed variables, respectively and χ^2-test for dichotomous variables. IOM, Institute of Medicine; SD, standard deviation.

Supplemental Table S2. Correlation coefficients between all measures of adiposity at 10 years (N=2,354).[a]

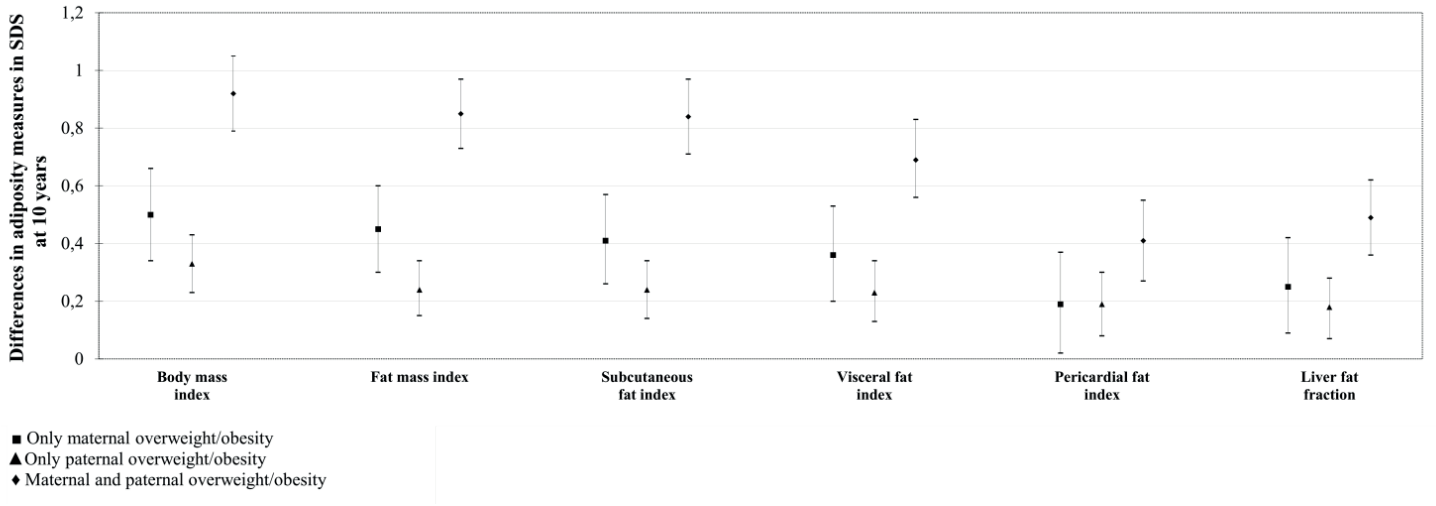
Measures of adiposity	Body mass index	Fat mass index	Subcutaneous fat index	Visceral fat index	Pericardial fat index	Liver fat fraction
Body mass index	1	0.81*	0.77*	0.58*	0.31*	0.38*
Fat mass index	0.81*	1	0.95*	0.69*	0.33*	0.42*
Subcutaneous fat index	0.77*	0.95*	1	0.74*	0.35*	0.45*
Visceral fat index	0.58*	0.69*	0.74*	1	0.47*	0.39*
Pericardial fat index	0.31*	0.33*	0.35*	0.47*	1	0.18*
Liver fat fraction	0.38*	0.42*	0.45*	0.39*	0.18*	1

[a] Values are Spearman correlation coefficients. *p-value <0.01

Supplemental Table S3. Maternal body mass index and childhood general and organ fat measures.[a]

	Measures of adiposity at 10 years in SDS					
	Body mass index (N=2,354)	Fat mass index (N=2,339)	Subcutaneous fat index (N=2,049)	Visceral fat index (N=2,052)	Pericardial fat index (N=2,123)	Liver fat fraction (N=2,319)
BMI (kg/m^2 in SDS)	0.35 (0.31, 0.39)*	0.32 (0.28, 0.36)*	0.30 (0.26, 0.34)*	0.25 (0.21, 0.29)*	0.12 (0.08, 0.16)*	0.18 (0.14, 0.22)*
Underweight (<18.5 kg/m^2)	-0.44 (-0.64, -0.24)*	-0.27 (-0.45, -0.08)*	-0.25 (-0.44, -0.05)**	-0.35 (-0.56, -0.15)*	-0.27 (-0.48, -0.06)**	-0.15 (-0.36, 0.05)
Normal weight (18.5 – 24.9 kg/m^2)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Overweight (25.0 – 29.9 kg/m^2)	0.53 (0.43, 0.63)*	0.48 (0.38, 0.57)*	0.47 (0.37, 0.57)*	0.36 (0.26, 0.47)*	0.15 (0.04, 0.26)*	0.25 (0.15, 0.35)*
Obesity (≥30.0 kg/m^2)	0.99 (0.83, 1.14)*	0.95 (0.81, 1.10)*	0.89 (0.73, 1.05)*	0.72 (0.55, 0.88)*	0.42 (0.25, 0.60)*	0.53 (0.37, 0.69)*

[a]Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal pre-pregnancy body mass index or for body mass index clinical groups as compared to the reference group (normal weight). Model includes child´s sex and age at outcome measurements (except for sex- and age-adjusted body mass index SDS). **p-value <0.05, *p-value <0.01. SDS; standard deviation scores.



Supplemental Figure S2. Parental body mass index and childhood general and organ fat measures (N=1,795). Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood outcomes in SDS for parental body mass index clinical groups as compared to the reference group (maternal and paternal normal weight). Model includes child´s sex and age at outcome measurements (except for sex- and age-adjusted body mass index SDS). SDS, standard deviation scores.

Supplemental Table S4. Maternal gestational weight gain and childhood general and organ fat measures.[a]

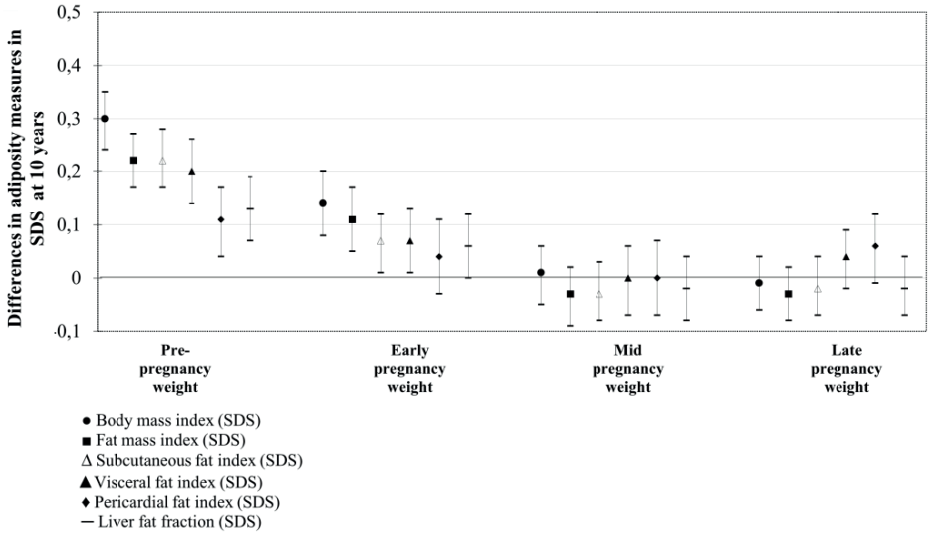
	Measures of adiposity at 10 years in SDS					
	Body mass index (N=1,462)	**Fat mass index (N=1,451)**	**Subcutaneous fat index (N=1,287)**	**Visceral fat index (N=1,288)**	**Pericardial fat index (N=1,336)**	**Liver fat fraction (N=1,444)**
Maximum weight gain per week (kg in SDS)	0.02 (-0.03, 0.07)	-0.03 (-0.08, 0.01)	-0.04 (-0.09, 0.01)	0.00 (-0.05, 0.06)	0.01 (-0.04, 0.07)	-0.02 (-0.07, 0.03)
Insufficient weight gain	-0.06 (-0.21, 0.08)	0.06 (-0.07, 0.19)	0.04 (-0.10, 0.17)	0.00 (-0.15, 0.15)	0.04 (-0.11, 0.19)	0.08 (-0.06, 0.22)
Sufficient weight gain	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Excessive weight gain	0.21 (0.10, 0.33)*	0.18 (0.07, 0.29)*	0.15 (0.04, 0.26)**	0.20 (0.08, 0.32)*	0.11 (-0.01, 0.23)	0.10 (-0.02, 0.21)

[a] Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal maximum weight gain per week or for IOM weight gain clinical groups as compared to the reference group (sufficient weight gain). Model includes child´s sex and age at outcome measurements (except for sex- and age-adjusted body mass index SDS). **p-value <0.05, *p-value <0.01. SDS; standard deviation scores.

Supplemental Table S5. Maternal weight gain until late pregnancy and childhood general and organ fat measures.[a]

	Measures of adiposity at 10 years in SDS[b]					
	Body mass index (N=1,316)	**Fat mass index (N=1,306)**	**Subcutaneous fat index (N=1,157)**	**Visceral fat index (N=1,158)**	**Pericardial fat index (N=1,207)**	**Liver fat fraction (N=1,301)**
Weight gain until late pregnancy per week (kg in SDS)	0.08 (0.03, 0.13)*	0.02 (-0.03, 0.07)	0.01 (-0.04, 0.06)	0.04 (-0.02, 0.09)	0.02 (-0.04, 0.08)	0.00 (-0.05, 0.06)
Insufficient weight gain	-0.13 (-0.28, 0.02)	-0.05 (-0.19, 0.08)	-0.02 (-0.16, 0.12)	-0.04 (-0.19, 0.12)	0.06 (-0.11, 0.22)	0.04 (-0.11, 0.18)
Sufficient weight gain	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Excessive weight gain	0.18 (0.06, 0.30)*	0.14 (0.03, 0.25)**	0.14 (0.02, 0.25)**	0.19 (0.06, 0.31)*	0.12 (-0.01, 0.25)	0.07 (-0.05, 0.19)
			MRI measures of adiposity at 10 years in SDS conditional on body mass index[c]			
			Subcutaneous fat index (N=1,157)	**Visceral fat index (N=1,158)**	**Pericardial fat index (N=1,207)**	**Liver fat fraction (N=1,301)**
Weight gain until late pregnancy per week (kg in SDS)			-0.09 (-0.14, -0.04)*	-0.01 (-0.07, 0.05)	0.00 (-0.06, 0.06)	-0.03 (-0.08, 0.02)
Insufficient weight gain			0.13 (0.00, 0.27)	0.06 (-0.10, 0.21)	0.11 (-0.06, 0.27)	0.10 (-0.05, 0.25)
Sufficient weight gain			*Reference*	*Reference*	*Reference*	*Reference*
Excessive weight gain			0.01 (-0.10, 0.12)	0.11 (-0.01, 0.24)	0.07 (-0.06, 0.20)	0.01 (-0.11, 0.13)

[a] Estimates are based on multiple imputed data. Model includes child´s sex and age at outcome measurements (except for sex- and age-adjusted body mass index SDS), maternal age, educational level, ethnicity, parity, smoking habits during pregnancy, and child's breastfeeding duration, timing of introduction of solid foods and television watching time. Models for weight gain until late pregnancy per week were additionally adjusted for pre-pregnancy body mass index. **p-value <0.05, *p-value <0.01. [b] Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal weight gain until late pregnancy per week or for IOM weight gain clinical groups as compared to the reference group (sufficient weight gain). [c] Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in the standardized residuals of the childhood outcomes (obtained by conditional regression analyses on body mass index at 10 years) per SDS change in maternal weight gain until late pregnancy per week or for IOM weight gain clinical groups as compared to the reference group (sufficient weight gain). SDS; standard deviation scores.



Supplemental Figure S3. Maternal pre-, early, mid, and late pregnancy weight with childhood general and organ fat measures (N=1,121). Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal pre-pregnancy weight and per change in standardized residuals of maternal early, mid, and late pregnancy weight obtained from conditional regression analyses. Model includes child´s sex and age at outcome measurements (except for sex- and age-adjusted body mass index SDS). SDS; standard deviation scores.

Chapter 3.1

Genome-wide association analysis identifies three new susceptibility loci for childhood body mass index

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Abstract

A large number of genetic loci are associated with adult body mass index. However, the genetics of childhood body mass index are largely unknown. We performed a meta-analysis of genome-wide association studies of childhood body mass index, using sex- and age-adjusted standard deviation scores. We included 35,668 children from 20 studies in the discovery phase and 11,873 children from 13 studies in the replication phase. In total, 15 loci reached genome-wide-significance (p-value $<5*10^{-8}$) in the joint discovery and replication analysis, of which 12 are previously identified loci in or close to *ADCY3*, *GNPDA2*, *TMEM18*, *SEC16B*, *FAIM2*, *FTO*, *TFAP2B*, *TNNI3K*, *MC4R*, *GPR61*, *LMX1B* and *OLFM4* associated with adult body mass index or childhood obesity. We identified three novel loci: rs13253111 near *ELP3*, rs8092503 near *RAB27B*, and rs13387838 near *ADAM23*. Per additional risk allele, body mass index increased 0.04 Standard Deviation Score (SDS) (Standard Error (SE) 0.007), 0.05 SDS (SE 0.008) and 0.14 SDS (SE 0.025), for rs13253111, rs8092503, and rs13387838, respectively. A genetic risk score combining all 15 SNPs showed that each additional average risk allele was associated with a 0.073 SDS (SE 0.011, p-value $=3.12*10^{-10}$) increase in childhood body mass index in a population of 1,955 children. This risk score explained 2% of the variance in childhood body mass index. This study highlights the shared genetic background between childhood and adult body mass index and adds three novel loci. These loci likely represent age-related differences in strength of the associations with body mass index.

Background

Childhood obesity is an important public health problem with severe consequences, including an increased risk of premature death.[1-5] Body mass index (BMI) has a strong genetic component with some reported heritability estimates being over 80%.[6-8] Large genome-wide association studies (GWAS) have revealed many genetic loci associated with BMI or adiposity in adults.[9-13] However, the genetic loci underlying BMI in children are less well known. The biological background of BMI may differ between children and adults. In addition, it may be that the relative contributions of the same genetic loci differ depending on age, for example due to different gene-environment-interactions or body fat distributions.[6,14,15] A limited number of loci has been identified to associate with dichotomous definitions of childhood obesity.[16-18] Also, the roles of specific known adult loci for BMI, such as *FTO* and *ADCY3*, have been described in children.[13,19] The age-specific effects are illustrated by longitudinal studies on the effects of the well-known adult BMI increasing risk allele of *FTO* with BMI throughout childhood.[15] It has been reported that adult BMI increasing risk allele is associated with lower BMI in infancy, an earlier adiposity rebound and a higher BMI from the age of five years onwards.[14,15,20] To date, studies did not present a large GWAS meta-analysis on the full spectrum of childhood BMI.[13,16-19]

To identify genetic loci influencing childhood BMI, we meta-analyzed 20 GWAS with a total of 35,668 children of European ancestry, combining data for around 2.5 million single nucleotide polymorphisms (SNPs) imputed to the HapMap imputation panel. We used as outcome sex- and age-adjusted standard deviation scores at the oldest age between 2 and 10 years.

Methods

Study populations

Characteristics of each discovery and replication study population can be found in Supplementary Table S1 and Supplementary Methods. The discovery analysis included 20 studies with an age range from 3 to 10 years: the Avon Longitudinal Study of Parents and Children (ALSPAC, 6,887 children), the Children's Hospital of Philadelphia (CHOP, 2,456 children), the Copenhagen Studies on Asthma in Childhood 2000 birth cohort (COPSAC2000, 309 children), the Danish National Birth Cohort (DNBC, 1,020 children), the Generation R Study (GenerationR, 2,226 children), the GOYA Study (GOYA, 199 children), the Helsinki Birth Cohort Study (HBCS, 1,674 children), the INfancia y Medio Ambiente Project (INMA, 756 children), the Leipzig study (Leipzig, 555 children), the Lifestyle –

Immune System – Allergy Study plus German Infant Study on the influence of Nutrition Intervention (LISA+GINI, 1,147 children), the Manchester Asthma and Allergy Study (MAAS, 801 children), the Norwegian Mother and Child Cohort Study (MoBa, 126 children), the Northern Finland Birth Cohort 1966 (NFBC 1966, 3,948 children), the Northern Finland Birth Cohort 1986 (NFBC 1986, 4,000 children), the Netherlands Twin Register (NTR, 1,810 children), the Physical Activity and Nutrition in Children Study (PANIC, 423 children), the Western Australian Pregnancy Cohort (Raine) Study (Raine, 1,458 children), the Special Turku coronary Risk factor Intervention Project (STRIP, 569 children), the Young Finns Study (YFS, 1,134 children), the British 1958 Birth Cohort Study, with two subcohorts which were entered into the meta-analysis separately (1958BC-T1DGC, 1,974 children, and 1958BC-WTCCC2, 2,196 children).

We included 13 replication studies. Eleven of these were cohort studies: 574 children from the Copenhagen Studies on Asthma in Childhood 2010 birth cohort (COPSAC2010), 676 additional children from the DNBC, 386 additional children from LISA+GINI, 3,152 children from the TEDS Study, 1,955 children from the Prevention and Incidence of Asthma and Mite Allergy birth cohort study (PIAMA), 1,665 children from the BREATHE Study, 447 children from the Bone Mineral Density in Childhood Study (BMDCS), 200 children from the TEENs of Attica: Genes and Environment (TEENAGE) study, additional imputed data on 857 children from the Leipzig Study, 480 additional children from PANIC, and additional imputed data for 569 children from STRIP. We also included two obesity case-control studies in the replication: the Danish Childhood Obesity Biobank (306 cases, 158 controls) and the French Young Study (304 cases, 144 controls). In the BREATHE Study, information was available about six SNPs only (rs8046312, rs12429545, rs13130484, rs3845265, rs543874, rs8084077).

All included children were of European ethnic origin. Sex- and age-adjusted standard deviation scores were created for BMI at the latest time point (oldest age, if multiple measurements existed) between 2 and 10 years using the same software across all studies (LMS growth; Pan H, Cole TJ, 2012. Available from: http://www.healthforallchildren.co.uk). Syndromic cases of obesity and children of non-European ethnic origin were excluded. In the case of twin pairs, only one twin was included, either randomly or based on genotyping or imputation quality.

Statistical approach

Cohort-specific genome-wide association analyses were first run in the discovery cohorts, using high-density Illumina or Affymetrix SNP arrays, followed by imputation to the HapMap CEU release 22 imputation panel. The MAAS study imputed to the combined 1000 Genomes

(1000G) Pilot + HapMap 3 (release June 2010/Feb 2009) panel. Before imputation, studies applied study-specific quality filters on samples and SNP call rate, minor allele frequency and Hardy-Weinberg disequilibrium (see Supplementary Table S1 for details). Leipzig (discovery sample), NFBC1986, STRIP (discovery sample), and PANIC (discovery sample) contributed unimputed data from the Metabochip. Linear regression models assuming an additive genetic model were run in each study, to assess the association of each SNP with SDS-BMI, adjusting for principal components if this was deemed needed in the individual studies. As SDS-BMI is age- and sex-specific, no further adjustments were made. Before the meta-analysis, we applied quality filters to each study, filtering out SNPs with a minor allele frequency below 1% and SNPs with poor imputation quality (MACH r2_hat ≤0.3, IMPUTE proper_info ≤0.4 or info ≤0.4). For studies contributing unimputed metabochip data to the discovery analysis, we excluded SNPs with a SNP call rate <0.95 or with a Hardy Weinberg Equilibrium p-value of ≤0.00001. We performed fixed effects inverse-variance weighted meta-analysis of all discovery samples using Metal.[21] Genomic control was applied to every study before the meta-analysis. Individual study lambdas ranged from 0.985 to 1.077 (Supplementary Table S2). The lambda of the discovery meta-analysis was 1.10. After the meta-analysis, SNPs for which information was available in only one study were removed.

The final dataset consisted of 2,499,691 autosomal SNPs. The most significant SNP for each of 43 genome-wide significant or suggestive loci (p-value $<5*10^{-6}$) was taken forward for replication in 13 replication cohorts. A locus was defined as a region 500 kb to either side of the most significant SNP. All replication cohorts had *in silico* data available. One of them only had non-imputed data (BREATHE), two (TEENAGE and TEDS) had data imputed to HapMap release 22, one cohort (PANIC) used exome chip data and the other nine performed imputation to 1000G. The replication samples of the STRIP and Leipzig studies only contributed 20 and 21 imputed SNPs, respectively, as the unimputed SNPs were part of the discovery analysis). Fixed effects inverse-variance meta-analysis was performed for these 43 SNPs combining the discovery samples and all replication samples, giving a joint analysis beta, standard error and p-value (Table 1 and Supplementary Table S2).

Sensitivity analyses

Allele frequency differences between the discovery and the replication samples were small and stayed within a range of seven percentage points for all SNPs, except for rs1573972, which had a minor allele frequency of 9% in the discovery analysis and 28% in the replication analysis. This was likely due to the inclusion of one study (MAAS) that had imputed to the combined HapMap+1000G panel, whereas all other studies with imputed data had imputed to HapMap. To increase homogeneity, we performed several sensitivity analyses. First, we reran the discovery meta-analysis excluding the MAAS study. This analysis

Table 1. Results of the discovery, replication and joint analyses for 43 loci with p-values <5*10^{-6} in the discovery phase

SNP	CHR	Position	Nearest gene	EA / Non-EA	EAF[a]	Beta[a]	SE[a]	p-value discovery	p-value replication	p-value joint
rs13130484[b]	4	44870448	*GNPDA2*	T/C	0.44	0.067	0.007	8.94*10^{-11}	4.29*10^{-18}	**1.58*10^{-23}**
rs11676272[b]	2	24995042	*ADCY3*	G/A	0.46	0.068	0.007	8.55*10^{-23}	0.020	**7.12*10^{-23}**
rs4854349[b]	2	637861	*TMEM18*	C/T	0.83	0.090	0.009	6.00*10^{-21}	0.005	**5.41*10^{-22}**
rs543874[b]	1	176156103	*SEC16B*	G/A	0.20	0.077	0.009	2.38*10^{-17}	8.77*10^{-4}	**2.20*10^{-19}**
rs7132908[b]	12	48549415	*FAIM2*	A/G	0.39	0.066	0.008	4.99*10^{-19}	0.043	**1.57*10^{-18}**
rs1421085[b]	16	52358455	*FTO*	C/T	0.41	0.059	0.007	3.20*10^{-19}	0.654	**4.53*10^{-16}**
rs12429545[c]	13	53000207	*OLFM4*	A/G	0.13	0.076	0.010	3.66*10^{-11}	1.01*10^{-4}	**2.08*10^{-14}**
rs987237[b]	6	50911009	*TFAP2B*	G/A	0.19	0.062	0.009	3.81*10^{-13}	0.224	**1.80*10^{-12}**
rs12041852[b]	1	74776088	*TNNI3K*	G/A	0.46	0.046	0.007	1.77*10^{-10}	0.142	**2.28*10^{-10}**
rs6567160[b]	18	55980115	*MC4R*	C/T	0.23	0.050	0.008	4.06*10^{-12}	0.996	**1.21*10^{-9}**
rs13253111	8	28117893	*ELP3*	A/G	0.57	0.042	0.007	4.13*10^{-9}	0.114	**4.89*10^{-9}**
rs8092503	18	50630485	*RAB27B*	G/A	0.27	0.045	0.008	8.55*10^{-8}	0.034	**8.17*10^{-9}**
rs3829849[b]	9	128430621	*LMX1B*	T/C	0.36	0.041	0.007	1.46*10^{-6}	0.001	**8.81*10^{-9}**
rs13387838	2	206989692	*ADAM23*	A/G	0.04	0.139	0.025	2.40*10^{-8}	0.306	**2.84*10^{-8}**
rs7550711[d]	1	109884409	*GPR61*	T/C	0.04	0.105	0.019	1.50*10^{-8}	0.401	**4.52*10^{-8}**
rs17309930[b]	11	27705069	*BDNF*	A/C	0.21	0.045	0.009	2.47*10^{-8}	0.540	1.41*10^{-7}
rs2590942[b]	1	72657869	*NEGR1*	T/G	0.82	0.047	0.009	3.88*10^{-9}	0.966	1.91*10^{-7}
rs13107325[b]	4	103407732	*SLC39A8*	T/C	0.07	0.081	0.016	1.19*10^{-8}	0.970	3.79*10^{-7}
rs10151686[b]	14	29536217	*PRKD1*	A/G	0.04	0.096	0.019	1.50*10^{-6}	0.109	6.99*10^{-7}
rs25832	5	66211438	*LOC375449*	A/G	0.71	0.039	0.008	2.41*10^{-6}	0.177	1.62*10^{-6}
rs7869969	9	95257268	*FAM120A*	G/A	0.33	0.036	0.008	4.43*10^{-7}	0.425	1.68*10^{-6}
rs11079830[c]	17	44037629	*HOXB6*	A/G	0.58	0.034	0.007	1.43*10^{-6}	0.254	1.98*10^{-6}
rs4569924	5	153520218	*GALNT10*	T/C	0.43	0.032	0.007	4.06*10^{-7}	0.823	3.48*10^{-6}

Table 1. *(Continued)*

SNP	CHR	Position	Nearest gene	EA / Non-EA	EAF[a]	Beta[a]	SE[a]	p-value discovery	p-value replication	p-value joint
rs8046312[b]	16	19886835	*GPR139*	A/C	0.81	0.042	0.009	$4.06*10^{-10}$	0.185	$3.97*10^{-6}$
rs1838856	2	113822060	*PAX8*	A/C	0.46	0.034	0.008	$1.85*10^{-6}$	0.588	$1.47*10^{-5}$
rs633143	1	179716108	*CACNA1E*	T/C	0.14	0.044	0.011	$3.40*10^{-6}$	0.648	$2.44*10^{-5}$
rs4923207	11	24713901	*LUZP2*	T/G	0.79	0.039	0.010	$1.60*10^{-6}$	0.834	$3.52*10^{-5}$
rs6971577	7	140350204	*MRPS33*	C/G	0.78	0.036	0.009	$1.17*10^{-6}$	0.690	$6.80*10^{-5}$
rs10866069	3	64366964	*ADAMTS9*	T/C	0.17	0.041	0.011	$3.05*10^{-6}$	0.687	$8.43*10^{-5}$
rs12457682	18	7216505	*LAMA1*	C/A	0.23	0.035	0.009	$3.81*10^{-6}$	0.942	$9.01*10^{-5}$
rs11165675[b]	1	96812556	*PTBP2*	A/G	0.27	0.031	0.008	$2.93*10^{-6}$	0.520	$1.01*10^{-4}$
rs12096993	1	217931859	*SLC30A10*	T/C	0.27	0.031	0.008	$1.38*10^{-6}$	0.583	$1.02*10^{-4}$
rs760931	1	1637388	*CDC2L1*	C/G	0.93	0.103	0.027	$3.15*10^{-6}$	0.160	$1.27*10^{-4}$
rs2968990	4	131098524	*C4orf33*	C/T	0.37	0.028	0.007	$1.67*10^{-6}$	0.487	$1.42*10^{-4}$
rs1247117	10	120418792	*C10orf46*	G/A	0.11	0.040	0.011	$3.47*10^{-6}$	0.339	$2.62*10^{-4}$
rs6580706	12	47959818	*TUBA1C*	C/G	0.34	0.031	0.009	$1.83*10^{-8}$	0.047	$3.68*10^{-4}$
rs8092620	18	41433991	*SLC14A2*	G/T	0.48	0.024	0.007	$3.31*10^{-6}$	0.199	$7.32*10^{-4}$
rs188584	3	62675007	*CADPS*	C/A	0.77	0.028	0.008	$3.23*10^{-6}$	0.139	0.001
rs4870949	8	126704776	*TRIB1*	T/C	0.07	0.164	0.054	$3.13*10^{-10}$	0.061	0.002
rs1573972	4	171559399	*AADAT*	C/T	0.19	0.030	0.013	$3.78*10^{-6}$	0.232	0.020
rs214821	20	2258291	*TGM3*	T/C	0.02	0.479	0.208	$3.38*10^{-6}$	0.575	0.021
rs8084077	18	49532928	*DCC*	T/C	0.73	0.014	0.008	$3.47*10^{-6}$	$2.72*10^{-5}$	0.062
rs3845265	18	63690108	*DSEL*	G/A	0.71	0.013	0.008	$8.86*10^{-7}$	$1.44*10^{-6}$	0.087

[a] From joint analysis. [b] Locus previously reported in[11]. [c] Locus previously reported in[11,16]. [d] Locus previously reported in[9,11]. CHR; Chromosome, EA; Effect Allele, EAF; Effect Allele Frequency, SE; Standard Error

did not materially change our findings, with one additional SNP (rs10055577) reaching the subthreshold level of significance (p-value =1.10*10^{-6}) and five SNPs (rs4870949, rs1838856, rs633143, rs10866069, and rs1573972) losing significance. None of these five SNPs had replicated in the primary analysis. Second, we reran the replication and joint meta-analysis including only those cohorts that imputed to 1000G. Results of this analysis were very similar to the primary analysis, with two additional replicated SNPs, rs17309930 near *BDNF* and rs13107325 in *SLC39A8*. Both of these are known loci for adult BMI.[11,13] Third, we reran the replication including only the HapMap-imputed and unimputed studies (TEDS, TEENAGE, and BREATHE). The results were very similar to those using all studies, with rs4870949 and rs2590942 now passing the significance threshold and rs8092503 and rs3829849 now just above it (results not shown). Rs1573972 was not replicated in any of the analyses. As results of the third and fourth sensitivity analyses were very similar to those including all replication cohorts, we used the latter as our main analysis for reasons of power.

Genetic risk score and percentage of variance explained

A weighted risk score was computed as the sum of the number of SDS-BMI-increasing alleles (dosage) weighted by the effect sizes from the discovery meta-analysis. Then, the score was rescaled to range from zero to the maximum number of SDS-BMI increasing alleles (30 alleles for 15 SNPs) and rounded to the nearest integer. The association of the risk score with SDS-BMI was assessed in one of the largest replication cohorts (PIAMA, N=1,955) by running a linear regression model. The variance in SDS-BMI explained by the risk score was estimated by the unadjusted R^2 of this model. The percentage of variance in adult BMI explained by the 15 SNPs was calculated using the published data from the recently published large meta-analysis of GWAs studies on adult BMI.[11] For each SNP, the variance explained was calculated as: 2*(adult effect size^2)*MAF*(1-MAF) and these variances were then summed to give the total percentage of variance in adult BMI explained by the 15 SNPs.[11,22]

LD score regression

LD score regression was used with the standard settings.[23] Changing the minor allele frequency filter from 0 to 0.05 did not change the results. Therefore, we report the results of the unfiltered analysis only.

eQTL analysis

eQTL analysis was conducted using most significant SNP from each of the 15 genome-wide significant loci from the joint analysis. There was no linkage disequilibrium between these SNPs. First, we assessed whether the top SNPs or their proxies, identified on the basis of R^2 >0.7, were associated with gene expression in whole-blood cells in a sample of 5,311 individuals.[24] Expression in this dataset was assessed using Illumina Whole-Genome Expression BeadChips (HumanHT-12). eQTLs were deemed *cis* when the distance between the SNP chromosomal position and the probe midpoint was less than 250 kb. eQTLs were mapped using Spearman's rank correlation, using imputation dosage values as genotypes. An FDR p-value of <0.05 was considered significant. Second, the 15 SNPs were introduced to the online eQTL database Genevar (www.sanger.ac.uk/resources/software/genevar) to explore their associations with expression transcripts of genes in proximity (<1 Mb distance) to the SNP in adipose tissue from 856 healthy female twins of the MuTHER resource.[25,26] We used Bonferroni correction for the significance threshold (p-value <0.003).

Data-driven Expression Prioritized Integration for Complex Traits (DEPICT)[27]

DEPICT was run using SNPs with p-value $<10^{-5}$ as yielding 56 independent DEPICT loci comprising 100 genes. DEPICT was run using default settings, that is using 500 permutations for bias adjustment, 20 replications for false discovery rate estimation, normalized expression data from 77,840 Affymetrix microarrays for gene set reconstitution (see reference 43 for details), 14,461 reconstituted gene sets for gene set enrichment analysis, and testing 209 tissue/cell types assembled from 37,427 Affymetrix U133 Plus 2.0 Array samples for enrichment in tissue/cell type expression.[27,28]

Results

Study characteristics are shown in Supplementary Table S1. Childhood BMI was transformed into sex- and age-adjusted standard deviation scores (SDS) (LMS growth; Pan H, Cole TJ, 2012. http://www.healthforallchildren.co.uk).

Meta-analysis of genome-wide association studies

Inverse-variance weighted fixed-effects meta-analysis revealed 861 SNPs with genome-wide significant or suggestive p-values ($<5*10^{-6}$). Two SNPs with high heterogeneity were not followed up (I^2 values of 89.4 and 96.0), leaving 859 SNPs representing 43 loci. A

locus was defined as a region of 500 kb to either side of the most significant SNP. The Manhattan and Quantile-Quantile plots of the discovery meta-analysis are shown in Figure 1 and Supplementary Figure S1, respectively. The lambda for the discovery meta-analysis was 1.10. LD score regression analysis showed that this slight inflation was mainly due to polygenicity of the trait, rather than to population stratification, cryptic relatedness or other confounding factors (intercept 1.01). Individual study lambdas are shown in Supplementary Table S2. All 43 loci were taken forward for replication in a sample of 11,873 children from 13 studies. Table 1 and Supplementary Tables S3 and S4 show the results of the discovery, replication, and joint analyses for the 43 genome-wide and suggestive loci.

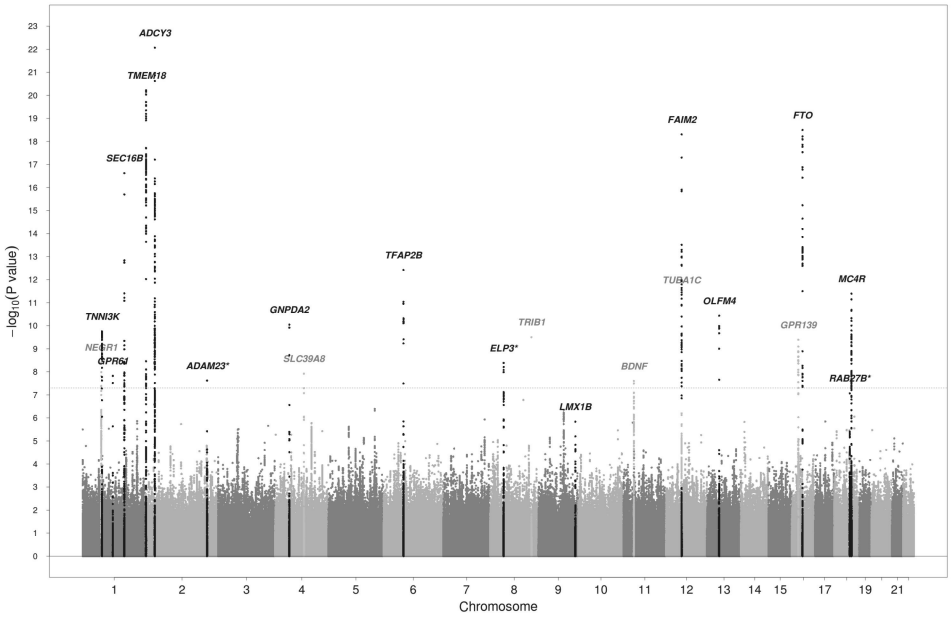

Figure 1. Manhattan plot of results of the discovery meta-analysis of 20 studies. Chromosomes are shown on the x-axis, the $-\log_{10}$ of the p-value on the y-axis. The grey dotted line represents the genome-wide significance cutoff of $5*10^{-8}$. Genes shown in black are the known loci that were significantly associated with childhood BMI in the joint discovery and replication analysis. Genes shown in grey were significant in the discovery, but not in the joint discovery and replication analysis. * indicates novel loci that were significantly associated with childhood BMI in the joint discovery and replication analysis. See also Table 1.

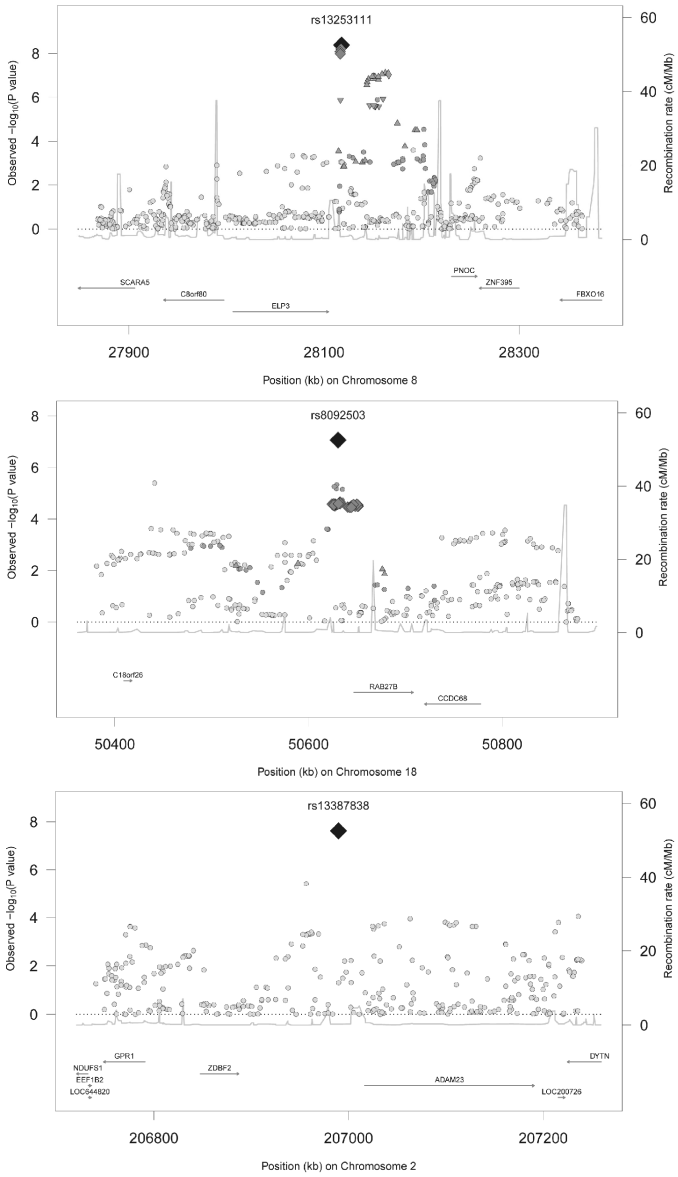

Figure 2. Regional plots of the three novel loci for childhood BMI. On the x-axis the position of SNPs on the chromosome is shown. On the left y-axis is the $-\log_{10}$ of the p-values from the discovery analysis, on the right y-axis is the estimated recombination rate (from HapMap), shown by the light grey line in the figure. The named SNP is the most significant SNP in the locus from the discovery meta-analysis. The linkage disequilibrium of all SNPs with the most significant SNP is shown by the symbols, with dark grey diamonds indicating an R^2 of ≥0.8, inversed dark grey triangles indicating an R^2 of 0.6-0.8, dark grey triangles indicating an R^2 of 0.4-0.6, dark grey circles indicating an R^2 of 0.2-0.4, and light grey circles indicating an R^2 of 0-0.2. Genes (from HapMap release 22) are plotted below the x-axis.

In total, 15 of these reached genome-wide significance in the joint analysis. Twelve out of these 15 had been reported previously for related phenotypes. SNPs in or close to *ADCY3*, *GNPDA2*, *TMEM18*, *SEC16B*, *FAIM2*, *FTO*, *TFAP2B*, *TNNI3K*, *MC4R*, *GPR61*, *LMX1B* and *OLFM4* are associated with adult BMI or childhood obesity.[11,13,16] We identified three novel loci: rs13253111 near *ELP3*, rs8092503 near *RAB27B*, and rs13387838 near *ADAM23*. Per additional risk allele, BMI increased 0.04 Standard Deviation Score (SDS) (Standard Error (SE) 0.007), 0.05 SDS (SE 0.008) and 0.14 SDS (SE 0.025), for rs13253111, rs8092503, and rs13387838, respectively. Figure 2 and Supplementary Figure S2 show the regional plots and the forest plots, respectively, for these loci.

Genetic risk score

We combined the 15 identified genome-wide significant SNPs into a genetic risk score that summed the number of BMI-increasing alleles weighted by their betas from the discovery analysis and rescaled to a range of 0 to 30, which is the maximum number of risk alleles. The risk score was associated with childhood BMI (p-value =3.12*10^{-10}) in 1,955 children from the PIAMA Study, one of our largest replication cohorts. For each additional average risk allele in the score, childhood BMI increased by 0.073 SDS (SE 0.011) (Figure 3). This risk score explained 2.0% of the variance in childhood BMI.

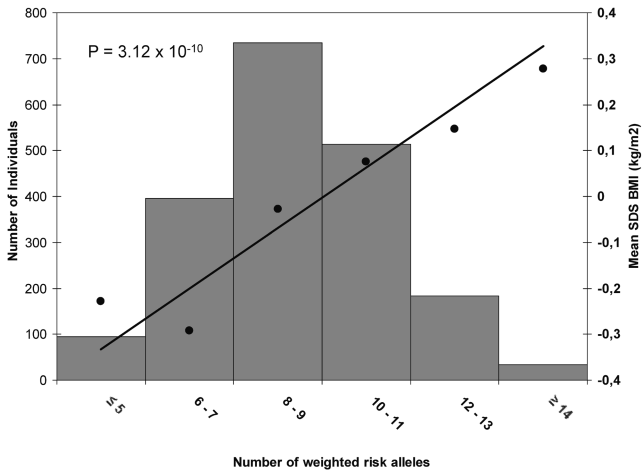

Figure 3. Association of the weighted risk score with BMI. Along the x axis, categories of the weighted risk score are presented, the mean standard deviation score (SDS)-BMI per group is shown on the right y axis, with the line representing the regression of the mean SDS-BMI values on the categories of the weighted risk score. The left y axis represents the number of children in each risk score category, shown in the histogram. The p-value is derived from the analysis of the continuous risk score. Analysis was performed in the PIAMA Study (N=1,955).

Associations with adult body mass index and childhood obesity

The genetic correlation between childhood BMI and adult BMI was 0.73. A lookup of the 15 SNPs associated with childhood BMI in a recently published GWAS meta-analysis on adult BMI in more than 300,000 participants, revealed that all SNPs showed evidence for association were nominally significantly associated with adult BMI, with p-values of 0.005, $5.76*10^{-5}$ and 0.003 for the novel SNPs rs13253111, rs8092503 and rs13387838, respectively. Also, the direction of the effect estimates for all 15 SNPs was the same in children and adults (Supplementary Table S5).[11] The 15 SNPs found in this study explained 0.94% of the variance in adult BMI in the GIANT consortium.[11]

A reverse lookup in our dataset of the 97 known genome-wide significant loci previously reported to be associated with adult BMI showed that 22 out of the 97 loci were significantly associated with childhood BMI index, using a Bonferroni-adjusted p-value cutoff of $5.2*10^{-4}$ for 97 SNPs. A total of 50 out of the 97 known adult BMI SNPs were nominally associated with childhood BMI (p-value <0.05). The direction of the effect estimates was the same in adults and children for 86 SNPs (p-value binomial sign test $<1.0*10^{-4}$; Supplementary Table S6).

We looked up the association of the three novel loci in a GWAS meta-analysis of childhood obesity. In this study, childhood obesity cases were defined as having a BMI ≥95th percentile, whereas childhood normal weight controls were defined as having a BMI <50th percentile. This meta-analysis included 22 studies, of which 16 were also included in our current meta-analysis. All three SNPs were associated with childhood obesity (p-values 0.01, 0.005, and $6.0*10^{-4}$ for rs13253111, rs8092503, and rs13387838, respectively).[16]

Functional analysis

To explore functionality, we first analyzed if the 15 identified SNPs affect messenger RNA expression (eQTLs). We analyzed eQTLs from peripheral blood samples from 5,311 individuals, which revealed two *cis*-eQTLs (false discovery rate (FDR) p-value <0.05) for rs11676272, the top SNP in one of the previously identified loci (*ADCY3*). One of these eQTLs was for *ADCY3*, and one was for *DNAJC27*.[24] Also, we found a *cis*-eQTL for *FAM125B* for rs3829849, which is located in *LMX1B* (Supplementary Table S7). eQTL analysis in adipose tissue, a more specific target tissue in relation to BMI, from 856 healthy female twins in the MuTHER resource in Genevar revealed two significant *cis*-eQTLs (distance to SNP <1 Mb) for rs11676272, for transcripts of *ADCY3* and *POMC*, with a Bonferroni-corrected p-value of <0.003.[25,26] The association of rs11676272 with expression of *ADCY3* was also validated in a second eQTL analysis in a smaller set of 206 lymphoblastoid cell lines.[29] We did not identify eQTLs related to our three novel loci.

Second, we performed functional analyses with the tool Data-Driven Expression Prioritized Integration for Complex Traits (DEPICT) using all SNPs with a p-value $<1*10^{-5}$ in the discovery analysis (see Materials and methods for details).[30] Gene prioritization analysis did not show prioritized genes, nor did the gene set enrichment analysis reveal evidence for enriched reconstituted gene sets and genes near the associated SNPs were not found to enrich for expression in a panel of 2009 tissue and cell types (FDR <0.05; Supplementary Tables S8a, b and c).

Discussion

In this GWAS meta-analysis of childhood BMI among more than 47,000 children, we identified 15 genome-wide significant loci, of which three loci, rs13253111 near *ELP3*, rs8092503 near *RAB27B*, and rs13387838 near *ADAM23*, have not been associated with adiposity related phenotypes before.

Large GWAS have revealed many genetic loci associated with BMI or adiposity in adults.[9-13] A recent meta-analysis in up to 339,224 individuals identified 97 BMI-associated loci, explaining 2.7% of the adult BMI variation. Pathway analyses showed that the central nervous system may play a large role in obesity susceptibility. The number of identified loci associated with BMI or obesity in childhood is scarce. Of the total of 15 loci associated with childhood BMI in the current study, 12 have previously been associated with adiposity outcomes in adults or children. All 12 loci are known to be associated with adult BMI.[11] Also, eight loci, including those in or near *ADCY3* (annotated to the nearby gene *POMC* in the previous paper), *TMEM18*, *SEC16B*, *FAIM2*, *FTO*, *TNNI3K*, *MC4R* and *OLFM4*, have previously been associated with childhood obesity.[16] All three novel loci were nominally associated with the more extreme outcome of childhood obesity in a largely overlapping population of child cohorts.[16]

A recent meta-analysis of two studies showed that the known loci *FTO*, *MC4R*, *ADCY3*, *OLFM4* are associated with BMI trajectories in childhood.[31] Their findings also suggested that a locus annotated to *FAM120AOS* influences childhood BMI, which could not be replicated in the current study. The lead SNP in this locus, rs944990, had a p-value of $1.61*10^{-5}$ in the current analysis. These findings suggest that the overlap between the genetic background of childhood and adult BMI is relatively large, but not complete.

Rs7550711 represents one of the 12 identified loci known to be associated with BMI or obesity in adults and children. Rs7550711 is a proxy for rs17024258 and rs17024393 (R^2=0.8 with both SNPs), which have previously been associated with adult obesity and

BMI, respectively, and annotated with the *GNAT2* gene. However, our proxy resides in *GPR61*, G protein-coupled receptor 61, the biology of which may be more relevant to BMI. Gpr61-deficient mice are obese and have hyperphagia, suggesting the role of Gpr61 in food intake regulation.[32] Further studies, including expression studies in relevant human tissues, are needed to establish the causal genes underlying this association.

We identified three loci, rs13253111 near *ELP3*, rs8092503 near *RAB27B*, and rs13387838 near *ADAM23*, which have not been associated with adiposity related phenotypes before in adulthood or childhood. The nearest genes to the novel loci have varying functions. *ELP3*, Elongator Acetyltransferase Complex, subunit 3, has a potential role in the migration of cortical projection neurons and in paternal demethylation after fertilization in mice.[33-35] *RAB27B*, RAS-associated protein RAB27B, encodes a membrane-bound protein with a role in secretory vesicle fusion and trafficking. It has been associated with pituitary hormone secretion, regulation of exocytosis of digestive enzyme containing granules from pancreatic acinar cells, and with gastric acid secretion.[36-38] Expression of *ADAM23*, A Disintegrin And Metalloproteinase Domain 23, may influence tumor progression and brain development.[39,40] It has also been described to be expressed in mouse adipose tissue and to have a potential role in adipogenesis *in vitro*.[41]

Two of our novel loci, rs13253111 near *ELP3* and rs13387838 near *ADAM23*, are close to rs4319045 and rs972540, respectively. Both these SNPs were reported as subthreshold results in the GWAS meta-analysis on adult BMI.[11] However, the linkage disequilibrium between the SNPs in both pairs is very low ($R^2 \leq 0.1$ for both) suggesting that these SNPs may represent different signals. It is important to note that, although both SNPs reached genome-wide significance in the joint discovery and replication analysis, the p-values in the replication stage were non-significant. This lack of significance may be due to the smaller sample size and lower power. Also, the joint p-values were slightly higher than the discovery p-values. Heterogeneity between the discovery and the replication stages was low to moderate, with I^2 values of 61.1 and 27.8 for rs13253111 and rs13387838, respectively (p-values >0.01 for both). These two signals need to be interpreted with some caution and further studies with larger sample sizes are needed to fully clarify the role of variants in these regions in the physiology of BMI.

Functional analysis showed *cis*-eQTLs for the lead SNPs in two of the known loci. Rs11676272 was associated with eQTLs in *ADCY3* and *DNAJC27*, also known as *RBJ*. Both these genes have been associated with adult BMI before and the association of rs11676272 with expression of *ADCY3* has been previously described in childhood BMI.[11,13,42] Rs3829849 was associated with an eQTL in *FAM125B*, or *MVB12B*, multivesicular body subunit 12B. This gene encodes a component of ESCRT-I (endosomal sorting complex required for transport

I), a plasma membrane complex with a role in vesicular trafficking, was recently described to be associated with intra-ocular pressure.[43] However, the LD of our SNPs with the peak markers for the *DNAJC27* (R^2=0.11) and the *FAM125B* (R^2=0.03) transcripts was low. Our analysis using DEPICT did not show enriched gene sets. This may reflect the relatively limited sample size in our analysis. Further studies are needed to determine the potential functional impact of all SNPs associated with childhood BMI.

Using LD score regression analysis with our meta-analysis results and the results from the recently published GWAs meta-analysis on adult BMI as input, we found that the genetic correlation between childhood and adult BMI was high.[11,23] The variance in adult BMI explained by the 15 SNPs identified in this study was lower than in children. The novel SNPs reported in this study may represent loci that specifically influence childhood BMI, but not adult BMI. An alternative explanation is that the effect sizes of these loci may be larger in children than in adults, which may explain the discovery in childhood studies but not in adult studies.[11] The large overlap between childhood and adult BMI loci suggests that many of these loci may not represent childhood-specific effects, but rather involvement of the same loci with differential effect sizes at different ages. Age-specific effects of genetic variants associated with BMI in children have been described for the *FTO* locus.[15] However, longitudinal studies with multiple measurements of BMI are needed to confirm and quantify such varying effects with age. In discussing the genetic overlap between childhood and adult BMI, it needs to be noted that, because of the differences in body proportions and body fat distribution, childhood BMI may be a different phenotype as compared to adult BMI. Our outcome was the conventional measure of BMI calculated as weight/height^2. Especially in early childhood, higher orders of magnitude for height may be more appropriate. Results from a previously published GWAS study on childhood BMI in two of the cohorts included in the current meta-analysis suggest that the results for SNPs close to *ADCY3* are different when higher orders of magnitude for height are being used.[37] Further studies are needed to identify loci related to more specific and directly assessed measures of adiposity and body fat distribution in young children.

In conclusion, we identified 15 loci associated with childhood BMI, of which three are novel. Our results highlight a considerable shared genetic background between childhood and adult BMI. The novel BMI-related loci may reflect childhood-specific genetic associations or differences in strength of associations between age groups.

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Supplement

Supplementary Table S1 can be found online.

Supplementary Table S2. Individual study lambdas

Study name	Lambda
ALSPAC	1.029
CHOP	0.995
COPSAC2000	1.008
DNBC	0.998
Generation R	1.045
GOYA	1.003
HBCS	1.010
INMA	1.015
Leipzig	1.042
LISAGINI	1.005
MAAS	1.004
MoBa	1.012
NFBC1966	1.048
NFBC1986	1.063
NTR	1.009
PANIC	1.077
Raine	1.018
STRIP	0.985
YFS	0.995
1958BC-T1DGC	1.003
1958 BC-WTCCC2	0.999

Supplementary Table S3. Results of the discovery, replication and combined analyses for all 43 loci with p-values <5*10^{-6} in the discovery phase

						Discovery analysis				Replication analysis			Joint analysis		
SNP	CHR	Position	Nearest gene	EA/ Non-EA	EAF[a]	Beta	SE	p-value	Hetero-geneity p-value	Beta	SE	p-value	Beta	SE	p-value
rs13130484[b]	4	44870448	*GNPDA2*	T/C	0.44	0.051	0.008	8.94*10^{-11}	0.996	0.116	0.013	4.29*10^{-18}	0.067	0.007	**1.58*10^{-23}**
rs11676272[b]	2	24995042	*ADCY3*	G/A	0.46	0.077	0.008	8.55*10^{-23}	0.395	0.036	0.015	0.020	0.068	0.007	**7.12*10^{-23}**
rs4854349[b]	2	637861	*TMEM18*	C/T	0.83	0.097	0.010	6.00*10^{-21}	0.810	0.059	0.021	0.005	0.090	0.009	**5.41*10^{-22}**
rs543874[b]	1	176156103	*SEC16B*	G/A	0.20	0.082	0.010	2.38*10^{-17}	0.210	0.059	0.018	8.77*10^{-4}	0.077	0.009	**2.20*10^{-19}**
rs7132908[b]	12	48549415	*FAIM2*	A/G	0.39	0.077	0.009	4.99*10^{-19}	0.440	0.031	0.015	0.043	0.066	0.008	**1.57*10^{-18}**
rs1421085[b]	16	52358455	*FTO*	C/T	0.41	0.073	0.008	3.20*10^{-19}	1.45*10^{-4}	0.007	0.016	0.654	0.059	0.007	**4.53*10^{-16}**
rs12429545[c]	13	53000207	*OLFM4*	A/G	0.13	0.075	0.011	3.66*10^{-11}	0.106	0.083	0.021	1.01*10^{-4}	0.076	0.010	**2.08*10^{-14}**
rs987237[b]	6	50911009	*TFAP2B*	G/A	0.19	0.071	0.010	3.81*10^{-13}	0.719	0.024	0.020	0.224	0.062	0.009	**1.80*10^{-12}**
rs12041852[b]	1	74776088	*TNNI3K*	G/A	0.46	0.053	0.008	1.77*10^{-10}	0.240	0.023	0.015	0.142	0.046	0.007	**2.28*10^{-10}**
rs6567160[b]	18	55980115	*MC4R*	C/T	0.23	0.065	0.009	4.06*10^{-12}	0.398	1.00*10^{-4}	0.018	0.996	0.050	0.008	**1.21*10^{-9}**
rs13253111	8	28117893	*ELP3*	A/G	0.57	0.049	0.008	4.13*10^{-9}	0.865	0.023	0.014	0.114	0.042	0.007	**4.89*10^{-9}**
rs8092503	18	50630485	*RAB27B*	G/A	0.27	0.047	0.009	8.55*10^{-8}	0.156	0.038	0.018	0.034	0.045	0.008	**8.17*10^{-9}**
rs3829849[b]	9	128430621	*LMX1B*	T/C	0.36	0.038	0.008	1.46*10^{-6}	0.560	0.052	0.016	0.001	0.041	0.007	**8.81*10^{-9}**
rs13387838	2	206989692	*ADAM23*	A/G	0.04	0.151	0.027	2.40*10^{-8}	0.092	0.067	0.066	0.306	0.139	0.025	**2.84*10^{-8}**
rs7550711[d]	1	109884409	*GPR61*	T/C	0.04	0.120	0.021	1.50*10^{-8}	0.487	0.041	0.042	0.401	0.105	0.019	**4.52*10^{-8}**
rs17309930[b]	11	27705069	*BDNF*	A/C	0.21	0.053	0.010	2.47*10^{-8}	0.972	0.012	0.019	0.540	0.045	0.009	1.41*10^{-7}
rs2590942[b]	1	72657869	*NEGR1*	T/G	0.82	0.060	0.010	3.88*10^{-9}	0.633	8.00*10^{-4}	0.019	0.966	0.047	0.009	1.91*10^{-7}
rs13107325[b]	4	103407732	*SLC39A8*	T/C	0.07	0.102	0.018	1.19*10^{-8}	0.446	0.001	0.034	0.970	0.081	0.016	3.79*10^{-7}
rs10151686[b]	14	29536217	*PRKD1*	A/G	0.04	0.107	0.022	1.50*10^{-6}	0.477	0.062	0.039	0.109	0.096	0.019	6.99*10^{-7}
rs25832	5	66211438	*LOC375449*	A/G	0.71	0.044	0.009	2.41*10^{-6}	0.562	0.022	0.017	0.177	0.039	0.008	1.62*10^{-6}
rs7869969	9	95257268	*FAM120A*	G/A	0.33	-0.041	0.008	1.43*10^{-6}	0.370	0.012	0.015	0.425	0.036	0.008	1.68*10^{-6}
rs11079830[c]	17	44037629	*HOXB6*	A/G	0.58	-0.044	0.009	4.43*10^{-7}	0.842	0.016	0.014	0.254	0.034	0.007	1.98*10^{-6}
rs4569924	5	153520218	*GALNT10*	T/C	0.43	0.040	0.008	4.06*10^{-7}	0.607	0.003	0.015	0.823	0.032	0.007	3.48*10^{-6}

Supplementary Table S3. *(Continued)*

						Discovery analysis				Replication analysis			Joint analysis		
SNP	CHR	Position	Nearest gene	EA/ Non-EA	EAF[a]	Beta	SE	p-value	Hetero-geneity p-value	Beta	SE	p-value	Beta	SE	p-value
rs8046312[b]	16	19886835	*GPR139*	A/C	0.81	0.067	0.011	$4.06*10^{-10}$	0.996	-0.023	0.017	0.185	0.042	0.009	$3.97*10^{-6}$
rs1838856	2	113822060	*PAX8*	A/C	0.46	0.044	0.009	$1.85*10^{-6}$	0.902	0.008	0.015	0.588	0.034	0.008	$1.47*10^{-5}$
rs633143	1	179716108	*CACNA1E*	T/C	0.14	-0.048	0.010	$3.81*10^{-6}$	0.977	0.009	0.021	0.648	0.044	0.011	$2.44*10^{-5}$
rs4923207	11	24713901	*LUZP2*	T/G	0.79	0.052	0.011	$1.60*10^{-6}$	0.547	-0.004	0.020	0.834	0.039	0.010	$3.52*10^{-5}$
rs6971577	7	140350204	*MRPS33*	C/G	0.78	0.051	0.010	$1.17*10^{-6}$	0.415	-0.007	0.018	0.690	0.036	0.009	$6.80*10^{-5}$
rs10866069	3	64366964	*ADAMTS9*	T/C	0.17	0.055	0.012	$3.05*10^{-6}$	0.073	-0.009	0.023	0.687	0.041	0.011	$8.43*10^{-5}$
rs12457682	18	7216505	*LAMA1*	C/A	0.23	-0.057	0.012	$3.40*10^{-6}$	0.269	0.001	0.017	0.942	0.035	0.009	$9.01*10^{-5}$
rs11165675[b]	1	96812556	*PTBP2*	A/G	0.27	-0.041	0.009	$1.67*10^{-6}$	0.835	-0.011	0.018	0.520	0.031	0.008	$1.01*10^{-4}$
rs12096993	1	217931859	*SLC30A10*	T/C	0.27	-0.058	0.013	$3.47*10^{-6}$	0.012	-0.009	0.016	0.583	0.031	0.008	$1.02*10^{-4}$
rs760931	1	1637388	*CDC2L1*	C/G	0.93	0.045	0.009	$1.38*10^{-6}$	0.169	0.046	0.033	0.160	0.103	0.027	$1.27*10^{-4}$
rs2968990	4	131098524	*C4orf33*	C/T	0.37	0.042	0.009	$1.67*10^{-6}$	0.835	-0.010	0.015	0.487	0.028	0.007	$1.42*10^{-4}$
rs1247117	10	120418792	*C10orf46*	G/A	0.11	0.058	0.013	$3.47*10^{-6}$	0.011	-0.022	0.023	0.339	0.040	0.011	$2.62*10^{-4}$
rs6580706	12	47959818	*TUBA1C*	C/G	0.34	0.058	0.010	$1.83*10^{-8}$	0.687	-0.031	0.016	0.047	0.031	0.009	$3.68*10^{-4}$
rs8092620	18	41433991	*SLC14A2*	G/T	0.48	0.039	0.008	$3.31*10^{-6}$	0.338	-0.018	0.014	0.199	0.024	0.007	$7.32*10^{-4}$
rs188584	3	62675007	*CADPS*	C/A	0.77	0.046	0.010	$3.23*10^{-6}$	0.408	-0.025	0.017	0.139	0.028	0.008	0.001
rs4870949	8	126704776	*TRIB1*	T/C	0.07	1.817[e]	0.289	$3.13*10^{-10}$	0.298	0.104	0.055	0.061	0.164	0.054	0.002
rs1573972	4	171559399	*AADAT*	C/T	0.19	0.087	0.019	$3.78*10^{-6}$	0.663	-0.022	0.018	0.232	0.030	0.013	0.020
rs214821	20	2258291	*TGM3*	T/C	0.02	1.627[e]	0.350	$3.38*10^{-6}$	0.600	-0.145	0.258	0.575	0.479	0.208	0.021
rs8084077	18	49532928	*DCC*	T/C	0.73	0.041	0.009	$3.47*10^{-6}$	0.119	-0.064	0.015	$2.72*10^{-5}$	0.014	0.008	0.062
rs3845265	18	63690108	*DSEL*	G/A	0.71	0.044	0.009	$8.86*10^{-7}$	0.600	-0.072	0.015	$1.44*10^{-6}$	0.013	0.008	0.087

[a] From joint analysis. [b] Locus previously reported in [11]. [c] Locus previously reported in [11,16]. [d] Locus previously reported in [9,11]. [e] Beta and SE based on two studies only (923 and 431 children for rs4870949 and rs214821, respectively)

CHR: Chromosome; EA: Effect Allele; EAF: Effect Allele Frequency; SE: Standard Error

Supplementary Table S4. Directions of effect for the individual discovery and replication studies for all 43 loci with p-values <5*10^{-6} in the discovery phase

SNP	CHR	Position	Nearest gene	EA/ Non-EA	Direction of effect discovery studies	Direction of effect replication studies	Beta (SE) joint analysis	p–value joint analysis
rs13130484[a]	4	44870448	*GNPDA2*	T/C	+++++-+++++++++++++-+	+-+-+-?+?--?+++	0.067 (0.007)	**1.58*10^{-23}**
rs11676272[a]	2	24995042	*ADCY3*	G/A	+++++?++++++++++-++++	++-+++?+?+-?++?	0.068 (0.007)	**7.12*10^{-23}**
rs4854349[a]	2	637861	*TMEM18*	C/T	++++++++++++++?+-++++	+++++++?+?++?++?	0.090 (0.009)	**5.41*10^{-22}**
rs543874[a]	1	176156103	*SEC16B*	G/A	+++++++-+++++++++-++++	++++++?+?++?-++	0.077 (0.009)	**2.20*10^{-19}**
rs7132908[s]	12	48549415	*FAIM2*	A/G	+++++?++++++++++-????	-++++-?+--++-+?	0.066 (0.008)	**1.57*10^{-18}**
rs1421085[a]	16	52358455	*FTO*	C/T	+?+++-+++++++-++-+++++	-+++-+?+?--?-+?	0.059 (0.007)	**4.53*10^{-16}**
rs12429545[b]	13	53000207	*OLFM4*	A/G	+++++-++++++++--+++--	+---+-?+?++?+++	0.076 (0.010)	**2.08*10^{-14}**
rs987237[a]	6	50911009	*TFAP2B*	G/A	++++++++++++++++++++-+	-+++-+?+?-+?+-?	0.062 (0.009)	**1.80*10^{-12}**
rs12041852[a]	1	74776088	*TNNI3K*	G/A	+++++-+++--+++++-??++	+-++-+?+?-+?-+?	0.046 (0.007)	**2.28*10^{-10}**
rs6567160[a]	18	55980115	*MC4R*	C/T	+++++-++++++++++++++	-+-+-+?+?+-?--?	0.050 (0.008)	**1.21*10^{-9}**
rs13253111	8	28117893	*ELP3*	A/G	++++++++++-++++++????	--++++++++---+?	0.042 (0.007)	**4.89*10^{-9}**
rs8092503	18	50630485	*RAB27B*	G/A	+++-+++++-+++++-+++++	+++++-+?+?--?++?	0.045 (0.008)	**8.17*10^{-9}**
rs3829849[a]	9	128430621	*LMX1B*	T/C	+++++--++++++--+-+++-	+++--+?+?++?++?	0.041 (0.007)	**8.81*10^{-9}**
rs13387838	2	206989692	*ADAM23*	A/G	+?+++-++++++++-??+???	+-?+++?+?--?+-?	0.139 (0.025)	**2.84*10^{-8}**
rs7550711[c]	1	109884409	*GPR61*	T/C	+?++++++++++++---++++	-++++-?+?++?+-?	0.105 (0.019)	**4.52*10^{-8}**
rs17309930[a]	11	27705069	*BDNF*	A/C	++++++++++-+++++?-+++	+-++++?+?--?-+?	0.045 (0.009)	1.41*10^{-7}
rs2590942[a]	1	72657869	*NEGR1*	T/G	+++-+-++++++++++-++++	-+--+-?+?+-?+-?	0.047 (0.009)	1.91*10^{-7}
rs13107325[a]	4	103407732	*SLC39A8*	T/C	+++++-++-++++++++++-?	++++--?+?+-?--?	0.081 (0.016)	3.79*10^{-7}
rs10151686[a]	14	29536217	*PRKD1*	A/G	++-++++++?-++-++--++++++	+-+-++?+?+-?++?	0.096 (0.019)	6.99*10^{-7}
rs25832	5	66211438	*LOC375449*	A/G	++++++++++++++++-????	+++-+-+++--?++?	0.039 (0.008)	1.62*10^{-6}
rs7869969	9	95257268	*FAM120A*	G/A	++++++++++++-++?????	-++++++++--+-+?	0.036 (0.008)	1.68*10^{-6}
rs11079830[b]	17	44037629	*HOXB6*	A/G	+++++-+++--++++++????	--++++++--+-++?	0.034 (0.007)	1.98*10^{-6}
rs4569924	5	153520218	*GALNT10*	T/C	++++--+-++++++-+?++++	+++-+-?+?+-?-+?	0.032 (0.007)	3.48*10^{-6}
rs8046312[a]	16	19886835	*GPR139*	A/C	++++++++++++++++++????	++++-+-+-+--++-	0.042 (0.009)	3.97*10^{-6}
rs1838856	2	113822060	*PAX8*	A/C	+?+++-+++++++?++?????	+++-+---?+++-0?	0.034 (0.008)	1.47*10^{-5}
rs633143	1	179716108	*CACNA1E*	T/C	++++---++++++++-????	+-+--+-++-+-+-?	0.044 (0.011)	2.44*10^{-5}

Supplementary Table S4. *(Continued)*

SNP	CHR	Position	Nearest gene	EA/ Non-EA	Direction of effect discovery studies	Direction of effect replication studies	Beta (SE) joint analysis	p–value joint analysis
rs4923207	11	24713901	*LUZP2*	T/G	++-+--++++++++++????	+----+--?++?-+?	0.039 (0.010)	$3.52*10^{-5}$
rs6971577	7	140350204	*MRPS33*	C/G	++++-++++++++++?????	---++++-+-++-+?	0.036 (0.009)	$6.80*10^{-5}$
rs10866069	3	64366964	*ADAMTS9*	T/C	++++-?++++-+-+++-????	+--+++--++++--?	0.041 (0.011)	$8.43*10^{-5}$
rs12457682	18	7216505	*LAMA1*	C/A	++++++++++++++++????	-+----++--+--+?	0.035 (0.009)	$9.01*10^{-5}$
rs11165675[a]	1	96812556	*PTBP2*	A/G	+++++-++++++--+--+-++-	----+-?+?--?-+?	0.031 (0.008)	$1.01*10^{-4}$
rs12096993	1	217931859	*SLC30A10*	T/C	++--++++++++++-+????	+-+---+-++-++-?	0.031 (0.008)	$1.02*10^{-4}$
rs760931	1	1637388	*CDC2L1*	C/G	??+???+???+?+????????	+-++++-+++--++??	0.103 (0.027)	$1.27*10^{-4}$
rs2968990	4	131098524	*C4orf33*	C/T	+++-+++++++-+-++++????	-++++-+------+?	0.028 (0.007)	$1.42*10^{-4}$
rs1247117	10	120418792	*C10orf46*	G/A	+-++++++++-+++-+++-+++	-++-+-?+?+-?--?	0.040 (0.011)	$2.62*10^{-4}$
rs6580706[a]	12	47959818	*TUBA1C*	C/G	+?++++++++++?+++????	++-+---+---+--?	0.031 (0.009)	$3.68*10^{-4}$
rs8092620	18	41433991	*SLC14A2*	G/T	+++++-+-++++++++-????	++-+--+--+---+?	0.024 (0.007)	$7.32*10^{-4}$
rs188584	3	62675007	*CADPS*	C/A	++++++-++++++++--????	--+--+---+-+--?	0.028 (0.008)	0.001
rs4870949	8	126704776	*TRIB1*	T/C	??????????+??????+????	+++-+--?++++???	0.164 (0.054)	0.002
rs1573972	4	171559399	*AADAT*	C/T	+++++-++++-+++-++????	+--+----++++++?	0.030 (0.013)	0.020
rs214821	20	2258291	*TGM3*	T/C	??????????+?????+????	??????-??+-????	0.479 (0.208)	0.021
rs8084077	18	49532928	*DCC*	T/C	+++-++++--++++--+++-+	-+++-+?-?+-?---	0.014 (0.008)	0.062
rs3845265	18	63690108	*DSEL*	G/A	+-++++++++++++++-????	--------++-?---	0.013 (0.008)	0.087

Direction of the effect for the effect allele for each individual study is shown: + indicates a positive effect estimate for the effect allele, - indicates a negative effect estimate for the effect allele, 0 indicates an effect estimate of 0, ? indicates no information available. Order of the studies in the discovery analysis: ALSPAC, Generation R, HBCS, DNBC, Raine, GOYA, YFS, LISA+GINI, NFBC 1966, MAAS, COPSAC2000, 1958BC-T1DGC, 1958BC-WTCCC2, CHOP, INMA, NTR, MoBa, Leipzig, NFBC 1986, STRIP, PANIC. Order of the studies in the replication analysis: COPSAC2010, BMDCS, DNBC (replication), LISA+GINI (replication), Danish Childhood Obesity Biobank controls, Danish Childhood Obesity Biobank cases, PANIC, PIAMA, STRIP, French Young Study cases, French Young Study controls, Leipzig (replication), TEDS, TEENAGE, BREATHE

[a] Locus previously reported in [11]. [b] Locus previously reported in [11,16]. [c] Locus previously reported in [9,11]. CHR: Chromosome; EA: Effect Allele; SE: Standard Error

Supplementary Table S5. Results of a lookup of the 15 replicated loci for childhood BMI in the published data of the meta-analysis of GWAs studies on adult BMI of the GIANT consortium

SNP	CHR	Position	Effect allele	Non-effect allele	Consistent direction of effect in children and adults	p-value (children)	p-value (adults)
rs11676272	2	24995042	G	A	Y	$7.12*10^{-23}$	$1.12*10^{-21}$
rs7132908	12	48549415	A	G	Y	$1.57*10^{-18}$	$1.23*10^{-13}$
rs13253111	8	28117893	A	G	Y	$4.89*10^{-9}$	$5.37*10^{-3}$
rs13387838	2	206989692	A	G	Y	$2.84*10^{-8}$	$2.52*10^{-3}$
rs8092503	18	50630485	G	A	Y	$8.17*10^{-9}$	$5.76*10^{-5}$
rs4854349	2	637861	C	T	Y	$5.41*10^{-22}$	$3.79*10^{-47}$
rs12429545	13	53000207	A	G	Y	$2.08*10^{-14}$	$1.09*10^{-12}$
rs12041852	1	74776088	G	A	Y	$2.28*10^{-10}$	$2.64*10^{-14}$
rs543874	1	176156103	G	A	Y	$2.20*10^{-19}$	$2.62*10^{-35}$
rs3829849	9	128430621	T	C	Y	$8.81*10^{-9}$	$2.17*10^{-6}$
rs987237	6	50911009	G	A	Y	$1.80*10^{-12}$	$1.96*10^{-28}$
rs7550711	1	109884409	T	C	Y	$4.52*10^{-8}$	$1.56*10^{-13}$
rs13130484	4	44870448	T	C	Y	$1.58*10^{-23}$	$4.24*10^{-38}$
rs6567160	18	55980115	C	T	Y	$1.21*10^{-9}$	$3.93*10^{-53}$
rs1421085	16	52358455	C	T	Y	$4.53*10^{-16}$	$8.83*10^{-151}$

Betas and standard errors are not given because BMI was analysed on different scales between children and adults

Supplementary Table S6. Results of a lookup of the 97 loci that were reported to be genome-wide significantly associated with adult BMI in the current childhood BMI meta-analysis

SNP	CHR	Position	Effect allele	Non-effect allele	Consistent direction of effect in children and adults	p-value (children)	p-value (adults)
rs1558902	16	52361075	A	T	Y	$3.88*10^{-14}$	$7.51*10^{-153}$
rs6567160	18	55980115	C	T	Y	$4.06*10^{-12}$	$3.93*10^{-53}$
rs13021737	2	622348	G	A	Y	$4.61*10^{-18}$	$1.11*10^{-50}$
rs10938397	4	44877284	G	A	Y	$1.88*10^{-9}$	$3.21*10^{-38}$
rs543874	1	176156103	G	A	Y	$2.38*10^{-17}$	$2.62*10^{-35}$
rs2207139	6	50953449	G	A	Y	$6.92*10^{-11}$	$4.13*10^{-29}$
rs11030104	11	27641093	A	G	Y	$9.82*10^{-7}$	$5.56*10^{-28}$
rs3101336	1	72523773	C	T	Y	$4.14*10^{-8}$	$2.66*10^{-26}$
rs7138803	12	48533735	A	G	Y	$4.98*10^{-18}$	$8.15*10^{-24}$
rs10182181	2	25003800	G	A	Y	$2.36*10^{-21}$	$8.78*10^{-24}$
rs3888190	16	28796987	A	C	Y	$6.84*10^{-5}$	$3.14*10^{-23}$
rs1516725	3	187306698	C	T	Y	0.113	$1.89*10^{-22}$
rs12446632	16	19842890	G	A	Y	$1.32*10^{-9}$	$1.48*10^{-18}$
rs2287019	19	50894012	C	T	Y	0.718	$4.59*10^{-18}$
rs16951275	15	65864222	T	C	Y	0.009	$1.91*10^{-17}$
rs3817334	11	47607569	T	C	Y	0.013	$5.15*10^{-17}$
rs2112347	5	75050998	T	G	Y	0.015	$6.19*10^{-17}$
rs12566985	1	74774781	G	A	Y	$2.21*10^{-10}$	$3.28*10^{-15}$
rs3810291	19	52260843	A	G	Y	$2.85*10^{-4}$	$4.81*10^{-15}$
rs7141420	14	78969207	T	C	Y	0.069	$1.23*10^{-14}$
rs13078960	3	85890280	G	T	Y	0.002	$1.74*10^{-14}$
rs10968576	9	28404339	G	A	Y	0.100	$6.61*10^{-14}$
rs17024393	1	109956211	C	T	Y	$3.08*10^{-8}$	$7.03*10^{-14}$
rs657452	1	49362434	A	G	Y	0.757	$5.48*10^{-13}$
rs12429545	13	53000207	A	G	Y	$3.66*10^{-11}$	$1.09*10^{-12}$
rs12286929	11	114527614	G	A	Y	0.024	$1.31*10^{-12}$
rs13107325	4	103407732	T	C	Y	$1.19*10^{-8}$	$1.83*10^{-12}$
rs11165643	1	96696685	T	C	Y	0.099	$2.07*10^{-12}$
rs7903146	10	114748339	C	T	Y	0.043	$1.11*10^{-11}$
rs10132280	14	24998019	C	A	Y	0.042	$1.14*10^{-11}$
rs17405819	8	76969139	T	C	Y	0.006	$2.07*10^{-11}$
rs6091540	20	50521269	C	T	Y	0.274	$2.15*10^{-11}$
rs1016287	2	59159129	T	C	N	0.415	$2.25*10^{-11}$
rs4256980	11	8630515	G	C	Y	$7.68*10^{-4}$	$2.90*10^{-11}$
rs17094222	10	102385430	C	T	Y	0.010	$5.94*10^{-11}$
rs12401738	1	78219349	A	G	Y	0.562	$1.15*10^{-10}$
rs7599312	2	213121476	G	A	N	0.669	$1.17*10^{-10}$

Supplementary Table S6. *(Continued)*

SNP	CHR	Position	Effect allele	Non-effect allele	Consistent direction of effect in children and adults	p-value (children)	p-value (adults)
rs2365389	3	61211502	C	T	Y	$7.60*10^{-4}$	$1.63*10^{-10}$
rs205262	6	34671142	G	A	N	0.490	$1.75*10^{-10}$
rs2820292	1	200050910	C	A	Y	0.882	$1.83*10^{-10}$
rs12885454	14	28806589	C	A	N	0.457	$1.94*10^{-10}$
rs9641123	7	93035668	C	G	Y	0.132	$2.08*10^{-10}$
rs12016871	13	26915782	T	C	N	0.793	$2.29*10^{-10}$
rs16851483	3	142758126	T	G	Y	0.001	$3.55*10^{-10}$
rs1167827	7	75001105	G	A	Y	0.038	$6.33*10^{-10}$
rs758747	16	3567359	T	C	Y	0.045	$7.47*10^{-10}$
rs1928295	9	119418304	T	C	Y	0.001	$7.91*10^{-10}$
rs9925964	16	31037396	A	G	Y	0.384	$8.11*10^{-10}$
rs11126666	2	26782315	A	G	Y	0.444	$1.33*10^{-9}$
rs2650492	16	28240912	A	G	Y	0.071	$1.92*10^{-9}$
rs6804842	3	25081441	G	A	Y	0.238	$2.48*10^{-9}$
rs12940622	17	76230166	G	A	Y	0.002	$2.49*10^{-9}$
rs7164727	15	70881044	T	C	Y	0.019	$3.92*10^{-9}$
rs11847697	14	29584863	T	C	Y	$1.94*10^{-5}$	$3.99*10^{-9}$
rs4740619	9	15624326	T	C	Y	0.272	$4.56*10^{-9}$
rs492400	2	219057996	C	T	Y	0.019	$6.78*10^{-9}$
rs13191362	6	162953340	A	G	Y	0.450	$7.34*10^{-9}$
rs3736485	15	49535902	A	G	Y	0.582	$7.41*10^{-9}$
rs17001654	4	77348592	G	C	Y	0.174	$7.76*10^{-9}$
rs11191560	10	104859028	C	T	Y	0.228	$8.45*10^{-9}$
rs2080454	16	47620091	C	A	Y	0.015	$8.60*10^{-9}$
rs7715256	5	153518086	G	T	Y	$4.85*10^{-7}$	$8.85*10^{-9}$
rs2176040	2	226801046	A	G	Y	0.567	$9.99*10^{-9}$
rs1528435	2	181259207	T	C	Y	0.059	$1.20*10^{-8}$
rs2075650	19	50087459	A	G	Y	0.332	$1.25*10^{-8}$
rs1000940	17	5223976	G	A	Y	0.174	$1.28*10^{-8}$
rs2033529	6	40456631	G	A	Y	0.028	$1.39*10^{-8}$
rs11583200	1	50332407	C	T	Y	0.081	$1.48*10^{-8}$
rs7239883	18	38401669	G	A	Y	0.286	$1.51*10^{-8}$
rs2836754	21	39213610	C	T	Y	0.018	$1.61*10^{-8}$
rs9400239	6	109084356	C	T	Y	0.038	$1.61*10^{-8}$
rs10733682	9	128500735	A	G	Y	0.051	$1.83*10^{-8}$
rs11688816	2	62906552	G	A	Y	0.039	$1.89*10^{-8}$
rs11057405	12	121347850	G	A	N	0.994	$2.02*10^{-8}$

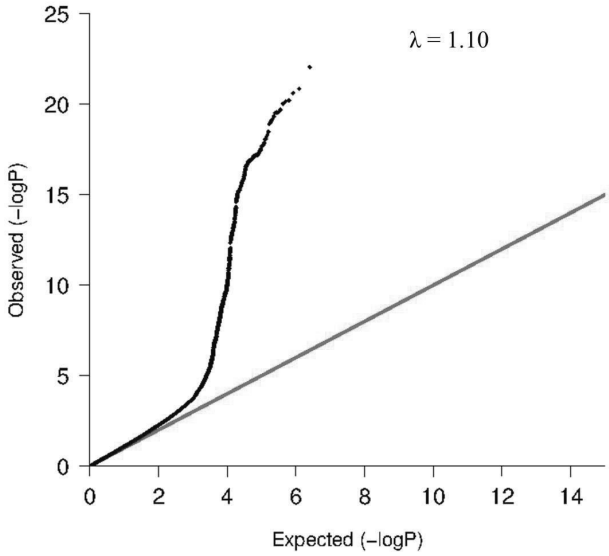
Supplementary Table S6. *(Continued)*

SNP	CHR	Position	Effect allele	Non-effect allele	Consistent direction of effect in children and adults	p-value (children)	p-value (adults)
rs9914578	17	1951886	G	C	Y	0.016	$2.07*10^{-8}$
rs977747	1	47457264	T	G	Y	0.095	$2.18*10^{-8}$
rs2121279	2	142759755	T	C	N	0.926	$2.31*10^{-8}$
rs29941	19	39001372	G	A	Y	$2.42*10^{-4}$	$2.41*10^{-8}$
rs11727676	4	145878514	T	C	Y	0.054	$2.55*10^{-8}$
rs3849570	3	81874802	A	C	N	0.261	$2.60*10^{-8}$
rs9374842	6	120227364	T	C	Y	0.021	$2.67*10^{-8}$
rs6477694	9	110972163	C	T	Y	0.051	$2.67*10^{-8}$
rs4787491	16	29922838	G	A	Y	0.01	$2.70*10^{-8}$
rs1441264	13	78478920	A	G	Y	$4.46*10^{-5}$	$2.96*10^{-8}$
rs7899106	10	87400884	G	A	Y	0.210	$2.96*10^{-8}$
rs2176598	11	43820854	T	C	N	0.560	$2.97*10^{-8}$
rs2245368	7	76446079	C	T	Y	0.256	$3.19*10^{-8}$
rs17203016	2	207963763	G	A	Y	0.545	$3.41*10^{-8}$
rs17724992	19	18315825	A	G	Y	0.004	$3.42*10^{-8}$
rs7243357	18	55034299	T	G	Y	0.396	$3.86*10^{-8}$
rs16907751	8	81538012	C	T	Y	0.729	$3.89*10^{-8}$
rs1808579	18	19358886	C	T	Y	$4.94*10^{-4}$	$4.17*10^{-8}$
rs13201877	6	137717234	G	A	N	0.951	$4.29*10^{-8}$
rs2033732	8	85242264	C	T	Y	0.125	$4.89*10^{-8}$
rs9540493	13	65103705	A	G	Y	0.039	$4.97*10^{-8}$
rs1460676	2	164275935	C	T	Y	0.213	$4.98*10^{-8}$
rs6465468	7	95007450	T	G	N	0.147	$4.98*10^{-8}$

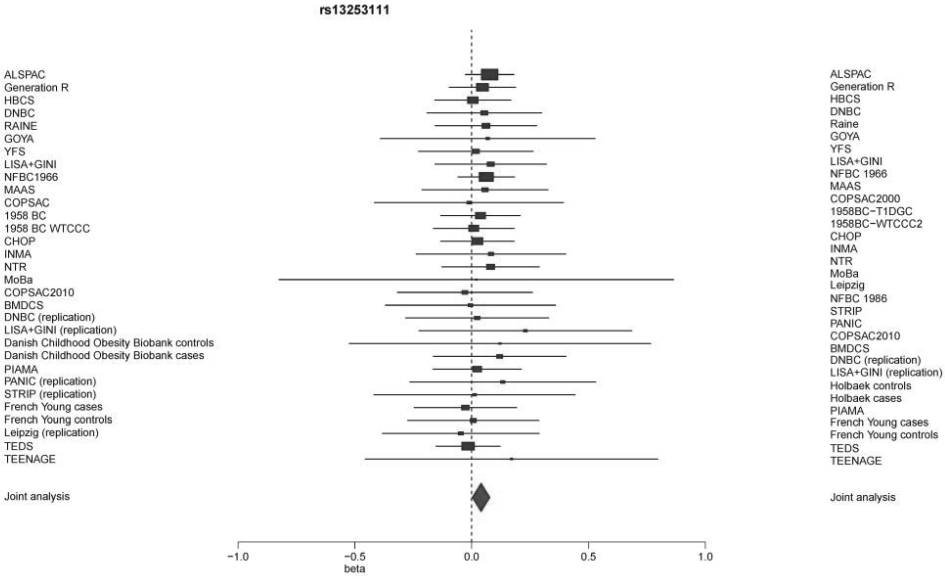
Supplementary Table S7. Significant results (FDR <0.05) of eQTL analyses of the 15 genome-wide significant hits in untransformed peripheral blood samples from 5,311 individuals

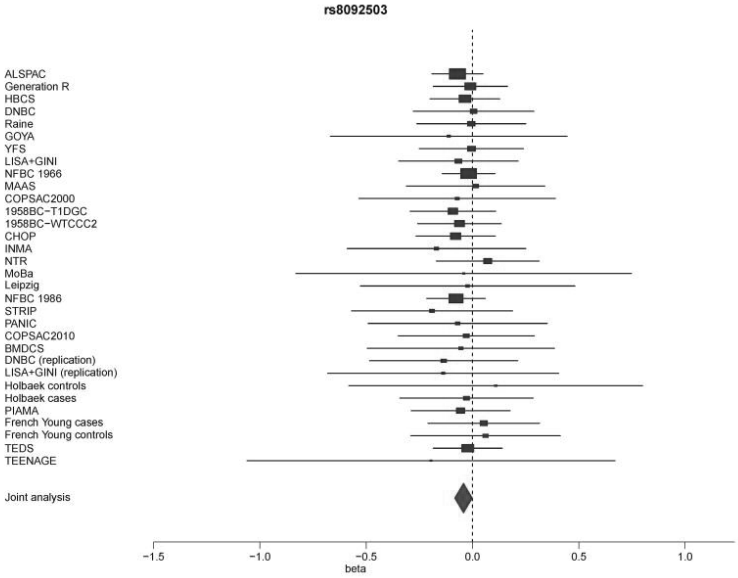
Primary SNP	Peak marker	Distance (bp) (bp)	p-value	Transcript	R^2	D'
rs11676272	rs6752378	8578	$1.50*10^{-17}$	*ADCY3*	0.97	1
rs11676272	rs1172293	26382	$3.34*10^{-8}$	*DNAJC27*	0.11	1
rs3829849	rs10122788	183968	$2.50*10^{-11}$	*FAM125B*	0.03	0.26

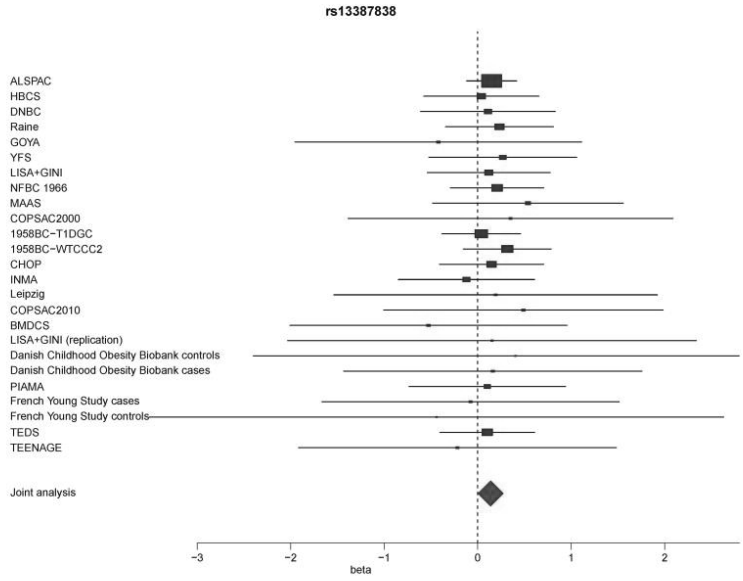
eQTLs: expression quantitative trait loci; p-value: p-value for the association of the primary SNP with the transcript; Distance: distance between the primary SNP and the peak marker; R^2 and D' values represent linkage disequilibrium between the primary SNP and the peak marker for that eQTL



Supplementary Figure S1. Quantile-Quantile plot of the SNPs in the discovery meta-analysis. The black line shows the Quantile-Quantile plot of all SNPs, with the expected $-\log_{10}$(p-values) on the x-axis and the observed $-\log_{10}$(p-values) on the y-axis


rs13253111
ALSPAC
Generation R
HBCS
DNBC
RAINE
GOYA
YFS
LISA+GINI
NFBC1966
MAAS
COPSAC
1958 BC
1958 BC WTCCC
CHOP
INMA
NTR
MoBa
COPSAC2010
BMDCS
DNBC (replication)
LISA+GINI (replication)
Danish Childhood Obesity Biobank controls
Danish Childhood Obesity Biobank cases
PIAMA
PANIC (replication)
STRIP (replication)
French Young cases
French Young controls
Leipzig (replication)
TEDS
TEENAGE
Joint analysis
ALSPAC
Generation R
HBCS
DNBC
Raine
GOYA
YFS
LISA+GINI
NFBC 1966
MAAS
COPSAC2000
1958BC−T1DGC
1958BC−WTCCC2
CHOP
INMA
NTR
MoBa
Leipzig
NFBC 1986
STRIP
PANIC
COPSAC2010
BMDCS
DNBC (replication)
LISA+GINI (replication)
Holbaek controls
Holbaek cases
PIAMA
French Young cases
French Young controls
TEDS
TEENAGE
Joint analysis
−1.0
−0.5
0.0
0.5
1.0
beta


rs8092503
ALSPAC
Generation R
HBCS
DNBC
Raine
GOYA
YFS
LISA+GINI
NFBC 1966
MAAS
COPSAC2000
1958BC−T1DGC
1958BC−WTCCC2
CHOP
INMA
NTR
MoBa
Leipzig
NFBC 1986
STRIP
PANIC
COPSAC2010
BMDCS
DNBC (replication)
LISA+GINI (replication)
Holbaek controls
Holbaek cases
PIAMA
French Young cases
French Young controls
TEDS
TEENAGE
Joint analysis
−1.5
−1.0
−0.5
0.0
0.5
1.0
beta



Supplementary Figure S2. Forest plots of the top SNP in each of the three novel loci. Individual discovery and replication studies are listed on the y-axis. Black squares represent betas, horizontal lines represent genome-wide (99.999995%) confidence inteals. Size of the box represents sample size. The black diamond represents the beta and genome-wide confidence interval of the joint analysis of all discovery and replication samples.

Supplementary methods per cohort and Supplementary Table S8 can be found online.

Chapter 3.2

Associations of genetic risk scores based on adult adiposity pathways with childhood growth and adiposity measures

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Abstract

Background: Results from genome-wide association studies (GWAS) identified many loci and biological pathways that influence adult body mass index (BMI). We aimed to identify if biological pathways related to adult BMI also affect infant growth and childhood adiposity measures.

Methods: We used data from a population-based prospective cohort study among 3,975 children with a mean age of 6 years. Genetic risk scores were constructed based on the 97 SNPs associated with adult BMI previously identified with GWAS and on 28 BMI related biological pathways based on subsets of these 97 SNPs. Outcomes were infant peak weight velocity, BMI at adiposity peak and age at adiposity peak, and childhood BMI, total fat mass percentage, android/gynoid fat ratio, and preperitoneal fat area. Analyses were performed using linear regression models.

Results: A higher overall adult BMI risk score was associated with infant BMI at adiposity peak and childhood BMI, total fat mass, android/gynoid fat ratio, and preperitoneal fat area (all p-values <0.05). Analyses focused on specific biological pathways showed that the membrane proteins genetic risk score was associated with infant peak weight velocity, and the genetic risk scores related to neuronal developmental processes, hypothalamic processes, cyclicAMP, WNT-signaling, membrane proteins, monogenic obesity and/or energy homeostasis, glucose homeostasis, cell cycle, and muscle biology pathways were associated with childhood adiposity measures (all p-values <0.05). None of the pathways were associated with childhood preperitoneal fat area.

Conclusions: A genetic risk score based on 97 SNPs related to adult BMI was associated with peak weight velocity during infancy and general and abdominal fat measurements at the age of 6 years. Risk scores based on genetic variants linked to specific biological pathways, including central nervous system and hypothalamic processes, influence body fat development from early life onwards.

Background

Childhood overweight and obesity are associated with various adverse short- and long-term consequences, including cardiovascular disease and type 2 diabetes.[1-4] Besides the well-known lifestyle-related risk factors, overweight and obesity have a strong genetic component with heritability estimates from twin studies reported to be up to 80%.[5,6] Large genome-wide association studies (GWAS) have identified many single nucleotide polymorphisms (SNPs) associated with body mass index (BMI) in adults.[7,8] Less is known about the genetic background of BMI in childhood. Three recent studies revealed a total of 15 genetic loci associated with childhood BMI, most of which are also associated with adult BMI.[9-11] We previously reported that a genetic risk score based on 29 SNPs related to adult BMI was associated with infant growth and childhood adiposity measures.[12] A recent GWAS increased the number of adult BMI associated SNPs to 97.[8] These SNPs are located in or close to genes linked to several biological pathways. In adults especially central nervous system processes seem to play a role.[8] The role of these pathways in body fat development during early life is not known yet. Thus far, GWAS in children did not report any specific biological pathways.[11] Knowledge on the biological pathways influencing BMI from early life onwards may help to better understand the development of overweight and obesity in children.

In this study, we used data from 3,975 children participating in a population-based cohort study to examine the associations of genetic risk scores for adult BMI, both overall and based on specific biological pathways, with infant weight growth patterns and childhood adiposity measures. For comparison, we also examined the associations of genetic risk scores based on the 49 SNPs related with adult waist-hip-ratio (WHR) and on the 15 SNPs associated with childhood BMI with the same infant and childhood outcomes.[11,13]

Methods

Study design and population

This study was embedded in the Generation R Study, a population-based, prospective cohort study from fetal life onwards in Rotterdam, the Netherlands.[14] All pregnant women with an expected delivery date between April 2002 and January 2006 and living in Rotterdam were asked to participate. The study was approved by the local Medical Ethical Committee and written consent was obtained for each participating child. GWA scans were available for 59% of all children (N=5,732).[15] The Generation R Study is a multi-ethnic cohort. Participants of European origin constitute the largest ethnic group (56%), and the

largest other groups are Surinamese (9%), Turkish (7%) and Moroccan (6%).[14] Our present study included all singleton live births with GWA data and information on at least one of the outcomes of interest (N=4,151). A participant flowchart is shown in Supplemental Figure S1.

Genetic variants and risk scores

DNA was isolated from cord blood or, in a small minority of children with missing cord blood samples, at 6 years of age. For genome-wide association analysis the Illumina 610 and 660W Quad platforms were used.[16] Stringent quality checks were performed in which individuals with low sample call rates (<97.5%) or sex mismatches were excluded. Imputation of genotypes to the cosmopolitan panel of HapMap ii (release 22) was done using MACH software.[17,18] Prior to imputation, we excluded SNPs with a high level of missing data (SNP call rate <98%), significant deviations from Hardy-Weinberg equilibrium (p-value $<1*10^{-6}$), or low minor allele frequencies (<0.1%). Information about the SNPs of interest for the current study was extracted from the GWAS dataset. The average imputation quality for all SNPs included in this study was 0.96, ranging from 0.55 to 1.00, demonstrating overall good imputation. For 93 out of the 97 known BMI SNPs information was available in our GWA dataset. We used proxies (R^2>0.96, D'=1) for the remaining four BMI SNPs: rs13012571 was used as a proxy for rs13021737, rs1978487 for rs9925964, rs6445197 for rs2365389, and rs9636202 for rs17724992. Thus, the total number of SNPs used in the analysis was 97 (Supplemental Table S1). These SNPs were combined into weighted BMI genetic risk scores (see below). The same procedure was used for the 49 WHR and 15 child BMI SNPs.[11,13] For 46 of the 49 WHR SNPs information was available in the GWA dataset. Rs4607103 was used as a proxy for rs2371767 (R^2=0.90, D'=1). For the WHR SNPs rs8042543 and rs6556301 no perfect proxy was available leading to a total number of SNPs of 47 for WHR. For all but one SNPs identified for childhood BMI, information was available in our dataset. We used rs3751812 as a proxy for rs1421085 (R^2=0.93, D'=0.97) (Supplemental Table S1.)

In the paper on adult BMI, the 97 adult BMI SNPs were categorized into pathway categories. The authors performed a literature search, which brought about 405 genes within 500kb on either side and with r^2>0.2 of the 97 SNPs.[8] Based on their biological function, these genes were then catagorized into 28 pathways. We used this same categorization, but we excluded categories consisting of one SNP only. For each pathway category, we combined SNPs into a weighted genetic risk score. Some SNPs were included in more than one category based on their biological function (Supplemental Table S2). The number of overlapping SNPs between the biological categories is shown in Supplemental Table S3. As a comparison we ran a pathway analysis using QIAGEN's Ingenuity® Pathway Analysis software (IPA) (IPA®, QIAGEN Redwood City,www.qiagen.com/ingenuity).

Infant weight growth and childhood general and abdominal adiposity

We used repeated growth measurements to derive infant peak weight velocity (PWV), BMI at adiposity peak (BMIAP) and age at adiposity peak (AGEAP), as described previously.[19-23] Briefly, the Reed1 model was used for boys and girls separately, to obtain PWV during infancy. BMIAP and AGEAP were obtained by fitting a cubic mixed effects model on log(BMI) from 2 weeks to 1.5 years of age while adjusting for sex.

At the median age of 6.0 years (95% range 5.7, 7.4) we measured general and abdominal adiposity measures as described in detail previously.[24] Briefly, BMI (kg/m^2) was calculated from height and weight measured without shoes and heavy clothing. Total, android, and gynoid fat mass were measured by Dual-energy X-ray absorptiometry (DXA) (iDXA, GE-Lunar, 2008, Madison, WI, USA).[24] Total fat mass (kg) was calculated as a percentage of total body weight (kg). Android/gynoid fat ratio provides the ratio of central body fat distribution in the abdomen (android fat) and hip (gynoid fat) regions.[25] Preperitoneal fat area, which is a measure of visceral abdominal fat, was measured by abdominal ultrasound.[24,26,27]

Statistical analysis

We constructed a weighted genetic risk score combining the 97 adult BMI SNPs summing the number of outcome increasing risk alleles from the GWA dosage data, weighted using effect estimates of risk increasing alleles in adults. The risk score was rescaled to standard deviation scores (SDS, (observed value-mean)/standard deviation (SD)). Similarly, we constructed genetic risk scores based on SNPs involved in 28 different biological categories, and based on 47 adult WHR SNPs and 15 childhood BMI SNPs. For the biological categories and the WHR SNPs, we used the effect estimates from the original papers as weights.[8,11,13] For the 15 childhood SNPs, weights were obtained from the GWAS meta-analysis without the Generation R data.[11] We used linear regression analyses to examine the associations of the risk scores with PWV, BMIAP, and AGEAP in infancy, and BMI, total fat mass percentage, android/gynoid fat ratio, and preperitoneal fat area in childhood. The variance explained by the risk scores was considered to be the increase in the unadjusted R^2 between the model containing all covariates and the risk score or separate SNPs, and the same model without the risk score. For all analyses, we natural logarithm transformed total fat mass, android/gynoid fat ratio, and preperitoneal fat area to obtain a normal distribution. Standard deviation scores were created for all outcome measures to allow comparison of effect estimates. For BMI, age-adjusted SD scores were created using the Dutch reference growth curves (Growth Analyzer 3.0, Dutch Growth Research Foundation, Rotterdam, the Netherlands).[12] To enable comparison with our current risk scores, we rescaled the previously published 29 adult BMI SNPs risk score to SD scores. All models

were adjusted for sex plus the first four principal components from the genetic data to adjust for ethnic background.[28] Models for general and abdominal adiposity measures were additionally adjusted for age except for BMI which was already age adjusted. Models for total fat mass, android/gynoid fat ratio, and preperitoneal fat area were additionally adjusted for height.[24] We also tested whether the associations of the child and adult BMI risk scores with the childhood adiposity outcomes were explained by infant growth by adding PWV and BMIAP separately to the regression models. For the analyses of the 28 biological pathways, we applied Bonferroni correction and considered a p-value of <0.0018 (0.05/28) as significant. All analyses were performed using the Statistical Package for the Social Sciences version 21.0 for Windows (SPSS; IBM, Chicago, IL, USA).

Results

Characteristics of the study population

Characteristics of all children are listed in Table 1. The children had a median age of 6.0 years (95% range 5.7, 7.4). The median BMI at that age was 15.8 (95% range 13.7, 21.2).

Infant weight growth patterns

The overall adult BMI genetic risk score was associated with BMIAP (Table 2; Figure 1a-c), but not with other infant weight growth measures. BMIAP increased by 0.048 SDS (95% confidence interval (CI) 0.015, 0.081) per SD increase in the genetic risk score. Of the 28 adult BMI genetic risk scores based on biological pathways, only the membrane proteins pathway genetic risk score was associated with PWV (p-value <0.002). Effect estimates for the unweighted and weighted 97 adult BMI SNPs risk scores were similar (Supplemental Table S4). As a comparison, the overall adult WHR genetic risk score was not associated with any infant growth measure (Table 2; Supplemental Figure S2a-c), whereas the childhood BMI genetic risk score was associated with PWV and BMIAP (0.048 SDS (95% CI 0.016, 0.079) and 0.051 SDS (95% CI 0.017, 0.084), respectively, per SD increase in the genetic risk score) (Table 2; Supplemental Figure S3a-c). The genetic risk score based on 29 adult BMI SNPs showed lower effect_estimates per SD increase than our 97 SNPs adult BMI risk score for PWV and BMIAP, and a higher effect estimate for AGEAP, although none of theassociations were significant for the 29 SNP genetic risk score (Supplemental Table S5). The largest variance explained by the adult BMI and pathway risk scores was obtained for the membrane proteins pathway with PWV (0.33%) (Supplemental Table S6).

Table 1. Characteristics of the study population. (N= 3,975)

Characteristics	Full group (N=9,975)	European (N=2,566)	Turkish (N=300)	Surinamese (N=287)	Moroccan (N=234)	Other (N=588)
Birth						
Boys	50.2%	49.6%	53.7%	53.3%	50.4%	49.2%
Gestational age at birth (weeks)[a]	40.1 (36.4; 42.3)	40.3 (33.3; 42.0)	40.0 (36.2; 42.3)	39.7 (35.7; 42.0)	40.6 (36.4; 42.2)	40.0 (36.4; 42.1)
Weight at birth (grams)	3458 (514)	3506 (514)	3402 (480)	3238 (536)	3496 (426)	3379 (506)
Infant						
Peak weight velocity (kg/year)	12.2 (2.1)	12.0 (2.0)	13.1 (2.4)	12.5 (2.2)	12.6 (2.1)	12.4 (2.2)
Body mass index at adiposity peak (kg/m^2)	17.6 (0.8)	17.5 (0.8)	17.9 (0.9)	17.5 (0.9)	17.8 (0.8)	17.7 (0.8)
Age at adiposity peak (years)	0.7 (0.04)	0.7 (0.04)	0.7 (0.04)	0.7 (0.04)	0.7 (0.04)	0.7 (0.04)
Childhood						
Age at visit (years)[a]	6.0 (5.7; 7.8)	6.0 (5.7; 7.5)	6.1 (5.7; 7.7)	6.1 (5.5; 8.2)	6.1 (5.7; 8.3)	6.1 (5.7; 8.2)
Height (cm)	119.6 (6.0)	119.5 (5.6)	119.0 (5.7)	119.9 (7.0)	119.1 (5.9)	120.1 (4.9)
Weight (kg)	23.3 (4.2)	22.9 (3.6)	24.5 (5.3)	23.5 (5.3)	23.9 (4.1)	24.0 (4.9)
Body mass index (kg/m^2)[a]	15.8 (13.7; 21.3)	15.7 (13.7; 19.8)	16.6 (13.6; 24.2)	15.7 (13.2; 23.3)	16.4 (14.0; 22.0)	16.2 (13.6; 22.0)
Total fat mass percentage[a]	24.0 (16.3; 38.6)	23.5 (16.4; 36.4)	26.6 (18.3; 43.5)	24.1 (14.8; 41.4)	25.9 (17.8; 39.9)	24.3 (15.9; 39.4)
Android-gynoid fat ratio[a]	0.2 (0.2; 0.4)	0.2 (0.2; 0.4)	0.3 (0.2; 0.5)	0.2 (0.2; 0.5)	0.2 (0.2; 0.4)	0.2 (0.1; 0.4)
Preperitoneal fat area (cm^2)[a]	0.4 (0.2; 1.2)	0.4 (0.2; 1.0)	0.5 (0.2; 1.9)	0.4 (0.2; 1.7)	0.4 (0.2; 1.6)	0.4 (0.2; 1.3)
Overweight (%)[b]	12.9	10.5	23.7	11.8	19.7	17.8
Obese (%)[b]	4.1	2.1	11.0	8.0	7.7	6.1

Values are means (standard deviations) unless otherwise specified
[a]Median (95% range) [b]The IOTF-classification was used to define overweight and obesity.[40]

Table 2. Associations of BMI, WHR, and childhood BMI genetic risk scores with infant growth (N= 2,955)[a]

Risk score (number of SNPs in risk score)	Peak weight velocity[b]		BMI at adiposity peak[b]		Age at adiposity peak[b]	
	Beta (CI 95%)	P-value	Beta (CI 95%)	P-value	Beta (CI 95%)	P-value
Main risk scores						
Adult BMI (N=97)	0.027 (-0.004; 0.058)	0.093	0.048 (0.015; 0.081)	**0.005**	0.015 (-0.021; 0.051)	0.418
Secondary risk scores						
Adult WHR (N=47)	-0.022 (-0.054; 0.010)	0.180	-0.010 (-0.044; 0.025)	0.587	-0.016 (-0.053; 0.022)	0.411
Child BMI (N=15)	0.038 (0.007; 0.070)	**0.018**	0.039 (0.006; 0.073)	**0.023**	0.027 (-0.010; 0.063)	0.153
ADULT BMI PATHWAY GENETIC RISK SCORES*						
Neuronal						
Neuronal developmental processes (N=29)	0.036 (0.003; 0.070)	0.031	0.049 (0.013; 0.084)	0.007	-0.020 (-0.058; 0.019)	0.311
Neurotransmission (N=10)	-0.009 (-0.040; 0.022)	0.558	-0.001 (-0.034; 0.032)	0.948	0.002 (-0.034; 0.038)	0.901
Hypothalamic expression and regulation (N=13)	0.001 (-0.030; 0.033)	0.932	0.008 (-0.025; 0.042)	0.637	0.023 (-0.013; 0.059)	0.203
Neuronal expression (N=12)	-0.034 (-0.065; -0.003)	0.034	-0.010 (-0.044; 0.024)	0.559	0.026 (-0.010; 0.062)	0.159
Lipid biosynthesis and metabolism (N=10)	0.002 (-0.030; 0.033)	0.918	0.006 (-0.028; 0.040)	0.358	0.020 (-0.017; 0.056)	0.291
Bone development (N=9)	0.017 (-0.014; 0.048)	0.290	0.017 (-0.017; 0.050)	0.336	0.001 (-0.035; 0.037)	0.957
Signaling						
MAPK1/extracellular signal-regulated kinases (N=9)	0.009 (-0.022; 0.040)	0.579	0.008 (-0.025; 0.042)	0.625	0.011 (-0.025; 0.047)	0.534
JAK (N=2)	-0.007 (-0.038; 0.025)	0.679	-0.005 (-0.038; 0.029)	0.779	-0.006 (-0.042; 0.030)	0.750
CyclicAMP (N=5)	-0.020 (-0.052; 0.013)	0.233	0.019 (-0.015; 0.054)	0.368	-0.016 (-0.053; 0.021)	0.391
WNTSignaling (N=6)	0.033 (0.001; 0.064)	0.041	0.017 (-0.016; 0.051)	0.311	0.019 (-0.017; 0.055)	0.293
G-protein coupled receptor						
Notch signaling (N=2)	0.010 (-0.021; 0.041)	0.531	0.009 (-0.024; 0.043)	0.581	0.012 (-0.024; 0.048)	0.508
Mitochondrial (N=8)	0.010 (-0.023; 0.043)	0.559	0.004 (-0.032; 0.039)	0.840	0.039 (0.001; 0.077)	0.046

Table 2. *(Continued)*

Risk score (number of SNPs in risk score)	Peak weight velocity[b]		BMI at adiposity peak[b]		Age at adiposity peak[b]	
	Beta (CI 95%)	P-value	Beta (CI 95%)	P-value	Beta (CI 95%)	P-value
Retinoic acid receptors (N=6)	0.019 (-0.013; 0.050)	0.245	0.025 (-0.009; 0.058)	0.144	0.019 (-0.017; 0.055)	0.308
Endocytosis/exocytosis (N=14)	0.004 (-0.027; 0.036)	0.778	0.005 (-0.028; 0.038)	0.776	0.007 (-0.029; 0.043)	0.699
Eye-related (N=5)	0.010 (-0.022; 0.042)	0.548	0.010 (-0.025; 0.045)	0.567	-0.030 (-0.067; 0.007)	0.116
Tumorigenesis (N=11)	0.018 (-0.015; 0.050)	0.285	0.018 (-0.017; 0.052)	0.320	-0.001 (-0.038; 0.036)	0.954
Apoptosis (N=13)	0.027 (-0.004; 0.059)	0.087	0.018 (-0.016; 0.052)	0.294	0.033 (-0.004; 0.069)	0.077
Membrane proteins (N=12)	0.057 (0.025; 0.088)	**$3.88*10^{-4}$**	0.048 (0.015; 0.082)	0.005	0.028 (-0.008; 0.065)	0.124
Hormone metabolism/regulation (N=4)	-0.009 (-0.041; 0.022)	0.564	-0.009 (-0.042; 0.025)	0.610	0.010 (-0.027; 0.046)	0.604
Purine/pyrimidine cycle (N=4)	0.009 (-0.022; 0.041)	0.557	0.039 (0.006; 0.073)	0.023	-0.025 (-0.061; 0.011)	0.178
Monogenic obesity/energy homeostasis (N=9)	-0.013 (-0.045; 0.018)	0.406	-0.014 (-0.048; 0.020)	0.413	0.026 (-0.011; 0.062)	0.168
Immune system (N=15)	0.045 (0.014; 0.076)	0.005	0.049 (0.015; 0.082)	0.004	-0.003 (-0.039; 0.033)	0.868
Limb development (N=3)	0.018 (-0.014; 0.049)	0.267	0.022 (-0.011; 0.056)	0.195	0.001 (-0.035; 0.037)	0.945
Ubiquitin pathways (N=6)	-0.006 (-0.038; 0.025)	0.684	0.007 (-0.027; 0.040)	0.693	-0.025 (-0.061; 0.011)	0.168
Glucose homeostasis/diabetes (N=11)	0.023 (-0.009; 0.054)	0.160	0.021 (-0.013; 0.055)	0.219	0.026 (-0.010; 0.063)	0.156
Cell cycle (N=23)	0.008 (-0.023; 0.039)	0.611	0.011 (-0.023; 0.044)	0.538	-0.001 (-0.037; 0.035)	0.959
DNARepair						
Nuclear trafficking (N=4)	-0.015 (-0.047; 0.017)	0.362	-0.023 (-0.057; 0.011)	0.187	-0.032 (-0.068; 0.005)	0.092
Muscle biology (N=6)	-0.011 (-0.043; 0.020)	0.479	-0.0003 (-0.034; 0.033)	0.985	0.014 (-0.022; 0.050)	0.446

[a]Analyses were performed in children with complete data on genetic variants, at least one outcome under study, and covariates [b]Values are linear regression coefficients for models adjusted for sex and the first four genetic principal components and represent the difference in standard deviation scores of the outcome measures for each additional average risk allele in the risk scores. *Significant after Bonferroni correction for the 28 pathways (p-value<0.0018)

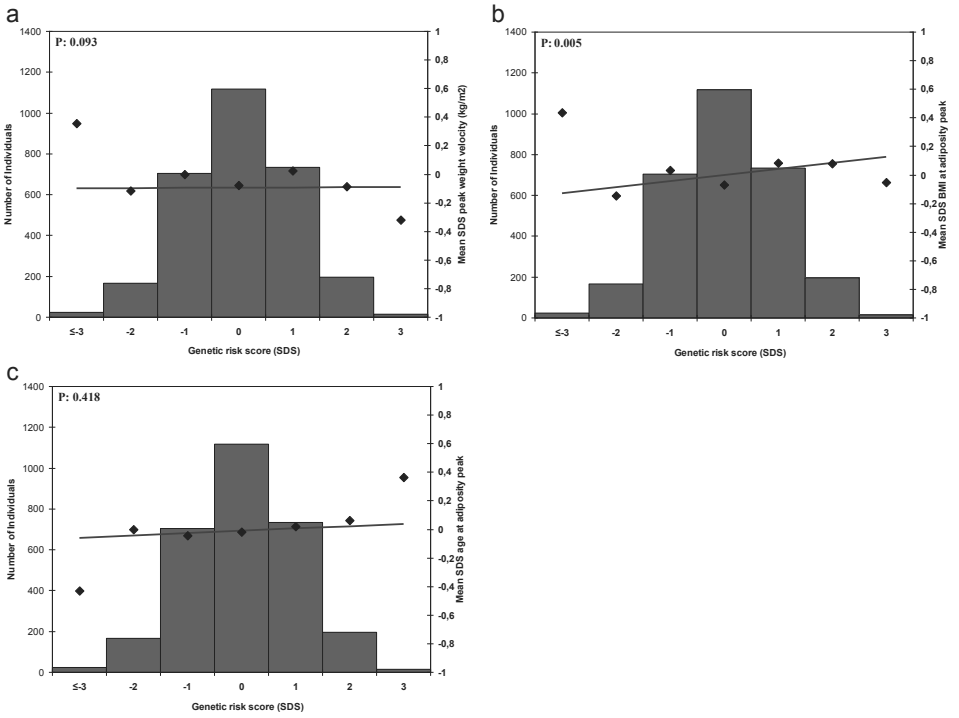

Figure 1. Association of adult body mass index genetic risk score with infant growth measures (N= 3,114).

The *x* axis represents the categories of the risk score (overall sum of risk alleles, weighted by previously reported effect estimates, rescaled to SDS. The risk score ranged from -4 to 3 SDS and was rounded to the nearest integer for clarity of presentation. The right *y* axis shows mean SDS and corresponds to the dots and the line representing the regression of the mean SDS values for each category of the risk score. The *y* axis on the left corresponds to the histogram representing the number of individuals in each risk-score category. P-value is based on the continuous risk score, as presented in **Table 2**. Graphs represent; **a:** peak weight velocity, **b:** BMI at adiposity peak , and **c:** age at adiposity peak.

General and abdominal adiposity at school-age

The overall adult BMI genetic risk score was associated with all childhood general and abdominal adiposity measures. For each SD increase in the genetic risk score, childhood BMI increased by 0.112 SDS (95% CI 0.084, 0.141), total fat mass increased by 0.092 SDS (95% CI 0.065, 0.119), android/gynoid fat ratio increased by 0.077 SDS (95% CI 0.045, 0.108), and increased preperitoneal fat area by 0.034 SDS (95% CI 0.001, 0.066) (Table 3; Figures 2a-d). Effect estimates for the unweighted and weighted 97 adult BMI SNPs risk scores were similar (Supplemental Table S4). Addition of PWV to the regression models did not materially change the effect estimates for the association of the BMI risk scores with BMI, total fat mass percentage, and android/gynoid fat ratio. However, the effect estimate

for the association of the adult BMI risk score with childhood preperitoneal fat area was no longer significant. We observed similar findings when we added BMIAP instead of PWV to these regression models. However, the effects on the associations of the BMI risk scores with BMI and total fat mass were somewhat larger. Effect estimates for the associations of the child BMI risk score with BMI and total fat mass were 10-15% lower after additional adjustment for PWV. Effect estimates for android/gynoid fat ratio and preperitoneal fat area did not materially change. We observed similar findings after additional adjustment for BMIAP (Supplemental Tables S7, S8).

Of the 28 adult BMI genetic risk scores based on the biological pathways, those based on neuronal developmental processes, hypothalamic expression and regulation, WNT-signaling, membrane proteins, monogenic obesity/energy homeostasis, glucose homeostasis/diabetes, and muscle biology were associated with childhood BMI (all p-values <0.0018). Genetic risk scores based on hypothalamic expression and regulation, cyclicAMP, membrane proteins, monogenic obesity/energy homeostasis, and cell cycle were associated with total fat mass, whereas for android/gynoid fat ratio only the genetic risk scores based on hypothalamic expression and regulation, membrane proteins, and monogenic obesity/energy homeostasis show significant associations (all p-values <0.0018). None of the pathways were associated with preperitoneal fat area (Table 3). We based our pathway risk scores on these biological categories to keep our analysis as close as possible to the analysis of the original paper as possible.[8] As comparison, we also ran a pathway analysis using IPA. Results were comparable regarding the major categories (eg. neurological development and function, cell cycle, lipid metabolism, apoptosis). However, the IPA software showed a larger subdivision with 74 different pathways instead of 28 as suggested by the GIANT consortium (Supplemental Table S9). The overall adult WHR genetic risk score was only associated with android/gynoid fat ratio (Table 3; Supplemental Figure S4a-d). The childhood BMI genetic risk score was associated with all childhood adiposity measures (Table 3; Supplemental Figure S5a-d). The genetic risk score based on 29 SNPs showed higher effect estimates per SD increase than our 97 SNPs adult BMI risk score for the childhood adiposity outcomes, especially for preperitoneal fat area (Supplemental Table S5). The 97 adult BMI SNPs explained 4.9% of childhood BMI when added into our model as individual SNPs. When the 97 SNPs were combined into the weighted risk score and added to our model, the risk score explained 1.4% of childhood BMI (Supplemental Table S10).

Table 3. Associations of BMI, WHR, and childhood BMI genetic risk scores with childhood adiposity (N= 3,975)[a, b]

	Body mass index[c,d]		Total fat mass[d,e,f]		Android/gynoid ratio[d,e,f]		Preperitoneal fat area[d,e,f]	
Risk score (number of SNPs in risk score)	**Beta (CI 95%)**	**P-value**	**Beta (CI 95%)**	**P-value**	**Beta (CI 95%)**	**P-value**	**Beta (CI 95%)**	**P-value**
Main risk scores								
Adult BMI (N=97)	0.112 (0.084; 0.141)	**$1.01*10^{-14}$**	0.092 (0.065; 0.119)	**$3.89*10^{-11}$**	0.077 (0.045; 0.108)	**$2.00*10^{-6}$**	0.034 (0.001; 0.066)	**0.042**
Secondary risk scores								
Adult WHR (N=47)	-0.012 (-0.042; 0.017)	0.405	-0.012 (-0.040 0.016)	0.402	0.073 (0.041; 0.105)	**$8.00*10^{-6}$**	0.029 (-0.004; 0.061)	0.088
Child BMI (N=15)	0.091 (0.063; 0.119)	**$3.43*10^{-10}$**	0.073 (0.046; 0.100)	**$1.40*10^{-7}$**	0.081 (0.050; 0.112)	**$3.75*10^{-7}$**	0.038 (0.006; 0.070)	**0.020**
Adult BMI pathway genetic risk scores*								
Neuronal								
Neuronal developmental processes (N=29)	0.018 (0.014; 0.023)	**2.25*10-5**	0.032 (0.003; 0.061)	0.031	0.038 (0.004; 0.071)	0.029	0.008 (-0.026; 0.042)	0.654
Neurotransmission (N=10)	0.013 (-0.015; 0.042)	0.370	-0.003 (-0.030; 0.024)	0.827	0.002 (-0.029; 0.034)	0.876	-0.009 (-0.040; 0.023)	0.595
Hypothalamic expression and regulation (N=13)	0.099 (0.071; 0.128)	**$5.81*10^{-12}$**	0.089 (0.062; 0.115)	**$1.29*10^{-10}$**	0.080 (0.049; 0.111)	**$5.30*10^{-7}$**	0.041 (0.009; 0.073)	0.013
Neuronal expression (N=12)	0.017 (-0.012; 0.046)	0.240	0.020 (-0.008; 0.047)	0.165	0.036 (0.004; 0.068)	0.027	0.009 (-0.023; 0.041)	0.583
	0.023 (-0.005; 0.052)	0.112	0.013 (-0.014; 0.041)	0.341	0.016 (-0.016; 0.048)	0.320	-0.001 (-0.033; 0.032)	0.972
Bone development (N=9)	0.018 (-0.011; 0.064)	0.226	0.006 (-0.021; 0.033)	0.656	0.015 (-0.016; 0.047)	0.340	0.004 (-0.028; 0.036)	0.811
Signaling								
MAPK1/extracellular signal-regulated kinases (N=9)	0.034 (0.006; 0.062)	0.018	0.037 (0.010; 0.064)	0.008	0.023 (-0.008; 0.054)	0.149	0.014 (-0.017; 0.046)	0.378
JAK (N=2)	0.033 (0.005; 0.062)	0.023	0.020 (-0.007; 0.047)	0.150	0.012 (-0.020; 0.043)	0.457	0.007 (-0.025; 0.039)	0.676
CyclicAMP (N=5)	0.046 (0.017; 0.075)	0.002	0.052 (0.024; 0.079)	**$2.75*10^{-4}$**	0.039 (0.006; 0.071)	0.019	0.026 (-0.007; 0.058)	0.123
WNTSignaling (N=6)	0.058 (0.030; 0.087)	**$6.10*10^{-5}$**	0.029 (0.002; 0.057)	0.034	0.039 (0.007; 0.070)	0.016	0.032 (0.000; 0.064)	0.047
G-protein coupled receptor								
Notch signaling (N=2)	-0.027 (-0.056; 0.001)	0.059	-0.028 (-0.055; 0.000)	0.046	-0.028 (-0.059; 0.003)	0.075	-0.028 (-0.060; 0.003)	0.080

Table 3. *(Continued)*

	Body mass index[c,d]		Total fat mass[d,e,f]		Android/gynoid ratio[d,e,f]		Preperitoneal fat area[d,e,f]	
Risk score (number of SNPs in risk score)	**Beta (CI 95%)**	**P-value**	**Beta (CI 95%)**	**P-value**	**Beta (CI 95%)**	**P-value**	**Beta (CI 95%)**	**P-value**
Mitochondrial (N=8)	0.040 (0.010; 0.070)	**0.009**	0.041 (0.012; 0.069)	0.005	-0.002 (-0.035; 0.031)	0.905	-0.003 (-0.037; 0.031)	0.877
Retinoic acid receptors (N=6)	0.045 (0.017; 0.074)	0.002	0.037 (0.010; 0.065)	0.007	0.016 (-0.015; 0.047)	0.313	0.017 (-0.015; 0.049)	0.293
Endocytosis/exocytosis (N=14)	-0.012 (-0.041; 0.016)	0.400	-0.003 (-0.030; 0.024)	0.840	-0.021 (-0.053; 0.010)	0.178	-0.020 (-0.051; 0.012)	0.218
Eye-related (N=5)	0.012 (-0.016; 0.041)	0.398	0.015 (-0.012; 0.043)	0.276	-0.012 (-0.044; 0.020)	0.456	0.003 (-0.029; 0.035)	0.845
Tumorigenesis (N=11)	0.041 (0.012; 0.070)	0.006	0.020 (-0.008; 0.048)	0.161	0.017 (-0.016; 0.049)	0.312	0.013 (-0.020; 0.046)	0.431
Apoptosis (N=13)	0.025 (-0.003; 0.054)	0.084	0.020 (-0.007; 0.047)	0.151	-0.008 (-0.039; 0.024)	0.621	-0.036 (-0.068; -0.004)	0.028
Membrane proteins (N=12)	0.075 (0.046; 0.103)	**$2.44*10^{-7}$**	0.044 (0.017; 0.071)	**0.002**	0.059 (0.028; 0.090)	**$1.93*10^{-4}$**	0.011 (-0.021; 0.044)	0.495
Hormone metabolism/regulation (N=4)	0.021 (-0.008; 0.049)	0.161	0.043 (0.015; 0.070)	0.002	0.026 (-0.005; 0.057)	0.103	0.004 (-0.028; 0.036)	0.812
Purine/pyrimidine cycle (N=4)	0.013 (-0.016; 0.041)	0.379	0.004 (-0.023; 0.031)	0.762	-0.017 (-0.048; 0.014)	0.285	-0.007 (-0.038; 0.024)	0.661
Monogenic obesity/energy homeostasis (N=9)	0.074 (0.045; 0.102)	**$4.74*10^{-7}$**	0.068 (0.041; 0.095)	**$1.00*10^{-6}$**	0.065 (0.034; 0.096)	**$5.00*10^{-5}$**	0.030 (-0.003; 0.062)	0.072
Immune system (N=15)	0.045 (0.017; 0.074)	0.002	0.037 (0.010; 0.065)	0.008	0.021 (-0.011; 0.052)	0.193	-0.008 (-0.040; 0.024)	0.620
Limb development (N=3)	0.035 (0.007; 0.064)	0.015	0.024 (-0.006; 0.049)	0.125	0.028 (-0.003; 0.060)	0.076	0.004 (-0.028; 0.036)	0.794
Ubiquitin pathways (N=6)	-0.007 (-0.036; 0.021)	0.617	0.006 (-0.021; 0.034)	0.656	-0.011 (-0.043; 0.020)	0.483	-0.015 (-0.047; 0.017)	0.359
Glucose homeostasis/diabetes (N=11)	0.050 (0.021; 0.079)	**0.001**	0.023 (-0.004; 0.051)	0.096	0.042 (0.011; 0.074)	0.008	0.009 (-0.023; 0.042)	0.575
Cell cycle (N=23)	0.044 (0.016; 0.073)	0.002	0.047 (0.019; 0.074)	**0.001**	0.024 (-0.007; 0.055)	0.135	0.007 (-0.025; 0.039)	0.684
DNARepair								
Nuclear trafficking (N=4)	-0.005 (-0.034; 0.023)	0.716	-0.009 (-0.036; 0.018)	0.518	-0.004 (-0.036; 0.028)	0.804	-0.008 (-0.041; 0.024)	0.608
Muscle biology (N=6)	0.048 (0.020; 0.077)	**0.001**	0.029 (0.002; 0.057)	0.038	0.025 (-0.007; 0.056)	0.127	0.022 (-0.010; 0.054)	0.181

[a]Analyses were performed in children with complete data on genetic variants, at least one outcome under study, and covariates [b]Values are linear regression coefficients for models adjusted for sex and the first four genetic principal components and represent the difference in standard deviation scores of the outcome measures for each additional average risk allele in the risk scores. [d]Values are additionally adjusted for age. [e]Values are additionally adjusted for height. [f]Regression coefficients are based on standard deviation scores of ln-transformed outcome measures.
* Significant after Bonferroni correction for the 28 pathways (p-value <0.0018)

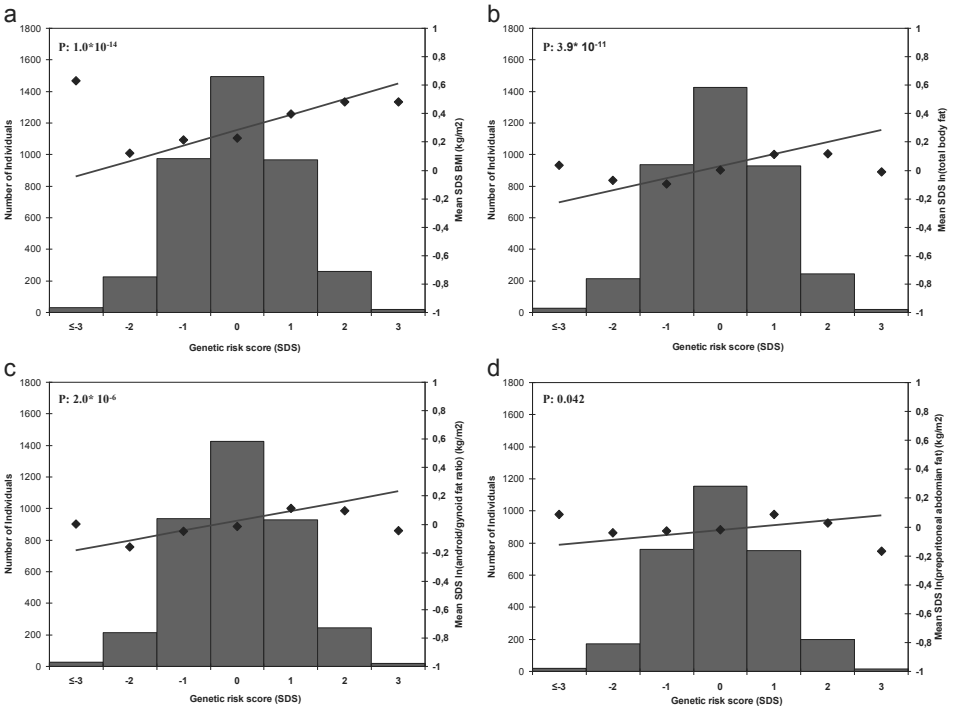

Figure 2. Association of adult body mass index genetic risk score with childhood adiposity measures (N= 4,151)

The *x* axis represents the categories of the risk score (overall sum of risk alleles, weighted by previous reported effect estimates, rescaled to SDS. The risk score ranged from -4 to 3 SDS and was rounded to the nearest integer for clarity of presentation). The right *y* axis shows the mean SDS and corresponds to the dots and a line representing the regression line of the mean SDS values for each category of the risk score. The *y* axis on the left corresponds to the histogram representing the number of individuals in each risk-score category. P-value is based on the continuous risk score, as presented in **Table 3**. Graph **a-d** represent; **a:** BMI in kg/m^2, **b:** ln(fat mass percentage), **c:** ln(android/gynoid fat ratio), and **d:** ln(preperitoneal fat area).

Discussion

We observed that a higher overall adult BMI genetic risk score based on 97 SNPs was associated with BMIAP during infancy, and with BMI, total fat mass, android/gynoid fat ratio, and preperitoneal fat area during childhood. A genetic risk score based on SNPs in or close to genes in the membrane proteins pathway was associated with infant PWV, whereas genetic risk scores based on pathways for neuronal developmental processes, hypothalamic processes, cyclicAMP, WNT-signaling, membrane proteins, monogenic obesity/energy homeostasis, glucose homeostasis, cell cycle, and muscle biology were associated with childhood adiposity measures. None of the pathway risk scores were associated with preperitoneal fat area.

Interpretation of main findings

Previous studies revealed a total of 97 loci related to adult BMI.[8] In a previous study, we reported on the association of a genetic risk score based on 29 adult BMI SNPs known at that time with infant growth and childhood adiposity measures.[12] This risk score was associated with a higher AGEAP and with a higher BMI, total fat mass, android/gynoid fat ratio, and preperitoneal fat area. In the current study, we aimed to identify the effects of updated and more detailed risk scores based on the 97 currently known loci and on subgroups of loci representing specific biological pathways on the same infant growth and childhood adiposity measures. Infant weight growth patterns are known to be strongly associated with BMI in childhood and adulthood, and childhood BMI is associated with obesity and cardiovascular disease in adulthood.[1-4,20,22,24] Thus, it is important to understand the molecular pathways underlying childhood adiposity.

Our results suggest a modest effect of the adult BMI risk score on infant weight growth measures. We observed an association of the overall adult BMI genetic risk score with BMIAP only. In our previous study, based on 29 adult BMI SNPs, the genetic risk score was associated with AGEAP only.[12] A recent study among 9,328 children reported an association of a genetic risk score of 32 adult BMI-associated SNPs, including the 29 included in our previous risk score, with BMIAP, which is in line with our current finding. Additionally, a weak inverse association was found of this risk score with AGEAP.[29] The difference in associations between the previously published 29 SNP adult BMI risk score and our current 97 SNP adult BMI risk score may imply that the increased number of SNPs in the current genetic risk score adds noise to the association of the 97 SNP adult BMI risk score with childhood adiposity outcomes. Also, the analyses were run in a slightly different population, as siblings were excluded for the current study. The added SNPs may be more representative of BMIAP. The childhood BMI genetic risk score was associated with infant

PWV and BMIAP, which are both strongly associated with increased risk of overweight in childhood.[23] The overall adult BMI genetic risk score was also associated with all childhood adiposity measures, which is in line with previous studies.[12,29,30] Some of these associations are partly explained by infant growth. The WHR risk score was associated with childhood android/gynoid fat ratio only, which is not surprising given the close relation of android/gynoid fat ratio to WHR. Results for the childhood BMI risk score were similar to the associations found with the adult BMI risk score, except that effect estimates were much larger for the child BMI risk score. Larger effect estimates may reflect stronger effects of the childhood-specific SNPs in children. ur results suggest that genetic risk scores based on adult BMI, WHR and childhood BMI influence childhood adiposity outcomes, and also BMI growth patterns from infancy onwards.

The 97 SNPs in our risk score explained 2.7% of the adult BMI variance in the original paper.[8] In the current study we found that the same SNPs, when added simultaneously to our regression model, account for 4.9% of childhood BMI suggesting a larger effect of these SNPs in childhood than in adulthood. This may be due to a relative increase in the effects of environmental factors over time. It should be noted that this estimate represents the upper bound of the phenotypic variation accounted for by the 97 SNPs, due to the method of entering all SNPs simultaneously to the model rather than combined into a risk score. When combined into a weighted risk score the 97 SNPs explained only 1.5% of childhood BMI. We previously reported on a genetic risk score combining only 29 adult BMI SNPs, which explained 2.4% of the variance in BMI in children of the Generation R Study.[12] Increasing the number of adult SNPs from 29 to 97 thus seemed to add noise to our risk score. It may be that some genetic loci show age-dependent associations with BMI, with different effects in children as compared to adults.[20,31] Previous work has described an inverse association of the fat mass and obesity related locus (*FTO*) with BMI before the age of 2.5 years, no association between 2.5 and 5 years, and a positive association from around the age of 5 years onwards. The association then strengthens with age, reaching its peak at the age of 20 years and subsequently weakens again.[20,31] A similar age dependent pattern has been observed for the melanocortin 4 receptor (*MC4R*) locus.[31]

Our results showed that during infancy only the membrane proteins pathway affects PWV. This pathway involves membrane proteins that play a role regulating different cell processes involved in e.g. apetite, cholesterol synthesis, and gene expression.[8] Our findings suggest that these processes are also important for weight growth during early life. None of the other pathways were associated with infant growth measures. In line with previous adult studies, a strong role was observed for central nervous system related processes in pathways associated with childhood adiposity measures.[8,32] Especially the hypothalamic expression and regulation pathway is suggested to be important, which is

confirmed in our analyses.[8,20] Mutations in some of the genes in the monogenic obesity/energy homeostasis related pathways are also suggested to act via central nervous system related processes.[32] The hypothalamic expression and regulation and the monogenic obesity/energy homeostasis pathways, as well as the membrane pathway were associated with childhood BMI, total fat mass, and android/gynoid fat ratio. The other pathways that were associated with BMI suggest a role for fasting/feeding related processes, glucose homeostasis, signaling, and diabetes related pathways. Our findings suggest a stronger role for the predefined categories during childhood than during infancy, which may be because the childhood measures are more closely related to adult BMI.

Reported total heritability estimates for childhood BMI from twin studies are as high as 80%. In the current study we found that a risk score based on the known SNPs only explained 1.5% of the variation in child BMI, emphasizing that a large part of the heritability remains to be discovered.[5,6] In addition to SNPs, other sources of (epi-)genetic variation, such as copy number variants (CNV) and differences in methylation, may also contribute.[33,34] A large part of the common CNVs have been efficiently tagged by SNPs in GWA studies.[35] However, associations of rarer CNVs with BMI and obesity showed mixed results.[34,36,37] Recently, methylation at specific sites in the DNA has been associated with BMI in adults and children.[33,38,39] Additional research in larger study populations is needed to further disentanble the (epi-)genetic background of BMI in children and adults.

Methodological considerations

The large number of participants and available detailed phenotypes is a major strength of the study. Of all children with genetic data, information on infant growth measures was available for 54%. Measures of childhood general and abdominal adiposity were available in 72% of all children. Children without information on infant growth measures had a higher BMI, total fat mass, android/gynoid fat ratio, and preperitoneal fat area (all p-values < 0.001) compared with the participants included in our analyses. This may have resulted in an underestimation of the association for the risk scores with infant growth and childhood adiposity measures. Detailed measurements of childhood abdominal adiposity were performed. Both DXA and abdominal ultrasound are considered valid methods for such measurements.[24,26] Not all SNPs were available in our GWAS dataset. We used a limited number of proxies in very high linkage disequilibrium to complete the sets of SNPs for adult BMI, WHR, and child BMI. No good proxies were available for two WHR SNPs. Given the high number of SNPs available, all risk scores are considered a good representative of the original set of adult SNPs. Although our population is relatively large, we still may have had limited power for these analyses, leaving a possibility of underestimating the number of associated pathways.

Conclusions

A genetic risk score based on 97 loci associated with adult BMI was associated with PWV during infancy and with general and abdominal fat measurements in childhood. Our results suggest that the genetic background and the pathways involved in adult and childhood adiposity at least partly overlap. Adult BMI related biological pathways involved in neuronal developmental processes, hypothalamic expression and regulation, cyclicAMP, WNT-signaling, membrane proteins, monogenic obesity/energy homeostasis, glucose homeostasis, and cell cycle likely influence adiposity from early life onwards. Further studies are needed to identify more (rare) loci and unravel the underlying mechanisms of childhood adiposity.

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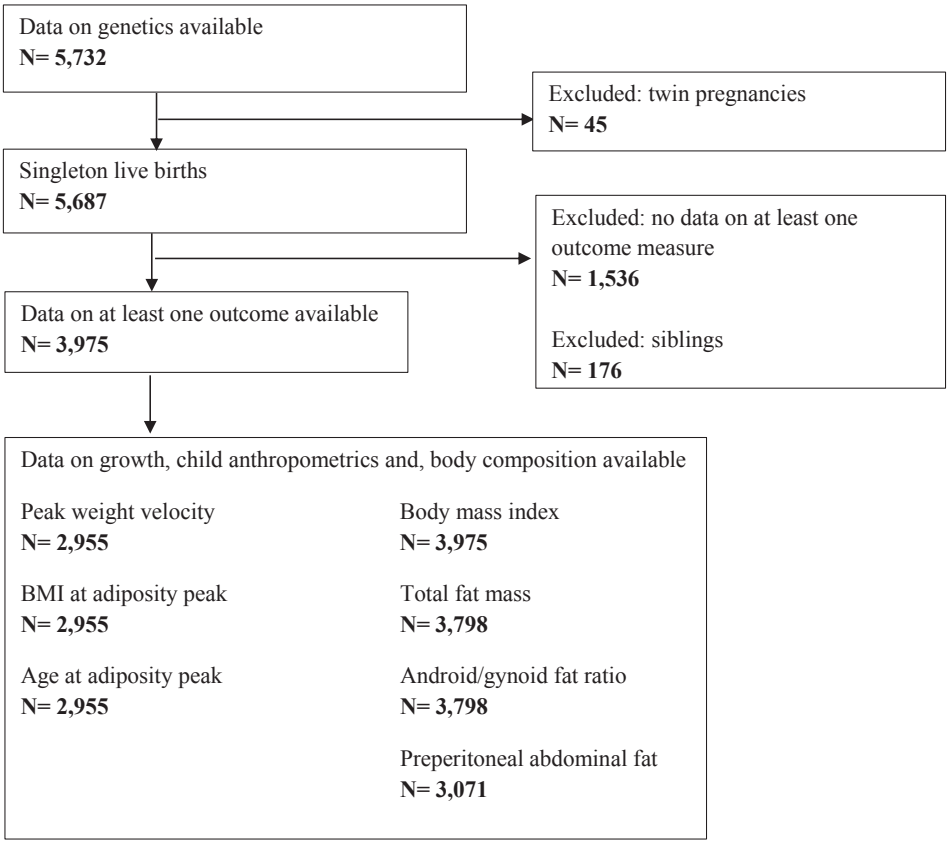
Supplement



Supplemental Figure S1. Flow chart of participants

Supplemental Table S1. List of SNPs included in the main BMI and WHR risk scores

Risk score *Gene name*	SNP name	Proxy	Chr.	Position	Risk score *Gene name*	SNP name	Proxy	Chr.	Position
Adult BMI									
RARB	rs6804842		3	25106437	*SCARB2*	rs17001654		4	77129568
TFAP2B	rs2207139		6	50845490	*MYST1*	rs9925964	rs1978487[a]	16	31129942
MAP2K5	rs16951275		15	68077168	*OLFM4*	rs12429545		13	54102206
FLJ45139	rs2836754		21	40291740	*C9orf93*	rs4740619		9	15634326
FTO	rs1558902		16	53803574	CALCR	rs9641123		7	93197732
NLRC3	rs758747		16	3627358	*NEGR1*	rs3101336		1	72751185
CADM1	rs12286929		11	115022404	*ATP2A1*	rs3888190		16	28889486
IFNGR1	rs13201877		6	137675541	*HNF4G*	rs17405819		8	76806584
RALYL	rs2033732		8	85079709	*KIAA1505*	rs2245368		7	76608143
GRID1	rs7899106		10	87410904	*PGPEP1*	rs17724992	rs9636202[a]	19	18449238
CBLN1	rs2080454		16	49062590	*FOXO3*	rs9400239		6	108977663
TAL1	rs977747		1	47684677	*SEC16B*	rs543874		1	177889480
NT5C2	rs11191560		10	104869038	*IRS1*	rs2176040		2	227092802
EPB41L4B	rs6477694		9	111932342	*PTBP2*	rs11165643		1	96924097
ZBTB10	rs16907751		8	81375457	*TMEM18*	rs13021737	rs13012571[a]	2	632550
DOC2A	rs4787491		16	30015337					
AGBL4	rs657452		1	49589847	**Adult WHR**				
HSD17B12	rs2176598		11	43864278	*BCL2*	rs12454712		18	58996864
TLR4	rs1928295		9	120378483	*BMP2*	rs979012		20	6571374
RBM26	rs1441264		13	79580919	*ADAMTS9*	rs2371767	rs4607103[b]	3	64686944
FUBP1	rs12401738		1	78446761	*SFXN2*	rs7917772		10	104477433
PRKD1	rs12885454		14	29736838	*SPATA5*	rs303084		4	124286398
BDNF	rs11030104		11	27684517	*WARS2*	rs2645294		1	119376110
NAV1	rs2820292		1	201784287	*SMAD6*	rs1440372		15	64820205
MTCH2	rs3817334		11	47650993	*MEIS1*	rs1385167		2	66054152
C5orf37	rs2112347		5	75015242	*VEGFA*	rs1358980		6	43872529
EHBP1	rs11688816		2	63053048	*EYA2*	rs6090583		20	44992238
RASA2	rs16851483		3	141275436	*DCST2*	rs905938		1	153258013
ZFP64	rs6091540		20	51087862	*CCDC92*	rs4765219		12	123006063
HHIP	rs11727676		4	145659064	*CMIP*	rs2925979		16	80092291
PACRG	rs13191362		6	163033350	*ITPR2*	rs10842707		12	26362631
FHIT	rs2365389	rs6445197[a]	3	61213993	*DNM3*	rs714515		1	170619613
MC4R	rs6567160		18	57829135	PEMT	rs4646404		17	17360924
KCTD15	rs29941		19	34309532	*SNX10*	rs1534696		7	26363764
ADCY3	rs10182181		2	25150296	*JUND*	rs12608504		19	18250135
QPCTL	rs2287019		19	46202172	*MSC*	rs12679556		8	72676782

Supplemental Table S1. *(Continued)*

Risk score *Gene name*	SNP name	Proxy	Chr.	Position	Risk score *Gene name*	SNP name	Proxy	Chr.	Position
LMX1B	rs10733682		9	129460914	*CALCRL*	rs1569135		2	187823643
SBK1	rs2650492		16	28333411	*LY86*	rs1294410		6	6683751
TCF7L2	rs7903146		10	114758349	*HMGA1*	rs1776897		6	34302989
RPL27A	rs4256980		11	8673939	*RFXDC2*	rs8030605		15	54291890
C6orf106	rs205262		6	34563164	*HOXC13*	rs1443512		12	52628951
LRP1B	rs2121279		2	143043285	*TNFAIP8*	rs1045241		5	118757185
SLC39A8	rs13107325		4	103188709	*BTNL2*	rs7759742		6	32489714
GNPDA2	rs10938397		4	45182527	*SCYL1BP1*	rs10919388		1	168639127
NPC1	rs1808579		18	21104888	*ABCA1*	rs10991437		9	106775741
UBE2E3	rs1528435		2	181550962	*HOXA11*	rs7801581		7	27190296
C19orf7	rs3810291		19	47569003	*RSPO3*	rs1936805		6	127493809
BCDIN3D	rs7138803		12	50247468	*CPEB4*	rs7705502		5	173253421
CADM2	rs13078960		3	85807590	*PPARG*	rs17819328		3	12464342
USP37	rs492400		2	219349752	*FAM13A1*	rs9991328		4	89932144
TOMM40	rs2075650		19	45395619	*PBRM1*	rs2276824		3	52612526
TNNI3K	rs12566985		1	75002193	*SLC30A10*	rs2820443		1	217820132
ASB4	rs6465468		7	95169514	FLRT1	rs11231693		11	63619188
CLIP1	rs11057405		12	122781897	*COBLL1*	rs10195252		2	165221337
MTIF3	rs12016871		NA	NA	*NMU*	rs3805389		4	56177507
STXBP6	rs10132280		14	25928179	*KCNJ2*	rs8066985		17	65964940
PRKD1	rs11847697		14	30515112	*NKX3-1*	rs7830933		8	23659269
HIF1AN	rs17094222		10	102395440	*CEBPA*	rs4081724		19	38516786
LINGO2	rs10968576		9	28414339	*NFE2L3*	rs10245353		7	25825139
FANCL	rs1016287		2	59305625	*MAP3K1*	rs9687846		5	55897651
CREB1	rs17203016		2	208255518	*ZNRF3*	rs2294239		22	27779477
KCNK3	rs11126666		2	26928811	*GDF5*	rs224333		20	33487376
LRFN2	rs2033529		6	40348653	*PLXND1*	rs10804591		3	130816923
ELAVL4	rs11583200		1	50559820	*FLJ16641*	rs17451107		3	158280303
SMG6	rs9914578		17	2005136					
HIP1	rs1167827		7	75163169	**Child BMI**				
ETV5	rs1516725		3	185824004	*GNPDA2*	rs13130484		4	44870448
ADPGK	rs7164727		15	73093991	*ADCY3*	rs11676272		2	24995042
GBE1	rs3849570		3	81792112	*TMEM18*	rs4854349		2	637861
MAN1A1	rs9374842		6	120185665	*SEC16B*	rs543874		1	176156103
RIT2	rs7239883		18	40147671	*FAIM2*	rs7132908		12	48549415
FIGN	rs1460676		2	164567689	*FTO*	rs1421085	rs3751812[c]	16	52358455
KIAA1303	rs12940622		17	78615571	*OLFM4*	rs12429545		13	53000207

Supplemental Table S1. *(Continued)*

Risk score *Gene name*	SNP name	Proxy	Chr.	Position	Risk score *Gene name*	SNP name	Proxy	Chr.	Position
ERBB4	rs7599312		2	213413231	*TFAP2B*	rs987237		6	50911009
DMXL2	rs3736485		15	51748610	*TNNI3K*	rs12041852		1	74776088
PCDH9	rs9540493		13	66205704	*MC4R*	rs6567160		18	55980115
RABEP1	rs1000940		17	5283252	*ELP3*	rs13253111		8	28117893
NRXN3	rs7141420		14	79899454	*RAB27B*	rs8092503		18	50630485
GALNT10	rs7715256		5	153537893	*LMX1B*	rs3829849		9	128430621
GPRC5B	rs12446632		16	19935389	*ADAM23*	rs13387838		2	206989692
GRP	rs7243357		18	56883319	GPR61	rs7550711		1	109884409
GNAT2	rs17024393		1	110154688					

[a] Linkage disequilibrium between proxies and original SNPs: R^2> 0.96, D'=1. [b] Linkage disequilibrium between proxy and original SNP: R^2= 0.90, D'=1. [c] Linkage disequilibrium between proxy and original SNP: R^2= 0.93, D'=0.97.

Supplemental Table S2 can be found online.

Supplemental Table S4. Associations of unweighted genetic risk score for BMI with infant growth and childhood adiposity[a,b]

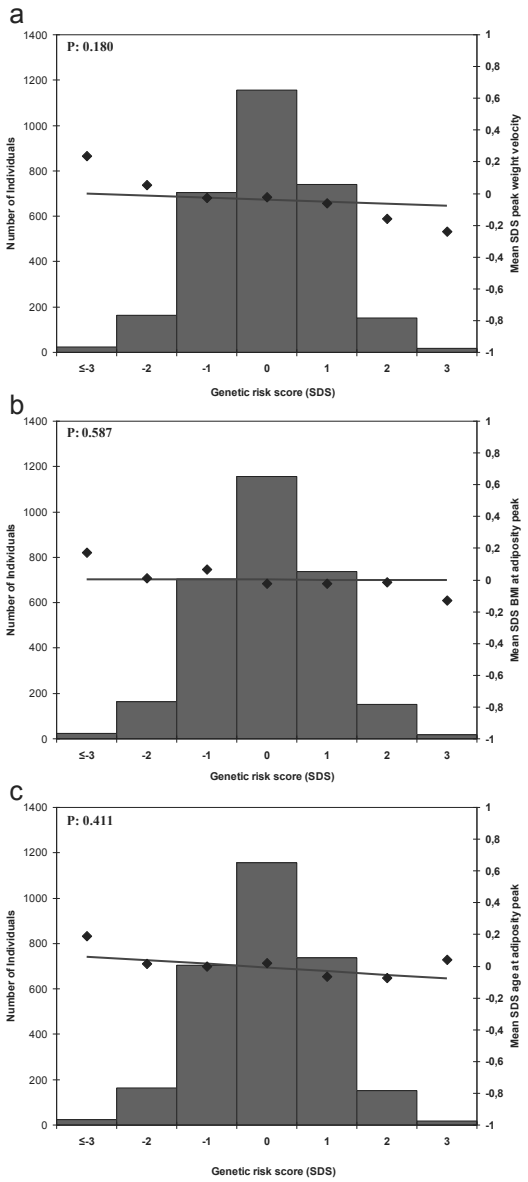
Adult BMI (N=97)	Peak weight velocity[c]	BMI at adiposity peak[c]	Age at adiposity peak[c]	Body mass index[c,d]	Total fat mass[d,e,f]	Android/gynoid ratio[d,e,f]	Preperitoneal fat area[d,e,f]
Beta	0.029	0.055	0.003	0.088	0.075	0.059	0.019
(CI 95%)	(-0.003, 0.060)	(0.022, 0.089)	(-0.034, 0.039)	(0.060, 0.117)	(0.047, 0.102)	(0.028, 0.091)	(-0.014, 0.051)
p-value	0.075	**0.001**	0.881	**$1.93*10^{-9}$**	**$1.11*10^{-7}$**	**$2.57*10^{-4}$**	0.264

[a]Analyses were performed in children with complete data on genetic variants, at least one outcome under study, and covariates [b]Values are linear regression coefficients for models adjusted for sex and the first four genetic principal components and represent the difference in standard deviation scores of the outcome measures for each additional average risk allele in the risk scores. [d]Values are additionally adjusted for age. [e]Values are additionally adjusted for height. [f]Regression coefficients are based on standard deviation scores of ln-transformed outcome measures.

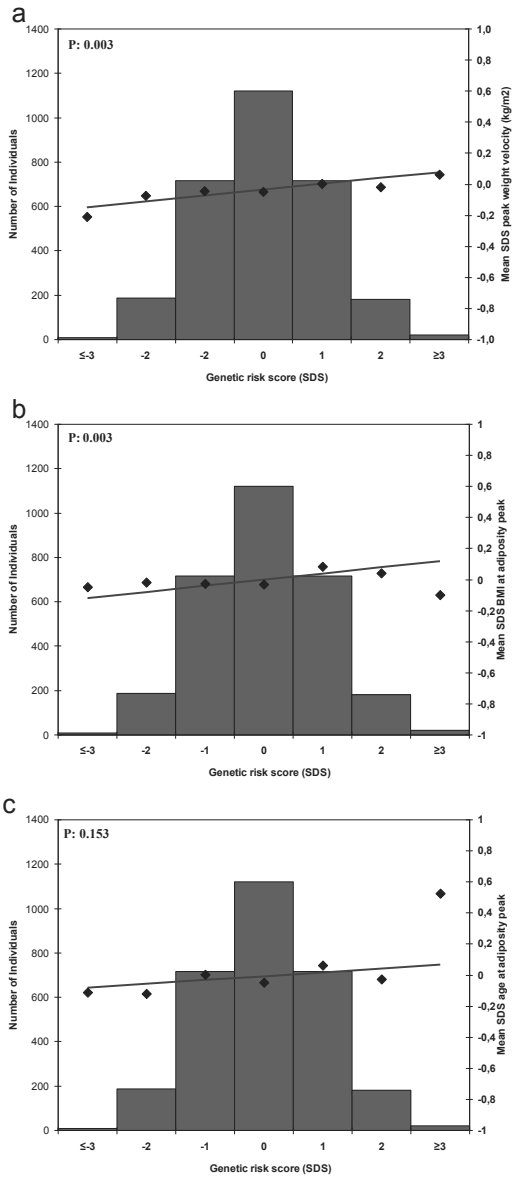
Supplemental Table S5. Associations of a 29 adult BMI SNPs genetic risk score with infant growth and childhood adiposity (N= 2,955)[a,b]

Adult BMI (N=29 SNPs)	Peak weight velocity[c]	BMI at adiposity peak[c]	Age at adiposity peak[c]	Body mass index[c,d]	Total fat mass[d,e,f]	Android/gynoid ratio[d,e,f]	Preperitoneal fat area[d,e,f]
Beta	0.011	0.031	0.032	0.149	0.121	0.109	0.070
(95% CI)	(-0.021, 0.042)	(-0.003, 0.065)	(-0.004, 0.069)	(0.121, 0.178)	(0.094, 0.148)	(0.077, 0.140)	(0.037, 0.103)
p-value	0.513	0.076	0.083	**$1.82*10^{-24}$**	**$6.98*10^{-18}$**	**$2.00*10^{-11}$**	**$2.70*10^{-5}$**

[a]Analyses were performed in children with complete data on genetic variants, at least one outcome under study, and covariates. Siblings were excluded for this analysis [b]Values are linear regression coefficients for models adjusted for sex and the first four genetic principal components and represent the difference in standard deviation scores of the outcome measures for each additional average risk allele in the risk scores. [d]Values are additionally adjusted for age. [e]Values are additionally adjusted for height. [f]Regression coefficients are based on standard deviation scores of ln-transformed outcome measures.



Supplemental Figure S2. Association of WHR risk score with average peak weight velocity (a), body mass index at adiposity peak (b), and age at adiposity peak (c) (N= 2,955). The x axis represents the categories of the risk score (overall sum of risk alleles, weighted by previous reported effect sizes, rescaled to SDS. The risk score ranged from -4 to 3 SDS and was rounded to the nearest integer for clarity of presentation). The right *y* axis shows the mean SDS and corresponds to the dots. The line represents the regression line of the mean SDS values on the categories of the risk score. The *y* axis on the left corresponds to the histogram representing the number of individuals in each risk-score category. The p-value is based on the continuous risk score, as presented in **Table 2**.



Supplemental Figure S3. Association of child BMI risk score with average peak weight velocity (a), body mass index at adiposity peak (b), and age at adiposity peak (c) (N= 2,955). The *x* axis represents the categories of the risk score (overall sum of risk alleles, weighted by previous reported effect sizes, rescaled to SDS. The risk score ranged from -4 to 4 SDS and was rounded to the nearest integer for clarity of presentation). The right *y* axis shows mean SDS and corresponds to the dots. The line represents the regression line of the mean SDS values on the categories of the risk score. The *y* axis on the left corresponds to the histogram representing the number of individuals in each risk-score category. The p-value is based on the continuous risk score, as presented in **Table 2**.

Supplemental Table S6. Phenotypic variance in measures of infant growth explained by genetic risk scores based on adult BMI and WHR SNPs (N= 2,955)

Risk score	Peak weight velocity	Body mass index at adiposity peak	Age at adiposity peak
Main risk scores			
Adult BMI (N=97)	0.07	**0.24**	0.02
Secondary risk scores			
Adult WHR (N=48)	0.05	0.01	0.02
Child BMI (N=15)	**0.15**	**0.16**	0.07
Adult BMI pathway genetic risk scores			
Neuronal			
Neuronal Developmental processes (N=29)	0.122	0.219	0.034
Neurotransmission (N=10)	0.009	<0.001	<0.001
Hypothalamic expression and regulatory function (N=13)	<0.001	0.007	0.054
Neuronal Expression (N=12)	0.118	0.010	0.066
Lipid biosynthesis and metabolism (N=10)	<0.001	0.004	0.037
Bone Development (N=9)	0.029	0.028	>0.001
Signaling			
Mitogen activated protein kinase1/Extracellular signal-regulated kinases (N=9)	0.008	0.007	0.013
JAK (N=2)	0.005	0.002	0.003
CyclicAMP (N=5)	0.037	0.038	0.024
WNTSignaling (N=6)	0.110	0.032	0.037
GPCR			
Notch Signaling (N=2)	0.010	0.009	0.015
Mitochondrial (N=8)	0.009	0.001	0.132
Retinoic Acid Receptors (N=6)	0.036	0.065	0.034
Endocytosis/Exocytosis (N=14)	0.002	0.002	0.005
Eye-related (N=5)	0.010	0.010	0.082
Tumorigenesis (N=11)	0.030	0.030	>0.001
Apoptosis (N=13)	0.077	0.034	0.104
Membrane Proteins (N=12)	**0.330**	0.243	0.078
Hormone metabolism/regulation (N=4)	0.009	0.008	0.009
Purine/Pyrimidine cycle (N=4)	0.009	0.159	0.060
Monogenic Obesity and/or Energy Homeostasis (N=9)	0.018	0.021	0.063
Immune System (N=15)	0.210	0.250	0.001
Limb Development (N=3)	0.032	0.219	>0.001
Ubiquitin pathways (N=6)	0.004	0.005	0.063
Glucose homeostasis and/or diabetes (N=11)	0.052	0.046	0.067
Cell cycle (N=23)	0.007	0.012	>0.001
DNARepair			
Nuclear trafficking (N=4)	0.022	0.053	0.095
Muscle Biology (N=6)	0.013	>0.001	0.019

Bold values represent explained variances for significant associations of the risk score with the outcome.

Supplemental Table S7. Associations of adult BMI genetic risk score with childhood adiposity, additionally adjusted for infant peak weight velocity (N= 3,975)[a, b]

Risk score (number of SNPs in risk score)	Body mass index[c,d]		Total fat mass[d,e,f]		Android/gynoid ratio[d,e,f]		Preperitoneal fat area[d,e,f]	
	Beta (CI 95%)	P-value	Beta (CI 95%)	P-value	Beta (CI 95%)	P-value	Beta (CI 95%)	P-value
Adult BMI (N=97)	0.107 (0.077; 0.137)	**$3.56*10^{-12}$**	0.088 (0.058; 0.117)	**$9.61*10^{-9}$**	0.079 (0.044; 0.114)	**$8.67*10^{-6}$**	0.020 (-0.016; 0.056)	0.274
Child BMI (N=15)	0.078 (0.048; 0.108)	**$3.59*10^{-7}$**	0.064 (0.034; 0.094)	**$3.20*10^{-5}$**	0.078 (0.043; 0.113)	**$1.30*10^{-5}$**	0.034 (-0.002; 0.069)	0.067

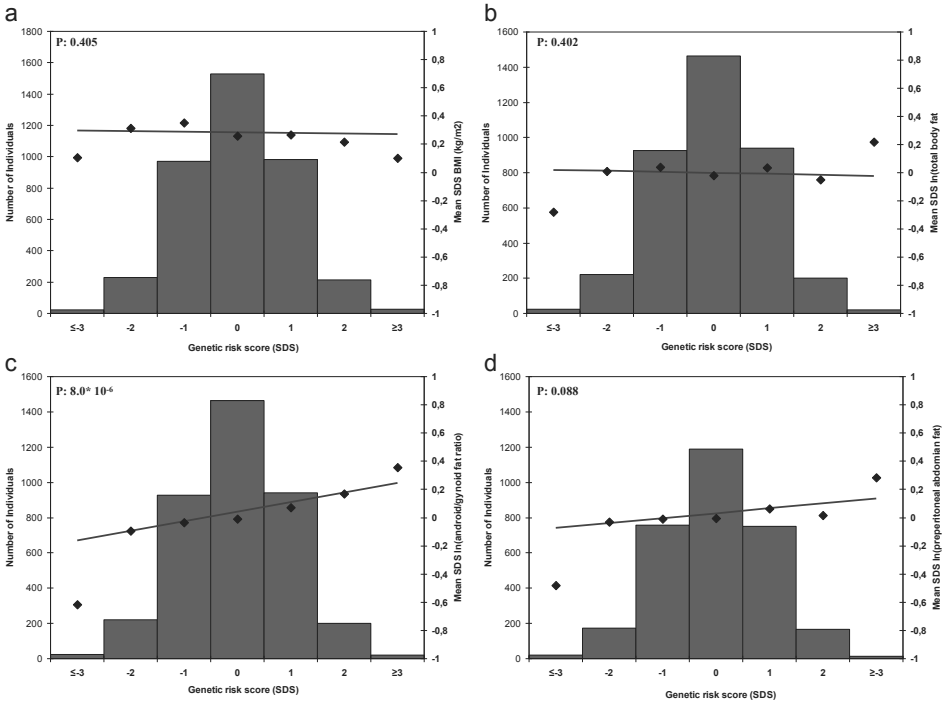
[a]Analyses were performed in children with complete data on genetic variants, at least one outcome under study, and covariates [b]Values are linear regression coefficients for models adjusted for sex, peak weight velocity, and the first four genetic principal components and represent the difference in standard deviation scores of the outcome measures for each additional average risk allele in the risk scores. [d]Values are additionally adjusted for age. [e]Values are additionally adjusted for height. [f]Regression coefficients are based on standard deviation scores of ln-transformed outcome measures.

Supplemental Table S8. Associations of adult BMI genetic risk score with childhood adiposity, additionally adjusted for BMI at adiposity peak (N= 3,975)[a, b]

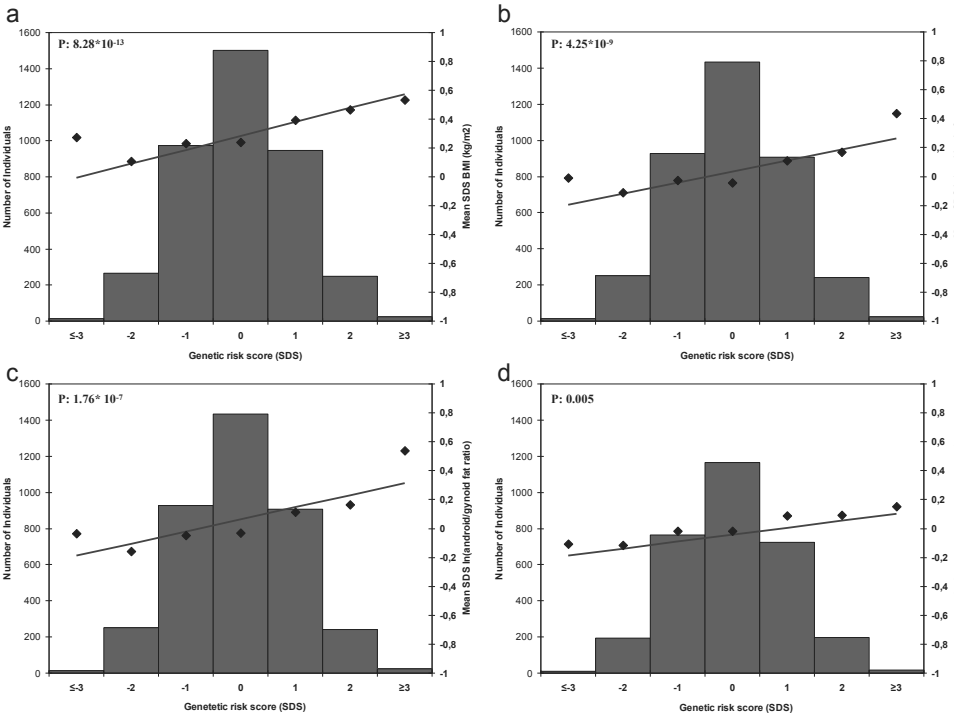
Risk score (number of SNPs in risk score)	Body mass index[c,d]		Total fat mass[d,e,f]		Android/gynoid ratio[d,e,f]		Preperitoneal fat area[d,e,f]	
	Beta (CI 95%)	P-value	Beta (CI 95%)	P-value	Beta (CI 95%)	P-value	Beta (CI 95%)	P-value
Adult BMI (N=97)	0.095 (0.066; 0.123)	**$4.76*10^{-11}$**	0.080 (0.050; 0.109)	**$1.78*10^{-7}$**	0.075 (0.040; 0.109)	**$3.00*10^{-5}$**	0.015 (-0.022; 0.051)	0.430
Child BMI (N=15)	0.070 (0.042; 0.098)	**$9.33*10^{-7}$**	0.061 (0.031; 0.090)	**$7.00*10^{-5}$**	0.076 (0.041; 0.111)	**$1.90*10^{-5}$**	0.033 (-0.003; 0.069)	0.073

[a]Analyses were performed in children with complete data on genetic variants, at least one outcome under study, and covariates [b]Values are linear regression coefficients for models adjusted for sex, BMI at adiposity peak, and the first four genetic principal components and represent the difference in standard deviation scores of the outcome measures for each additional average risk allele in the risk scores. [d]Values are additionally adjusted for age. [e]Values are additionally adjusted for height. [f]Regression coefficients are based on standard deviation scores of ln-transformed outcome measures.

Supplemental Table S9 can be found online.



Supplemental Figure S4. Association of WHR risk score with average BMI (a), total fat mass (b), android/gynoid fat ratio (c), and preperitoneal fat area (d) (N= 3,975). The *x* axis represents the categories of the risk score (overall sum of risk alleles, weighted by previous reported effect sizes, rescaled to SD-scores. The risk score ranged from -4 to 4 SDS and was rounded to the nearest integer for clarity of presentation). The right *y* axis shows the mean SDS and corresponds to the dots. The line represents the regression line of the mean SDS values on the categories of the risk score. The *y* axis on the left corresponds to the histogram representing the number of individuals in each risk-score category. The p-value is based on the continuous risk score, as presented in **Table 3**.



Supplemental Figure S5. Association of child BMI risk score with average BMI (a), total fat mass (b), android/gynoid fat ratio (c), and preperitoneal fat area (d) (N= 3,975). The *x* axis represents the categories of the risk score (overall sum of risk alleles, weighted by previous reported effect sizes, rescaled to SD-scores. The risk score ranged from -4 to 4 SDS and was rounded to the nearest integer for clarity of presentation). The right *y* axis shows the mean SDS and corresponds to the dots and a line representing the regression line of the mean SDS values for each category of the risk score. The *y* axis on the left corresponds to the histogram representing the number of individuals in each risk-score category. The p-value is based on the continuous risk score, as presented in **Table 3**.

Supplemental Table S10. Phenotypic variance in measures of childhood body composition explained by genetic risk scores based on adult BMI and WHR SNPs (N= 3,975).

Risk score	Body mass index	Total fat mass	Android/ gynoid ratio	Preperitoneal fat area
Main risk scores				
Adult BMI (N=97)	**1.43**	**0.81**	0.57	0.11
Secondary risk scores				
Adult WHR (N=48)	0.02	0.01	**0.50**	0.08
Child BMI (N=15)	**0.95**	**0.52**	0.64	0.14
Risk scores based on 97 adult BMI				
Neuronal				
Neuronal Developmental Processes (N=29)	**0.328**	0.087	0.119	0.005
Neurotransmission (N=10)	0.019	0.001	0.001	0.007
Hypothalamic Expression and Regulatory Function (N=13)	**1.136**	**0.767**	**0.626**	0.159
Neuronal Expression (N=12)	0.033	0.036	0.123	0.008
Lipid Biosynthesis and Metabolism (N=10)	0.061	0.017	0.025	<0.001
Bone Development (N=9)	0.035	0.004	0.023	0.002
Signaling				
Mitogen Activated Protein Kinase1/Extracellular Signal-regulated Kinases (N=9)	0.135	0.132	0.052	0.020
JAK (N=2)	0.125	0.039	0.014	0.005
CyclicAMP (N=5)	0.229	**0.247**	0.138	0.061
WNTS ignaling (N=6)	**0.387**	0.083	0.145	0.101
GPCR				
Notch Signaling (N=2)	0.086	0.074	0.079	0.079
Mitochondrial (N=8)	**0.165**	0.144	<0.001	0.001
Retinoic Acid Receptors (N=6)	0.232	0.136	0.025	0.029
Endocytosis/Exocytosis (N=14)	0.017	0.001	0.045	0.039
Eye-related (N=5)	0.017	0.022	0.014	0.001
Tumorigenesis (N=11)	0.180	0.037	0.025	0.016
Apoptosis (N=13)	0.072	0.038	0.006	0.124
Membrane Proteins (N=12)	**0.640**	**0.187**	**0.346**	0.012
Hormone Metabolism/Regulation (N=4)	0.047	0.175	0.066	0.002
Purine/Pyrimidine Cycle (N=4)	0.019	0.002	0.029	0.005
Monogenic Obesity and/or Energy Homeostasis (N=9)	**0.610**	0.442	**0.410**	0.084
Immune System (N=15)	0.235	0.133	0.042	0.006
Limb Development (N=3)	0.142	0.044	0.079	0.002
Ubiquitin Pathways (N=6)	0.006	0.004	0.012	0.022
Glucose Homeostasis and/or Diabetes (N=11)	**0.282**	0.052	0.173	0.008
Cell Cycle (N=23)	0.226	**0.210**	0.056	0.004
DNARepair				
Nuclear Trafficking (N=4)	0.003	0.008	0.002	0.007
Muscle Biology (N=6)	**0.262**	0.081	0.058	0.046

Bold values represent explained variances for significant associations of the risk score with the outcome.

Chapter 3.3

Associations of adult genetic risk scores for adiposity with childhood abdominal, liver and pericardial fat assessed by Magnetic Resonance Imaging

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Int J Obes (Lond). 2018;42:897-904

Abstract

BACKGROUND: Genome-wide association studies (GWAS) identified single nucleotide polymorphisms (SNPs) involved in adult fat distribution. Whether these SNPs also affect abdominal and organ-specific fat accumulation in children is unknown.

METHODS: In a population-based prospective cohort study among 1,995 children (median age: 9.8 years, 95% range 9.4, 10.8), We tested the associations of six genetic risk scores based on previously identified SNPs for childhood BMI, adult BMI, liver fat, WHR, pericardial fat mass, visceral- and subcutaneous adipose tissue ratio (VAT/SAT ratio), and four individual SAT and VAT associated SNPs, for association with SAT (N=1,746), VAT (N=1,742), VAT/SAT ratio (N=1,738), liver fat fraction (N=1,950), and pericardial fat mass (N=1,803) measured by Magnetic Resonance Imaging.

RESULTS: Per additional risk allele in the childhood BMI genetic risk score, SAT increased 0.020 standard deviation scores (SDS), (95% confidence interval (CI) 0.009, 0.031, p-value: $3.28*10^{-4}$) and VAT increased 0.021 SDS, 95% CI: 0.009, 0.032, p-value: $4.68*10^{-4}$). The adult BMI risk score was positively associated with SAT (0.022 SDS increase, CI: 0.015, 0.029, p-value: $1.33*10^{-9}$), VAT (0.017 SDS increase, CI: 0.010, 0.025, p-value: $7.00*10^{-6}$), and negatively with VAT/SAT ratio (-0.012 SDS decrease, CI: -0.019, -0.006, p-value: $2.88*10^{-4}$). The liver fat risk score was associated with liver fat fraction (0.121 SDS, CI: 0.086, 0.157, p-value: $2.65*10^{-11}$). Rs7185735 (SAT), was associated with SAT (0.151 SDS, CI: 0.087, 0.214, p-value: $3.00*10^{-6}$) and VAT/SAT ratio (-0.126 SDS, CI: -0.186, -0.065, p-value: $4.70*10^{-5}$). After stratification by sex the associations of the adult BMI risk score with SAT and VAT and of the liver fat risk score with liver fat fraction remained in both sexes. Associations of the childhood BMI risk score with SAT, and the adult BMI risk score with VAT/SAT ratio were present among boys only, whereas the association of the pericardial fat risk score with pericardial fat was present among girls only.

CONCLUSIONS: Genetic variants associated with BMI, body fat distribution, liver and pericardial fat already affect body fat distribution in childhood.

Background

Childhood overweight and obesity are related to short- and long-term complications, such as type 2 diabetes and cardiovascular disease.[1-4] Besides body mass index (BMI), body fat distribution is also considered to be important.[5] Especially abdominal fat, which can be stored as either subcutaneous (SAT) or visceral adipose tissue fat (VAT), is gaining interest.[6-8] In preadolescence on average lower levels of SAT and VAT are present than in adolescence and adulthood.[9] Previously, BMI was shown to be a relatively good measure for predicting SAT, but less so for VAT.[10,11] Also, fat accumulation in the liver and around the heart are suggested to play a role in metabolic disease.[12,13] A fatty liver is associated with dyslipidemia and dysglycemia, whereas pericardial fat is associated with coronary artery disease.[14,15] All four fat accumulation sites are heritable with heritability estimates of ranging from 30-60%.[11,16-18] Thus, fat distribution in particular areas, besides BMI, may affect the risk of metabolic disease and has a clear genetic component.

Recent large genome-wide associations studies (GWAS) have identified 97 single nucleotide polymorphisms (SNPs) associated with adult BMI and 15 SNPs associated with childhood BMI.[19-21] We have previously reported that genetic risk scores based on these SNPs were associated with infant growth and childhood adiposity measures determined using Dual-energy X-ray absorptiometry and ultrasound.[22] Although these 2-dimensional imaging techniques can estimate preperitoneal fat as a proxy of VAT, they are unable to accurately determine SAT, VAT, liver fat fraction, and pericardial fat.[23] Magnetic Resonance Imaging (MRI) can distinguish SAT, VAT, liver fat fraction, and pericardial fat more precisely and accurately by 3-dimensional measurements.[23,24] Other GWAS have identified SNPs for adult waist-hip ratio (WHR), SAT, VAT, VAT/SAT ratio, liver fat, and pericardial fat.[11,17,25-27] The genetic background of body fat distribution in children is largely unknown.

We hypothesized that genetic variants associated with childhood and adult BMI and more specific fat measures in adults are associated with fat accumulation in children. We tested in a population-based prospective cohort among 1,995 children whether genetic risk scores based on known variants are associated with SAT, VAT, liver fat fraction and pericardial fat assessed by MRI.

Methods

Study design and population

This study was embedded within the Generation R Study, a prospective population-based cohort in Rotterdam, the Netherlands.[24] All pregnant women residing in Rotterdam with a delivery date between April 2002 and January 2006 were invited to participate. The Medical Ethical Committee of Erasmus MC approved the study and an informed consent was obtained for all children. A total of 5,862 children participated in the follow-up at age ten years. GWA scans were available for 3,692 children.[28] Of these, MRI scans were available for 2,593 children (70%). All twins were excluded and only one non-twin sibling was selected per mother, based on data completeness or, if equal, randomly. The current study was limited to children with information on at least one of the outcomes (N=1,995). Figure 1 shows a participant flow chart.

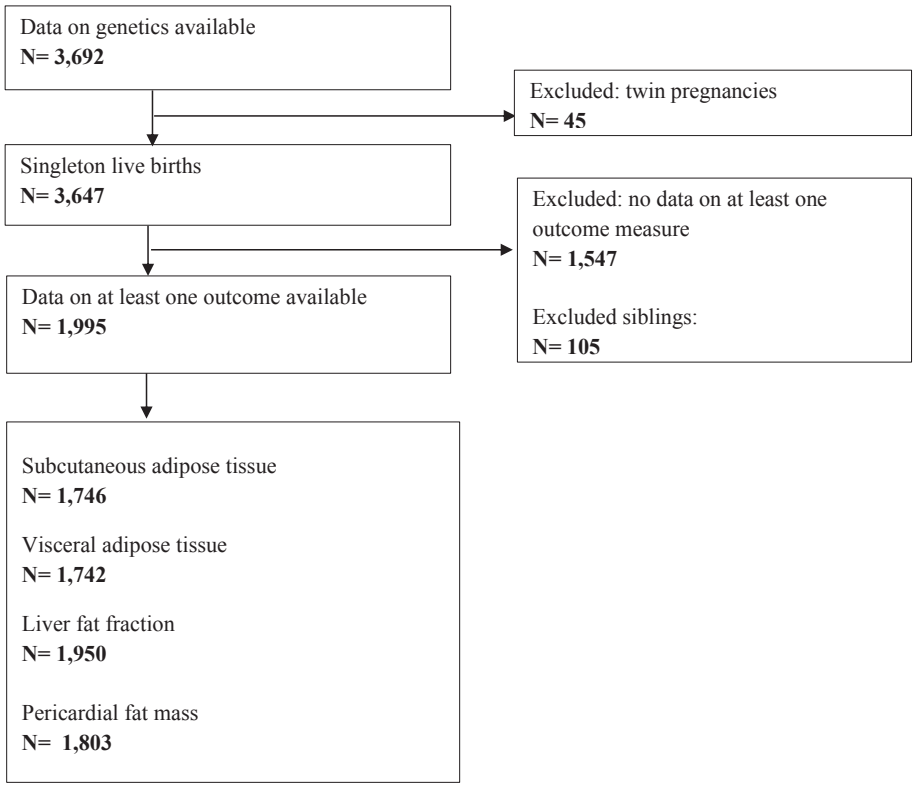

Figure 1 Flowchart of participants

Genetic risk scores and separate SNPs

DNA was isolated from cord blood or, for a small subgroup without cord blood samples, from blood samples taken at age 6 years.[28] For genome-wide association analysis the Illumina 610 and 660W Quad platforms were used.[24] Stringent quality checks were performed excluding individuals with low sample call rates or sex mismatches. Imputation of genotypes to the cosmopolitan panel of HapMap ii (release 22) was done using MACH software.[29,30] Prior to imputation, SNPs with a call rate <98%, significant deviations from Hardy-Weinberg equilibrium (p-value $<1*10^{-6}$), or minor allele frequencies <0.1% were excluded. Information about the SNPs for the present study was extracted from our GWAS dataset. We constructed a total of four weighted and two unweighted risk scores. Each score summed the number of outcome increasing risk alleles from the GWA dosage data, and SNPs were weighted individually using effect sizes from the original GWAS.[22] For BMI, we constructed two weighted genetic risk scores, combining 15 childhood BMI SNPs in one and 97 adult BMI SNPs in the other.[20,21] For the 15 childhood SNPs, weights were recalculated from the previous GWAS meta-analysis excluding the Generation R Study data, as these were part of the discovery dataset.[21] For one of the 15 childhood BMI SNPs, rs1421085, no information was available in our GWA dataset. We used rs3751812 as a proxy (R^2=0.93, D'=0.97). For four of the 97 adult BMI SNPs we used proxies (all R^2>0.96, D'=1): rs13012571 was used as a proxy for rs13021737, rs1978487 for rs9925964, rs6445197 for rs2365389, and rs9636202 for rs17724992. The other two weighted risk scores were created for WHR and liver fat.[25,26] For 46 of the 49 WHR SNPs information was available in the GWA dataset. Rs4607103 was used as a proxy for rs2371767 (R^2=0.90, D'=1). For the WHR SNPs rs8042543 and rs6556301 no good proxy was available leading to 47 WHR SNPs. As no effect estimates from previous GWAS were available for the pericardial fat mass and VAT/SAT ratio associated SNPs, unweighted risk scores were constructed based on three SNPs each for pericardial fat mass and VAT/SAT ratio.[27] A list of the SNPs included in the scores, a matrix listing the overlapping SNPs, and a matrix presenting the Pearson correlations between the risk scores are provided in Supplemental Tables S1a, S1b, and S1c. Previous GWAS identified one SNP for each of SAT (rs7185735), VAT and VAT adjusted for BMI (VATadjBMI) (rs2842895), SAT in women (rs2123685), and VAT and VATadjBMI in women (rs10060123).[27] For these phenotypes, we could therefore not create risk scores and we tested these SNPs separately.

Measures of adiposity at 10 years

MRI has been described as an accurate and reproducible technique and is considered the gold standard for the measurement of intra-abdominal and organ fat deposition.[23,31-33] Adiposity measures were obtained from MRI scans as described previously.[24] All children

were scanned using a 3.0 Tesla MRI (MR 750w, GE Healthcare, Milwaukee, WI, USA) using standard imaging and positioning protocols. Pericardial fat imaging in short axis orientation was performed using an ECG triggered black-blood prepared thin slice single shot fast spin echo acquisition (BB SSFSE) with multi-breath-hold approach. An axial 3-point Dixon acquisition for fat and water separation (IDEAL IQ) was used for liver fat and liver fat fraction imaging an axial abdominal scan from lower liver to pelvis and a coronal scan centered at the head of the femurs were performed with a 2-point DIXON acquisition (LavaFlex).[34]

The obtained fat scans were analyzed by the Precision Image Analysis company (PIA, Kirkland, Washington, United States), using the sliceOmatic (TomoVision, Magog, Canada) software package. All extraneous structures and any image artifacts were removed manually.[23] Pericardial fat included both epicardial- and paracardial fat directly attached to the pericardium, ranging from the apex to the left ventricular outflow tract. Total subcutaneous and visceral fat volumes ranged from the dome of the liver to the superior part of the femoral head. Fat masses were obtained by multiplying the total volumes by the specific gravity of adipose tissue, 0.9 g/ml. Liver fat fraction was determined by taking four samples of at least 4 cm^2 from the central portion of the hepatic volume. Subsequently, the mean signal intensities of these four samples were averaged to generate an overall mean liver fat fraction estimation. A more extensive description of the MRI measurement protocols can be found in Supplemental file S2). BMI (kg/m^2) was calculated from height and weight measured without shoes and heavy clothing.[35]

Statistical analysis

To examine whether the genetic risk scores were associated with the childhood adiposity measures we used linear regression analyses. To facilitate comparison of the effect estimates we created standard deviation (SD) scores for all outcomes. Sex- and age-adjusted SD scores (SDS) were constructed for BMI using the Dutch reference growth curves (Growth Analyser Research Calculation Tools, Version 4.0 http://www.growthanalyser.org). In order to make all outcomes except liver fat fraction independent of height, we estimated the optimal adjustment by log-log regression analyses.[36] All MRI adiposity measures except liver fat fraction and height were log-transformed, using natural logarithm (ln). Log-MRI adiposity measures were regressed on log-height. The regression slope then corresponds to the power to which height should be raised to calculate an index uncorrelated with height. Thus, we divided subcutaneous fat mass by $height^4$, visceral fat mass by $height^3$, and pericardial fat mass by $height^3$. All height-adjusted outcomes approached a normal distribution after ln-transformation. All models were adjusted for sex and age except models with BMI as an outcome since BMI SDS were already adjusted for sex and age. All models included the first four genetic principal components to adjust for ancestry. A

sensitivity analysis was performed adjusting all outcomes for BMI by conditional regression analysis to examine whether the associations were independent of BMI. Standardized residuals were obtained for each fat outcome from the regression of those outcomes on BMI. These standardized residuals were then used as outcome measures.[37] In addition, we repeated the analyses for SAT and VAT adjusting for VAT and SAT, respectively. Since body fat distribution may be different among boys and girls we planned a priori to stratify on sex.[38,39] Sex-specific associations were examined by adding the interaction term for the risk score with sex to the models. The variance explained by the risk scores was considered to be the increase in the unadjusted R^2 between the model containing all covariates and the risk score or individual SNP, and the same model without the risk score/SNP. We applied Bonferroni correction to account for multiple testing, correcting for all ten exposures. We considered a p-value of smaller than 0.05/10=0.005 significant. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 21.0 for Windows (IBM, Chicago, IL, USA). A power calculation was performed for all risk scores on the smallest and largest sample size used for analyses using a one sample, two sided test in R version 3.3.2, library 'pwr' (Supplemental Table S2).

Results

Participant characteristics

Characteristics of the participants are listed in Table 1. MRI scans were performed at a median age of 9.8 years (95% range 9.4, 10.8). The median BMI of the children was 16.9 kg/m^2 (95% range 14.0, 24.0).

Genetic risk scores and adiposity measures

The risk score for childhood BMI was associated with an increase in SAT, and VAT (Table 2). The adult BMI risk score was associated with an increase in SAT and VAT, and with a decrease in VAT/SAT ratio. The adult fatty liver risk score was solely associated with an increase in liver fat fraction, showing a relatively large increase of 0.121 SDS (95% CI: 0.086, 0.157) in liver fat fraction per additional average risk allele in the risk score. No associations were observed for the VAT/SAT ratio and pericardial fat risk scores (Table 2). Both the childhood and the adult BMI risk scores were associated with childhood BMI. Unweighted risk scores showed comparable results, except for the childhood BMI risk score for which the effect estimates were around twice as high as for the weighted risk score for all outcomes other than pericardial fat mass (Supplemental Table S3). Rs7185735 (SAT) was associated with an increase in SAT, and a decrease in VAT/SAT ratio and was also

associated with childhood BMI (Table 2). The childhood and adult BMI risk scores, the WHR and VAT/SAT ratio risk scores and the VAT/SAT ratio and fatty liver risk scores were correlated (Supplemental Table S1c).

Table 1. Characteristics of the study population. (N=1,995)

Characteristics	
Birth	
Boys (%)	979 (49.1)
Gestational age at birth (weeks)	40.1 (36.3; 42.3)
Weight at birth (grams) [a]	3 466 (510)
Childhood	
Age at visit (years)	9.8 (9.4; 10.8)
Height (cm) [a]	141.8 (6.6)
Weight (kg) [a]	35.3 (7.0)
Body mass index (kg/m^2)	16.9 (14.0; 24.0)
Overweight (%) [b]	273 (13.7)
Obese (%) [b]	55 (2.8)
Subcutaneous adipose tissue (grams)	1,291 (603; 5 246)
Visceral adipose tissue (grams)	369 (161; 981)
Liver fat fraction (%)	2.0 (1.3; 4.9)
Pericardial fat mass (grams)	11 (5; 23)

Values are medians (95% range) unless otherwise specified
[a] Means (standard deviations)
[b] The IOTF-classification was used to define overweight and obesity

After adjusting the outcomes for BMI the associations of the BMI risk scores with the outcomes were no longer present (Supplemental Table S4). The adult fatty liver risk score remained associated with liver fat fraction (0.128 SDS increase in liver fat percentage per additional risk allele, CI: 0.095, 0.161) and a new association was observed for the adult pericardial fat risk score with pericardial fat (0.074 SDS increase, CI: 0.028, 0.120). Rs7185735 (SAT) remained associated with SATadjBMI albeit with a lower effect estimate (Supplemental Table S4). Associations of the risk scores and individual SNPs with SAT adjusted for VAT and with VAT adjusted for SAT are shown in Supplemental Table S5.

There was a nominally significant interaction with sex only for the associations of the liver fat risk score with SAT and VAT/SAT ratio, for rs7185735 (SAT) with VAT/SAT ratio, and for rs10060123 (VAT and VATadjBMI in girls) with liver fat (data not shown). After stratification

by sex the associations that remained in both sexes were those of the adult BMI risk score with SAT and VAT, with a slightly higher effect estimate for SAT in boys compared to girls, and for the liver fat risk score with liver fat fraction, with a higher effect estimate in girls (Table 3). The childhood and adult BMI risk scores remained associated with BMI in both sexes, with a slightly higher effect estimate for the childhood BMI risk score in boys compared to girls. Associations in boys only were those of the childhood BMI risk score with SAT and of the adult BMI risk score with VAT/SAT ratio. Associations in girls only were those of the pericardial fat risk score with pericardial fat and of rs7185735 (SAT) with BMI (Table 3).

The highest variance explained for the MRI fat measures was 2.2%, for the adult liver risk score with liver fat fraction in the full group. This was even higher for girls (3.1%) (Supplemental Tables S6 and S7). A power calculation showed limited power to detect small effect estimates. The smallest effect that could be detected per additional risk allele with 80% power was 0.013 for the adult BMI risk score (N=1,993) (Supplemental Table S2).

Table 2. Associations of genetic risk scores with MRI adiposity measures (N=1,995)[a,b]

Risk score (number of SNPs in risk score)	SAT (N=1,746)		VAT (N=1,742)		VAT/SAT ratio (N=1,738)	
	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value
Childhood BMI (N=15)	0.020	**$3.28*10^{-4}$**	0.021	**$4.68*10^{-4}$**	-0.005	0.335
	(0.009; 0.031)		(0.009; 0.032)		(-0.015; 0.005)	
Adult BMI (N=97)	0.022	**$1.33*10^{-9}$**	0.017	**$7.00*10^{-6}$**	-0.012	**$2.88*10^{-4}$**
	(0.015; 0.029)		(0.010; 0.025)		(-0.019; -0.006)	
Adult WHR (N=49)	-0.010	0.056	-0.002	0.716	0.011	0.019
	(-0.019; 0.000)		(-0.012; 0.009)		(0.002;0.020)	
VAT/SAT ratio (N=3)*	-0.006	0.768	-0.008	0.694	0.001	0.966
	(-0.044; 0.032)		(-0.049; 0.033)		(-0.036; 0.037)	
Adult fatty liver (N=5)	0.003	0.853	0.003	0.863	0.001	0.966
	(-0.032; 0.039)		(-0.035; 0.042)		(-0.036; 0.037)	
Adult pericardial fat (N=3)*	-0.024	0.283	0.022	0.358	0.056	0.008
	(-0.068; 0.020)		(-0.025; 0.069)		(0.015; 0.098)	
rs7185735 (SAT)	0.151	**$3.00*10^{-6}$**	0.093	0.007	-0.126	**$4.70*10^{-5}$**
	(0.087; 0.214)		(0.025; 0.161)		(-0.186; -0.065)	
rs2123685 (SAT female)	-0.034	0.707	-0.167	0.081	-0.126	0.142
	(-0.210; 0.143)		(-0.355; 0.020)		(-0.293; 0.042)	
rs2842895 (VAT and VATadjBMI)	0.021	0.531	-0.001	0.970	-0.021	0.503
	(-0.044; 0.086)		(-0.071; 0.068)		(-0.083; 0.041)	
rs10060123 (VAT and VATadjBMI female)	-0.049	0.185	-0.068	0.081	-0.011	0.758
	(-0.121; 0.023)		(-0.145; 0.009)		(-0.079; 0.058)	

[a] Analyses were performed in children with complete data on genetic variants and covariates and at least one outcome under study
[b] Values are linear regression coefficients for models adjusted for sex, age, and the first four principal components and represent the difference in standard deviation scores of the outcome measures for each additional average risk allele in the risk scores.
Bold values represent nominally significant outcomes
*Unweighted risk scores since no information on the effect estimates was available. Bold values represent significant outcomes after Bonferroni correction for 10 analyses (p-value <0.005)

	Liver fat fraction (N=1,950)		Pericardial fat mass (N=1,803)		BMI @ 10 (N=1,993)	
	Beta (CI 95%)	**p-value**	**Beta (CI 95%)**	**p-value**	**Beta (CI 95%)**	**p-value**
	0.009	0.098	0.004	0.541	0.030	**$1.60*10^{-7}$**
	(-0.002; 0.020)		(-0.008; 0.015)		(0.019; 0.041)	
	0.004	0.247	0.009	0.013	0.027	**$6.18*10^{-13}$**
	(-0.003; 0.011)		(0.002; 0.017)		(0.019; 0.034)	
	-0.002	0.737	-0.010	0.055	-0.010	0.052
	(-0.011; 0.008)		(-0.020; 0.000)		(-0.020; 0.000)	
	-0.012	0.554	-0.013	0.513	-0.008	0.702
	(-0.050; 0.026)		(-0.053; 0.027)		(-0.047; 0.031)	
	0.122	**$2.54*10^{-11}$**	0.010	0.590	-0.008	0.702
	(0.086; 0.158)		(-0.028; 0.048)		(-0.047; 0.031)	
	-0.007	0.761	0.061	0.009	-0.028	0.226
	(-0.050; 0.037)		(0.015; 0.107)		(-0.072; 0.017)	
	0.044	0.178	0.038	0.269	0.115	**0.001**
	(-0.020; 0.107)		(-0.029; 0.105)		(0.050; 0.181)	
	0.075	0.403	-0.060	0.522	0.094	0.311
	(-0.101; 0.250)		(-0.243; 0.123)		(-0.088; 0.275)	
	-0.002	0.943	0.053	0.126	0.024	0.473
	(-0.067; 0.062)		(-0.015; 0.122)		(-0.042; 0.091)	
	-0.028	0.444	-0.068	0.079	-0.055	0.141
	(-0.099; 0.044)		(-0.143; 0.008)		(-0.129; 0.018)	

Table 3. Associations of genetic risk scores with MRI adiposity measures in boys (N=979) and girls (N=1,016) separately.[a,b]

Risk score (number of SNPs in risk score)	SAT		VAT		VAT/SAT ratio	
	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value
Boys	**N=856**		**N=851**		**N=851**	
Childhood BMI (N=15)	0.024 (0.008; 0.040)	**0.004**	0.022 (0.005; 0.040)	0.013	-0.008 (-0.023; 0.007)	0.301
Adult BMI (N=97)	0.025 (0.015; 0.035)	**$1.00*10^{-6}$**	0.018 (0.007; 0.029)	**0.001**	-0.015 (-0.025; -0.006)	**0.002**
Adult WHR (N=49)	-0.009 (-0.024; 0.005)	0.214	-0.004 (-0.020; 0.011)	0.596	0.006 (-0.008; 0.019)	0.390
VAT/SAT ratio (N=3)*	0.028 (-0.029; 0.085)	0.329	0.019 (-0.042; 0.080)	0.549	-0.017 (-0.070; 0.036)	0.525
Adult fatty liver (N=5)	0.033 (-0.018; 0.084)	0.202	0.024 (-0.031; 0.078)	0.398	-0.034 (-0.081; 0.014)	0.166
Adult pericardial fat (N=3)*	0.004 (-0.059; 0.069)	0.891	0.046 (-0.023; 0.114)	0.190	0.046 (-0.013; 0.105)	0.130
rs7185735 (SAT)	0.106 (0.012; 0.200)	0.028	0.075 (-0.027; 0.176)	0.148	-0.068 (-0.020; 0.156)	0.130
rs2123685 (SAT female)	-0.039 (-0.293; 0.215)	0.764	-0.200 (-0.471; 0.072)	0.149	-0.145 (-0.380; 0.091)	0.227
rs2842895 (VAT and VATadjBMI)	0.001 (-0.093; 0.086)	0.981	-0.036 (-0.138; 0.066)	0.487	-0.031 (-0.119; 0.057)	0.489
rs10060123 (VAT and VATadjBMI female)	-0.070 (-0.176; 0.035)	0.191	-0.071 (-0.184; 0.042)	0.220	0.023 (-0.075; 0.122)	0.640
Girls	**N=890**		**N=891**		**N=887**	
Childhood BMI (N=15)	0.016 (0.001; 0.031)	0.031	0.019 (0.004; 0.035)	0.014	-0.002 (-0.017; 0.012)	0.731
Adult BMI (N=97)	0.018 (0.009; 0.028)	**$2.65*10^{-4}$**	0.016 (0.006; 0.027)	**0.002**	-0.010 (-0.020; 0.000)	0.043
Adult WHR (N=49)	-0.010 (-0.023; 0.004)	0.149	0.0003 (-0.014; 0.014)	0.963	0.016 (0.003; 0.029)	0.014
VAT/SAT ratio (N=3)*	-0.037 (-0.088; 0.015)	0.16	-0.033 (-0.087; 0.022)	0.241	0.017 (-0.033; 0.067)	0.502
Adult fatty liver (N=5)	-0.033 (-0.084; 0.017)	0.198	-0.021 (-0.075; 0.032)	0.432	0.025 (-0.024; 0.074)	0.318
Adult pericardial fat (N=3)*	-0.045 (-0.106; 0.016)	0.147	0.004 (-0.061; 0.069)	0.905	0.063 (0.004; 0.122)	0.038
rs7185735 (SAT)	0.197 (0.112; 0.283)	**$6.00*10^{-6}$**	0.110 (0.019; 0.201)	0.018	-0.186 (-0.103; -0.269)	**$1.20*10^{-5}$**
rs2123685 (SAT female)	-0.034 (-0.279; 0.212)	0.788	-0.138 (-0.399; 0.122)	0.298	-0.103 (-0.341; 0.136)	0.397
rs2842895 (VAT and VATadjBMI)	0.035 (-0.055; 0.124)	0.449	0.031 (-0.064; 0.126)	0.525	-0.005 (-0.092; 0.082)	0.917
rs10060123 (VAT and VATadjBMI female)	-0.027 (-0.125; 0.072)	0.596	-0.066 (-0.170; 0.039)	0.216	-0.047 (-0.142; 0.049)	0.337

[a] Analyses were performed in children with complete data on genetic variants and covariates and at least one outcome under study
[b] Values are linear regression coefficients for models adjusted for sex, age, and the first four principal components and represent the difference in standard deviation scores of the outcome measures for each additional average risk allele in the risk scores. Bold values represent nominally significant outcomes *Unweighted risk scores since no information on the effect estimates was available. Bold values represent significant outcomes after Bonferroni correction for 10 analyses (p-value <0.005)

Liver fat fraction		Pericardial fat mass		BMI @ 10	
Beta (CI 95%)	**p-value**	**Beta (CI 95%)**	**p-value**	**Beta (CI 95%)**	**p-value**
N=958		**N=884**		**N=977**	
0.015	0.052	-0.005	0.545	0.038	**$9.00*10^{-6}$**
(0.000; 0.031)		(-0.022; 0.012)		(0.021; 0.054)	
0.007	0.167	0.010	0.061	0.029	**$5.95*10^{-8}$**
(-0.003; 0.017)		(0.000; 0.021)		(0.018; 0.039)	
-0.003	0.695	-0.012	0.123	-0.013	0.078
(-0.016; 0.011)		(-0.027; 0.003)		(-0.028; 0.001)	
-0.014	0.598	0.006	0.838	0.023	0.442
(-0.068; 0.039)		(-0.053; 0.065)		(-0.035; 0.081)	
0.094	**$1.65*10^{-6}$**	0.001	0.976	0.003	0.904
(0.045; 0.143)		(-0.053; 0.055)		(-0.050; 0.057)	
-0.003	0.917	0.028	0.402	-0.025	0.458
(-0.064; 0.057)		(-0.038; 0.095)		(-0.090; 0.040)	
0.046	0.312	0.075	0.137	0.071	0.155
(-0.136; 0.043)		(-0.024; 0.174)		(-0.027; 0.168)	
-0.047	0.708	-0.178	0.183	0.150	0.270
(-0.190; 0.197)		(-0.439; 0.084)		(-0.117; 0.416)	
-0.036	0.432	0.025	0.614	-0.010	0.837
(-0.054; 0.125)		(-0.073; 0.124)		(-0.107; 0.087)	
-0.111	0.028	-0.055	0.322	-0.070	0.206
(-0.210; 0.012)		(-0.165; 0.054)		(-0.177; 0.038)	
N=992		**N=919**		**N=1,016**	
0.003	0.715	0.011	0.147	0.023	**0.002**
(-0.013; 0.018)		(-0.004; 0.027)		(0.008; 0.038)	
0.001	0.905	0.009	0.108	0.024	**$2.0*10^{-6}$**
(-0.010; 0.011)		(-0.002; 0.019)		(0.014; 0.034)	
-0.001	0.917	-0.008	0.253	-0.007	0.334
(-0.015; 0.013)		(-0.022; 0.006)		(-0.020; 0.007)	
-0.012	0.660	-0.030	0.273	-0.038	0.161
(-0.066; 0.042)		(-0.085; 0.024)		(-0.090; 0.015)	
0.148	**$2.09*10^{-8}$**	0.017	0.546	-0.04	0.12
(0.097; 0.200)		(-0.036; 0.070)		(-0.091; 0.011)	
-0.007	0.834	0.100	**0.002**	-0.024	0.434
(-0.070; 0.056)		(0.036; 0.164)		(-0.086; 0.037)	
0.041	0.368	0.006	0.900	0.160	**$3.71*10^{-4}$**
(-0.132; 0.049)		(-0.086; 0.098)		(0.072; 0.247)	
0.193	0.132	0.085	0.621	0.034	0.787
(-0.059; 0.445)		(-0.192; 0.322)		(-0.212; 0.280)	
0.022	0.642	0.082	0.092	0.050	0.283
(-0.115; 0.071)		(-0.013; 0.177)		(-0.041; 0.141)	
0.049	0.351	-0.078	0.142	-0.040	0.433
(-0.054; 0.151)		(-0.182; 0.026)		(-0.140; 0.060)	

Discussion

In this study a higher childhood BMI genetic risk score was associated with a higher SAT and VAT. The adult BMI genetic risk score was additionally associated with a lower VAT/SAT ratio. A higher adult liver fat risk score was associated with a higher liver fat fraction. Rs7185735, previously associated with SAT in adults, was associated with SAT and VAT/SAT ratio. The associations of the adult BMI risk score with SAT and VAT, and of the liver fat risk score with liver fat fraction were found in both boys and girls. The association of the adult BMI risk score with VAT/SAT ratio remained significant in boys only, whereas stratification by sex revealed an association for the pericardial risk score with pericardial fat mass in girls only. The associations of rs7185735 (SAT) with SAT and VAT/SAT ratio were significant in girls only.

Interpretation of main findings

Childhood overweight and obesity are risk factors for later cardiometabolic disease.[1-4] Previous studies have shown, that SNPs associated with BMI in adulthood already exert their effects during childhood, although sometimes smaller or even in the opposite direction.[20-22,40] In addition to BMI the distribution and storage of fat in specific locations may also contribute to the cardiometabolic risk.[5] Understanding the pathophysiology of body fat distribution from early life onwards may give insight into the mechanisms underlying cardiometabolic disease. The SNPs identified specifically for adult WHR, SAT, VAT, liver fat, and pericardial fat may also play a role in the distribution of body fat from early life onwards. To the best of our knowledge, our study is the first to investigate associations of adult body fat SNPs with childhood fat distribution assessed by MRI.

In this study we observed that the childhood and adult risk scores for BMI, a general adiposity measure, were associated with both SAT and VAT, but not with the more specific liver fat fraction or pericardial fat mass. This is not surprising, as a higher BMI is often accompanied by an increased SAT and/or VAT.[6] A stronger association was observed for the adult BMI risk score with BMI than with SAT and VAT, suggesting that not all SNPs identified to play a role in BMI necessarily play a role in SAT and/or VAT and that these represent different phenotypes. The risk of cardiometabolic disease is not the same for SAT and VAT. SAT is deemed less pathogenic than VAT.[41] The adult BMI risk score was additionally associated with VAT/SAT ratio. The direction of effect was opposite to that expected, suggesting that a genetic risk for increased BMI is associated with a lower VAT/SAT ratio. Possibly this inversed effect is caused by the stronger association of the adult BMI risk score with SAT than with VAT. This is also reflected in the analyses of SATadjVAT and VATadjSAT, where the adult BMI risk score showed an association with SATadjVAT, but

not with VATadjSAT. It is known that SNPs in BMI-associated genetic regions, for example the *FTO* and *MC4R* regions, may have opposite or null effects on BMI in early childhood.[42] This is supported by the fact that the childhood BMI risk score was not associated with VAT/SAT ratio in our dataset, although we had limited power to detect a small effect size. Both BMI risk scores were associated with BMI, which is in line with previous results in younger children.[22] This indicates that the effect of the risk score on overall BMI is positive at this age, but that the relative effects on specific sites of fat accumulation in children may differ from those in adults.

The use of BMI as an overall measure of adiposity does not take into account body fat accumulation in specific locations.[5,7] The exact location and extent of fat accumulation in the body may provide a more precise determination of metabolic risk.[5] Waist-hip ratio is considered more representative than BMI for abdominal adiposity, which includes SAT and VAT.[38] We did not observe associations of the adult WHR risk score with the childhood adiposity measures, although the association with VAT/SAT ratio was nominally significant. Contrary to our expectations, we did not observe any associations for the VAT/SAT ratio risk score with any of the MRI measures. This may be because the effect of the SNPs included in this score is null in childhood, because our power to detect a small effect size was limited, or because only an unweighted risk score was available for this phenotype. However, the difference in effect estimates between weighted and unweighted risk scores was shown to be small in most analyses, except for the childhood BMI risk score. Therefore, we do not expect the use of the unweighted risk score to have a strong effect on the results. A higher adult fatty liver risk score was associated with an increased liver fat fraction indicating that at least some of the SNPs in the risk score affect liver fat fraction from childhood onwards. We could not draw this conclusion for the adult pericardial fat risk score with any of the MRI measures, which may again be due to the risk score being unweighted, the relatively young age of the children or limited power. After adjusting the outcomes for BMI the associations of the BMI risk scores with the abdominal fat measures were no longer present suggesting that the BMI associated SNPs affect overall fat accumulation and may not represent fat accumulation in these specific sites. By adjusting our outcomes for BMI we may also have lost some power which possibly hampered our ability to detect small effect sizes. We also found associations of the adult fatty liver risk score with liver fat fraction only and of the adult pericardial fat risk score with pericardial fat only, indicating that these risk scores seem to affect fat accumulation specifically in the liver and pericardium already in childhood and that these phenotypes are established via biological pathways distinct from those involved in more general adiposity measures. This is in line with the correlations between the genetic risk scores of the general adiposity measures.

The weak but significant correlation of the VAT/SAT ratio and fatty liver risk scores is likely caused by one overlapping locus indicating some shared genetic background, which is biologically plausible but not reflected in the associations with the phenotypes.[43]

Multiple previous GWAS have identified SNPs for SAT and VAT.[11,27] For the current study we used the largest GWAS on SAT and VAT to date which revealed four separate SNPs for these phenotypes.[27] Rs7185735, associated with SAT in adults, was also associated with SAT and VAT/SAT ratio in children. After adjusting our outcomes for BMI the association with SAT remained but showed a lower effect estimate. The association with VAT/SAT ratio attenuated and remained borderline significant. Rs7185735 is located in the genetic region coding for *FTO*. This region was the first robustly identified region associated with BMI, which is also reflected with the observed association of rs7185735 with BMI.[44] More recently, it was suggested that SNPs located in *FTO* actually influence the expression of *IRX3* and *IRX5* that are involved in adipocyte lipid accumulation.[45,46]

Sex is associated with body fat distribution.[38,39] To examine this in more detail, we stratified our analyses on sex. Results showed a difference for the associations of the adult BMI risk score with VAT/SAT ratio, which remained in boys only, and for rs7185735 (SAT) which seemed to affect SAT, VAT/SAT ratio, and BMI in girls only. Previous work has shown sexual dimorphism for adiposity measures such as an increased VAT/SAT ratio, but this was not observed in children below the age of 16.[47] We also showed that the association of the adult BMI risk score with VAT/SAT ratio in the full group was driven by the association in boys. The associations for the pericardial fat risk score with pericardial fat, and for rs7185735 (SAT) with VAT/SAT ratio were present in girls only. No associations were found for the individual SNPs identified by GWAS for VAT, or SAT and VAT in girls only. This may be due to limited power or because these specific SNPs have little or no effect in children. We also observed slightly higher effect estimates for some of our associations in either boys or girls, indicating that some adiposity associated SNPs may affect the accumulation of body fat differently in boys and girls.

Further research should be performed to examine whether the current associations can be replicated in other cohorts. Larger study populations may reveal additional associations. A larger study population will also provide more power to be able to investigate which individual SNPs in the risk scores are specifically affecting certain measures of body fat distribution.

Methodological considerations

Genetic information was available in 63% of our total sample size. Children without genetic information had a slightly lower VAT (p-value =0.001), and pericardial fat mass (p-value <0.001). We consider it unlikely that these differences have influenced our results. We did not find any differences regarding the other adiposity outcomes (p-value >0.05) (data not shown). Children without MRI measurements had a higher BMI and lower height than children with MRI measurements (p-value <0.05). Both children without genetic data and children without MRI measurements had a slightly lower gestational age at birth and a lower socio-economic status (p-value <0.05) than children with these data. These differences might reduce the generalizability of our findings. Not all SNPs were available in our GWAS dataset. We used a limited number of proxy SNPs for both BMI and the WHR risk scores in very high linkage disequilibrium (LD). Given the high number of SNPs available and the high LD for the proxies, both risk scores are considered a good representation of the original set of SNPs.[20,21,26] Although our population was relatively large, power may still have been limited, therefore our (lack of) findings with some of the adiposity outcomes should be interpreted with caution.

Conclusion

Our results suggest that genetic variants associated with childhood and adult BMI, adult body fat distribution, liver and pericardial fat already affect body fat distribution in childhood. We also found that adiposity associated genetic variants may regulate the distribution of fat in the body differently in boys and girls already before puberty.

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Supplement

Supplemental Table S1a. SNPs included in the genetic risk scores

Risk score	SNP name	Proxy	Chr.	Position	Coded allele
Adult BMI					
RARB	rs6804842		3	25106437	G
TFAP2B	rs2207139		6	50845490	G
MAP2K5	rs16951275		15	68077168	T
FLJ45139	rs2836754		21	40291740	C
FTO	rs1558902		16	53803574	A
NLRC3	rs758747		16	3627358	T
CADM1	rs12286929		11	115022404	G
IFNGR1	rs13201877		6	137675541	G
RALYL	rs2033732		8	85079709	C
GRID1	rs7899106		10	87410904	G
CBLN1	rs2080454		16	49062590	C
TAL1	rs977747		1	47684677	T
NT5C2	rs11191560		10	104869038	C
EPB41L4B	rs6477694		9	111932342	C
ZBTB10	rs16907751		8	81375457	C
DOC2A	rs4787491		16	30015337	G
AGBL4	rs657452		1	49589847	A
HSD17B12	rs2176598		11	43864278	T
TLR4	rs1928295		9	120378483	T
RBM26	rs1441264		13	79580919	A
FUBP1	rs12401738		1	78446761	A
PRKD1	rs12885454		14	29736838	C
BDNF	rs11030104		11	27684517	A
NAV1	rs2820292		1	201784287	C
MTCH2	rs3817334		11	47650993	T
C5orf37	rs2112347		5	75015242	T
EHBP1	rs11688816		2	63053048	G
RASA2	rs16851483		3	141275436	T
ZFP64	rs6091540		20	51087862	C
HHIP	rs11727676		4	145659064	T
PACRG	rs13191362		6	163033350	A
FHIT	rs2365389	rs6445197[a]	3	61213993	C
MC4R	rs6567160		18	57829135	C
KCTD15	rs29941		19	34309532	G
ADCY3	rs10182181		2	25150296	G
QPCTL	rs2287019		19	46202172	C
LMX1B	rs10733682		9	129460914	A
SBK1	rs2650492		16	28333411	A

Supplemental Table S1a. *(Continued)*

Risk score	SNP name	Proxy	Chr.	Position	Coded allele
TCF7L2	rs7903146		10	114758349	C
RPL27A	rs4256980		11	8673939	G
C6orf106	rs205262		6	34563164	G
LRP1B	rs2121279		2	143043285	T
SLC39A8	rs13107325		4	103188709	T
GNPDA2	rs10938397		4	45182527	G
NPC1	rs1808579		18	21104888	C
UBE2E3	rs1528435		2	181550962	T
C19orf7	rs3810291		19	47569003	A
BCDIN3D	rs7138803		12	50247468	A
CADM2	rs13078960		3	85807590	G
USP37	rs492400		2	219349752	C
TOMM40	rs2075650		19	45395619	A
TNNI3K	rs12566985		1	75002193	G
ASB4	rs6465468		7	95169514	T
CLIP1	rs11057405		12	122781897	G
MTIF3	rs12016871		NA	NA	T
STXBP6	rs10132280		14	25928179	C
PRKD1	rs11847697		14	30515112	T
HIF1AN	rs17094222		10	102395440	C
LINGO2	rs10968576		9	28414339	G
FANCL	rs1016287		2	59305625	T
CREB1	rs17203016		2	208255518	G
KCNK3	rs11126666		2	26928811	A
LRFN2	rs2033529		6	40348653	G
ELAVL4	rs11583200		1	50559820	C
SMG6	rs9914578		17	2005136	G
HIP1	rs1167827		7	75163169	G
ETV5	rs1516725		3	185824004	C
ADPGK	rs7164727		15	73093991	T
GBE1	rs3849570		3	81792112	A
MAN1A1	rs9374842		6	120185665	T
RIT2	rs7239883		18	40147671	G
FIGN	rs1460676		2	164567689	C
KIAA1303	rs12940622		17	78615571	G
ERBB4	rs7599312		2	213413231	G
DMXL2	rs3736485		15	51748610	A
PCDH9	rs9540493		13	66205704	A
RABEP1	rs1000940		17	5283252	G
NRXN3	rs7141420		14	79899454	T
GALNT10	rs7715256		5	153537893	G
GPRC5B	rs12446632		16	19935389	G

Supplemental Table S1a. *(Continued)*

Risk score	SNP name	Proxy	Chr.	Position	Coded allele
GRP	rs7243357		18	56883319	T
GNAT2	rs17024393		1	110154688	C
SCARB2	rs17001654		4	77129568	G
MYST1	rs9925964	rs1978487[a]	16	31129942	C
OLFM4	rs12429545		13	54102206	A
C9orf93	rs4740619		9	15634326	T
CALCR	rs9641123		7	93197732	C
NEGR1	rs3101336		1	72751185	C
ATP2A1	rs3888190		16	28889486	A
HNF4G	rs17405819		8	76806584	T
KIAA1505	rs2245368		7	76608143	C
PGPEP1	rs17724992	rs9636202[a]	19	18449238	A
FOXO3	rs9400239		6	108977663	C
SEC16B	rs543874		1	177889480	G
IRS1	rs2176040		2	227092802	A
PTBP2	rs11165643		1	96924097	T
TMEM18	rs13021737	rs13012571[a]	2	632550	G
Adult WHR					
BCL2	rs12454712		18	58996864	T
BMP2	rs979012		20	6571374	T
ADAMTS9	rs2371767	rs4607103[b]	3	64686944	T
SFXN2	rs7917772		10	104477433	A
SPATA5	rs303084		4	124286398	A
WARS2	rs2645294		1	119376110	T
SMAD6	rs1440372		15	64820205	C
MEIS1	rs1385167		2	66054152	G
VEGFA	rs1358980		6	43872529	T
EYA2	rs6090583		20	44992238	A
DCST2	rs905938		1	153258013	T
CCDC92	rs4765219		12	123006063	C
CMIP	rs2925979		16	80092291	T
ITPR2	rs10842707		12	26362631	T
DNM3	rs714515		1	170619613	G
PEMT	rs4646404		17	17360924	G
SNX10	rs1534696		7	26363764	C
JUND	rs12608504		19	18250135	A
MSC	rs12679556		8	72676782	G
CALCRL	rs1569135		2	187823643	A
LY86	rs1294410		6	6683751	C
HMGA1	rs1776897		6	34302989	G
RFXDC2	rs8030605		15	54291890	A
HOXC13	rs1443512		12	52628951	A

Supplemental Table S1a. *(Continued)*

Risk score	SNP name	Proxy	Chr.	Position	Coded allele
TNFAIP8	rs1045241		5	118757185	C
BTNL2	rs7759742		6	32489714	A
SCYL1BP1	rs10919388		1	168639127	C
ABCA1	rs10991437		9	106775741	A
HOXA11	rs7801581		7	27190296	T
RSPO3	rs1936805		6	127493809	T
CPEB4	rs7705502		5	173253421	A
PPARG	rs17819328		3	12464342	G
FAM13A1	rs9991328		4	89932144	T
PBRM1	rs2276824		3	52612526	C
SLC30A10	rs2820443		1	217820132	T
FLRT1	rs11231693		11	63619188	A
COBLL1	rs10195252		2	165221337	T
NMU	rs3805389		4	56177507	A
KCNJ2	rs8066985		17	65964940	A
NKX3-1	rs7830933		8	23659269	A
CEBPA	rs4081724		19	38516786	G
NFE2L3	rs10245353		7	25825139	A
MAP3K1	rs9687846		5	55897651	A
ZNRF3	rs2294239		22	27779477	A
GDF5	rs224333		20	33487376	G
PLXND1	rs10804591		3	130816923	A
FLJ16641	rs17451107		3	158280303	T
Child BMI					
GNPDA2	rs13130484		4	44870448	T
ADCY3	rs11676272		2	24995042	G
TMEM18	rs4854349		2	637861	C
SEC16B	rs543874		1	176156103	G
FAIM2	rs7132908		12	48549415	A
FTO	rs1421085	rs3751812[b]	16	52358455	G
OLFM4	rs12429545		13	53000207	A
TFAP2B	rs987237		6	50911009	G
TNNI3K	rs12041852		1	74776088	G
MC4R	rs6567160		18	55980115	C
ELP3	rs13253111		8	28117893	A
RAB27B	rs8092503		18	50630485	G
LMX1B	rs3829849		9	128430621	T
ADAM23	rs13387838		2	206989692	A
GPR61	rs7550711		1	109884409	T
VAT/SAT ratio					
LY86	rs912056		6	6681196	T
LYPLAL1	rs6689335		1	217695305	C

Supplemental Table S1a. *(Continued)*

Risk score	SNP name	Proxy	Chr.	Position	Coded allele
UBE2E2	rs7374732		3	23178458	C
Liver fat					
LYPLAL1	rs12137855		1	217515001	C
GCKR	rs780094		2	27594741	T
PPP1R3B	rs4240624		8	9221641	A
NCAN	rs2228603		19	19190924	T
PNPLA3	rs738409		22	42656060	G
Pericardial fat					
TRIB2	rs10198628		2	12881948	G
ENSA	rs6587515		1	148875512	G
EBF1	rs1650505		5	157962312	G
SAT SNP					
FTO	rs7185735		16	52380152	G
SAT SNP female					
GSDMB	rs2123685		17	35307415	T
VAT and VATadjBMI SNP					
RREB1	rs2842895		6	7051315	C
VAT and VATadjBMI SNP female					
GRAMD3	rs10060123		5	125711809	C

[a] Linkage disequilibrium between proxies and original SNPs: R^2>0.96, D'=1

[b] Linkage disequilibrium between proxy and original SNP: R^2=0.93, D'=0.97

Supplemental Table S1b. Matrix presentation of the number of overlapping SNPs and/or loci per risk score / phenotype

SNPs overlap	**Child BMI (N=15)**	**Adult BMI (N=97)**	**Adult WHR (N=49)**	**VAT/SAT ratio (N=3)**	**Adult fatty liver (N=5)**	**Adult pericardial fat (N=3)**	**SAT SNP**	**SAT SNP female**	**VAT and VATadjBMI SNP**	**VAT and VATadjBMI SNP female**
Child BMI (N=15)	**15**									
Adult BMI (N=97)	3	**97**								
Adult WHR (N=49)	0	0	**49**							
VAT/SAT ratio (N=3)	0	0	0	**3**						
Adult fatty liver (N=5)	0	0	0	0	**5**					
Adult pericardial fat (N=3)	0	0	0	0	0	**3**				
SAT SNP	0	0	0	0	0	0	**1**			
SAT SNP female	0	0	0	0	0	0	0	**1**		
VAT and VATadjBMI SNP	0	0	0	0	0	0	0	0	**1**	
VAT and VATadjBMI SNP female	0	0	0	0	0	0	0	0	0	**1**

Loci* overlap	**Child BMI (N=15)**	**Adult BMI (N=97)**	**Adult WHR (N=49)**	**VAT/SAT ratio (N=3)**	**Adult fatty liver (N=5)**	**Adult pericardial fat (N=3)**	**SAT SNP**	**SAT SNP female**	**VAT and VATadjBMI SNP**	**VAT and VATadjBMI SNP female**
Child BMI (N=15)	**15**									
Adult BMI (N=97)	6	**97**								
Adult WHR (N=49)	0	5	**49**							
VAT/SAT ratio (N=3)	0	0	2	**3**						
Adult fatty liver (N=5)	0	0	0	1	**5**					
Adult pericardial fat (N=3)	0	0	0	0	0	**3**				
SAT SNP	1	0	0	0	0	0	**1**			
SAT SNP female	0	0	0	0	0	0	0	**1**		
VAT and VATadjBMI SNP	0	0	1	1	0	0	0	0	**1**	
VAT and VATadjBMI SNP female	0	0	0	0	0	0	0	0	0	**1**

* A locus was defined as the region +/- 500,000 base pairs from the location of the SNP

Supplemental Table S1c. Matrix presentation of the correlation between the genetic risk scores

		Child BMI (N=15)	Adult BMI (N=97)	Adult WHR (N=49)	VAT/SAT ratio (N=3)	Adult fatty liver (N=5)	Adult pericardial fat (N=3)
Child BMI (N=15)	Pearson Correlation	**1**					
	Sig. (2-tailed)						
	N	**1867**					
Adult BMI (N=97)	Pearson Correlation	.196**	**1**				
	Sig. (2-tailed)	.000					
	N	1867	**1995**				
Adult WHR (N=49)	Pearson Correlation	.022	-.016	**1**			
	Sig. (2-tailed)	.341	.470				
	N	1867	1995	**1995**			
VAT/SAT ratio (N=3)	Pearson Correlation	-.025	.022	.127**	**1**		
	Sig. (2-tailed)	.283	.317	.000			
	N	1867	1995	1995	**1995**		
Adult fatty liver (N=5)	Pearson Correlation	-.014	.013	.013	.057*	**1**	
	Sig. (2-tailed)	.537	.563	.548	.011		
	N	1867	1995	1995	1995	**1995**	
Adult pericardial fat (N=3)	Pearson Correlation	.023	.017	-.011	-.025	-.028	**1**
	Sig. (2-tailed)	.318	.444	.627	.269	.210	
	N	1867	1995	1995	1995	1995	**1995**

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Supplemental Table S2. Power calculation

		N=1,738		N=1,993	
	Std. Deviation	**Beta detectable per SD***	**Beta detectable per additional risk allele***	**Beta detectable per SD***	**Beta detectable per additional risk allele***
Childhood BMI (N=15)	4.09	0.088	0.021	0.082	0.020
Adult BMI (N=97)	6.17		0.014		0.013
Adult WHR (N=49)	4.47		0.020		0.018
VAT/SAT ratio (N=3)*	1.16		0.076		0.071
Adult fatty liver N=5)	1.20		0.073		0.068
Adult pericardial fat (N=3)*	0.98		0.090		0.084

*Unweighted risk scores since no information on the effect estimates was available
* Based on a power of 80% with a significance level of 0.005

Supplemental Table S3. Associations of unweighted genetic risk scores with MRI adiposity measures (N=1,995)

Risk score (number of SNPs in risk score)	SAT (N=1,746)		VAT (N=1,742)		VAT/SAT ratio (N=1,738)		Liver fat percentage (N=1,950)		Pericardial fat mass (N=1,803)		BMI @ 10 (N=1,993)	
	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value
Main risk scores												
Child BMI (N=15)	0.047 (0.027; 0.066)	**$5.00*10^{-6}$**	0.046 (0.025; 0.067)	**$2.40*10^{-5}$**	-0.014 (-0.033; 0.005)	0.154	0.018 (-0.003; 0.038)	0.086	0.004 (-0.017; 0.025)	0.680	0.069 (0.048; 0.089)	**$7.00*10^{-6}$**
Adult BMI (N=97)	0.016 (0.009; 0.023)	**$9.00*10^{-6}$**	0.013 (0.005; 0.020)	**0.001**	-0.009 (-0.016; -0.002)	0.010	0.003 (-0.004; 0.010)	0.438	0.007 (0.000; 0.015)	0.066	0.022 (0.015; 0.029)	**$4.07*10^{-9}$**
Adult WHR (N=49)	-0.008 (-0.018; 0.002)	0.114	-0.001 (-0.011; 0.010)	0.909	0.011 (0.001; 0.020)	0.028	-0.002 (-0.012; 0.008)	0.649	-0.011 (-0.021; 0.000)	0.046	-0.008 (-0.018; 0.002)	0.116
VAT/SAT ratio (N=3)	-0.006 (-0.044; 0.032)	0.768	-0.008 (-0.049; 0.033)	0.694	0.,001 (-0.036; 0.037)	0.966	-0.012 (-0.050; 0.026)	0,.554	-0.013 (-0.053; 0.027)	0.513	-0.008 (-0.047; 0.031)	0.702
Adult fatty liver N=5)	0.002 (-0.035; 0.038)	0.922	0.006 (-0.033; 0.045)	0.755	0.003 (-0.031; 0.038)	0.845	0.105 (0.069; 0.141)	**$1.57*10^{-8}$**	0.021 (-0.018; 0.059)	0.292	-0.016 (-0.054; 0.021)	0.394
Adult pericardial fat (N=3)	-0.024 (-0.068; 0.020)	0.283	0.022 (-0.025; 0.069)	0.358	0.056 (0.015; 0.098)	0.008	-0.007 (-0.050; 0.037)	0.761	0.061 (0.015; 0.107)	0.009	-0.028 (-0.072; 0.017)	0.226

Bold values represent significant outcomes after Bonferroni correction for 10 analyses (p-value <0.005)

Supplemental Table S4. Associations of the BMI genetic risk scores with MRI adiposity measures adjusted for BMI (N=1,995)

Risk score (number of SNPs in risk score)	SATadjBMI (N=1,746)		VATadjBMI (N=1,742)		VAT/SAT ratio adjBMI (N=1,738)		Liver fat percentage adjBMI (N=1,950)		Pericardial fat mass adjBMI (N=1,803)	
	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value
Main risk scores										
Child BMI (N=15)	-0.004	0.434	0.004	0.545	0.006	0.243	0.000	0.924	-0.005	0.363
	(-0.014; 0.006)		(-0.008; 0.015)		(-0.004; 0.017)		(-0.010; 0.011)		(-0.017; 0.006)	
Adult BMI (N=97)	0.004	0.209	0.002	0.565	-0.003	0.350	-0.004	0.216	0.002	0.681
	(-0.002; 0.010)		(-0.005; 0.010)		(-0.010; 0.004)		(-0.011; 0.002)		(-0.006; 0.009)	
Adult WHR (N=49)	0.0004	0.929	0.007	0.181	0.006	0.243	0.002	0.608	-0.007	0.188
	(-0.008; 0.009)		(-0.003; 0.017)		(-0.004; 0.015)		(-0.007; 0.011)		(-0.017; 0.003)	
VAT/SAT ratio (N=3)*	0.002	0.888	-0.001	0.945	-0.002	0.929	-0.010	0.573	-0.012	0.565
	(-0.032; 0.037)		(-0.041; 0.038)		(-0.039; 0.035)		(-0.046; 0.025)		(-0.052; 0.028)	
Adult fatty liver (N=5)	0.019	0.247	0.011	0.549	-0.01	0.569	0.128	**$4.73*10^{-14}$**	0.021	0.278
	(-0.013; 0.051)		(-0.026; 0.049)		(-0.045; 0.025)		(0.095; 0.161)		(-0.017; 0.059)	
Adult pericardial fat (N=3)*	-0.013	0.521	0.038	0.101	0.058	0.007	-0.001	0.945	0.074	**0.002**
	(-0.052; 0.027)		(-0.007; 0.084)		(0.016; 0.100)		(-0.042; 0.039)		(0.028; 0.120)	
rs7185735 (SAT)	0.12	**$3.70*10^{-5}$**	0.036	0.282	-0.086	0.006	0.003	0.918	0.0002	0.997
	(0.063; 0.177)		(-0.030; 0.103)		(-0.148; -0.025)		(0.056; 0.063)		(-0.067; 0.068)	
rs2123685 (SAT female)	-0.085	0.294	-0.225	0.016	-0.178	0.041	0.064	0.445	-0.087	0.354
	(-0.243; 0.074)		(-0.408; -0.041)		(-0.348290; -0.007)		(-0.100; 0.228)		(-0.270; 0.097)	
rs2842895 (VAT and VATadjBMI)	0.018	0.549	-0.004	0.905	-0.032	0.326	-0.007	0.815	0.048	0.173
	(-0.041; 0.076)		(-0.072; 0.064)		(-0. 094; 0.031)		(-0. 068; 0.053)		(-0.021; 0.116)	
rs10060123 (VAT and VATadjBMI female)	-0.024	0.464	-0.051	0.180	-0.034	0.342	-0.010	0.777	-0.050	0.197
	(-0. 089; 0.041)		(-0. 126; 0.024)		(-0.103; 0.036)		(-0. 076; 0.057)		(-0. 125; 0.026)	

*Unweighted risk scores since no information on the effect estimates was available

Bold values represent significant outcomes after Bonferroni correction for 10 analyses (p-value <0.005)

Supplemental Table S5. Associations of the BMI genetic risk scores with SAT and VAT measures adjusted for VAT and SAT (N=1,995)

Risk score (number of SNPs in risk score)	SATadjVAT(N=1,736)**		VATadjSAT (N=1,736)**	
	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value
Main risk scores				
Child BMI (N=15)	0.008	0.152	0.008	0.179
	(-0.003; 0.018)		(-0.004; 0.019)	
Adult BMI (N=97)	0.014	**$2.90*10^{-5}$**	0.0002	0.962
	(0.008; 0.021)		(-0.007; 0.007)	
Adult WHR (N=49)	-0.011	0.018	0.007	0.168
	(-0.021; -0.002)		(-0.003; 0.017)	
VAT/SAT ratio (N=3)*	-0.002	0.899	-0.004	0.841
	(-0.039; 0.034)		(-0.043; 0.035)	
Adult fatty liver (N=5)	0.007	0.708	-0.002	0.912
	(-0.028; 0.041)		(-0.039; 0.035)	
Adult pericardial fat (N=3)*	-0.057	0.008	0.057	0.014
	(-0.099; -0.015)		(0.011; 0.102)	
rs7185735 (SAT)	0.132	**$2.20*10^{-5}$**	-0.042	0.212
	(0.071; 0.193)		(-0.107; 0.024)	
rs2123685 (SAT female)	0.128	0.136	-0.208	0.024
	(-0.040; 0.297)		(-0.389; -0.027)	
rs2842895 (VAT and VATadjBMI)	0.024	0.444	-0.019	0.580
	(-0.038; 0.087)		(-0.086; 0.048)	
rs10060123 (VAT and VATadjBMI female)	0.006	0.855	-0.049	0.194
	(-0.063; 0.075)		(-0.123; 0.025)	

*Unweighted risk scores since no information on the effect estimates was available
Bold values represent significant outcomes after Bonferroni correction for 10 analyses (p-value <0.005)
** SATadjVAT: SAT adjusted for VAT
VATadjSAT: VAT adjusted for SAT

Supplemental Table S6. Phenotypic variance in MRI fat measures explained by genetic risk scores based on adult and childhood BMI, and liver fat, and separate SNPs in the full group (N=1,995)

Risk score (SNPs in risk score)	SAT (N=1,746) Explained variance (%)	VAT (N=1,742) Explained variance (%)	VAT/SAT ratio (N=1,738) Explained variance (%)	Liver fat percentage (N=1,950) Explained variance (%)	Pericardial fat mass (N=1,803) Explained variance (%)	BMI @ 10 (N=1,993) Explained variance (%)
Childhood BMI (N=15)	**1.167**	**0.636**	0.874	0.464	<0.001	**1.765**
Adult BMI (N=97)	**1.769**	**1.107**	**0.566**	0.068	0.331	**2.458**
Adult WHR (N=49)	0.177	0.007	0.238	0.006	0.197	0.181
VAT/SAT ratio (N=3)*	0.002	0.002	<0.001	0.019	0.016	0.103
Adult fatty liver N=5)	0.056	0.047	0.006	**2.222**	0.364	0.046
Adult pericardial fat (N=3)*	0.004	0.009	0.303	0.005	0.023	0.195
rs7185735 (SAT)	**1.042**	0.400	**0.714**	0.092	0.066	**0.669**
rs2123685 (SAT female)	0.007	0.168	0.093	0.035	0.022	0.130
rs2842895 (VAT and VATadjBM)	0.019	0.000	0.019	<0.001	0.126	0.153
rs10060123 (VAT and VATadjBMI female)	0.085	0.168	0.004	0.030	0.166	0.144

Bold values represent explained variances for significant associations of the risk scores / SNPs with the outcome
*Unweighted risk scores since no information on the effect estimates was available.

Supplemental Table S7. Phenotypic variance in MRI fat measures explained by genetic risk scores based on adult and childhood BMI, and liver fat, and separate SNPs in boys (N=979) and girls (N=1,016) separately

Risk score (SNPs in risk score)	SAT (N=1,746) Explained variance (%)	VAT (N=1,742) Explained variance (%)	VAT/SAT ratio (N=1,738) Explained variance (%)	Liver fat percentage (N=1,950) Explained variance (%)	Pericardial fat mass (N=1,803) Explained variance (%)	BMI @ 10 (N=1,993) Explained variance (%)
Boys						
Childhood BMI (N=15)	**0.936**	0.404	0.804	0.352	<0.001	**2.261**
Adult BMI (N=97)	**2.509**	**1.202**	**0.972**	0.195	0.384	**2.931**
Adult WHR (N=49)	0.166	0.032	0.073	0.016	0.259	0.338
VAT/SAT ratio (N=3)*	0.103	0.041	0.040	0.028	0.005	0.087
Adult fatty liver N=5)	0.175	0.081	0.190	**1.440**	<0.001	0.029
Adult pericardial fat (N=3)*	0.002	0.196	0.228	0.001	0.077	0.083
rs7185735 (SAT)	0.521	0.239	0.228	0.104	0.241	0.230
rs2123685 (SAT female)	0.010	0.238	0.145	0.014	0.194	0.149
rs2842895 (VAT and VATadjBM)	0.000	0.055	0.048	0.063	0.028	0.032
rs10060123 (VAT and VATadjBMI female)	0.184	0.172	0.022	0.492	0.107	0.188
Girls						
Childhood BMI (N=15)	1.550	0.828	1.406	0.468	<0.001	**1.682**
Adult BMI (N=97)	**1.369**	**1.007**	0.376	0.002	0.276	**1.813**
Adult WHR (N=49)	0.215	<0.001	0.553	0.001	0.139	<0.001
VAT/SAT ratio (N=3)*	0.204	0.151	0.041	0.019	0.128	<0.001
Adult fatty liver N=5)	0.172	0.068	0.091	**3.096**	0.039	0.012
Adult pericardial fat (N=3)*	0.218	0.002	0.395	0.004	**0.993**	<0.001
rs7185735 (SAT)	**2.086**	0.615	**1.746**	0.081	0.002	**0.944**
rs2123685 (SAT female)	0.007	0.118	0.066	0.227	0.026	<0.001
rs2842895 (VAT and VATadjBM)	0.059	0.044	0.001	0.022	0.301	<0.001
rs10060123 (VAT and VATadjBMI female)	0.029	0.167	0.085	0.087	0.230	<0.001

Bold values represent explained variances for significant associations of the risk scores / SNPs with the outcome
*Unweighted risk scores since no information on the effect estimates was available.

Supplemental File S1: Additional description of the used methods

Measures of adiposity at 10 years

MRI has been described as an accurate and reproducible technique and considered the gold standard for the measurement of intra-abdominal and organ fat deposition.[23,31-33] Adiposity measures were obtained from MRI scans as described previously.[24] All children were scanned using a 3.0 Tesla MRI (Discovery MR750w, GE Healthcare, Milwaukee, WI, USA) for body fat imaging using standard imaging and positioning protocols. They wore light clothing without metal objects while undergoing the body scan.[48] The scanner was operated by trained research technicians and all imaging data were collected according to standardized protocols. Scans in the thorax, liver and abdomen were performed instructing the participant to perform breath-hold maneuvers in expiration with a maximum duration of 11 seconds. For imaging fat around the heart, a multi-breath-hold approach was used, using an ECG triggered black-blood prepared thin slice single shot fast spin echo acquisition (BB SSFSE). The slice orientation was copied directly from the short axis series of functional heart scans performed in the protocol of the study. A liver fat scan was subsequently performed using an axial volume and a special 3-point proton density weighted DIXON technique (IDEAL IQ) that could provide not only fat images of the upper abdomen, but more importantly was capable of generating a precise fat fraction image demonstrating if liver fat was present.[49] The IDEAL IQ scan is based on a carefully tuned 6-echo echo planar imaging (EPI) acquisition. Abdominal fat scans followed using an axial volume comprising the lower liver, abdomen and part of the upper pelvis using a proton density weighted 2-point DIXON acquisition (LavaFlex). Finally, a high resolution free-breathing coronally acquired scan centered at the head of the femurs was performed using a T1-weighted 2-point DIXON technique (LavaFlex). For both IDEAL IQ and LavaFlex measurements, water, fat, in-phase and out-of-phase 3D volumes were reconstructed. The obtained fat scans were subsequently analyzed by the Precision Image Analysis company (PIA, Kirkland, Washington, United States). Pericardial, subcutaneous, visceral, and liver fat were quantified using the sliceOmatic (TomoVision, Magog, Canada) software package. All extraneous structures and any image artifacts were removed manually.[23] Pericardial fat included both epicardial- and paracardial fat directly attached to the pericardium. Pericardial fat volume was quantified using the summation of discs method, ranging from the apex to the left ventricular outflow tract and was subsequently multiplied by the specific gravity of adipose tissue, 0.9 g/ml. Total subcutaneous and visceral fat volumes were generated by summing the volumes of the liver fat-only and abdominal fat-only scans. The liver set was used to quantify fat from the dome of the liver to the most inferior part of the right kidney, the abdominal set was used from there to the superior part of the femoral head. If the abdominal scan did not reach the femoral head, the femoral fat-

only scan was used to make up for any volume left behind. Subcutaneous and visceral fat masses were obtained by multiplying the total volumes by the specific gravity of adipose tissue, 0.9 g/ml. Liver fat fraction was determined by taking four samples of at least 4 cm^2 from the central portion of the hepatic volume. Subsequently, the mean signal intensities were averaged to generate an overall mean liver fat fraction estimation.

Chapter 3.4

Influence of genetic variants associated with body mass index on eating behaviour in childhood

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Abstract

Background: Childhood eating behaviours are associated with body mass index (BMI). Recent genome-wide association studies have identified many single nucleotide polymorphisms (SNPs) associated with adult and childhood BMI. We hypothesized that these SNPs also influence eating behaviour.

Methods: In a population-based prospective cohort study among 3,179 children (mean age (standard deviation): 4.0 (0.1) years), we tested two weighted genetic risk scores, based on 15 childhood and 97 adult BMI SNPs, and ten individual appetite and/or satiety related SNPs for association with food fussiness, food responsiveness, enjoyment of food, satiety responsiveness, slowness in eating.

Results: The 15 SNP-based childhood BMI genetic risk score was not associated with the eating behaviour subscales. The 97 SNP-based adult BMI genetic risk score was nominally associated with satiety responsiveness (β: -0.007 standard deviation, 95% confidence interval (CI) -0.013, 0.000). Of the ten individual SNPs, rs11030104 in BDNF and rs10733682 in *LMX1B* were nominally associated with satiety responsiveness (β: -0.057 standard deviation, 95% CI -0.112, -0.002).

Conclusions: Our findings do not strongly support the hypothesis that BMI associated SNPs also influence eating behaviour at this age. A potential role for BMI SNPs in satiety responsiveness during childhood was observed, however, no associations with the other eating behaviour subscales.

Background

Eating behaviour is associated with body mass index (BMI) in children.[1,2] Childhood eating behaviour includes food approach behaviours: food responsiveness and enjoyment of food, and food avoidance behaviours: satiety responsiveness, food fussiness and slowness in eating.[3] Food approach behaviours have been associated with higher BMI, whereas food fussiness, a high satiety responsiveness and slow eating have been associated with a lower fat free mass, lower BMI, and underweight.[1,4-8] However, food fussiness has occasionally also been linked with overweight between the ages of two and six years.[9] Eating behaviours have a genetic background, with fussy eating showing a heritability of 85%, satiety responsiveness of up to 72%, slowness in eating of up to 84%, food responsiveness of up to 59%, and enjoyment of food of up to 75% in twin studies.[10-13] However, their exact genetic architecture remains unclear.[10,11,14] Genetic variants associated with BMI may exert their effects by affecting eating behaviour.

Recently, a total of 15 childhood BMI associated single nucleotide polymorphisms (SNPs) have been described.[15] In addition, a very large genome-wide association study identified 97 SNPs related to BMI in adults.[16] Twelve of the childhood and adult BMI genetic loci overlapped . The SNPs associated with childhood or adult BMI are located in or near genes involved in widely varying processes, including energy homeostasis, satiety, appetite control, and glucose homeostasis.[16] Some of these processes may influence eating behaviour. Based on literature review ten out of the 97 adult BMI SNPs, located in or near *MC4R*, *GNPDA2*, *ADCY3/POMC*, *LMX1B*, *SCG3*, *BBS4*, *TUB*, *FTO*, *BDNF*, and *SH2B1*, may also be associated with eating behaviour through a potential role in the regulation of satiety and/or appetite.[14,16-27]

We hypothesized that genetic variants involved in childhood and adult BMI are also associated with eating behaviour. We examined in a population-based prospective cohort among 3,179 preschool children the associations of genetic risks scores based on childhood or adult BMI related SNPs and of eight individual SNPs located in or near genes with a potential role in appetite and/or satiety with the eating behaviours food fussiness, satiety responsiveness, slowness in eating, food responsiveness and enjoyment of food.

Methods

Study design and population

This study was embedded within the Generation R Study, a prospective population-based cohort in Rotterdam, the Netherlands.[28] All pregnant women residing in Rotterdam with a delivery date between April 2002 and January 2006 were invited to participate. The Medical Ethical Committee of the Erasmus Medical Center, Rotterdam, approved the study and informed consent was obtained for participating children. Information about child and family characteristics was obtained by postal questionnaires filled out by parents, and from medical records of hospitals, midwives, and community Child Health Centers. Children and their parents are in ongoing follow-up. A detailed design has been described previously.[28] The Generation R cohort is a multi-ethnic cohort. Participants of European origin constitute the largest ethnic group (56%). The largest other groups are Surinamese

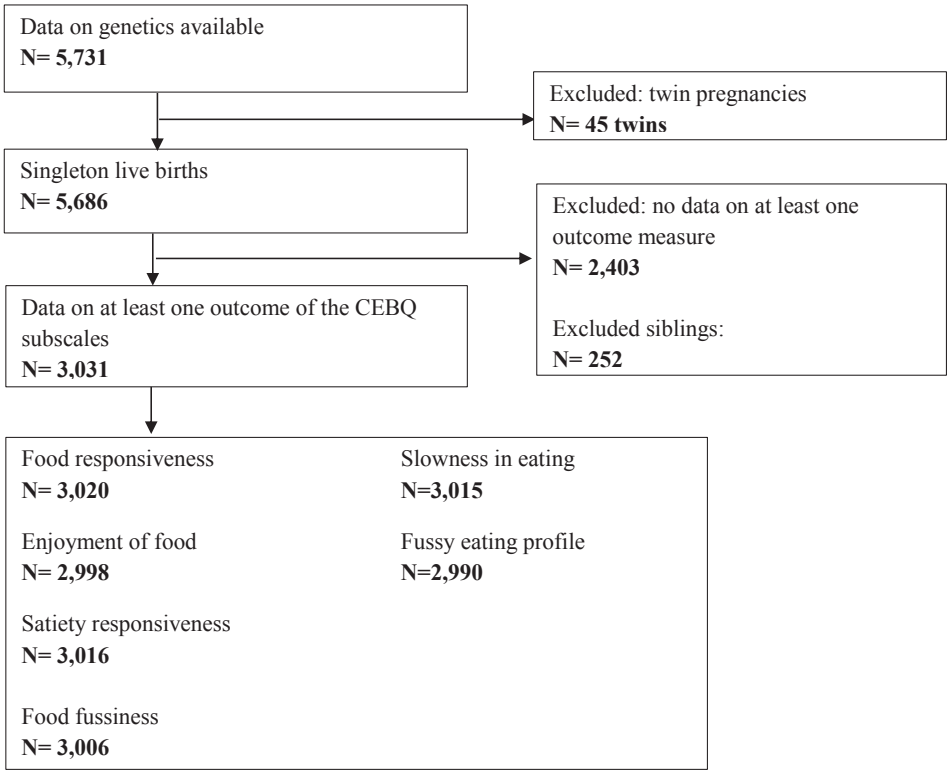

Figure 1. Flowchart of participants.

(9%), Turkish (7%) and Moroccan participants (6%).[28] Consent for participation during the preschool phase was obtained for 7,295 children and their parents. GWA scans were available for 5,731 children.[29] Of these, 3,207 children (56%) had information on eating behaviour. Our present study was limited to singleton live births with information on at least one eating behaviour outcome (N=3,179). We excluded all twins and one of each non-twin sibling pair, based on completeness of data and, if equal between the siblings, randomly. A participant flowchart is shown in Figure 1.

Genetic risk scores

DNA was isolated from blood samples collected at birth (cord blood) or, for a small subgroup with missing cord blood samples, at 6 years of age.[29] For genome-wide association analysis the Illumina 610 and 660W Quad platforms were used.[28] Stringent quality checks were performed in which individuals with low sample call rates or sex mismatches were excluded. Imputation of genotypes to the cosmopolitan panel of HapMap ii (release 22) was done using MACH software.[30] Prior to imputation, we excluded SNPs with a high level of missing data (SNP call rate <98%), significant deviations from Hardy-Weinberg equilibrium (p-value $<1*10^{-6}$), or low minor allele frequencies (<0.1%). Information about the SNPs of interest for the current study was extracted from the GWAS dataset. We constructed two weighted genetic risk scores, combining the 15 childhood BMI SNPs and 97 adult BMI SNPs, respectively.[15,16] Each score summed the BMI increasing risk alleles from the GWA dosage data, weighted using effect estimates from the original GWAS, as described previously.[31] For the 15 childhood SNPs, weights were recalculated from the GWAS meta-analysis without the Generation R Study data, as these were part of the discovery dataset.[15] As no information on rs1421085 was available in our GWA dataset, we used rs3751812 as a proxy in the child BMI score (R^2=0.93, D'=0.97). For adult BMI we used four proxies (R^2>0.96, D'=1): rs13012571 was used as a proxy for rs13021737, rs1978487 for rs9925964, rs6445197 for rs2365389, and rs9636202 for rs17724992. A list of the SNPs included in the risk scores is provided in the supplemental material (Supplemental Table S1). The SNPs analyzed separately were rs6567160 (*MC4R*), rs10938397 (*GNPDA2*), rs10182181 (*ADCY3*/*POMC*), rs10733682 (*LMX1B*), rs3736485 (*SCG3*), rs7164727 (*BBS4*), rs4256980 (*TUB*), rs1558902 (*FTO*), rs11030104 (*BDNF*), and rs3888190 (*SH2B1*). In the previous GWAs paper, a manual literature search was performed into the potential roles of all genes within a region of 500 kb on either side (1Mb total) of the 97 SNPs found to be associated to adult BMI.[16] This showed that eight genes may play a role in appetite and satiety control.[14,16-25,32] Additional literature search showed that *FTO* and *BDNF* may also play a role in appetite and satiety control, providing a total of ten SNPs to test separately.[26,27] All genes have an r^2>0.2 with the BMI SNP to which they are linked.[16]

Eating behaviour and BMI in childhood

Eating behaviour was assessed with the Children's Eating Behaviour Questionnaire (CEBQ) aged 4 years, as previously described.[33] The CEBQ is a validated, multi-dimensional parent report questionnaire designed to measure differences in children's eating behaviours.[3] The CEBQ has good psychometric properties in terms of internal reliability, test-retest reliability, factor structure and concurrent validity with actual eating behaviour.[3,34,35] The CEBQ consists of eight scales, each containing three to six items. Parents rated the frequency of their children's eating behaviour on a Likert scale from 1 (never) to 5 (always). Mean item scores were calculated for each scale. The CEBQ has good internal reliability, test-retest reliability, and factor structure.[3,34] We used five different CEBQ-subscales, food responsiveness , enjoyment of food, food fussiness, satiety responsiveness, and slowness in eating as our outcomes.[33] We also tested the association of the five eating behaviours with BMI to examine if a phenotypic association was present.

We also assessed the association of the genetic risk scores and individual SNPs with childhood BMI. BMI (kg/m^2) was calculated from height and weight measured without shoes and heavy clothing, as described previously.[36] We selected children with BMI data at the age closest to 4 years since eating behaviour was measured at this age. Children with BMI data had a median age of 3.8 years (95% range 3.7, 4.1). Subjects with overweight or obesity were defined according to the IOTF-classification (Table 1).[37]

Statistical analysis

To determine whether the genetic risk scores and the individual SNPs were associated with the five CEBQ-subscales we performed linear regression analyses. We also tested the risk scores and separate SNPs for association with childhood BMI at four years of age. We created SD-scores for all outcomes to facilitate comparison of effect estimates. For BMI, sex- and age-adjusted SD-scores were constructed using the Dutch reference growth curves (Growth Analyser Research Calculation Tools, Version 4.0 http://www.growthanalyser.org). All analyses were performed in the full group of children, and repeated in children of European ancestry as a sensitivity analysis. A child was classified as European if it was within four standard deviations from the Hapmap CEU panel mean value for all four first principal components. All outcomes except food responsiveness were normally distributed, but given our large sample size, we considered our model sufficiently robust to continue using the linear approach for food responsiveness. All models were sex and age adjusted except for BMI since SD scores were already sex and age adjusted. All models were also adjusted for ethnic background using the first four principal components from the genetic data . Principal components were calculated separately for the full- and the

European-ancestry group. The phenotypic variance explained was calculated as the increase in unadjusted R^2 between the full models and the same models without the risk score or separate SNPs. We applied Bonferroni correction based on the 2 risk scores and 10 separate SNPs and considered a p-value of <0.004 (0.05/12) as significant. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 21.0 for Windows (IBM, Chicago, IL, USA).

Results

Characteristics of the study population

Characteristics of all children are listed in Table 1. Information on eating behaviour was collected at a median age of 4.0 years (95% range 4.0, 4.3). The median BMI of the children was 15.9 kg/m^2 (95% range 13.6, 19.2) at a median age of 3.8 years (95% range 3.0, 4.0).

Genetic risk scores and eating behaviour

All five eating behaviours were significantly associated with BMI at four years (data not shown). The childhood BMI risk score was not associated with any of the five eating behaviour subscales, whereas the adult BMI genetic risk score was nominally associated with satiety responsiveness, but not with the other subscales (Table 2). A decrease of 0.007 SDS (95% CI -0.013, 0.000) for each additional average risk allele in the genetic risk score was observed for satiety responsiveness. In European children the childhood BMI risk score was nominally associated with satiety responsiveness, while the adult BMI genetic risk score was not associated with any of the five eating behaviour subscales (Table 3).

Of the separate SNPs, rs10733682 (in *LMX1B*) and rs11030104 (in *BDNF*) were nominally associated with satiety responsiveness, showing a decrease in satiety responsiveness of 0.057 SDS (95% CI -0.112, -0.002) and 0.087 SDS (95% CI -0.155, -0.020), respectively, for each additional copy of the risk allele. Rs6567160 (near *MC4R*), rs10938397 (near *GNPDA2*), rs10182181 (near *ADCY/POMC*), rs3736485 (near *SCG3*), rs7164727 (near *BBS4*), rs4256980 (near *TUB*), rs1558902 (in *FTO*), and rs3888190 (near *SH2B1*) were not associated with the subscales (Table 2). In European children none of the separate SNPs was associated with the eating behaviour subscales (Table 3).

Table 1. Characteristics of the study population (N=3,179).

Characteristics	All children (N=3,179)	European children (N=2,136)
Birth		
Boys (%)	1,527 (50.4)	1,089 (51.0)
Gestational age at birth (weeks)	40.1 (36.6;42.4)	40.3 (36.6;42.4)
Weight at birth (grams) [a,b]	3476 (511)	3554 (514)
SD-score (mean, standard deviation)	0.0 (1.0)	0.1 (1.0)
Childhood		
Age at visit (years)	3.8 (3.0;4.0)	3.8 (3.7;4.1)
Height (cm) [a,c]	103.2 (4.2)	103.7 (4.1)
SD-score (mean, standard deviation)	0.1 (1.0)	0.2 (1.0)
Weight (kg) [a,c]	17.0 (2.2)	17.0 (2.0)
SD-score (mean, standard deviation)	0.0 (1.1)	0.0 (1.0)
Body mass index (kg/m^2) [d]	15.9 (13.6;19.2)	15.7 (13.7;18.5)
SD-score (mean, standard deviation)	0.1 (1.0)	0.1 (0.9)
Normal weight (%) [e]	2528 (77.7)	1446 (81.1)
Overweight/obesity (%) [e]	358 (11.0)	151 (8.5)
Eating behaviour		
Age at questionnaire (years)	4.0 (4.0;4.3)	4.0 (4.0;4.3)
Food responsiveness [a,f]	1.8 (0.7)	1.8 (0.7)
Enjoyment of food [a,f]	3.4 (0.7)	3.4 (0.7)
Satiety responsiveness [a,f]	3.1 (0.7)	3.1 (0.6)
Food fussiness [a,f]	3.0 (0.8)	3.0 (0.8)
Slowness in eating [a]	3.1 (0.8)	3.1 (0.7)

Values are medians (95% range) unless otherwise specified
[a] Means (standard deviations) [b] Sweden 1991 (Sweden) / Niklasson was used as a reference to calculate standard deviation (SD) scores [c] Sweden 2002 (Sweden) / Albertsson-Wikland was used as a reference to calculate SD-scores [d] Netherlands, the 1997 (Netherlands, the) / Fredriks was used as a reference to calculate SD-scores [e] The IOTF-classification was used to define overweight and obesity [f] Values represent mean item scores range [1-5]

The childhood and adult BMI risk scores were associated with childhood BMI (Table 2). Rs10182181 (near *ADCY3/POMC*) and rs4256980 (near *TUB*) were nominally associated with childhood BMI. Associations with BMI in European children were similar, except for rs4256980 which was not associated (Table 3). The highest values of variance explained were obtained for the associations of the risk scores with BMI (0.5%). For the eating behaviours the highest variance explained was obtained for the childhood BMI risk scores with satiety responsiveness in the European group (0.3%) (Supplemental Tables S2, S3).

Table 2. Associations of genetic risk scores and separate satiety/appetite SNPs with measures of eating behaviour (N= 3,870)[a,b]

Risk score (SNPs in risk score)	Food responsiveness (N=3,020)		Enjoyment of food (N=2,998)		Satiety responsiveness (N=3,016)		Food fussiness (N=3,006)		Slowness in eating (N=3,015)		BMI (N=2,413)	
	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value
Main risk scores												
Child BMI (N=15)	0.007 (-0.012; 0.026)	0.469	-0.003 (-0.022; 0.016)	0.771	-0.010 (-0.029; 0.009)	0.317	0.000 (-0.020; 0.020)	0.998	-0.009 (-0.028; 0.011)	0.377	0.040 (0.019; 0.060)	**$1.25*10^{-4*}$**
Adult BMI (N=97)	0.005 (-0.001; 0.011)	0.123	0.004 (-0.002; 0.010)	0.224	-0.007 (-0.013; 0.000)	**0.037**	-0.001 (-0.007; 0.005)	0.775	-0.005 (-0.012; 0.001)	0.088	0.013 (0.007; 0.019)	**$3.50*10^{-5*}$**
Separate SNPs												
rs6567160 (*MC4R*)	0.048 (-0.013; 0.110)	0.125	0.016 (-0.046; 0.078)	0.610	0.015 (-0.047; 0.077)	0.638	0.021 (-0.042; 0.085)	0.510	-0.001 (-0.064; 0.061)	0.970	-0.045 (-0.107; 0.018)	0.161
rs10938397 (*GNPDA2*)	-0.006 (-0.061; 0.049)	0.830	-0.012 (-0.067; 0.044)	0.860	-0.002 (-0.058; 0.053)	0.939	-0.008 (-0.065; 0.049)	0.779	0.014 (-0.042; 0.070)	0.627	-0.052 (-0.106; 0.003)	0.064
rs10182181 (*ADCY3/POMC*)	0.023 (-0.032; 0.077)	0.413	0.0003 (-0.054; 0.055)	0.990	-0.032 (-0.087; 0.023)	0.252	-0.011 (-0.067; 0.045)	0.710	-0.019 (-0.074; 0.037)	0.512	0.076 (0.022; 0.129)	**0.006**
rs10733682 (*LMX1B*)	-0.041 (-0.096; 0.013)	0.137	-0.009 (-0.065; 0.046)	0.736	-0.057 (-0.112; -0.002)	**0.042**	0.010 (-0.047; 0.066)	0.733	-0.041 (-0.096; 0.015)	0.149	0.012 (-0.043; 0.066)	0.671
rs3736485 (*SCG3*)	-0.018 (-0.073; 0.037)	0.530	-0.033 (-0.088; 0.022)	0.238	0.005 (-0.051; 0.060)	0.867	0.025 (-0.031; 0.082)	0.385	-0.006 (-0.062; 0.050)	0.835	-0.022 (-0.077; 0.032)	0.420
rs7164727 (*BBS4*)	-0.014 (-0.072; 0.043)	0.629	-0.006 (-0.063; 0.052)	0.845	0.000 (-0.058; 0.058)	0.989	0.001 (-0.059; 0.060)	0.985	0.025 (-0.034; 0.083)	0.408	-0.021 (-0.078; 0.036)	0.476
rs4256980 (*TUB*)	-0.034 (-0.089; 0.021)	0.228	-0.023 (-0.078; 0.033)	0.425	0.047 (-0.009; 0.102)	0.097	-0.005 (-0.062; 0.051)	0.852	0.050 (-0.005; 0.106)	0.076	-0.065 (-0.119; -0.011)	**0.019**
rs3888190 (*SH2B1*)	0.035 (-0.021; 0.091)	0.218	-0.002 (-0.058; 0.055)	0.958	0.000 (-0.056; 0.057)	0.988	-0.001 (-0.059; 0.056)	0.959	-0.030 (-0.087; 0.027)	0.299	-0.015 (-0.071; 0.040)	0.589
rs1558902 (*FTO*)	-0.055 (-0.113; 0.003)	0.061	-0.051 (-0.109; 0.007)	0.085	0.020 (-0.039; 0.078)	0.506	0.034 (-0.026; 0.093)	0.269	0.024 (-0.035; 0.083)	0.430	-0.029 (-0.086; 0.028)	0.324
rs11030104 (*BDNF*)	0.022 (-0.046; 0.089)	0.528	0.020 (-0.048; 0.087)	0.565	-0.087 (-0.155; -0.020)	**0.012**	-0.018 (-0.088; 0.051)	0.605	-0.096 (-0.165;-0.028)	0.299	0.040 (-0.028; 0.107)	0.248

[a]Analyses were performed in children with complete data on genetic variants and covariates and at least one outcome under study[b]Values are linear regression coefficients for models adjusted for sex, age, and the first four principal components and represent the difference in standard deviation scores of the outcome measures for each additional average risk allele in the risk scores. Bold values represent nominally significant outcomes *Significant after Bonferroni correction for 12 analyses (p-value <0.004)

Table 3. Associations of genetic risk scores and separate satiety/appetite SNPs with measures of eating behaviour in European children (N= 2,136)[a,b]

Risk score (SNPs in risk score)	Food responsiveness (N=1,916)		Enjoyment of food (N=1,904)		Satiety responsiveness (N=1,913)		Food fussiness (N=1,907)		Slowness in eating (N=1,914)		BMI (N=1,784)	
	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value	Beta (CI 95%)	p-value
Main risk scores												
Child BMI (N=15)	0.008 (-0.014; 0.031)	0.468	0.008 (-0.013; 0.030)	0.454	-0.023 (-0.046; -0.001)	**0.039**	-0.015 (-0.038; 0.008)	0.202	-0.015 (-0.037; 0.007)	0.192	0.028 (0.007; 0.050)	**0.010**
Adult BMI (N=97)	0.005 (-0.002; 0.013)	0.166	0.004 (-0.004; 0.011)	0.323	-0.004 (-0.012; 0.003)	0.265	-0.002 (-0.010; 0.006)	0.599	-0.007 (-0.015; 0.000)	0.066	0.008 (0.001; 0.016)	**0.025**
Separate SNPs												
rs6567160 (*MC4R*)	0.075 (0.000; 0.149)	0.050	0.013 (-0.060; 0.086)	0.725	0.003 (-0.071; 0.078)	0.932	-0.007 (-0.085; 0.071)	0.866	-0.014 (-0.088; 0.060)	0.704	0.022 (-0.051; 0.095)	0.559
rs10938397 (*GNPDA2*)	-0.022 (-0.089; 0.045)	0.519	-0.033 (-0.098; 0.032)	0.320	-0.013 (-0.080; 0.054)	0.706	0.033 (-0.036; 0.103)	0.347	0.046 (-0.020; 0.112)	0.173	-0.023 (-0.088; 0.042)	0.483
rs10182181 (*ADCY3/POMC*)	0.026 (-0.041; 0.093)	0.453	0.005 (-0.060; 0.070)	0.883	-0.058 (-0.125; 0.009)	0.090	-0.002 (-0.072; 0.068)	0.953	-0.015 (-0.081; 0.051)	0.660	0.087 (0.023; 0.151)	**0.008**
rs10733682 (*LMX1B*)	-0.054 (-0.121; 0.013)	0.117	0.018 (-0.048; 0.083)	0.594	-0.047 (-0.114; 0.020)	0.171	0.024 (-0.046; 0.094)	0.496	-0.034 (-0.100; 0.033)	0.320	0.030 (-0.035; 0.095)	0.362
rs3736485 (*SCG3*)	-0.013 (-0.081; 0.055)	0.698	-0.055 (-0.121; 0.011)	0.101	0.007 (-0.061; 0.075)	0.840	0.037 (-0.034; 0.108)	0.303	-0.017 (-0.084; 0.051)	0.629	-0.064 (-0.131; 0.002)	0.057
rs7164727 (*BBS4*)	-0.032 (-0.104; 0.039)	0.377	0.005 (-0.064; 0.075)	0.884	-0.027 (-0.099; 0.044)	0.450	-0.024 (-0.098; 0.050)	0.530	0.034 (-0.036; 0.105)	0.341	-0.050 (-0.120; 0.019)	0.156
rs4256980 (*TUB*)	-0.005 (-0.075; 0.064)	0.883	-0.013 (-0.080; 0.055)	0.709	0.032 (-0.037; 0.101)	0.368	0.001 (-0.071; 0.073)	0.983	0.024 (-0.044; 0.092)	0.492	-0.023 (-0.090; 0.045)	0.509
rs3888190 (*SH2B1*)	0.027 (-0.041; 0.094)	0.439	-0.004 (-0.070; 0.061)	0.898	-0.048 (-0.115; 0.019)	0.162	0.006 (-0.064; 0.077)	0.857	-0.055 (-0.122; 0.011)	0.104	-0.003 (-0.069; 0.062)	0.917
rs1558902 (*FTO*)	-0.048 (-0.118; 0.022)	0.177	-0.026 (-0.094; 0.042)	0.449	-0.017 (-0.087; 0.053)	0.631	0.004 (-0.068; 0.077)	0.905	0.020 (-0.049; 0.089)	0.565	-0.015 (-0.083; 0.052)	0.655
rs11030104 (*BDNF*)	0.030 (-0.052; 0.112)	0.470	0.005 (-0.064; 0.075)	0.969	-0.047 (-0.129; 0.035)	0.259	-0.006 (-0.091; 0.080)	0.898	-0.076 (-0.157; 0.004)	0.064	0.073 (-0.007; 0.152)	0.072

[a]Analyses were performed in children with complete data on genetic variants and covariates and at least one outcome under study [b]Values are linear regression coefficients for models adjusted for sex, age, and the first four principal components and represent the difference in standard deviation scores of the outcome measures for each additional average risk allele in the risk scores. Bold values represent nominally significant outcome

Discussion

We observed that a higher overall adult BMI genetic risk score and the individual SNPs rs10733682 (in *LMX1B*) and rs11030104 (in *BDNF*) were nominally associated with a lower satiety responsiveness, but not with any of the other eating behaviours. The childhood BMI risk score and the other individual BMI SNPs were not associated with any of the eating behaviours. In European children solely the childhood BMI risk score was nominally associated with a lower satiety responsiveness.

Interpretation of main findings

Fussy eating has been suggested to be associated with BMI, but results of previous studies are inconclusive. Whereas most studies reported a lower BMI, weight and/or body fat percentage in fussy eaters, other studies did not find any association or even described an association with a higher weight.[1,4-9] In the Generation R Study, we previously observed an association of fussy eating with a lower BMI.[6] More consistent results were reported for the other CEBQ-subscales. A higher food responsiveness and lower satiety responsiveness have been associated with faster infant growth.[38] Other studies reported a higher food responsiveness and enjoyment of food and a lower satiety responsiveness, food fussiness, and slowness in eating in children with a higher weight.[1,2,7,34] As some of the genetic variants associated with BMI have been suggested to exert their effects by influencing satiety or appetite, we examined whether genetic variants underlying BMI also influenced eating behaviours in our population.

We found a nominal association for the adult BMI risk score with satiety responsiveness. The inverse direction of the association of the genetic risk score with satiety responsiveness is in line with previous research.[27] In 2,258 children aged 10 years, a recent study combined 28 childhood and adult obesity SNPs derived from two meta-analyses into a weighted genetic risk score and found an inverse association of the genetic risk score with satiety responsiveness.[27] Another, smaller, study in 652 children aged 6 years found an inverse association of a genetic risk score of 32 adult BMI SNPs with SE.[39] We did not find an association of the genetic risk scores with food responsiveness, enjoyment of food, food fussiness and slowness in eating. In the European group the adult BMI risk score was not associated with satiety responsiveness, although the effect estimate was similar to that in the full group. This may be due to the smaller sample size of the European ancestry subgroup resulting in loss of power. Nevertheless, the childhood BMI risk score was associated with satiety responsiveness in the European group, with relatively large

effect estimates, which may indicate the presence of SNPs that have stronger effects in this ethnicity. Overall, due to the relatively small sample size and the small effect per SNP, the statistical power may have been limited in both groups.

Although there is evidence in the literature for the role of *MC4R*, *GNPDA2*, *ADCY3/POMC*, *SCG3*, *BBS4*, *TUB*, *FTO*, and *SH2B1* in regulating appetite and/or satiety, we did not observe an association of SNPs in or near these genes with the eating behaviours.[14,16-24,27] Again, this may be due to the relatively small sample size of our population. We did observe a nominally significant association of the genetic variants in *LMX1B* and *BDNF* with satiety responsiveness. *LMX1B* has a role in serotonergic neuron development, which are located in the central nervous system.[24] Serotonergic neurons are responsible for the production of serotonin, which affects appetite.[32] Mutations located in the *BDNF* gene have been shown to result in insatiable appetite and severe obesity.[26]

As expected, both risk scores were associated with BMI at 4 years.[16,31] Effect estimates and explained variances were smaller at 4 years (0.040 (95% CI 0.019, 0.060), R^2: 0.5% for the childhood risk score and 0.013 (95% CI 0.007, 0.019), R^2: 0.6% for the adult risk score) than previously observed at 6 years (0.091 (95% CI 0.063, 0.119), R^2: 1.0% and 0.112 (95% CI 0.084, 0.141), R^2: 1.4%, respectively).[31] This may be due to some SNPs having an age-dependent effect on BMI, with smaller or absent effects at this young age. The effect on BMI of the *MC4R* locus, for example, strengthens with age through childhood and adolescence and weakens again after the age of 20 years.[40] A similar effect has been suggested for *FTO*.[40] For the individual appetite and/or satiety related SNPs, we only observed a nominal association for the SNPs near *ADCY3/POMC* and *TUB* with BMI. The individual SNPs were chosen for their suggested role in satiety and/or appetite. Perhaps, since some of these SNPs were not identified in studies specifically examining childhood BMI, not all of the individual SNPs play a role in children. *LMX1B* had a relatively large effect estimate when compared with those of the risk scores including all SNPs. Possibly some of the other BMI SNPs add noise to the risk score. Alternatively, it is known that some SNPs have an age-dependent effect on BMI as discussed above. As such, it may be that some of these SNPs do play a role in children, but that their effects are too small to detect in our sample size. However, a direct comparison of effect estimates is difficult, given that the effect of the genetic risk score is shown per additional average allele in the risk score. Furthermore, it is possible that different body sizes are associated with different eating behaviours. Hence, it cannot be excluded that the risk scores and individual SNPs influence eating behaviour through an influence on BMI. However, the two individual SNPs that were associated with satiety responsiveness were not primarily associated with BMI in our population. In addition, the associations of these SNPs with satiety responsiveness remained after adjustment for BMI (data not shown), which makes this assumption less

likely. The association of the genetic risk score with satiety responsiveness was no longer significant after adjustment for BMI (data not shown), potentially reflecting the stronger association of the risk score with BMI than with satiety_responsiveness to begin with, the large number of SNPs involved in various functional pathways in the score, or a potential pathway of the effect of the risk score through BMI.

Further research is needed to examine whether the associations of the BMI risk scores, rs10733682, and rs11030104 with satiety responsiveness can be replicated in other, larger populations. A larger study population may also reveal further associations with the other eating behaviours. We measured eating behaviours in early life. At that age, children may have limited control over the amount of food intake.[3] Nevertheless, continuity of the eating behaviours has been shown between 2.5 and 9 years.[11] Therefore, further research is needed to show potential effects at older ages.

Methodological considerations

Genetic information was available in 78.6% of our total sample size of children participating in the preschool phase of the study. No significant differences were present regarding the eating behaviours in the groups with and without genetic information available (p-value >0.05, data not shown). The CEBQ-subscales were based upon validated questionnaires filled out by the parents rather than with objective observations of children's eating habits.[33] Non-responders included more families with a lower socio-economic status and younger mothers (p-value <0.001) possibly reducing the generalizability of our findings. Not all SNPs were available in our GWAS dataset. We used a limited number of proxy SNPs in very high linkage disequilibrium (LD) to complete the sets of SNPs. Given the high number of SNPs available and the high LD for the proxies, both risk scores are considered a good representation of the original set of SNPs.[15,16] Although our population is relatively large, power may still have been limited, so our lack of findings with some of the eating behaviour scales should be interpreted with caution.

Conclusion

No strong associations were found for BMI SNPs with eating behaviour at this young age. We did observe a potential role for a genetic risk score of adult BMI associated SNPs in satiety responsiveness during childhood, which was in line with previous research.[27] Results in European children were comparable. Further research is needed to explore this in more detail.

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SUPPLEMENTAL

Supplemental Table S1. SNPs included in the genetic risk scores of adult BMI and childhood BMI.

Risk score	SNP name	Proxy	Chr.	Position
Adult BMI				
RARB	rs6804842		3	25106437
TFAP2B	rs2207139		6	50845490
MAP2K5	rs16951275		15	68077168
FLJ45139	rs2836754		21	40291740
FTO	rs1558902		16	53803574
NLRC3	rs758747		16	3627358
CADM1	rs12286929		11	115022404
IFNGR1	rs13201877		6	137675541
RALYL	rs2033732		8	85079709
GRID1	rs7899106		10	87410904
CBLN1	rs2080454		16	49062590
TAL1	rs977747		1	47684677
NT5C2	rs11191560		10	104869038
EPB41L4B	rs6477694		9	111932342
ZBTB10	rs16907751		8	81375457
DOC2A	rs4787491		16	30015337
AGBL4	rs657452		1	49589847
HSD17B12	rs2176598		11	43864278
TLR4	rs1928295		9	120378483
RBM26	rs1441264		13	79580919
FUBP1	rs12401738		1	78446761
PRKD1	rs12885454		14	29736838
BDNF	rs11030104		11	27684517
NAV1	rs2820292		1	201784287
MTCH2	rs3817334		11	47650993
C5orf37	rs2112347		5	75015242
EHBP1	rs11688816		2	63053048
RASA2	rs16851483		3	141275436
ZFP64	rs6091540		20	51087862
HHIP	rs11727676		4	145659064
PACRG	rs13191362		6	163033350
FHIT	rs2365389	rs6445197[a]	3	61213993
MC4R	rs6567160		18	57829135
KCTD15	rs29941		19	34309532
ADCY3	rs10182181		2	25150296
QPCTL	rs2287019		19	46202172
LMX1B	rs10733682		9	129460914

Supplemental Table S1. *(Continued)*

Risk score	SNP name	Proxy	Chr.	Position
SBK1	rs2650492		16	28333411
TCF7L2	rs7903146		10	114758349
RPL27A	rs4256980		11	8673939
C6orf106	rs205262		6	34563164
LRP1B	rs2121279		2	143043285
SLC39A8	rs13107325		4	103188709
GNPDA2	rs10938397		4	45182527
NPC1	rs1808579		18	21104888
UBE2E3	rs1528435		2	181550962
C19orf7	rs3810291		19	47569003
BCDIN3D	rs7138803		12	50247468
CADM2	rs13078960		3	85807590
USP37	rs492400		2	219349752
TOMM40	rs2075650		19	45395619
TNNI3K	rs12566985		1	75002193
ASB4	rs6465468		7	95169514
CLIP1	rs11057405		12	122781897
MTIF3	rs12016871		NA	NA
STXBP6	rs10132280		14	25928179
PRKD1	rs11847697		14	30515112
HIF1AN	rs17094222		10	102395440
LINGO2	rs10968576		9	28414339
FANCL	rs1016287		2	59305625
CREB1	rs17203016		2	208255518
KCNK3	rs11126666		2	26928811
LRFN2	rs2033529		6	40348653
ELAVL4	rs11583200		1	50559820
SMG6	rs9914578		17	2005136
HIP1	rs1167827		7	75163169
ETV5	rs1516725		3	185824004
ADPGK	rs7164727		15	73093991
GBE1	rs3849570		3	81792112
MAN1A1	rs9374842		6	120185665
RIT2	rs7239883		18	40147671
FIGN	rs1460676		2	164567689
KIAA1303	rs12940622		17	78615571
ERBB4	rs7599312		2	213413231
DMXL2	rs3736485		15	51748610
PCDH9	rs9540493		13	66205704
RABEP1	rs1000940		17	5283252
NRXN3	rs7141420		14	79899454
GALNT10	rs7715256		5	153537893

Supplemental Table S1. *(Continued)*

Risk score	SNP name	Proxy	Chr.	Position
GPRC5B	rs12446632		16	19935389
GRP	rs7243357		18	56883319
GNAT2	rs17024393		1	110154688
SCARB2	rs17001654		4	77129568
MYST1	rs9925964	rs1978487[a]	16	31129942
OLFM4	rs12429545		13	54102206
C9orf93	rs4740619		9	15634326
CALCR	rs9641123		7	93197732
NEGR1	rs3101336		1	72751185
ATP2A1	rs3888190		16	28889486
HNF4G	rs17405819		8	76806584
KIAA1505	rs2245368		7	76608143
PGPEP1	rs17724992	rs9636202[a]	19	18449238
FOXO3	rs9400239		6	108977663
SEC16B	rs543874		1	177889480
IRS1	rs2176040		2	227092802
PTBP2	rs11165643		1	96924097
TMEM18	rs13021737	rs13012571[a]	2	632550
Child BMI				
GNPDA2	rs13130484		4	44870448
ADCY3	rs11676272		2	24995042
TMEM18	rs4854349		2	637861
SEC16B	rs543874		1	176156103
FAIM2	rs7132908		12	48549415
FTO	rs1421085	rs3751812[b]	16	52358455
OLFM4	rs12429545		13	53000207
TFAP2B	rs987237		6	50911009
TNNI3K	rs12041852		1	74776088
MC4R	rs6567160		18	55980115
ELP3	rs13253111		8	28117893
RAB27B	rs8092503		18	50630485
LMX1B	rs3829849		9	128430621
ADAM23	rs13387838		2	206989692
GPR61	rs7550711		1	109884409

[a] Linkage disequilibrium between proxies and original SNPs: R^2>0.96, D'=1

[b] Linkage disequilibrium between proxy and original SNP: R^2= 0.93, D'=0.97

Supplemental Table S2. Phenotypic variance in eating behaviours explained by genetic risk scores based on adult and childhood BMI and satiety/appetite related SNPs in the full group

Risk score (SNPs in risk score)	Food responsiveness (N=3,020) Explained variance (%)	Enjoyment of food (N=2,998) Explained variance (%)	Satiety responsiveness (N=3,016) Explained variance (%)	Food fussiness (N=3,006) Explained variance (%)	Slowness in eating (N=3,015) Explained variance (%)	BMI (N=2,413) Explained variance (%)
Main risk scores						
Child BMI (N=15)	0.090	0.056	0.164	0.003	0.112	**0.514**
Adult BMI (N=97)	0.020	0.003	**0.038**	<0.001	0.030	**0.545**
Separate SNPs						
rs6567160 (*MC4R*)	0.090	0.010	0.008	0.017	0.073	0.073
rs10938397 (*GNPDA2*)	0.002	0.006	<0.001	0.003	0.011	0.009
rs10182181 (*ADCY3*/*POMC*)	0.025	<0.001	0.050	0.005	0.017	**0.269**
rs10733682 (*LMX1B*)	0.084	0.004	**0.156**	0.004	0.080	<0.001
rs3736485 (*SCG3*)	0.015	0.053	0.001	0.029	0.002	0.024
rs7164727 (BBS4)	0.009	0.002	<0.001	<0.001	0.026	<0.001
rs4256980 (TUB)	0.055	0.024	0.104	0.001	0.121	**0.202**
rs3888190 (*SH2B1*)	0.058	<0.001	<0.001	<0.001	0.042	0.013
rs1558902 (*FTO*)	0.133	0.113	0.017	0.047	0.024	<0.001
rs11030104 (*BDNF*)	0.015	0.013	**0.241**	0.010	0.296	<0.001

Bold values represent explained variances for significant associations of the risk scores / SNPs with the outcome

Supplemental Table S3. Phenotypic variance in eating behaviours explained by genetic risk scores based on adult and childhood BMI and satiety/appetite related SNPs in the European group

Risk score (SNPs in risk score)	Food responsiveness (N=1,916) Explained variance (%)	Enjoyment of food (N=1,904) Explained variance (%)	Satiety responsiveness (N=1,913) Explained variance (%)	Food fussiness (N=1,907) Explained variance (%)	Slowness in eating (N=1,914) Explained variance (%)	BMI (N=1,784) Explained variance (%)
Main risk scores						
Child BMI (N=15)	0.032	0.034	**0.256**	0.099	0.103	**0.435**
Adult BMI (N=97)	0.116	0.060	0.075	0.017	0.205	**0.331**
Separate SNPs						
rs6567160 (*MC4R*)	0.232	0.008	0.000	0.002	0.009	0.023
rs10938397 (*GNPDA2*)	0.025	0.060	0.009	0.054	0.113	0.033
rs10182181 (*ADCY3/POMC*)	0.034	0.001	0.173	0.000	0.012	**0.473**
rs10733682 (*LMX1B*)	0.149	0.017	0.113	0.028	0.060	0.055
rs3736485 (*SCG3*)	0.009	0.164	0.003	0.065	0.014	0.241
rs7164727 (BBS4)	0.047	0.001	0.034	0.024	0.055	0.134
rs4256980 (TUB)	0.001	0.009	0.049	0.000	0.029	0.029
rs3888190 (*SH2B1*)	0.036	0.001	0.118	0.002	0.160	0.001
rs1558902 (*FTO*)	0.111	0.035	0.014	0.001	0.020	0.013
rs11030104 (*BDNF*)	0.032	0.000	0.077	0.001	0.208	0.215

Bold values represent explained variances for significant associations of the risk scores / SNPs with the outcome

Chapter 3.5

Associations of liver enzymes and steatosis related genetic variants with alanine-aminotransferase concentrations in children

C. Monnereau, V.W.V. Jaddoe, J.F. Felix

Submitted

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Chapter 4.1

Maternal BMI at the start of pregnancy and offspring epigenome-wide DNA methylation: findings from the pregnancy and childhood epigenetics (PACE) consortium

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Abstract

Pre-pregnancy maternal obesity is associated with adverse offspring outcomes at birth and later in life. Individual studies have shown that epigenetic modifications such as DNA methylation could contribute. Within the Pregnancy and Childhood Epigenetics (PACE) Consortium, we meta-analysed the association between pre-pregnancy maternal BMI and methylation at over 450,000 sites in newborn blood DNA, across 19 cohorts (9,340 mother-newborn pairs). We attempted to infer causality by comparing the effects of maternal versus paternal BMI and incorporating genetic variation. In four additional cohorts (1,817 mother-child pairs), we meta-analysed the association between maternal BMI at the start of pregnancy and blood methyla-tion in adolescents. In newborns, maternal BMI was associated with small (<0.2% per BMI unit (1 kg/m^2), p-value $<1.06*10^{-7}$) methylation variation at 9,044 sites throughout the genome. Adjustment for estimated cell proportions greatly attenuated the number of significant CpGs to 104, including 86 sites common to the unadjusted model. At 72/86 sites, the direction of the association was the same in newborns and adolescents, suggesting persistence of signals. However, we found evidence for a6causal intrauterine effect of maternal BMI on newborn methylation at just 8/86 sites. In conclusion, this well-powered anal-ysis identified robust associations between maternal adiposity and variations in newborn blood DNA methylation, but these small effects may be better explained by genetic or lifestyle factors than a causal intrauterine mechanism. This high-lights the need for large-scale collaborative approaches and the application of causal inference techniques in epigenetic epidemiology.

Background

Offspring of mothers with a high body mass index (BMI) at the start of pregnancy have a higher risk of obesity and obesity-related disorders in later life.[1] Maternal obesity in pregnancy is also associated with other offspring outcomes, including neurodevelopmental and respiratory outcomes.[2–5] These associations might be explained by shared mother-child genetic or postnatal environmental influences, or they could also reflect a causal intrauterine mechanism leading to early programming of adverse health in the offspring.[6]

Disentangling the genetic and shared postnatal environmental effects from a causal intrauterine effect is difficult, but there are a number of causal inference approaches that may be useful.[7] For example, some studies have used a negative control design whereby the association between maternal adiposity and offspring outcome is compared to the association between paternal adiposity and the same outcome. The key assumption of the negative control design is that both exposures share the same postnatal environmental and genetic confounders. A systematic review of such studies, together with subsequent studies not included in the review, have found only limited support for specific effects of maternal adiposity on offspring adiposity beyond birth.[8-12] To our knowledge, similar causal inference techniques have not yet been applied to study maternal effects of adiposity in pregnancy on other aspects of offspring health.

If there is a causal intrauterine effect of maternal adiposity on offspring health outcomes, the mechanism is unclear. Epigenetic modifications, such as DNA methylation, might partly mediate associations between maternal and offspring phenotypes by causing changes to gene expression that are mitotically heritable.[6,13-15] Differential DNA methylation has been reported when assessing offspring exposed in utero to extreme maternal undernutrition, maternal morbid obesity and less extreme maternal underweight and maternal obesity, in comparison to those not exposed; yet weak or no evidence has been found for associations between continuous maternal BMI and offspring DNA methylation, whether globally, at specific loci identified in array or at candidate genes.[16-26] However, individual studies were limited in sample size and thus underpowered to detect differential methylation. Meta-analysis of results from multiple individual cohorts increases sample size and power to detect differential methylation, but this approach has rarely been employed in the field of epigenetic epidemiology.

Comprising many birth cohorts from around the world, the Pregnancy and Childhood Epigenetics (PACE) Consortium was established to facilitate meta-analysis of epigenome-wide studies relevant to maternal and childhood health and disease.[25] In this PACE study, we meta-analysed harmonised cohort-specific epigenome-wide data on associations between

maternal BMI at the start of pregnancy and DNA methylation in the blood of newborns. We then conducted further analyses (Figure 1) to explore whether these associations could be reproduced in adolescent samples, and implemented causal inference methods to evaluate the potential confounding effects of shared environment and genetic variation.

METHODS

Figure 1 gives an outline of the design of this study.

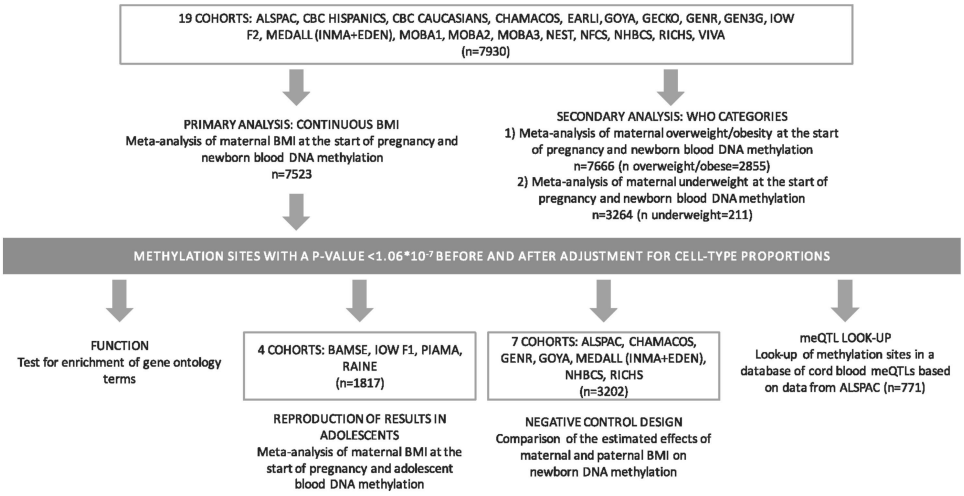

Figure 1. An overview of the study design.

Participating cohorts

A total of 23 independent cohorts participated. Detailed methods for each cohort are provided (Supplementary Methods) and summarised in Supplementary Table S1.

Nineteen cohorts participated in the meta-analysis of maternal BMI at the start of pregnancy and newborn blood DNA methylation: The Avon Longitudinal Study of Parents and Children (ALSPAC)[27-29]; two independent datasets from the Californian Birth Cohort (CBC_Hispanics and CBC_Caucasians)[30]; Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS); Early Autism Risk Longitudinal Investigation (EARLI)[31]; the Genome-Wide Population-based Association Study of Extremely Overweight Young Adults (GOYA), which is a sample from the Danish National Birth Cohort[32,33]; Groningen

Expert Center for Kids with Obesity (GECKO); Generation R (GENR)[34]; Genetics of Glycemic Regulation in Gestation and Growth (GEN3G)[35]; the Isle of Wight Birth Cohort third generation (IOW F2)[36]; two cohorts from the FP7 project Mechanisms of the Development of Allergy (MEDALL), INfancia y Medio Ambiente (INMA)[37] and a study on the pre- and early postnatal; determinants of child health and development (EDEN)[38], were pooled and analysed as a single cohort referred to as MEDALL; three independent data-sets from the Norwegian Mother and Child Cohort Study (MOBA1, MOBA2, MOBA3)[39,40]; the Norway Facial Clefts Study (NFCS), the Newborn Epigenetic Study (NEST)[41,42]; the New Hampshire Birth Cohort Study (NHBCS); the Rhode Island Child Health Study (RICHS)[43] and Project Viva (Viva).

An additional four independent cohorts participated in the meta-analysis of maternal BMI at the start of pregnancy and off-spring whole blood DNA methylation at adolescence (ages 15-18): the Children Allergy Milieu Stockholm Epidemiology cohort (BAMSE)[44] , IOW birth cohort second generation (IOW F1), the Prevention and Incidence of Asthma and Mite Allergy birth cohort (PIAMA), the Western Australia Pregnancy Cohort (RAINE).

All cohorts acquired ethics approval and informed consent from participants prior to data collection through local ethics committees. Full details are provided in the Supplementary Methods.

Maternal BMI at the start of pregnancy

In each cohort, maternal BMI [weight (kg)/height (m^2)] was calculated from either self-reported or measured height and weight, either before pregnancy or early in the first trimester (Supplementary Table S1). Cohorts were asked to double check values 5 standard deviations from the mean to ensure that they were not data entry errors. Primarily, we were interested in the effects of maternal BMI as a continuous variable, but also investigated World Health Organization categories of maternal overweight or obesity (25.0 kg/m^2), and under-weight (<18.5 kg/m^2), compared to a normal weight reference group (18.5–24.9 kg/m^2).

Covariates

All cohorts ran models adjusted for maternal age (years), maternal social class (variable defined by each individual cohort), maternal smoking status (the preferred categorization was into three groups: no smoking in pregnancy, stopped smoking in early pregnancy, smoking throughout pregnancy, but a binary categorization of any versus no smoking was also acceptable) and parity (the preferred categorization was into two groups: no previous

children, one or more previous children). We did not adjust for or stratify by sex of the child because sex cannot be a true confounder of any association between maternal pre-pregnancy BMI and offspring methylation; although it has a large influence on methylation, it cannot feasibly alter pre-pregnancy BMI. Furthermore, because the intrauterine hormonal environment is likely to be different for males and females, and could also be influenced by maternal BMI, we would risk introducing collider bias by adjusting for sex, which would be strongly correlated with sex-associated hormonal environment on the causal pathway between maternal BMI and methylation.

Each cohort also adjusted for technical covariates using methods suitable for that cohort (Supplementary Table S1). Certain cohorts also included additional covariates to correct for study design/sampling factors where needed (Supplementary Table S1). For GOYA, which is a case-control study where case mothers have a BMI >32 kg/m^2 and control mothers have a BMI anywhere within the normal distribution, we restricted the continuous maternal BMI models to a randomly selected sub-group with a normal BMI distribution to avoid confounding by substructure. Binary comparison models were run using the whole GOYA cohort with no additional adjustment for substructure.

We hypothesised that BMI might influence newborn blood cellular composition, so each cohort additionally adjusted for cell proportions by including the estimated variables as covariates. All cohorts independently estimated cell counts using the estimateCellCounts function in the minfi R package, which is based on the method developed by Houseman.[45,46] The cohort-specific analyses, as well as the meta-analyses, were completed before a cord blood reference set was widely available, so cohorts used an adult whole blood reference to estimate cell counts.[47] This estimated the proportion of B-cells, CD8+ T-cells, CD4+ T-cells, granulocytes, NK-cells and monocytes in each sample. NHBCS, RICHS and Project Viva included five estimated cell types (omitting granulocytes) and all other cohorts included six. When cord blood references became available, a sensitivity analysis was run in ALSPAC adjusting for cell proportions estimated using these reference sets.[48-50] One of these reference sets includes nucleated red blood cells, which can contribute greatly to cord blood DNA methylation profiles.[50]

Methylation measurements and quality control

Each cohort conducted its own laboratory measurements. DNA from newborn or adolescent blood samples underwent bisulfite conversion using the EZ-96 DNA Methylation kit (Zymo Research Corporation, Irvine, USA). For all cohorts, DNA methylation was measured using the Illumina InfiniumVR HumanMethylation450 BeadChip assay at Illumina or in cohort-specific laboratories.[51,52] Each cohort also conducted its own quality

control and normalisation of methylation data, as detailed in the Supplementary Methods (Supplementary Figure S2) and summarised in Supplementary Table S1. In all analyses, cohorts used normalised, untransformed beta-values, which are on a scale of 0 (completely unmethylated) to 1 (completely methylated).

Cohort-specific statistical analyses

Each cohort performed independent epigenome-wide association studies (EWAS) according to a common, pre-specified analysis plan. Models were run using M-type multiple robust linear regression [rlm in the MASS R package] in an attempt to control for potential heteroscedasticity and/or influential outliers in the methylation data.[53] In the primary analysis, continuous maternal BMI at the start of pregnancy was modelled as the exposure and offspring individual CpG-level methylation (untransformed beta-values) was modelled as the outcome, with adjustment for covariates and estimated cell counts. In secondary models, we modelled the exposure as binary variables comparing WHO BMI categories to a normal weight reference group. We also explored the impact of cellular composition by comparing models run with and without adjustment for estimated cell counts.

Meta-analysis

Cohorts uploaded their EWAS results files to a server at the University of Bristol, where we performed fixed-effects meta-analysis weighted by the inverse of the variance with METAL.[54] A shadow meta-analyses was also conducted independently by authors at the Erasmus University in Rotterdam to minimise the likelihood of human error. All downstream analyses were conducted using R version 2.5.1 or later.[55] We excluded control probes (N=65), and probes mapped to the X (N=11,232) or Y (N=416) chromosomes. This left a total of 473,864 CpGs measured in at least one cohort (218,350 [46%] of these were measured in all 19 cohorts, 393,986 [83%] were measured in at least 18 cohorts). Multiple testing was accounted for using the Bonferroni method. CpGs with a Bonferroni-corrected p-value <0.05, i.e. p-value $<1.06*10^{-7}$, in both the cell proportion-unadjusted and cell proportion-adjusted models were taken forward for further analysis. To assess heterogeneity, we generated forest plots, and ran random effects models and "leave-one-out" analyses using the metafor R package.[56] We compared our Bonferroni-significant probes to a list of potentially problematic probes published by Naeem et al. We did not remove these probes as this would risk removing potentially interesting effects. However, we tested whether these probes contained large numbers of outlying values by performing dip tests for multimodality using the diptest package, where a p-value >0.05 suggests

the distribution is unimodal.[57,58] Kolmogorov-Smirnov tests were used to compare the distribution of p-values to that expected by chance and were conducted using the core R function ks.test().

Enrichment and functional analysis

Sites were annotated using the IlluminaHumanMethylation450k.db R package, with enhanced annotation for nearest genes within 10 Mb of each site, as previously described.[59,60]These annotations were then updated using the R package mygene.[61] Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using the missMethyl R package.[62] This takes into account the differing number of sites associated with each gene on the 450k array. P-values for enrichment were adjusted for multiple testing using the FDR method.

Reproduction of maternal BMI-related differential DNA methylation in adolescence

Four cohorts independently performed robust linear regression to assess the association between maternal BMI at the start of pregnancy and (mixed gender) adolescent whole blood DNA methylation. Each of these cohorts ran models adjusted for maternal smoking, maternal age, socioeconomic status, parity during the index pregnancy and estimated cell counts. Results were uploaded to the server at the University of Bristol where they were summarised using fixed effects meta-analysis in the metafor package.[56] A look-up of maternal BMI-related sites identified in the newborn meta-analysis (N=86 with p-value $<1.06*10^{-7}$ in the cell-adjusted and cell-unadjusted models) was performed and FDR correction applied to account for multiple testing. These "reproduction" cohorts were completely independent from the original "discovery" cohorts.

Negative control design

In an attempt to examine a potential causal effect of maternal BMI on newborn blood methylation at identified sites, we used a negative control design.[7] In this analysis, estimates for associations between maternal BMI and offspring DNA methylation were compared to the equivalent estimates for paternal BMI (the negative control), with adjustment for the other parent's BMI.

Seven cohorts (ALSPAC, CHAMACOS, Generation R, GOYA, MEDALL [INMA and EDEN pooled], NHBCS, RICHS) with the necessary data independently performed robust linear regression to assess the association between paternal BMI (kg/m^2) and newborn blood

DNA methylation at sites identified as associated with maternal BMI. Each cohort ran models adjusted for maternal smoking, age, socioeconomic status, parity and estimated cell counts. We also explored the independent effect of maternal and paternal BMI in mutually adjusted models. Results for each of the seven cohorts were uploaded to the server at the University of Bristol where they were summarised using fixed effects inverse-variance weighted meta-analysis and compared to meta-analysed results of the maternal effect in these seven cohorts. The criteria for evidence of an intrauterine effect were, in the mutually adjusted models, 1) maternal BMI and paternal BMI show the same direction of association with offspring methylation (i.e. paternal BMI is not having an independent effect in the opposite direction to the effect of maternal BMI), 2) the magnitude of association with offspring methylation is larger for maternal BMI than for paternal BMI, 3) there is evidence of heterogeneity (an I^2 value >40) in a meta-analysis of the maternal and paternal mutually-adjusted estimates. We also calculated heterogeneity p-values between the mutually adjusted maternal and paternal BMI estimates using the metafor R package.[56]

Identification of methylation quantitative trait loci (meQTLs)

We performed a look-up of maternal BMI-associated methylation sites in an online catalogue of both cis- (within 100 kb) and trans- methylation quantitative trait loci (meQTLs) identified in an ALSPAC study (http://mqtldb.org/; date last accessed July 29, 2017).[63] The meQTLs were identified in cord blood of 771 children at birth using 395,625 methylation probes and 8,074,398 SNP loci after adjustment for sex, the top ten ancestry principal components, bisulfite conversion batch and estimated cell counts. A p-value threshold of $1*10^{-7}$ was used to define meQTLs.[63] We compared the list of meQTLs to results of an adult BMI GWAS published by the GIANT consortium.[64,65] meQTLs were considered nominally associated with BMI if the GWAS p-value was <0.05. FDR correction for multiple testing was also performed.

Results

Study characteristics

We meta-analysed results from 19 independent cohorts to test the association between maternal BMI at the start of pregnancy and epigenome-wide newborn blood DNA methylation. A summary of methods used by each cohort is provided in Supplementary Table S1, with a more detailed description in the Supplementary Methods. Supplementary Table S2 lists sample sizes and summarises EWAS results for each cohort and meta-analysis. For our primary model, with continuous maternal BMI as the exposure, we

Table 1. Characteristics of each cohort included in the meta-analysis of the association between maternal pre-pregnancy BMI and offspring blood DNA methylation at birth. BMI is categorised according to WHO guideline

Cohort	N in continuous BMI model	Mean maternal BMI (SD) in continuous BMI model	Mean maternal age (SD) in continuous BMI model	Total N obese	Total N over weight	Total N under weight	Total N normal weight	Ethnicity
ALSPAC	788	22.8 (3.6)	29.7 (4.4)	37	106	26	619	European
CBC (Hispanic)	132	24.2 (5.7)	27.2 (5.7)	15	27	11	79	Hispanic
CBC (White)	155	23.3 (3.9)	32.0 (5.7)	8	34	0[a]	108	European
CHAMACOS	368	26.9 (5.1)	25.3 (5.0)	80	141	3[a]	144	Hispanic
EARLI	211	27.8 (6.9)	34.0 (4.7)	69	51	3[a]	88	European/Mixed
GECKO	176	24.2 (3.9)	30.4 (4.0)	14	45	$_{3}$a	114	European
GEN3G	170	24.8 (5.6)	28.0 (4.1)	25	33	$_{3}$a	109	European
Generation R	875	24.5 (4.2)	31.5 (4.2)	90	202	13[a]	570	European
GOYA[b]	545	23.1 (3.2)	29.5 (4.1)	466	106	16	387	European
IOW F2	53	27.7 (7.3)	21.5 (1.4)	19[a]	11[a]	0[a]	23	European
MEDALL (INMA+EDEN)	330	24.1 (5.1)	30.6 (4.5)	37	62	26	205	European
MoBa1	1034	24.0 (4.6)	29.9 (4.3)	98	215	67	688	European
MoBa2	647	24.2 (4.4)	30.0 (4.5)	72	136	18	431	European
MoBa3	231	24.2 (4.3)	29.6 (4.4)	25	49	$_{5}$a	152	European
NEST	384	27.6 (8.9)	28.8 (6.4)	108	76	19[a]	181	Mixed
NFCS	867	23.5 (4.1)	29.1 (4.9)	70	157	37	603	European
NHBCS	118	24.4 (4.2)	31.0 (4.4)	12	29	3[a]	74	European
RICHS	96	25.8 (6.9)	28.3 (5.5)	21	21	10	44	European
Project Viva	343	24.3 (4.9)	33.1 (4.5)	41	77	10[a]	215	European
Meta-analysis	7523							

[a]Included in the continuous BMI model, but excluded from the categorical analyses due to low sample sizes.

[b]A subset of the GOYA cohort (545) was included in the continuous BMI model. The entire cohort (975) was included in the binary BMI models.

analysed results from 7,523 mother-child pairs. The overall sample size-weighted mean maternal BMI was 24.4 kg/m^2 (range of cohort-specific means: 22.8, 27.8). In secondary analyses, we examined World Health Organisation categories for maternal BMI, comparing normal weight women (N=4,834) to i) overweight or obese women combined (N=2,885 women, of whom 1,299 were obese) and ii) underweight women (N=211 women). The majority of participants were of European ancestry. Table 1 summarizes the characteristics of each cohort.

Maternal BMI at the start of pregnancy is associated with widespread but small differences in newborn blood DNA methylation

When treated as a continuous variable, maternal BMI at the start of pregnancy was associated with differential methylation in newborn blood at 9,044 sites (Supplementary Table S3) before and 104 sites (Supplementary Table S4) after adjustment for cell-counts (Bonferroni correction for 473,864 tests p-value <1.06*10^{-7}); 86 sites were common to both models. Before adjustment for cell-counts, lambdas (λ), a measure of p-value inflation, were generally high and QQ plots showed inflation of p-values in most cohorts (Table 2, Supplementary Table S2 and Supplementary Figures pages 2–5). Values for λ were closer to 1 for most cohorts after adjustment for estimated cell counts. In a meta-analysis of results from two of the larger cohorts, ALSPAC and Generation R (λ =1.60), k was not substantially further reduced after removal of potential outliers using the Tukey method (λ =1.58) or additional adjustment for 10 ancestry principal components (λ =1.67).[66]

Sites associated with maternal BMI were spread over the genome and did not tend to be restricted to certain regions (Figure 2). Effect sizes were very small, with the median absolute effect at the genome-wide significant sites being a difference in methylation beta value of 0.0003 per one unit (kg/m^2) increase in maternal BMI (i.e. a 0.03% absolute change, range: 0.15% decrease to 0.13% increase). At most of the Bonferroni-significant sites (8,899/9,044 and 96/104), higher maternal BMI was associated with lower newborn blood methylation.

Results from the primary model, where the exposure was continuous BMI, were consistent with those from a binary comparison of maternal overweight/obesity (BMI >25) with normal weight (BMI 18.5 to 25): the Spearman's coefficient for correlation between regression coefficients was 0.70. Maternal over-weight/obesity was associated with differential newborn blood methylation at 4,037 sites (Supplementary Table S5) before and 159 sites (Supplementary Table S6) after cell-adjustment (p-value <1.06*10^{-7}), compared with normal weight. The crossover between these 159 sites and the 104 identified with p-value

<1.06*10^{-7} in the cell-adjusted continuous model was just 21/104, but 150/159 were associated with continuous BMI after correction for multiple testing at 159 sites (FDR-corrected p-value <0.05). The direction of effect for the binary comparison was consistent with that for the continuous exposure at all 159 sites. As expected, the magnitude of effect was larger when BMI was binary than when BMI was continuous, but the median effect at sites with p-value <1.06*10^{-7} was still small (0.31% decrease in mean methylation beta value in the overweight/obese group com-pared to the normal weight group).

Eight sites (Supplementary Table S7) were associated with maternal underweight (BMI <18.5) compared to nor-mal weight with p-value <1.06*10^{-7}, but this analysis was likely underpowered given the small number of underweight women (N=211), and there was large inter-study heterogeneity in results (I^2 median 62.3, range 0 to 91.3). Given these results, we did not explore the association between maternal underweight and offspring methylation any further.

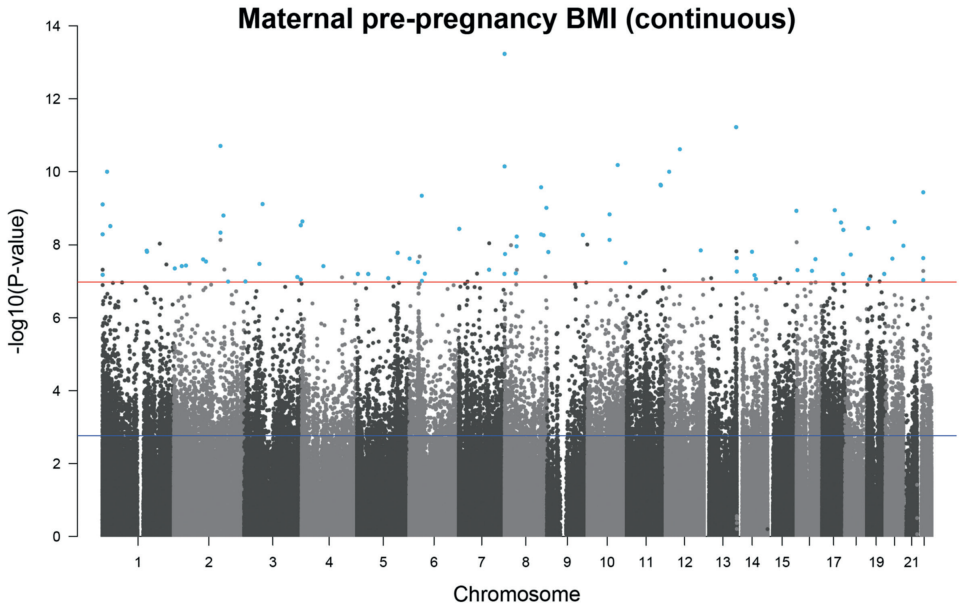

Figure 2. A Manhattan plot for the meta-analysis of associations between maternal pre-pregnancy BMI and offspring DNA methylation at birth after adjustment for maternal covariates and estimated cell counts. The red line shows the Bonferroni threshold for multiple testing. Methylation sites that surpassed the Bonferroni-correction threshold (p-value <1.06*10^{-7}) before and after adjustment for estimated cell counts are highlighted in blue.

Table 2. Summary of cohort-specific and meta-analysis results for EWAS of continuous maternal pre-pregnancy BMI and newborn blood DNA methylation

Cohort	N	Lambda (before adjusting for cells)	Bonferroni hits (before adjusting for cells)	Lambda (after adjusting for cells)	Bonferroni hits (after adjusting for cells)
ALSPAC	788	1.53	12	1.18	1
CBC (Hispanic)	132	1.05	12	0.96	7
CBC2 (White)	155	1.80	31	1.19	3
CHAMACOS	368	1.34	1	0.87	0
EARLI	211	0.88	0	0.89	2
GECKO	176	1.75	14	1.15	2
GEN3G	170	1.13	10	1.04	10
GENR	875	1.86	248	1.96	11
GOYA	545	1.87	2	1.01	1
IOW F2	53	1.08	0	1.05	0
MEDALL (INMA+EDEN)	330	1.24	0	0.92	0
MoBa1	1034	4.69	39	2.74	1
MoBa2	647	2.70	8	2.76	14
MoBa3	231	1.03	0	0.78	1
NEST	384	0.76	0	0.93	0
NFCS	867	0.95	0	0.98	0
NHBCS	118	1.02	2	1.17	4
RICHS	96	1.89	14	2.92	33
VIVA	343	1.27	8	1.49	7
FE Meta-analysis	7523	3.27	9044	2.41	104
RE Meta-analysis			1825		25

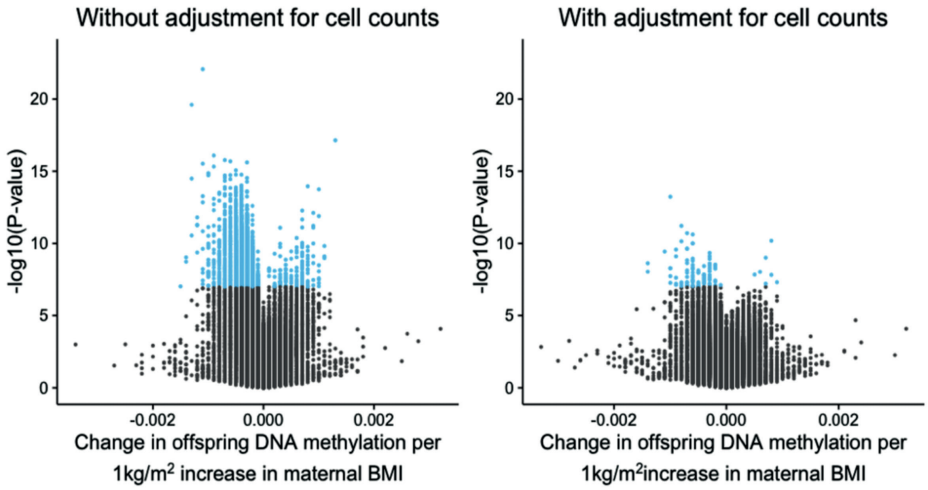

Figure 3. Volcano plots to illustrate the large increase in p-values after adjusting for estimated cell counts. Methylation sites that reached the Bonferroni threshold for multiple testing ($1.06*10^{-7}$) are highlighted in blue.

Adjusting for cellular heterogeneity greatly attenuates associations between maternal BMI and newborn blood DNA methylation

As mentioned above, adjusting for estimated cell proportions in newborn blood samples greatly reduced the number of sites associated with maternal BMI with p-values $<1.06*10^{-7}$ (Figure 3). This reduction in signal was seen in all meta-analyses and most individual cohort analyses (Table 2). At all 9,044 sites associated with continuous maternal BMI, adjusting for cell counts shifted the effect size towards the null. The median relative change in estimate after adjustment was 52% and 9,007/9,044 sites attenuated by 10% or more. After adjustment, the precision of the estimates at 8,984/9,044 sites was increased (i.e. the standard error was reduced). Taken together, this suggests that much of the association between maternal BMI at the start of pregnancy and newborn DNA methylation is due to varying cell type proportions.

Surprisingly, however, estimated cell proportions were not strongly correlated with maternal BMI in any of the five cohorts that supplied these data (Supplementary Table S8). Given this, we hypothesised that large changes in estimates might indicate measurement error in estimated cell counts, and that this measurement error might be due to an adult whole blood reference panel being used to estimate cell counts in cord/newborn blood samples. However, we found little evidence for this: cord blood reference panels by Andrews and Bakulski, Gervin et al. and deGoede et al. became available after we had finalised the meta-

analysis results.[48-50] When we used each of these references to estimate cell proportions in ALSPAC cord blood samples, regression coefficients and p-values were similar to those obtained when an adult reference panel was used in this cohort. Of the 86 sites where maternal BMI was associated with newborn methylation before and after adjustment for cell counts in the meta-analysis (p-value $<1.06*10^{-7}$), 15 were associated with maternal BMI with p-value <0.05 in ALSPAC when an adult reference panel was used. Of these 15 sites, 12 sites also had p-value <0.05 when any of the cord blood reference panels were used. The percentage change in estimates between models using the adult and cord blood reference panels was under 10% at 14/15 sites using the Andrews and Bakulski reference (median percentage change in estimates: 4.1), under 10% at 14/15 sites using the Gervin et al. reference (median percent-age change in estimates: 3.4) and under 10% at 12/15 using the deGoede reference (median percentage change in estimates: 3.7). Furthermore, cell counts estimated using any of the three cord blood references correlated relatively well with each other (median Spearman's correlation coefficient: 0.67, range: 0.05 to 0.95), but were not correlated with maternal BMI (median Spearman's correlation coefficient: 0.007, range: 0.10 to 0.15) (Supplementary Table S8). Although maternal BMI was not associated with estimated cell proportions in our data, others have observed that maternal BMI is associated with cord blood cellular heterogeneity, in addition, some random variability in cell distribution across the range of maternal BMI can be expected.[67,68] Therefore, we believe that adjustment is appropriate and indeed necessary.

Further analysis of 86 sites where maternal BMI is associated with newborn DNA methylation both before and after adjustment for cell counts

For further analysis, we selected the 86 sites where maternal BMI at the start of pregnancy was associated with offspring newborn blood DNA methylation both before and after adjustment for estimated cell proportions (Table 3), and performed subsequent analyses using the cell-adjusted model. We used three main strategies to determine the robustness of our findings at these 86 sites:

Firstly, we assessed inter-study heterogeneity and influence of individual studies. There was weak to moderate heterogeneity at most sites; I^2 was less than 40% at 57/86 sites (median 31.2%, range 0.0 to 70.6%) and 31/86 sites had a heterogeneity p-value <0.05. In a comparison of estimates from random- and fixed-effects meta-analysis models, the percentage change in estimates was <10% for 72/86 sites (median percentage change in estimates: 2.8). In the random effects model, the largest p-value at the 86 sites was 0.0058 and 20/86 sites had p-value $<1.06*10^{-7}$, despite lower power compared to the fixed effects

Table 3. Methylation sites where continuous maternal pre-pregnancy BMI was associated with offspring newborn blood methylation with a Bonferroni-corrected p-value <0.05 (p-value <1.06*10^{-7}) before and after adjustment for cell counts

			Cell-unadjusted model			Cell-adjusted model		
CHR	CpG site	Gene	b[a]	SE	p-value	b[a]	SE	p-value
7	cg12009398	VIPR2	1.31*10^{-3}	1.42*10^{-4}	2.49*10^{-20}	1.01*10^{-3}	1.34*10^{-4}	5.88*10^{-14}
13	cg09285795	SOX1	7.75*10^{-4}	1.10*10^{-4}	2.09*10^{-12}	7.71*10^{-4}	1.12*10^{-4}	5.99*10^{-12}
2	cg23080818	RBMS1	8.76*10^{-4}	1.08*10^{-4}	4.76*10^{-16}	6.98*10^{-4}	1.04*10^{-4}	1.96*10^{-11}
12	cg25213362	TMPRSS12	6.71*10^{-4}	8.91*10^{-5}	4.93*10^{-14}	5.89*10^{-4}	8.82*10^{-5}	2.42*10^{-11}
10	cg17782974	TRIM8	1.29*10^{-3}	1.50*10^{-4}	7.18*10^{-18}	8.15*10^{-4}	1.25*10^{-4}	6.54*10^{-11}
7	cg05086444	VIPR2	8.32*10^{-4}	1.30*10^{-4}	1.79*10^{-10}	8.17*10^{-4}	1.25*10^{-4}	7.14*10^{-11}
1	cg03258665	EPHA2	1.10*10^{-3}	1.12*10^{-4}	8.65*10^{-23}	5.68*10^{-4}	8.78*10^{-5}	9.97*10^{-11}
12	cg20065216	DUSP16	6.63*10^{-4}	9.88*10^{-5}	1.97*10^{-11}	6.03*10^{-4}	9.32*10^{-5}	9.98*10^{-11}
11	cg26434090	DSCAML1	7.94*10^{-4}	1.07*10^{-4}	1.10*10^{-13}	6.97*10^{-4}	1.10*10^{-4}	2.27*10^{-10}
11	cg18268562	FOXR1	6.27*10^{-4}	1.01*10^{-4}	4.72*10^{-10}	6.51*10^{-4}	1.03*10^{-4}	2.36*10^{-10}
8	cg00285394	SQLE	8.68*10^{-4}	1.39*10^{-4}	4.57*10^{-10}	8.96*10^{-4}	1.42*10^{-4}	2.66*10^{-10}
22	cg27179375	POM121L1P	1.33*10^{-3}	2.00*10^{-4}	2.85*10^{-11}	1.07*10^{-3}	1.71*10^{-4}	3.65*10^{-10}
6	cg05586134	PTCRA	4.81*10^{-4}	6.32*10^{-5}	2.80*10^{-14}	3.32*10^{-4}	5.33*10^{-5}	4.54*10^{-10}
3	cg07357021	PRICKLE2	3.94*10^{-4}	5.08*10^{-5}	9.48*10^{-15}	2.82*10^{-4}	4.59*10^{-5}	7.68*10^{-10}
1	cg21778193	MIR200B	1.09*10^{-3}	1.48*10^{-4}	1.42*10^{-13}	8.75*10^{-4}	1.42*10^{-4}	7.86*10^{-10}
8	cg04836151	LY6H	9.72*10^{-4}	1.37*10^{-4}	1.27*10^{-12}	7.03*10^{-4}	1.15*10^{-4}	9.77*10^{-10}
17	cg09243648	SP6	9.32*10^{-4}	1.25*10^{-4}	8.14*10^{-14}	6.00*10^{-4}	9.85*10^{-5}	1.13*10^{-9}
15	cg07822775	PCSK6	5.55*10^{-4}	6.98*10^{-5}	1.86*10^{-15}	3.44*10^{-4}	5.65*10^{-5}	1.18*10^{-9}
10	cg14906690	KAT6B	8.09*10^{-4}	1.07*10^{-4}	3.76*10^{-14}	7.06*10^{-4}	1.17*10^{-4}	1.47*10^{-9}
2	cg05309280	GORASP2	5.62*10^{-4}	1.01*10^{-4}	2.63*10^{-8}	6.07*10^{-4}	1.01*10^{-4}	1.58*10^{-9}
4	cg10635092	ZFYVE28	7.89*10^{-4}	1.27*10^{-4}	5.12*10^{-10}	7.11*10^{-4}	1.19*10^{-4}	2.30*10^{-9}
20	cg13403462	NECAB3	1.37*10^{-3}	2.23*10^{-4}	9.21*10^{-10}	1.38*10^{-3}	2.30*10^{-4}	2.36*10^{-9}
17	cg10187674	ABCA5	2.98*10^{-4}	5.39*10^{-5}	3.05*10^{-8}	3.21*10^{-4}	5.39*10^{-5}	2.47*10^{-9}
3	cg19762797	XXYLT1	3.38*10^{-4}	4.32*10^{-5}	5.28*10^{-15}	2.13*10^{-4}	3.58*10^{-5}	2.92*10^{-9}
1	cg09230763	MAP3K6	9.16*10^{-4}	1.10*10^{-4}	8.18*10^{-17}	6.04*10^{-4}	1.02*10^{-4}	3.09*10^{-9}

Table 3. *(Continued)*

			Cell-unadjusted model			Cell-adjusted model		
CHR	CpG site	Gene	b[a]	SE	p-value	b[a]	SE	p-value
19	cg18156417	MAP2K2	$3.49*10^{-4}$	$5.11*10^{-5}$	$8.01*10^{-12}$	$2.82*10^{-4}$	$4.77*10^{-5}$	$3.51*10^{-9}$
7	cg26220185	MAD1L1	$6.65*10^{-4}$	$9.48*10^{-5}$	$2.23*10^{-12}$	$4.06*10^{-4}$	$6.88*10^{-5}$	$3.68*10^{-9}$
17	cg13540311	SEPT9	$3.33*10^{-4}$	$4.70*10^{-5}$	$1.40*10^{-12}$	$2.46*10^{-4}$	$4.17*10^{-5}$	$3.92*10^{-9}$
2	cg16877087	RBMS1	$5.85*10^{-4}$	$9.39*10^{-5}$	$4.72*10^{-10}$	$5.62*10^{-4}$	$9.59*10^{-5}$	$4.66*10^{-9}$
1	cg20594982	AGRN	$1.20*10^{-3}$	$1.70*10^{-4}$	$1.57*10^{-12}$	$9.95*10^{-4}$	$1.70*10^{-4}$	$5.17*10^{-9}$
8	cg14660676	SQLE	$1.09*10^{-3}$	$1.72*10^{-4}$	$2.36*10^{-10}$	$1.05*10^{-3}$	$1.80*10^{-4}$	$5.21*10^{-9}$
9	cg09723488	LHX6	$5.87*10^{-4}$	$7.14*10^{-5}$	$2.06*10^{-16}$	$3.41*10^{-4}$	$5.84*10^{-5}$	$5.38*10^{-9}$
8	cg13176454	ST3GAL1	$2.98*10^{-4}$	$3.77*10^{-5}$	$2.92*10^{-15}$	$2.00*10^{-4}$	$3.43*10^{-5}$	$5.49*10^{-9}$
8	cg14030674	ANK1	$1.08*10^{-3}$	$1.32*10^{-4}$	$3.01*10^{-16}$	$5.50*10^{-4}$	$9.46*10^{-5}$	$5.97*10^{-9}$
10	cg27102629	KAT6B	$9.89*10^{-4}$	$1.24*10^{-4}$	$1.90*10^{-15}$	$6.68*10^{-4}$	$1.16*10^{-4}$	$7.37*10^{-9}$
2	cg06399427	RBMS1	$5.17*10^{-4}$	$8.11*10^{-5}$	$1.88*10^{-10}$	$4.76*10^{-4}$	$8.23*10^{-5}$	$7.39*10^{-9}$
16	cg01979489	PDIA2	$4.74*10^{-4}$	$6.71*10^{-5}$	$1.57*10^{-12}$	$3.24*10^{-4}$	$5.63*10^{-5}$	$8.54*10^{-9}$
7	cg05837990	CDHR3	$1.44*10^{-3}$	$2.39*10^{-4}$	$1.83*10^{-9}$	$1.40*10^{-3}$	$2.43*10^{-4}$	$9.12*10^{-9}$
9	cg21241902	NSMF	$6.10*10^{-4}$	$8.93*10^{-5}$	$8.16*10^{-12}$	$4.87*10^{-4}$	$8.49*10^{-5}$	$9.88*10^{-9}$
8	cg00729699	DMTN	$7.83*10^{-4}$	$1.15*10^{-4}$	$1.16*10^{-11}$	$5.52*10^{-4}$	$9.64*10^{-5}$	$1.03*10^{-8}$
20	cg03719642	UCKL1	$6.86*10^{-4}$	$1.18*10^{-4}$	$5.46*10^{-9}$	$6.83*10^{-4}$	$1.19*10^{-4}$	$1.07*10^{-8}$
8	cg18144647	SFRP1	$5.55*10^{-4}$	$9.28*10^{-5}$	$2.14*10^{-9}$	$5.22*10^{-4}$	$9.13*10^{-5}$	$1.11*10^{-8}$
12	cg21814615	KNTC1	$4.62*10^{-4}$	$7.19*10^{-5}$	$1.34*10^{-10}$	$3.64*10^{-4}$	$6.41*10^{-5}$	$1.43*10^{-8}$
1	cg14528056	GBAP1	$6.87*10^{-4}$	$1.14*10^{-4}$	$1.75*10^{-9}$	$5.14*10^{-4}$	$9.08*10^{-5}$	$1.45*10^{-8}$
1	cg22820188	LMNA	$7.08*10^{-4}$	$8.59*10^{-5}$	$1.69*10^{-16}$	$4.38*10^{-4}$	$7.74*10^{-5}$	$1.56*10^{-8}$
14	cg08289937	DDHD1	$3.54*10^{-4}$	$4.74*10^{-5}$	$7.43*10^{-14}$	$2.51*10^{-4}$	$4.44*10^{-5}$	$1.56*10^{-8}$
9	cg21186778	RCL1	$7.09*10^{-4}$	$9.24*10^{-5}$	$1.67*10^{-14}$	$3.63*10^{-4}$	$6.43*10^{-5}$	$1.58*10^{-8}$
5	cg17514558	PCDHB19P	$6.14*10^{-4}$	$1.09*10^{-4}$	$1.69*10^{-8}$	$6.46*10^{-4}$	$1.14*10^{-4}$	$1.67*10^{-8}$
8	cg15240102	LOC286083	$7.30*10^{-4}$	$9.23*10^{-5}$	$2.74*10^{-15}$	$4.00*10^{-4}$	$7.10*10^{-5}$	$1.80*10^{-8}$
18	cg21026022	CABYR	$1.21*10^{-3}$	$1.75*10^{-4}$	$5.52*10^{-12}$	$9.95*10^{-4}$	$1.77*10^{-4}$	$1.87*10^{-8}$
13	cg18995031	RASA3	$4.11*10^{-4}$	$5.70*10^{-5}$	$5.85*10^{-13}$	$2.84*10^{-4}$	$5.09*10^{-5}$	$2.31*10^{-8}$

Table 3. *(Continued)*

			Cell-unadjusted model			Cell-adjusted model		
CHR	CpG site	Gene	b[a]	SE	p-value	b[a]	SE	p-value
22	cg04027757	POM121L1P	$7.26*10^{-4}$	$1.27*10^{-4}$	$9.59*10^{-9}$	$6.45*10^{-4}$	$1.15*10^{-4}$	$2.33*10^{-8}$
6	cg01963618	LINC01622	$4.79*10^{-4}$	$6.77*10^{-5}$	$1.39*10^{-12}$	$3.51*10^{-4}$	$6.29*10^{-5}$	$2.40*10^{-8}$
20	cg21445553	GGTLC1	$1.09*10^{-3}$	$1.87*10^{-4}$	$5.90*10^{-9}$	$9.50*10^{-4}$	$1.70*10^{-4}$	$2.41*10^{-8}$
16	cg05976575	CMTM2	$4.72*10^{-4}$	$6.35*10^{-5}$	$1.15*10^{-13}$	$3.21*10^{-4}$	$5.75*10^{-5}$	$2.49*10^{-8}$
2	cg13758186	CREG2	$4.86*10^{-4}$	$7.71*10^{-5}$	$2.90*10^{-10}$	$3.89*10^{-4}$	$6.98*10^{-5}$	$2.52*10^{-8}$
2	cg20710902	BUB1	$3.44*10^{-4}$	$5.63*10^{-5}$	$1.04*10^{-9}$	$2.74*10^{-4}$	$4.94*10^{-5}$	$2.88*10^{-8}$
6	cg03046925	GPX6	$4.25*10^{-4}$	$6.26*10^{-5}$	$1.06*10^{-11}$	$2.80*10^{-4}$	$5.05*10^{-5}$	$2.98*10^{-8}$
10	cg18330571	EBF3	$4.92*10^{-4}$	$7.14*10^{-5}$	$5.75*10^{-12}$	$3.68*10^{-4}$	$6.65*10^{-5}$	$3.15*10^{-8}$
3	cg11156132	PRKCD	$6.83*10^{-4}$	$9.30*10^{-5}$	$1.97*10^{-13}$	$3.21*10^{-4}$	$5.82*10^{-5}$	$3.36*10^{-8}$
2	cg18499001	LOC388942	$2.04*10^{-4}$	$3.75*10^{-5}$	$4.98*10^{-8}$	$2.12*10^{-4}$	$3.85*10^{-5}$	$3.72*10^{-8}$
2	cg05113927	UCN	$6.23*10^{-4}$	$1.11*10^{-4}$	$1.83*10^{-8}$	$6.10*10^{-4}$	$1.11*10^{-4}$	$3.85*10^{-8}$
4	cg22670329	CXCL6	$4.29*10^{-4}$	$7.97*10^{-5}$	$7.32*10^{-8}$	$4.16*10^{-4}$	$7.56*10^{-5}$	$3.86*10^{-8}$
2	cg15913725	TSSC1	$2.76*10^{-4}$	$4.46*10^{-5}$	$5.89*10^{-10}$	$2.37*10^{-4}$	$4.32*10^{-5}$	$4.46*10^{-8}$
7	cg01881287	EFCAB10	$8.12*10^{-4}$	$1.29*10^{-4}$	$3.40*10^{-10}$	$7.22*10^{-4}$	$1.32*10^{-4}$	$4.84*10^{-8}$
16	cg05635274	PRSS21	$5.84*10^{-4}$	$9.30*10^{-5}$	$3.24*10^{-10}$	$4.82*10^{-4}$	$8.84*10^{-5}$	$4.95*10^{-8}$
16	cg03221837	IRX3	$5.48*10^{-4}$	$7.77*10^{-5}$	$1.73*10^{-12}$	$4.22*10^{-4}$	$7.76*10^{-5}$	$5.20*10^{-8}$
13	cg13557773	RASA3	$9.98*10^{-4}$	$1.74*10^{-4}$	$9.82*10^{-9}$	$9.53*10^{-4}$	$1.75*10^{-4}$	$5.42*10^{-8}$
8	cg14434213	RNF5P1	$8.52*10^{-4}$	$1.42*10^{-4}$	$2.21*10^{-9}$	$7.83*10^{-4}$	$1.45*10^{-4}$	$6.04*10^{-8}$
6	cg05659486	LRRC1	$6.47*10^{-4}$	$8.98*10^{-5}$	$5.82*10^{-13}$	$4.10*10^{-4}$	$7.58*10^{-5}$	$6.22*10^{-8}$
5	cg15029475	C5orf38	$7.23*10^{-4}$	$1.01*10^{-4}$	$7.55*10^{-13}$	$4.88*10^{-4}$	$9.01*10^{-5}$	$6.32*10^{-8}$
19	cg22545168	LAIR1	$4.79*10^{-4}$	$6.92*10^{-5}$	$4.62*10^{-12}$	$3.49*10^{-4}$	$6.45*10^{-5}$	$6.32*10^{-8}$
5	cg23111106	OSMR	$4.73*10^{-4}$	$6.81*10^{-5}$	$3.77*10^{-12}$	$3.16*10^{-4}$	$5.85*10^{-5}$	$6.34*10^{-8}$
7	cg23749005	PTPRN2	$1.00*10^{-3}$	$1.66*10^{-4}$	$1.36*10^{-9}$	$8.95*10^{-4}$	$1.65*10^{-4}$	$6.34*10^{-8}$
17	cg21937867	PRCD	$4.63*10^{-4}$	$6.56*10^{-5}$	$1.70*10^{-12}$	$2.88*10^{-4}$	$5.33*10^{-5}$	$6.39*10^{-8}$
1	cg04972348	MIR200B	$1.30*10^{-3}$	$1.65*10^{-4}$	$3.23*10^{-15}$	$8.61*10^{-4}$	$1.59*10^{-4}$	$6.66*10^{-8}$
14	cg05881436	SNAPC1	$3.59*10^{-4}$	$5.77*10^{-5}$	$4.93*10^{-10}$	$2.78*10^{-4}$	$5.16*10^{-5}$	$6.84*10^{-8}$

Table 3. *(Continued)*

			Cell-unadjusted model			Cell-adjusted model		
CHR	**CpG site**	**Gene**	**b[a]**	**SE**	**p-value**	**b[a]**	**SE**	**p-value**
3	cg23166970	MCCC1	$1.32*10^{-4}$	$2.42*10^{-5}$	$4.87*10^{-8}$	$1.29*10^{-4}$	$2.39*10^{-5}$	$7.66*10^{-8}$
5	cg08407524	LINC01023	$3.49*10^{-4}$	$5.16*10^{-5}$	$1.27*10^{-11}$	$2.33*10^{-4}$	$4.34*10^{-5}$	$8.28*10^{-8}$
14	cg01428678	GPHN	$1.89*10^{-4}$	$3.38*10^{-5}$	$2.19*10^{-8}$	$1.85*10^{-4}$	$3.45*10^{-5}$	$8.69*10^{-8}$
19	cg26284544	TGFBR3L	$8.41*10^{-4}$	$1.44*10^{-4}$	$5.39*10^{-9}$	$7.64*10^{-4}$	$1.43*10^{-4}$	$8.79*10^{-8}$
3	cg12155036	LINC00887	$6.84*10^{-4}$	$1.22*10^{-4}$	$2.04*10^{-8}$	$6.49*10^{-4}$	$1.21*10^{-4}$	$9.00*10^{-8}$
22	cg25432807	POM121L1P	$6.07*10^{-4}$	$1.10*10^{-4}$	$3.08*10^{-8}$	$5.35*10^{-4}$	$1.00*10^{-4}$	$9.32*10^{-8}$
6	cg25521481	TTBK1	$7.08*10^{-4}$	$1.01*10^{-4}$	$2.53*10^{-12}$	$5.34*10^{-4}$	$1.00*10^{-4}$	$9.78*10^{-8}$
3	cg25185429	ITPR1	$3.02*10^{-4}$	$3.96*10^{-5}$	$2.44*10^{-14}$	$1.79*10^{-4}$	$3.37*10^{-5}$	$1.02*10^{-7}$
2	cg01517690	ZSWIM2	$6.24*10^{-4}$	$1.08*10^{-4}$	$7.55*10^{-9}$	$5.52*10^{-4}$	$1.04*10^{-4}$	$1.02*10^{-7}$

[a] Difference in newborn DNA methylation beta value per 1 kg/m^2 increase in maternal pre-pregnancy BMI.

model. Forest plots and results of a leave-one-out analysis showed that results from most cohorts agreed on the direction of effect at the 86 top sites and no single cohort consistently had a disproportionately large influence on the meta-analysis (Supplementary Figures, pages 6–37).

Secondly, we performed a sensitivity analysis restricting the meta-analysis to 15/19 cohorts comprising participants of European origin only. The results from this sensitivity analysis were consistent with those of the main analysis. The Spearman's correlation coefficient for regression coefficients was 0.91, and the percentage change in estimates was >10% for 47/86 sites (median percentage change in estimates: 9.7%). While this modest difference could reflect confounding by ancestry, it might also occur because the cohorts of non-European ancestry tended to have a higher mean maternal BMI and were more variable compared to the European ancestry cohorts (Table 1).

Thirdly, we compared the 86 sites to a list of 190,672 probes on the Illumina 450k platform that Naeem et al. suggested might give spurious readings (Supplementary Table S9).[69] Forty-two sites were on this list: seven located in regions containing SNPs, 11 in regions containing repeat sequences and four in regions where insertions or deletions are found. These sites may be more likely to contain outlier values that influence results, however diptests for multimodality and visual inspection of density plots of methylation beta values in ALSPAC and GOYA did not support this (p-value >0.05; Supplementary Figures, pages 38–51).[57] Additionally, all cohort-specific analyses were conducted using robust linear regression, which is designed to be robust to outliers in the outcome variable (methylation). Other reasons that probes had been flagged by Naeem et al. as potentially problematic were that they hybridise to multiple genomic loci (four sites), did not produce results consistent with those produced by whole-genome bisulfite sequencing (nine sites) and were particularly susceptible to errors in bisulfite conversion (four sites).

Maternal BMI-associated newborn blood methylation sites are not enriched for certain biological processes or pathways

Maternal BMI-associated newborn blood methylation sites were spread throughout the genome and did not appear to cluster in certain chromosomal regions. The 86 maternal BMI-associated methylation sites are near 77 gene regions, and there were several instances where multiple sites mapped to the same gene: RBMS1 [3 sites], POM121L1P [3 sites], VIPR2 [2 sites], SQLE [2 sites], RASA3 [2 sites], MIR200B [2 sites], KAT6B [2 sites]. The list of 77 genes was not enriched for any gene ontology (GO) term (Supplementary Table S10) or KEGG pathway (Supplementary Table S11) after FDR-correction for multiple testing, but this analysis was likely underpowered.

Associations between maternal BMI at the start of pregnancy and newborn DNA methylation were reproduced in the whole blood of adolescents at most sites

In order to assess whether associations at birth are also present in later childhood, four cohorts (BAMSE, IOW birth cohort [IOW F1], PIAMA, and RAINE; total N=1,817 mother-child pairs) contributed results to a meta-analysis of maternal BMI at the start of pregnancy and methylation in the whole blood of adolescent offspring (age range: 15 to 18 years, weighted mean: 17 years). Cohorts are summarised in Table 4. These cohorts were completely independent of those that contributed results to the newborn analysis, therefore we were able to assess reproducibility of our newborn results later in life. All models discussed here were corrected for estimated cell counts. Full results are provided in Supplementary Table S12.

There was evidence for reproducible associations at most of the 86 sites: the direction of association at adolescence was the same as that at birth for 72/86 sites (Spearman correlation coefficient: 0.67). Twenty-two of these 72 sites had a p-value <0.05 at adolescence, despite the much smaller sample size. Although no associations survived correction for multiple testing at 86 sites, 22/72 sites with nominal p-values <0.05 is higher than the 5% expected by chance alone (Kolmogorov p-value=$3.3*10^{-16}$). Across the 72 sites where effects were in the same direction, the effect estimates in the adolescence analysis were a median of 2.25 times smaller (i.e. closer to the null) than the effect estimates in the newborn analysis (range: 2889 times smaller to 1.35 times larger) but at some sites, estimates at both time points were remarkably similar (Figure 4). It is also of particular note that six of the top ten sites with the largest effect size were the same at birth and adolescence. These sites were cg05837990 (CDHR3), cg13403462 (ACTL10/NECAB3), cg27179375 (POM121L1P), cg12009398 (VIPR2), cg20594982 (AGRN) and cg21445553 (GGTLC1). One of the top ten sites with the smallest p-values was also common to both analyses: cg05086444 (VIPR2).

Table 4. Characteristics of each cohort included in the meta-analysis of the effect of maternal pre-pregnancy BMI on offspring DNA methylation at adolescence

Cohort	N	Mean maternal BMI (SD)	Mean maternal age (SD)	Mean adolescent age (SD)	Ethnicity
BAMSE	221	23.2 (3.4)	31.2 (4.3)	16.6 (0.3)	European
IOW F1	279	24.4 (4.0)	27.3 (5.2)	18.0 (0.0)	European
PIAMA	583	22.6 (3.1)	30.9 (3.7)	16.3 (0.2)	European
RAINE	734	22.4 (4.4)	29.1 (5.8)	17.3 (0.6)	European
Meta-analysis	1817				

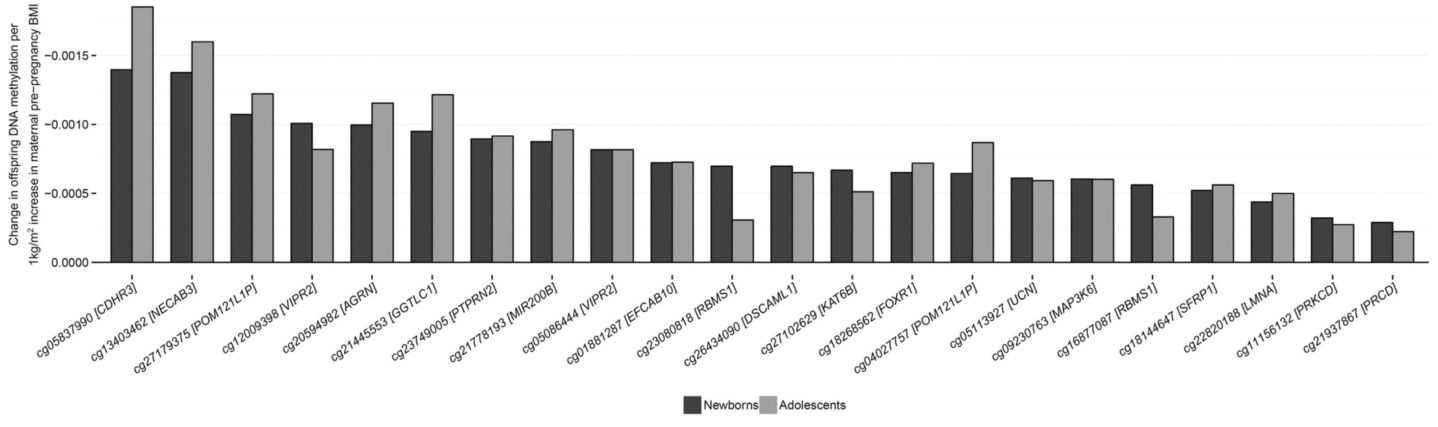

Figure 4. Comparison of estimates of the effect of maternal BMI on offspring DNA methylation at birth and at adolescence. Of the 86 sites where maternal BMI at the start of pregnancy was associated with newborn blood methylation, 72 had the same direction of association in the analysis of adolescents. Plotted here are the 22/86 methylation sites with a p-value <0.05 in the analysis of adolescents, ordered by effect size in newborns.

Negative control design supports a causal intrauterine effect of maternal BMI on newborn blood methylation at nine sites

We used a negative control design in an attempt to disentangle a potential causal, intrauterine effect of maternal BMI on newborn blood methylation from the effect of confounding by shared genetics or postnatal environment.[7] The logic is that paternal and maternal exposures may both be associated with offspring methylation due to shared familial confounding factors or by inheritance of parental genotypes, but paternal BMI would not normally be expected to affect the intrauterine environment. Therefore, if there is a causal intrauterine influence, only maternal BMI would be expected to be independently associated with methylation. Evidence for an intrauterine effect is stronger where estimates for associations between maternal BMI and offspring DNA methylation are greater than the equivalent estimates for paternal BMI, whereas consistent maternal and paternal estimates provides evidence for confounding by genetic or shared postnatal environmental factors.

It is also important to adjust the maternal estimate for paternal BMI, and vice versa, because maternal and paternal BMI are somewhat correlated due to assortative mating. For example, in the cohorts that contributed to this study, Spearman's correlation coefficients between maternal and paternal BMI ranged from 0.18 to 0.25 (p-value <0.001).

Seven cohorts contributed results to this negative control analysis: ALSPAC (N=619), CHAMACOS (N=180), Generation R (N=829), GOYA (N=422), MEDALL (INMA and EDEN pooled N=316), NHBCS (N=96) and RICHS (N=92). The total number of families included in the meta-analysis of the mutually adjusted models was 2,554. Results for all models are provided in Supplementary Table S13.

Based on the above criteria, we found some evidence for a causal intrauterine effect of maternal BMI on newborn blood methylation at some sites: At 64 of 86 sites, the paternal and maternal effect estimates were in the same direction, i.e. we could be more certain that no independent paternal-specific effect exists. At 40 of these 64 sites, the maternal BMI estimate was greater than the paternal BMI estimate after mutual adjustment (median 2.19 times greater, range 1.01 to 142.4 times greater). At nine of these 40 sites, there was some evidence of heterogeneity between the mutually adjusted maternal and paternal BMI estimates (I^2>40; Supplementary Table S14). These criteria were used to define support for a possible maternal specific, intrauterine effect. Therefore, at 77/86 sites, evidence from this negative control study was more supportive of the association between maternal BMI and newborn blood methylation being explained by genetic or shared prenatal environmental factors than a causal intrauterine effect. Figure 5 displays the results for

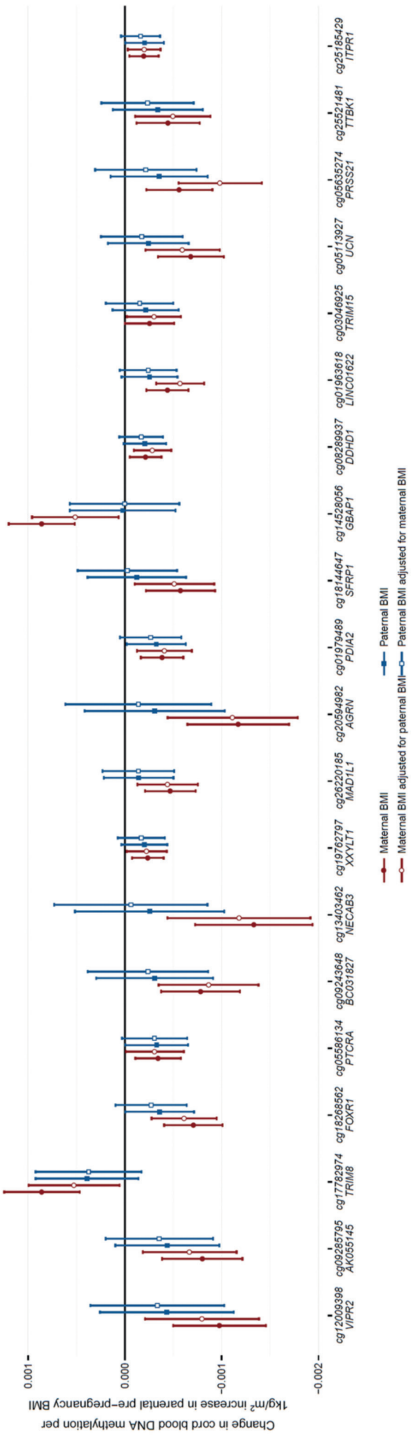

Figure 5. Comparison of estimates of the effect of maternal and paternal BMI on newborn DNA methylation. Of the 86 sites where maternal BMI at the start of pregnancy was associated with newborn blood methylation, we found 20 sites (plotted here) where the estimated effect of maternal BMI, adjusted for paternal BMI, had a p-value <0.05 and was in the same direction and greater than the estimated effect of paternal BMI, adjusted for maternal BMI. Sites are ordered by p-value in the full maternal BMI meta-analysis.

Table 5. A summary of the 8 sites where there is strongest evidence for a causal intrauterine effect of maternal BMI on newborn blood DNA methylation

CHR	CPG	Coordinate	Nearest gene	Illumina annotated gene	Relation to CPG island	Relation to gene	b[a] at birth	p-value at birth	b[a] at adolescence	p-value at adolescence
17	cg09243648	45944464	SP6			Body; 1st Exon	$6.0*10^{-4}$	1.1*10-09	2.0*10-05	$8.9*10^{-1}$
20	cg13403462	32256071	NECAB3	ACTL10;NECAB3	South shore		$1.4*10^{-3}$	2.4*10-09	$1.6*10^{-3}$	$4.1*10^{-3}$
1	cg20594982	976707	AGRN	AGRN	Island	Body	$1.0*10^{-3}$	5.2*10-09	$1.2*10^{-3}$	$1.5*10^{-3}$
8	cg18144647	41113257	SFRP1				$5.2*10^{-4}$	$1.1*10^{-8}$	$5.6*10^{-4}$	$9.1*10^{-3}$
1	cg14528056	155194782	GBAP1	GBAP1	North shelf	Body	$5.1*10^{-4}$	$1.5*10^{-8}$	$2.9*10^{-4}$	$1.7*10^{-1}$
6	cg01963618	1102332	LINC01622	LOC285768		TSS1500	$3.5*10^{-4}$	$2.4*10^{-8}$	$1.8*10^{-4}$	$1.9*10^{-1}$
2	cg05113927	27531244	UCN	UCN	Island	TSS200	$6.1*10^{-4}$	$3.9*10^{-8}$	$5.9*10^{-4}$	$9.4*10^{-3}$
16	cg05635274	2866901	PRSS21	PRSS21	North shore	TSS1500	$4.8*10^{-4}$	$5.0*10^{-8}$	$3.7*10^{-4}$	$2.1*10^{-1}$

[a] Difference in offspring DNA methylation beta value per 1 kg/m^2 increase in maternal pre-pregnancy BMI.

the 20 sites where the mutually adjusted maternal and paternal BMI estimates were in the same direction, with the maternal effect being larger than the paternal effect and having a p-value <0.05 (Figure 5).

meQTLs at maternal BMI-associated cord blood methylation sites provide further support for confounding by genetics at four sites

To explore the genetic influence on DNA methylation at the 86 maternal BMI-associated cord blood methylation sites, we performed a look-up in an online catalogue of methylation quantitative trait loci (meQTL) that were previously identified using ALSPAC data.[63] We identified 821 meQTLs where genetic variation was associated with cord blood DNA methylation at 27/86 sites with p-value $<1*10^{-7}$. Of these 821 meQTLs, 68 were within 1 Mb of the methylation site (cis) and 753 were outside of this window (trans).

If an meQTL is also associated with maternal BMI, this could suggest that the association between maternal BMI and new-born methylation is confounded by shared genetics. Of the 821 identified meQTLs, data for 225 were available in the results of the largest adult BMI GWAS meta-analysis to date, conducted by the GIANT consortium.[64] Of these, 17/225 were nominally associated (p-value <0.05) with BMI in GIANT. These 17 meQTLs were associated with cis methylation at four CpGs: 11 with cg03258665 (EPHA2), four with cg00285394 (SQLE), one with cg03719642 (UCKL1) and one with cg18268562 (FOXR1). Therefore, there is some evidence that associations between maternal BMI and methylation at these four sites are confounded by shared genetics. For most of the meQTLs, the associations SNP-BMI and SNP-methylation were in opposite directions. Thus, the same effect allele was associated with higher BMI (effect estimates ranging 0.007 to 0.015) and lower methylation (effect estimates ranging 0.523 to 0.235). Only in the rs8567-cg03719642 association was the effect allele associated with lower BMI (effect estimate: 0.012) and higher methylation (effect estimate: 0.287).

Using a combination of evidence, we identified eight sites where maternal BMI may have a causal intrauterine effect on newborn blood methylation

As described above, by employing a negative control design, we found nine sites where the estimated effect of maternal BMI was stronger than that of paternal BMI. One of these sites (cg18268562 at FOXR1) is an meQTL that was nominally associated with BMI in GIANT. Therefore, we find strongest support for a causal intrauterine effect of maternal BMI at the start of pregnancy on newborn blood methylation at just eight sites (Table 5). At the remaining 78 of our top 86 sites, the apparent associations between maternal BMI and

newborn blood methylation might be more appropriately explained by shared mother-offspring genetic and postnatal environmental factors. These findings are summarised in Supplementary Table S14.

DISCUSSION

We found that maternal BMI at the start of pregnancy is associated with small variation in newborn blood DNA methylation at 86 sites throughout the genome, after adjusting for cell proportions. At around a quarter of these 86 sites, we found nominal associations between maternal pre-pregnancy BMI and DNA methylation in an independent cohort of adolescents, sometimes with remarkably consistent effect sizes to those found in neonates. However, when we employed two causal inference strategies, we found supporting evidence for a causal intrauterine effect at only eight sites. Taken together, our results suggest that the effects of maternal pre-pregnancy adiposity on neonatal blood DNA methylation are primarily related to variations in the cellular distributions in cord blood, as well as shared environment and genetic variation. Although there may be a causal intrauterine effect at some sites, the biological significance of such small effects is unclear.

Our findings are in contrast to some previous studies that have reported strong associations between maternal BMI/ adiposity and DNA methylation in neonates.[21,24-26,70,71] However, in these smaller studies there has been a lack of consistency in terms of the specific loci identified. Although we replicated, at look-up level of significance, an inverse association between maternal BMI and newborn blood methylation at cg01422136 (ZCCHC10) that was reported in a study of African American and Haitian mother-child pairs from the Boston Birth Cohort, this association was not epigenome-wide significant in our study (p-value =0.0016).[24] We did not replicate specific associations reported in other previous studies of maternal BMI and newborn blood methylation, including some that were reported in individual studies from the PACE consortium.[21,25,26,70,71] This lack of consistency highlights the potential presence of false positive findings in small EWAS studies and the importance of meta-analysis for improving power and reproducibility.

The 86 Bonferroni-significant sites were robust across cohorts and after adjustment for cell proportions, so they are unlikely to have arisen due to chance, study-specific biases or technical aspects of the array, which should be independent of our exposure. However, effect sizes were very small; all were less than a 0.15% change in methylation per one-unit increase in maternal BMI. The biological significance of such small effects is unclear and could not be further explored in this study due to lack of genome-wide data on downstream

gene and protein expression. One reason we may not have observed larger effect sizes is that the studied cohorts consisted mostly of women whose weight fell within the WHO BMI category of normal weight. Perhaps the largest effects only exist at the extremities of the BMI distribution, as is the case with some other maternal BMI-associated offspring phenotypes, including offspring BMI.[6] However, we also found relatively small effects in our binary exposure model comparing methylation in offspring of women who were overweight or obese to methylation in offspring of women who were normal weight at the start of pregnancy.

Without integration with gene expression data, it is impossible for us to truly infer (either way) whether maternal BMI-associated variation in methylation at our 86 sites is functionally important. The 77 mapped genes were not enriched for any GO term or KEGG pathway, which could suggest that there is little or no significant biological effect. However, this analysis was likely underpowered and it is worth noting that, individually, some of the 77 genes that map to our 86 sites have functions that could potentially link maternal adiposity to offspring health outcomes, either through shared genetic factors or an epigenetic effect on gene regulation. These may be useful candidates for future studies that are better placed to explore the biological significance of the methylation sites we have identified. For example, GWAS studies have identified that variants at some of our differentially methylated loci are associated with adiposity-related traits: total energy total energy expenditure [CDHR3], energy intake [PTPRN2], lipoprotein-a levels [DSCAML1], adiponectin levels [CREG2], and type 2 diabetes [ANK1, RBMS1].[72-77] In studies of DNA methylation, greater whole blood methylation at cg17782974 (TRIM8) was associated with higher BMI in elderly participants in the Lothian Birth Cohort study and higher maternal BMI in our study.[78] Another 450k study found that several sites at PTPRN2 were hypermethylated in subcutaneous adipose tissue of women before gastric-bypass compared to the same women after gastric-bypass and associated weight-loss, whereas we found that higher maternal BMI at the start of pregnancy was associated with hypomethylation at PTPRN2 in newborn blood.[79] We also found that higher maternal BMI at the start of pregnancy was associated with lower newborn methylation at a site (cg03221837) near IRX3. More copies of the risk allele at the obesity-associated SNP FTO is associated with higher blood expression of IRX3 in humans, and IRX3-deficient mice have been shown to have a 25-30% reduction in body weight.[80] However, it is important to note that although IRX3 was the nearest gene to the maternal BMI-associated methylation site in our study, the site was actually 299,591 bp downstream from the gene. Finally, we were particularly interested to find two sites (cg12009398, cg05086444) on the gene body of VIPR2 where greater maternal BMI was associated with lower methylation. The associations were consistent in adolescents, with p-values <0.008, although we did not find any evidence that the associations were causal. VIPR2 encodes vasoactive intestinal peptide receptor 2 (VIPR2), which functions as a neuro-transmitter

and as a neuroendocrine hormone. A GWA analysis in 1,000 participants found that the vasoactive intestinal peptide (VIP) pathway was strongly associated with fat mass and with BMI, suggesting that the VIP pathway may play an important role in the development of obesity.[81] In a study using the 450k array, lower VIPR2 methylation was found in the saliva of children with attention deficit hyperactivity disorder (ADHD), relative to controls, albeit at different sites than those identified in the present study.[82] Given previously identified associations between maternal BMI and offspring ADHD, further work is warranted to explore the extent to which VIPR2 gene function (driven either by genetic variation or regulation by methylation) might explain associations between maternal adiposity and neurodevelopment of the offspring.[83-86]

Of the 86 sites where maternal BMI was associated with methylation in the blood of newborns, 72 showed the same direction of association in the blood of an independent smaller sample of adolescents. At some sites, effect estimates were remarkably consistent between the two age groups. Of particular note, six of the top 10 sites with the largest effect size in the cell-adjusted newborn analysis also had the largest effect size amongst adolescents. This consistency from birth to adolescence could be explained as either i) an intrauterine influence of maternal pre-pregnancy BMI on variation in offspring DNA methylation that persists to adolescence, ii) confounding by shared familial genetic and/or environmental influences on maternal BMI and offspring methylation that remain stable over time, or iii) the possibility that both maternal pre-/early-pregnancy and the adolescent's own BMI have independent effects on the child's methylation. We did not adjust for adolescent's BMI because that may introduce a collider that would bias the association between shared familial factors and maternal BMI away from the null.

We were interested in whether the 86 maternal BMI-associated sites represented a causal intrauterine effect of maternal adiposity on offspring methylation, or if associations were better explained by confounding by shared environment or genetics. By employing a negative control design, we found nine sites where the estimated effect of maternal BMI was larger than that of paternal BMI, after mutual adjustment. Maternal and paternal BMI were not strongly correlated in any of the cohorts that took part in this analysis (Spearman's R ranging 0.13 to 0.25), so collinearity in the mutually adjusted models is unlikely to bias interpretation of results. This is sup-ported by the observation that standard errors did not increase substantially between the unadjusted and adjusted models. At one of the nine sites (cg18269562 mapping to FOXR1), cord blood methylation has previously been strongly associated (p-value $<1*10^{-7}$) with common genetic variants.[63] This meQTL was also nominally associated (p-value <0.05) with BMI according to the GIANT consortium adult BMI GWAS meta-analysis.[64,65] We considered that the association between maternal BMI and newborn methylation at this site was likely driven by a shared genetic effect.

Therefore, we could be more confident of a causal intrauterine effect of maternal adiposity on methylation of blood DNA in newborns at only 8/86 sites. At the remaining 78/ 86 sites, shared genetic and/or prenatal environmental factors, which would be expected to be the same whether the exposure were maternal or paternal BMI, may have larger influences on newborn blood methylation than maternal BMI at the start of pregnancy.

Our findings are in line with studies reporting that a large proportion of variation in DNA methylation is explained by genetics. One study estimated that at around 50% of CpG sites on the Illumina 450k array methylation has a substantial genetic component.[87] Another study of DNA methylation using the same platform in 237 neonates found that, of 1,423 genomic regions that were highly variable across individuals, 25% were best explained by genotype alone and 75% by an interaction of genotype with different in utero environmental factors (including maternal BMI).[88] These studies, along with our own, highlight complex relationships between genetic inheritance, intrauterine environmental exposures and offspring epi-genetics. In light of this, we recommend that where the exposure is genetically heritable, extra care should be taken to avoid over-interpreting EWAS results as representing causal environmental effects.[89] Causal analysis techniques, such as the negative control and meQTL analyses conducted in this study, will be useful in this regard.

Regardless of whether maternal BMI has a biologically significant, causal effect on newborn blood DNA methylation, the robust, and seemingly persistent, associations we identified in our study suggests that, as has been shown for maternal smoking, blood DNA methylation could be a useful indicator of maternal BMI during pregnancy.[90] Such an indicator would be useful in studies where maternal BMI data are missing. Likewise, newborn blood methylation at maternal BMI-associated sites might also be predictive of offspring outcomes, capturing both genetic and environmental influences of maternal adiposity.

Although our findings suggest no strong effect of maternal pre-pregnancy adiposity (as measured by BMI) on offspring methylation in blood, this does not preclude the possibility that there is an effect of maternal adiposity measured in different ways and/or on offspring methylation in different tissues. It will be interesting to explore in further work how maternal adiposity-associated exposures during pregnancy, such as gestational weight gain, maternal hypertension and hyperglycemia, influence offspring DNA methylation. Such pregnancy exposures may be more likely to have a pronounced intrauterine effect on offspring methylation and/or developmental programming of health outcomes than maternal adiposity at the start of pregnancy. Although previous studies in ALSPAC and MoBa did not identify any sites where gestational weight gain was associated with cord blood methylation, the question should be revisited in a consortium context.[21,91] Further

exploration is also warranted to assess the degree to which methylation in blood correlates with that in other tissues. DNA methylation shows strong tissue-specificity, for example, one study found that BMI was associated with DNA methylation in adipose tissue, but not in peripheral blood leukocytes.[92] Conversely, a large EWAS found that BMI was associated with methylation at HIF3A in both blood and adipose tissues.[93] The causal effect of maternal BMI on newborn methylation may be stronger in tissues other than blood. However, we note that in the context of this study, offspring blood might be considered a mechanistically relevant tissue: blood cellular heterogeneity and leukocyte methylation are strongly associated with inflammation, which is considered chronic amongst those with obesity.

There are several strengths to our study, including the large sample size comprised of established cohorts, the use of robust statistical methods, the comprehensive analysis of results and the application of causal inference techniques. Potential limitations include: i) adiposity is a complex trait that is only crudely and indirectly measured by BMI, therefore an investigation of more specific measures of adiposity might yield different results, ii) cohorts collected data on BMI in different ways (measured/self-reported) at different times (pre-pregnancy/ early pregnancy). However, measured and self-reported BMI before and during early pregnancy are strongly correlated, so we do not believe this will bias our results substantially.[94] iii) The analysis was completed before the widespread availability of any cord blood reference panels for estimations of cell counts, so all cohorts used an adult whole blood reference panel, which may introduce measurement error in cell count estimates.[48] However, in ALSPAC, one of the largest participating cohorts, we found that adjusting for cell counts generated using any one of three recently released cord blood reference panels produced results consistent with those produced using the adult whole blood reference. Nevertheless, we consider that there is likely to be at least some degree of residual influence of cell heterogeneity in our results. iv) We had very limited data with repeat measures in the same individuals at birth and adolescence, so we did not explore change in methylation over time in a longitudinal model. v) Cohorts used different methods to normalise data. However, a previous PACE analysis found that results obtained using raw betas were similar to those obtained using normalized betas generated with various methods, which indicates that this did not impact the inferences drawn from the meta-analysis, and at any rate, bias would tend to limit power rather than introduce spurious associations.[59] vi) Although we have presented two lines of evidence (consistent maternal and paternal estimates and the presence of meQTLs) that provide support for a genetic component in explaining associations between maternal BMI and newborn blood methylation at some sites, we were unable to formally quantify the relative contribution of genetics and the intrauterine environment. Techniques that attempt to do so, such as M-GCTA, require genetic and methylation data on larger sample sizes than

were available in any individual cohort.[95] vii) The Illumina 450k array only covers 1.7% of CpG sites on the human genome, and most of these are located in promoter regions. We found robust associations between maternal BMI and newborn DNA methylation despite this low coverage and bias. We therefore encourage more studies on this topic using more advanced EWAS platforms (such as the Illumina EPIC array). viii) Finally, it is possible that epigenetic markers other than DNA methylation in cord blood may be more closely associated with maternal BMI at the start of pregnancy, but this was not explored in this study.

In conclusion, in this well-powered study, we observed robust associations between maternal pre/early-pregnancy BMI and DNA methylation at 86 sites in the blood of newborns, some of which were reproduced in adolescents. However, effect sizes were very small, there was no evidence of biological functional enrichment, and causal inference strategies provided support for causal effects at just 8/86 sites. This study highlights that although some small studies report strong associations between prenatal exposures and epigenetics, large-scale collaborative efforts are necessary to identify robust associations, and causal inference strategies are needed to assess whether such associations are likely to be explained by a direct intrauterine effect or more likely due to genetic or shared environmental factors.

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Supplement

Supplementary Tables, Figures and cohort specific Methods are available at HMG online.

Chapter 4.2

Meta-analysis of epigenome-wide association studies in neonates reveals widespread differential DNA methylation associated with birthweight

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Chapter 5

General discussion

Introduction

Overweight and obesity are defined as the excessive or abnormal accumulation of fat in the body.[1] This may lead to various health problems including type 2 diabetes and cardiovascular disease.[2-4] The prevalence of adults, but also children, with overweight or obesity has reached a high level plateau in many high income countries, while still rising in low income countries.[1,5] This rapid increase in prevalence for low income countries, for example Asian countries, even seems to affect children more than adults.[5]

Adiposity is a complex disease, meaning that multiple factors contribute to its development. Well-known contributors to the risk of developing overweight or obesity are environmental and lifestyle factors such as a poor diet, physical inactivity, alcohol intake, smoking, and stress.[1,6] The risk of overweight and obesity may already be affected when the child is still in the womb. Maternal factors during pregnancy such as overweight, diabetes, smoking or the use of certain medications have been shown to be associated with risk of obesity in the child.[7] Promotion of a healthy pregnancy is therefore assumed to be important in reducing the risk of offspring adiposity.[7,8] However, this assumption only holds true when these factors appear causally related to childhood obesity. The exact mechanisms underlying these associations are still not entirely clear. DNA methylation has been put forward as a potential pathway.[9,10]

In addition to the well-known environmental and lifestyle factors, less is known about the genetic background of adiposity. BMI tracks from childhood into adulthood, indicating that in general children with a high BMI tend to have a high BMI as adults as well. Therefore, it is of great importance that we understand the factors that contribute to the onset of childhood overweight and obesity.[2,11,12] Many studies have focused on different candidate genes in relation to the development of overweight in early life and adulthood. Large genome-wide association studies on adiposity traits have also been performed in adults to identify genetic variants contributing to adiposity. Such large genome-wide association studies are very informative in unraveling the genetic background of adiposity. Nevertheless, none had been performed in children thus far.

The general aim of this thesis was to assess the associations of maternal lifestyle related, genetic and epigenetic factors with maternal adiposity and birthweight. The main findings have been presented and discussed in the previous chapters of this thesis. This chapter will provide a more general discussion of these findings, consider the general methodological issues, and provide suggestions for future research.

Interpretation of main findings

Maternal adiposity and offspring outcomes

Part of the origins of childhood adiposity may lie before birth.[10] Maternal pre-pregnancy BMI and gestational weight gain have been shown to be associated with offspring adiposity.[7,13,14] Within the Generation R Study it was shown previously that overweight and obesity of the mother is associated with offspring overweight and obesity at the ages of 4 and 6 years.[15,16] A weaker association was observed for gestational weight gain with offspring obesity.[15] Our results also showed associations of maternal pre-pregnancy BMI and gestational weight gain with childhood adiposity at the age of 10 years. We observed associations of maternal pre-pregnancy BMI with subcutaneous and visceral fat index in their children independent of childhood BMI. This suggests that the association is specific for accumulation in these abdominal depots and does not only represent the overall measure of BMI. The associations found for pre-pregnancy BMI with pericardial and liver fat index were not independent of BMI, suggesting that they are actually driven by BMI. In line with our findings a previous study showed that the offspring of obese mothers with gestational diabetes had increased liver fat already at the first month of life.[17] This finding was found to be independent of neonatal subcutaneous fat. Another study reported that a higher maternal BMI was associated with increased abdominal fat, independent of weight, and increased liver fat during infancy.[18] The latter association was not tested for independence of weight.

Maternal obesity before pregnancy was also associated with increased visceral fat in 1,228 Greek infants.[19] Interestingly, our results did not confirm earlier findings in the Generation R Study, which reported that maternal pre-pregnancy BMI was not associated with subcutaneous nor with preperitoneal fat at the age of 6 years, after correcting for the child's BMI.[16] These differences may be due to the difference in the age of the children at the time of the measurements or to the use of different imaging techniques. At the age of 6 years adiposity measures were determined using 2-dimensional imaging techniques such as dual-energy X-ray absorptiometry and ultrasound. Although these techniques can estimate preperitoneal fat as a proxy of VAT, they are unable to accurately distinguish different types of abdominal and organ fat.[20] A more recent and accurate approach that has been considered the golden standard is the use of 3-dimensional measurements by Magnetic Resonance Imaging (MRI), which is able to distinguish SAT, VAT, liver fat fraction and pericardial fat.[20,21] These measures are considered better markers for cardio-metabolic risk than the measures determined by the 2-dimensional imaging techniques.[22] We did not observe a consistent association of gestational weight gain with the specific fat measures, which was in line with previous results from the same cohort after additional

adjustment for childhood BMI at the age of 6 years.[8] We can therefore conclude from our observational study that gestational weight gain is less clearly associated with fat accumulation in organ specific depots than maternal BMI before pregnancy. A recent study showed little evidence for a causal effect of maternal BMI on offspring risk for fatness in childhood and adolescence.[23] This study used the Mendelian Randomization method to examine causality. When performing a Mendelian randomization analysis genetic variants are used as instrumental variable to examine the causal relationship between exposure and outcome.[24] Therefore, finding little evidence for a causal effect of maternal BMI on offspring fatness indicates that promoting a healthy life style for all family members may have a larger impact on offspring adiposity than a focus on maternal BMI only.

The exact mechanisms underlying fat accumulation at different sites of the body and the role of maternal obesity in this is not fully understood yet. Several processes, such as adipogenesis, hypothalamic appetite and feeding regulation, physical activity, and energy metabolism may be programmed when the fetus is still in the womb.[7,25] Therefore, the *in utero* period is of great interest for future adiposity research.

Genetics of childhood adiposity

Genome-wide analysis on childhood BMI

Genetic variants may play an important role in the development of childhood adiposity. A large GWAS on adult BMI in over 339,000 adults has estimated the contribution of common genetic variants to be up to 21%. The 97 SNPs identified in that study explain only 2.7% of the genetic variation in adult BMI.[26] Recently, an even larger GWAS on BMI in 681,275 adults, currently available as a pre-print only, identified 716 SNPs associated with adult BMI, explaining 5% of the genetic variation.[27] Despite the large number of SNPs identified for adult BMI, the modest percentage of variance explained by these SNPs indicates that additional research is still necessary to unravel the exact genetic background of overweight and obesity. Less is known about the genetic background of childhood adiposity. At the start of the work presented in this thesis, several studies have been performed on childhood obesity and BMI-associated phenotypes such as age at adiposity rebound, BMI at adiposity peak, and waist-hip ratio, but not on the full range of childhood BMI as a continuous measure.[28,29] We performed a large GWAS meta-analysis on childhood BMI including over 35,000 children aged 2-10 years.[30] We identified 15 SNPs of which 12 had been previously identified for adult BMI and/or childhood obesity, whereas three SNPs represented novel loci (Table 1). The number of SNPs identified for childhood BMI is much lower than that for adults. This may in part be explained by the lower sample size included in the childhood GWAS meta-analysis, a roughly 20 times difference. A larger sample size means more power to discover genetic variants with relatively small effect

sizes. Another explanation could be that only some of the SNPs identified for adult BMI are already important from childhood onwards, explaining the lower number of associated SNPs in children compared to adults. It would be plausible that largely the same loci are involved at both stages of life with potentially differing effect sizes per age. The variance in childhood BMI explained by the genetic risk score based on the 97 known adult BMI SNPs was lower than for a smaller set of 29 adult BMI SNPs from a previous smaller GWAS study, 1.5% and 2.4%, respectively.[31,32] We may therefore conclude that increasing our risk score to 97 SNPs introduced noise or that the lower variance explained is due to the differing magnitude or direction of effect sizes in children and adults. These age-specific effect sizes are observed for example for the *MC4R* locus showing a weak or even inverse association with BMI in early life as compared to adulthood.[29,33]

BMI based genetic risk scores and childhood growth measures

Childhood BMI is associated with obesity and cardiovascular disease in adulthood.[1-3] In addition, strong associations have been shown between infant weight growth patterns and BMI throughout life.[34-36] Additional knowledge on the genetic background and pathways involved in early-life adiposity may thus be important in understanding the pathology of obesity and cardiovascular disease. Previous work from the Generation R Study showed that a genetic risk score based on 29 adult BMI SNPs was associated with age at adiposity peak (AGEAP) in infancy and with BMI, total fat mass, android/gynoid fat ratio, and preperitoneal fat area at the age of 6 years.[31] The work performed in this thesis suggests that genetic risk scores based on adult BMI (97 SNPs based risk score), waist-hip ratio (WHR) and childhood BMI influence childhood adiposity outcomes from infancy onwards.[32] We found an association of the adult BMI risk score with BMI at adiposity peak (BMIAP), instead of with AGEAP which was found associated with the 29 adult BMI SNPs risk score in the same cohort as described above.[31] This discrepancy in association may be because the added SNPs may have been more representative of BMIAP than of AGEAP.[32] The associations observed for the adult and childhood BMI risk scores with childhood adiposity measures of BMI at 6 years, total fat mass, android/gynoid fat ratio, and preperitoneal fat area were comparable with previous findings.[31,32] Effect estimates were larger for the childhood BMI risk score as compared to the adult risk score, likely since the childhood risk score is more specific as the SNPs were both identified and tested for their association with BMI in children.[32]

Childhood BMI associated pathways

Previous adult BMI studies have highlighted the possible role of central nervous system (CNS) related processes in the predisposition for obesity.[26,37] One study found that several genes annotated to BMI-associated SNPs were either highly expressed or known to act in the

CNS.[37] Another study performed a pathway analysis based on 97 adult BMI-associated loci and observed a possible role for CNS related processes too.[26] The results presented in this thesis were in line with these previous studies, showing a strong role for the hypothalamic expression and regulation pathway, but also for the membrane proteins pathway and the monogenic obesity pathway. The pathways were more strongly associated with childhood adiposity measures than with infant adiposity measures, possibly due to the fact that the childhood measures are more closely related to adult BMI.

Body fat associated genetic variants and childhood fat distribution assessed by Magnetic Resonance Imaging

In the Generation R Study specific measurements of abdominal, liver, and pericardial fat assessed by MRI are available for children aged 10 years. We were the first to investigate the associations of SNPs identified for childhood and adult body fat with childhood fat distribution assessed by MRI. We observed an association for both the adult and the childhood BMI risk scores with SAT and VAT. The observed associations for the adult BMI risk score with SAT and VAT were less strong than its association with BMI, which suggests that not all BMI-associated SNPs necessarily play a role in SAT and/or VAT and that SAT and VAT may thus represent at least partially different phenotypes than BMI. Since VAT represents a more pathogenic phenotype than SAT in terms of later-life cardiometabolic disease, we would expect a higher BMI to be associated with higher VAT/SAT ratio.[38] However, our results showed the opposite, a higher genetic risk for increased BMI was associated with a lower VAT/SAT ratio. This may be explained by the stronger association of the adult BMI risk score with SAT than with VAT. The risk score based on SNPs identified for adult liver fat was found associated with childhood liver fat indicating that (some of) these SNPs included affect liver fat fraction already at a young age. We obtained similar results for the adult pericardial fat risk score with childhood pericardial fat, but only after adjusting our outcome measurement for BMI, indicating a specific association of the pericardial SNPs with childhood pericardial fat. Since the BMI risk scores were not associated with liver and pericardial fat, the accumulation of fat in the liver and pericardium during childhood may occur via pathways differing from those involved in the more general fat measures. Previous literature shows that sex has an influence on body fat distribution.[39-41] After stratifying on sex we indeed observed differences in associations with the adiposity measures. Interestingly, we showed that the association of the adult BMI risk score with VAT/SAT ratio we found in the full group was driven by the association in boys, which was not observed previously in prepubertal children.[41] Thus, genetic variants associated with BMI, body fat distribution, liver and pericardial fat may already affect body fat distribution during childhood.

Liver and steatosis associated genetic variants and childhood alanine transferase concentrations

Fat accumulation in the liver may lead to the development of cardiometabolic disease.[42] Once liver disease or damage is present, high concentrations of liver enzymes such as alanine-aminotransferase (ALT) may be measured in the blood.[43,44] A GWAS in 484 Korean children tried to identify SNPs associated with ALT concentrations in children, but only found subthreshold hits, probably due to a relatively low power.[45] In another GWAS in European-ancestry adults with a larger sample size of 45,596 four SNPs were identified that did not match the subthreshold hits of p-value $<1*10^{-5}$ in the Korean childhood study.[46] Reasons for this may be power, differences in ethnicity or different SNPs that play a role in adults and children.[46] We constructed a risk score based on the adult ALT concentration SNPs and observed an association of this risk score with ALT concentrations in childhood. This finding suggests that although these adult loci were not identified in the Korean GWAS in children, one or multiple of the adult SNPs already influence ALT concentrations from early life onwards. A plausible explanation would be that fatty liver disease, not caused by excessive alcohol consumption (non-alcoholic fatty liver disease (NAFLD)) tracks, which could be the result of a shared genetic background between children and adults as suggested by our results. However, although studies on the natural history of NAFLD are available in literature a gap in knowledge on the course of NAFLD between the ages 20-39 is unfortunately present preventing us to state with certainty that NALFD tracks.[47-49]

BMI associated genetic variants and eating behaviour in childhood

Previous studies have shown associations of childhood eating behaviour with weight, growth, and BMI. Thus, it may be that BMI-associated SNPs exert their effects via changes in eating behaviour.[50-57] We showed that a higher adult BMI risk score was nominally associated with a lower satiety response in children, which was in line with previous research.[58] The association diminished after additionally adjusting satiety responsiveness for BMI. This may be explained by the risk score being stronger associated with BMI and adjusting for BMI may therefore take away the weak association with satiety responsiveness, but it may also reflect a possible pathway from the SNP to eating behaviour through BMI. We also suggested a possibly stronger effect of certain childhood SNPs in Europeans since in this group the childhood BMI risk score but not the adult BMI risk score was associated with satiety responsiveness. The genetic variants in *LMX1B* and *BDNF* were also found nominally associated with satiety responsiveness independent of BMI. These genes were already known for their role in appetite regulation in adults.[59-61] Interestingly, *LMX1B* is involved in the development of serotonergic neurons, located in the CNS, again emphasizing the role of the CNS in BMI-related processes.[61] Overall, despite the association with satiety responsiveness, we were not able to find strong evidence that genetic variants associated

with BMI underlie eating behaviour. Although eating behaviour has been shown to be relatively stable between 2.5 and 9 years of age, in most cases food intake will still be controlled by the parents at these ages instead of by the children themselves.[62,63] The effect of BMI-associated genes on eating behaviour may therefore be more visible in older children, who have more control over what they eat.

Epigenetics of maternal adiposity and birthweight

Maternal prepregnancy BMI and offspring DNA methylation

Several studies have shown associations of maternal (pre)pregnancy BMI and cardiometabolic or developmental disorders in the offspring.[16,64-67] Common genetic variation may underlie part of these associations. Changes in DNA methylation may also play a role. Multiple relatively small studies have found associations of maternal BMI or adiposity with differential methylation at specific sites in the offspring.[68-73] In our study on maternal pre-pregnancy BMI and offspring methylation we were not able to replicate any of these reported associations on a genome-wide significant level, indicating that the potential for false positive findings should be considered when performing or interpreting small EWAS. In our larger sample size of 9,340 mother-newborn pairs, we identified a total of 86 robustly associated methylation sites, with small effect sizes. Nevertheless, small effect sizes may not necessarily mean little biological impact and small changes in methylation may influence gene transcription.[74] On the other hand it could also be that we did not find the sites for which effects may be larger, because the majority of our population concerned women within the normal weight category and not within the extremes, where larger effects may be expected. For some of the differentially methylated sites identified in our study, genetic variants identified in GWAS are located in the same loci that are associated with adiposity related traits, suggesting biological relevance of those loci.[75-78] We also investigated the consistency of the direction of the effect sizes over time and observed that for 72/86 sites the direction of effect remained stable from birth to adolescence. Even though none of the 72 hits survived multiple testing, p-values for 22/72 sites were below 0.05. Persistence of effects may suggest an intrauterine influence of maternal pre-pregnancy BMI on offspring DNA methylation persisting into adolescence, confounding by shared familial genetics or shared environment, or that both maternal BMI and the offspring's own BMI independently influence offspring methylation. Also, making this even more complex, a substantial part of observed variation in DNA methylation may be explained by genetics.[79] As discussed previously in this thesis, age-dependent effects have been observed for genetic variants located in for example the *FTO* and *MC4R* locus.[29,33] Possibly, such age-related genetic effects also influence DNA methylation and its subsequent effect on associated phenotypes.[80,81]

Causality is difficult to prove in methylation studies. When interested in a direct causal intrauterine effect, we would think that maternal, but not paternal, BMI would have such an effect. When comparing associations of maternal BMI with offspring methylation with those of paternal BMI, we found nine sites at which the effect of maternal BMI was stronger than that of paternal BMI making it more plausible that a direct intrauterine effect was present. For one out of these nine sites there was evidence that the effect was caused by a genetic effect shared between mother and child. For the remaining 8 sites we could state with more certainty that a causal intrauterine effect of maternal pre-pregnancy BMI on offspring DNA methylation was present (Table 1). We may therefore conclude that the majority of the observed associations may be better explained by genetic or lifestyle factors than a direct intrauterine effect.

Cord blood DNA methylation and birthweight

In addition to maternal pre-pregnancy BMI, other intrauterine exposures such as maternal smoking, hyperglycaemia and hypertension may also influence offspring health.[82-88] Observational studies have shown birthweight to be associated with later life health consequences such as cardio-metabolic and mental health, certain types of cancer and mortality.[85-88] Likely, birthweight is a proxy for a suboptimal intrauterine environment which leads to both a lower birthweight and adverse health in later life.[89] In an EWAs in 8,825 children, we found an association of DNA methylation in neonatal blood with birthweight at 1,071 methylation sites, of which 955 cytosine phosphate guanine (CpG) sites were considered suitable for further analysis. In contrast with the EWAS on maternal BMI and offspring DNA methylation, our results were largely in line with previous small EWAS on birthweight in three populations, which were also all part of our meta-analysis.[90-92] We did not find persistence of the association of DNA methylation with birthweight into adulthood, suggesting that the long term health effects are unlikely to be caused by persistence of differential methylation at specific sites at birth. Possibly these early methylation patterns result in structural or functional alterations in organs, which may predispose individuals to later poorer health. Alternatively, our lack of findings on persistence may be caused by limited power in the adult sample. In line with our findings, one of the three previous EWAS on birthweight compared cord blood methylation with methylation at the age of 7 and 17, but none of the methylation sites associated with birthweight persisted in to adolescence.[91] Mendelian Randomization analysis did not show a causal association with birthweight. For this Mendelian Randomization analysis 135 *cis*-methylation quantitative trait loci (*cis*-mQTLs; able to regulate methylation levels at specific CpGs) were used as instruments for a subset (127/955) of the birthweight associated CpGs. We did find a potential causal association for 9/127 CpGs with ten later-life diseases. However, results

should be interpreted with caution since genetic instruments were only available for a minority of the identified CpGs and for most of the 127 CpGs we only had one genetic variant available, making it impossible to distinguish causality from horizontal pleiotropy.

Table 1. Overview of genome-wide significant hits identified in this thesis using GWAS and EWAS.

SNP / CpG	Chromosome	Position	Nearest gene	Beta[a]	SE[a]	p-value
SNPs associated with childhood BMI						
rs13130484[b]	4	44870448	*GNPDA2*	0.067	0.007	$1.58*10^{-23}$
rs11676272[b]	2	24995042	*ADCY3*	0.068	0.007	$7.12*10^{-23}$
rs4854349[b]	2	637861	*TMEM18*	0.09	0.009	$5.41*10^{-22}$
rs543874[b]	1	176156103	*SEC16B*	0.077	0.009	$2.20*10^{-19}$
rs7132908[b]	12	48549415	*FAIM2*	0.066	0.008	$1.57*10^{-18}$
rs1421085[b]	16	52358455	*FTO*	0.059	0.007	$4.53*10^{-16}$
rs12429545[c]	13	53000207	*OLFM4*	0.076	0.01	$2.08*10^{-14}$
rs987237[b]	6	50911009	*TFAP2B*	0.062	0.009	$1.80*10^{-12}$
rs12041852[b]	1	74776088	*TNNI3K*	0.046	0.007	$2.28*10^{-10}$
rs6567160[b]	18	55980115	*MC4R*	0.05	0.008	$1.21*10^{-9}$
rs13253111	8	28117893	*ELP3*	0.042	0.007	$4.89*10^{-9}$
rs8092503	18	50630485	*RAB27B*	0.045	0.008	$8.17*10^{-9}$
rs3829849	9	128430621	*LMX1B*	0.041	0.007	$8.81*10^{-9}$
rs13387838	2	206989692	*ADAM23*	0.139	0.025	$2.84*10^{-8}$
rs7550711[d]	1	109884409	*GPR61*	0.105	0.019	$4.52*10^{-8}$
CpGs associated with maternal prepregnancy BMI and showing evidence for a causal relationship						
cg09243648	17	45944464	*SP6*	$-6.0*10^{-4}$	$9.85*10^{-5}$	$1.1*10^{-9}$
cg13403462	20	32256071	*NECAB3*	$-1.4*10^{-3}$	$2.30*10^{-4}$	$2.4*10^{-9}$
cg20594982	1	976707	*AGRN*	$-1.0*10^{-3}$	$1.70*10^{-4}$	$5.2*10^{-9}$
cg18144647	8	41113257	*SFRP1*	$-5.2*10^{-4}$	$9.13*10^{-5}$	$1.1*10^{-8}$
cg14528056	1	155194782	*GBAP1*	$5.1*10^{-4}$	$9.08*10^{-5}$	$1.5*10^{-8}$
cg01963618	6	1102332	*LINC01622*	$-3.5*10^{-4}$	$6.29*10^{-5}$	$2.4*10^{-8}$
cg05113927	2	27531244	*UCN*	$-6.1*10^{-4}$	$1.11*10^{-4}$	$3.9*10^{-8}$
cg05635274	16	2866901	*PRSS21*	$-4.8*10^{-4}$	$8.84*10^{-5}$	$5.0*10^{-8}$
CpGs associated with birthweight and showing evidence for a causal relationship						
None						

a From joint analysis. b Locus previously reported in [26]. c Locus previously reported in [26,93]. d Locus previously reported in [26,94]. e Difference in newborn DNA methylation beta value per 1 kg/m2 increase in maternal pre-pregnancy BMI.
CHR: Chromosome; CpG: cytosine phosphate guanine site; SE: Standard Error; SNP: single nucleotide polymorphism

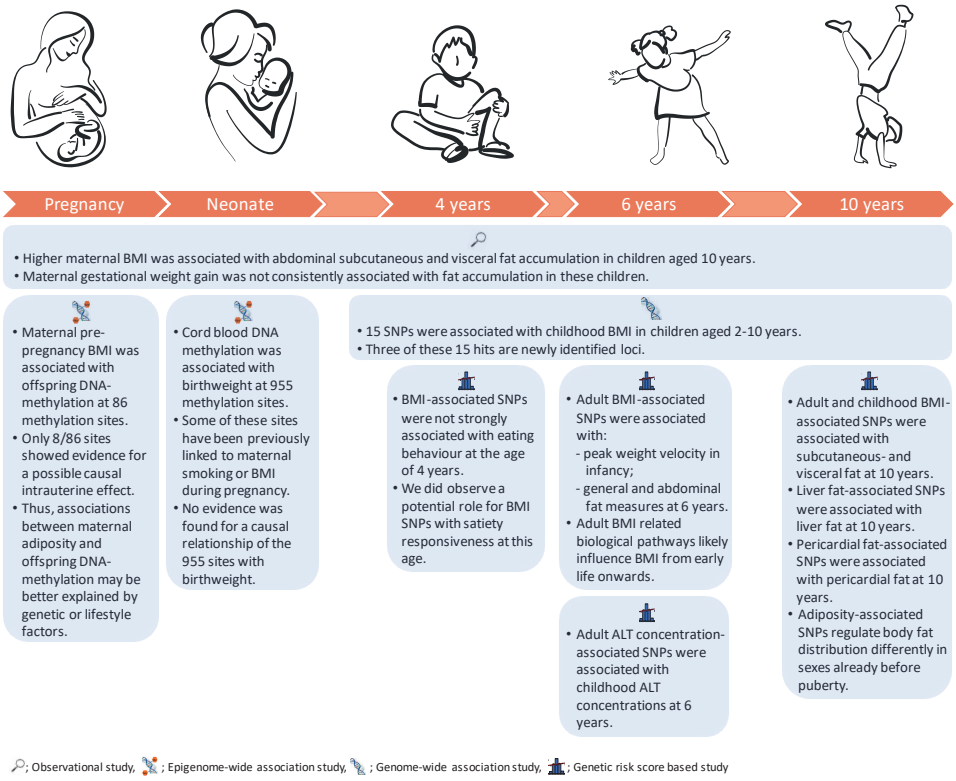

Figure 1. Presentation of the most important findings presented in this thesis.

Methodological considerations

The studies presented in this thesis were performed within the Generation R Study and the Early Growth Genetics (EGG) and the Pregnancy And Childhood Epigenetics (PACE) consortia. Study-specific strengths and limitations have been discussed in Chapters 2, 3, and 4 of this thesis. In the following paragraphs several general methodological issues regarding selection bias, information bias, confounding, causality, and specific issues in genetic and epigenetic studies will be addressed.

Selection bias

Selection bias may result from the procedure used to select study participants or factors influencing the study participation, leading to a difference in the relation between exposure and outcome for the subjects included in the analyses and the subjects that were eligible,

but not included.[95] This bias may have occurred within the Generation R Study due to either non-responders at baseline or loss to follow-up. Of all children eligible at birth 61% participated at baseline. When compared to the general population of Rotterdam, the included mothers were less often of ethnic minorities, had a higher socio-economic status, and less often had medical complications.[96] Genetic data were available for 59% of our total sample. Epigenetic data were obtained from a subgroup of Dutch children, comprising 14% of our total sample. Children with (epi)genetic data available had mothers with a slightly higher educational level and a slightly lower BMI, and had a slightly higher gestational age and birthweight than children without (epi)genetic data available. Results of analyses in the Generation R Study may therefore reflect a relatively healthier population than the general population of Rotterdam, thus potentially affecting external validity. Loss to follow-up occurred over time with participation rates of 85% of the original cohort at the age of 6 years and 76% at the age of 10 years. Reduction of the number of participants may lead to selection bias in case the loss of participants concerns a specific subgroup in which associations of exposures and outcomes may be different and overall leads to a loss of statistical power. Because of the slightly healthier population in the analyses presented in this thesis we may have reported a slight underestimation of the actual associations, which may have reduced the generalizability of our findings.

Information bias

Information bias arises from measurement error and can also be referred to as misclassification when analyzing discrete variables. Two types of information bias can occur: differential and non-differential misclassification. Differential misclassification may be present when the probability of being misclassified is non-random and dependent on the outcome or exposure. As a result, the outcome will be either an under- or overestimation of the true value. With non-differential misclassification participants have the same probability to be assigned to the wrong category. Non-differential misclassification is a random error not dependent on the exposure or outcome.[95] In most cases this will lead to an underestimation of the true value. The data used in this thesis were collected longitudinally and thus without previous knowledge of the research questions, making differential misclassification of the exposure less likely, whereas non-differential classification might have occurred. However, some differential misclassification may be present in this thesis when using self-reported pre-pregnancy BMI and maximum gestational weight gain as an exposure. Mothers with a higher weight may have underreported their BMI or weight gain, possibly resulting in an underestimation of the true effect. Similarly, over- and underreporting may be present in the parent reported child eating behaviours which were used as outcomes in our study. For all outcome measures used in this thesis measurement errors may have occurred. Anthropometric measures such as BMI or waist circumference

may have a slightly higher chance of measurement error as compared to the data obtained using dual X-ray absorptiometry, abdominal ultrasound or magnetic resonance imaging techniques which have been reported highly reproducible and accurate.[20,97-100] However, skilled personnel as well as advanced and well known methods were used to gather these data. Information bias may also be present in (epi)genetic studies presented in this thesis and will be discussed below.

Confounding

Confounding is present when all or part of the observed association between the exposure and the outcome can be accounted for by another variable affecting the outcome without being affected by the exposure itself.[101] When a confounder is present in an analysis it may influence the obtained effect estimate prohibiting finding the true effect of exposure on outcome. For genetic studies confounding is limited since the DNA is in principle not influenced by external factors. One factor necessary to correct for would be population stratification, since observed associations with the outcome may differ between subjects of different ancestral or demographic background within the study sample.[102] For the epigenetic studies in this thesis confounding may play a larger role since external factors introduced by for example genetics, technical measurement error (batch effects) or environmental factors may influence both the exposure and the outcome. We took into account many potential confounders, based on literature, the availability of data measurement in other cohorts, and confounding effect tested in our study sample, for which information was available in our dataset. In the risk score analyses our effect estimates were only mildly affected by adjustment for potential confounders such as height, which were included to correct for a potential remaining effect of these variables on our outcome measures. This may indicate that the observed effect estimates are close to representing the true effect. However for all studies in this thesis, the possibility of residual confounding by unknown or unmeasured variables should be taken into account.

Causality

For the studies presented in this thesis causality remains to be tested. For the epigenetic studies we explored causality by discriminating between the effects caused by paternal and maternal BMI on offspring DNA methylation and by performing an exploratory Mendelian Randomization analysis on the association of DNA methylation and birthweight. However, these studies should be interpreted with caution and further investigation is still necessary. The Bradford Hill criteria are often used to assess causality. The Bradford Hill criteria include examination of the strength, consistency, specificity, temporality, biological gradient, plausibility, coherence, experimental evidence, and analogy.[103] The

associations observed in this thesis are relatively weak, but weak associations may still be causal.[103] Our findings show consistency with other studies and the temporality of our studies is supported by the longitudinal nature of our data collection. In case of cross-sectional analyses, such as the EWAS on birthweight, we are unable to state with certainty that the exposure occurred before the outcome. In some of the studies in this thesis we found a possible biological gradient. A higher maternal BMI was associated with higher childhood BMI, subcutaneous, visceral and pericardial fat mass and liver fat fraction. We also discussed plausible mechanisms underlying our results and reported on coherence with animal studies. In GWAS genetic variants found are not necessarily located in or near the causal genes, although the identified SNPs are generally annotated to the nearest gene. Additional work such as functional analysis and animal models should be next steps in inferring which is the causal gene linked to the SNP. In contrast to GWAS, EWAS are more susceptible to confounding due to the dynamic nature of this process. Since DNA methylation may be an exposure as well as an outcome, or just a biomarker for the actual outcome of interest, causality is even more complex.[104] In the EWAS on maternal pre-pregnancy BMI and offspring DNA methylation we did try to infer a causal relationship by using a negative control design.[105] For eight CpG sites we found some evidence for a causal intrauterine effect of maternal BMI on offspring DNA methylation.

As explained above, another method to examine causality is an approach Mendelian Randomization. In our EWAS on DNA methylation and birthweight we applied Mendelian Randomization, using mQTLs as instrumental variables for the birthweight associated CpGs. For ten CpGs we found evidence for a possible causal association, but for none of the CpGs we were able to prove causality. In this thesis we were unable to test specificity, experimental evidence, and analogy. Considering that many of the Bradford Hill criteria for causality were addressed and some evidence was present for potential causality resulting from Mendelian Randomization analysis we have provided some careful evidence indicating causality.

Methodological issues in genetic and epigenetic studies

GWAS are a method often used to identify common variants associated with a phenotype of interest. Although GWAS enable us to examine possible associations at millions of sites in the genome, this concerns only the common genetic variants (allele frequencies >1%). The variance in BMI explained by common genetic variants identified so far is relatively low.[26] Possibly, identification of rarer genetic variants will give us more insight into the missing part of the genetic background of complex traits.[106] Rare genetic variants may have larger effect sizes than common genetic variants. Unfortunately, most studies focusing on rare variants are still underpowered.[106] In order to avoid the so called 'winner's curse',

which refers to overestimation of the true effect in the original study, replication of the obtained results should always be performed.[102] Another pitfall that should be considered is population stratification. Obtained associations with the outcome may differ between subjects of different ancestral or demographic background within the study sample.[102] To take this effect into account, we adjusted for principal components and applied genomic control in all GWAS presented in this thesis.[107] Since in GWAS a large number of independent tests are performed the possibility of obtaining false positive findings is relatively large. To reduce this type I error we applied the conservative Bonferroni correction to our data setting our statistical significance level to $0.05/1{,}000{,}000=5*10^{-8}$, where 1,000,000 represents the number of independent tests performed.

Limitations are also present for EWAS. One of the difficulties that has to be taken into account is the target tissue used for analysis. DNA methylation is tissue-specific and other tissues may be more representative of the phenotypes of interest than blood, which was used in the analyses in this thesis. Buccal samples can be used for methylation analysis too, are non-invasive and biological stability is slightly better for buccal than for blood samples, making them attractive for methylation analyses.[108] In population studies, especially those involving young participants, it is not possible to take tissue samples beyond blood or buccal samples.[108,109] Previous studies underline the difficulties in DNA methylation in relation to BMI between blood and other tissues. One study did not find an association of own BMI with DNA methylation in peripheral blood, but did find an association in adipose tissue.[110] Another study found an association of own BMI with *HIF3A* methylation in both these tissues.[111] For the DNA methylation studies in this thesis only blood samples were available and we cannot draw conclusions about the role of the identified DNA methylation sites in other tissues. In general, even if the DNA methylation sites identified in blood are not of mechanistic importance, DNA methylation at these sites may still be used as an indicator of an exposure. In addition, the composition of blood cells differs per sample and since DNA methylation is also cell type specific it is necessary to correct for the cell type composition. We therefore adjusted our analyses according to the statistical method suggested by Houseman and colleagues using a reference set based on the blood cell type composition of six adult males.[112,113] Recently three cord blood reference panels became available.[114-116] Sensitivity analyses in one of the individual cohorts (n=788) of the EWAS populations presented in this thesis did not show large differences when using any of the cord blood reference sets as compared to results obtained using the adult reference set.[114-117] We therefore consider it unlikely that the use of this reference set has introduced a larger bias than if the cord blood reference set had been used instead. A final methodological issue is the fact that the arrays used for the DNA methylation studies

described in this thesis cover 485,000 sites which is only 2% of the total DNA methylation sites.[109,118] The more recent EPIC array includes around 850,000 sites, including more enhancer regions, although overall coverage remains limited.[119]

Future perspectives

In this thesis, we identified 15 common genetic variants associated with childhood BMI using GWAS. Three out of these 15 variants were newly identified and together the variants explained 2% of the genetic variance in childhood BMI. For adult BMI the genetic variants identified thus far explain 5% of the variance in adult BMI, whereas a recent GWAS has estimated the variance explained by common genetic variation up to 21%, thus more remains to be discovered.[26,27] Additional information may lie within the rarer variants, gene-gene and gene-environment interactions, possibly associated with (childhood) adiposity. Techniques available to analyse the whole genome and to identify rarer variants are constantly evolving and will possibly lead to a better understanding of complex phenotypes. Further knowledge on BMI-associated genetic variants and interactions that are present will help us understand the underlying pathways and mechanisms for BMI but also fat storage and metabolism in more detail. Also, since age-related effects have been shown for certain adiposity loci, additional age ranges should be analysed to see whether effects of SNPs vary with age and how this may differ for different SNPs. Additional analyses on the causality of the obtained associations as well as on the underlying mechanisms may subsequently give us further insight into how genetic and epigenetic factors affect cardiometabolic health. To achieve this, analyses in larger numbers of children are needed. This way a better comparison can be made between SNPs associated with adiposity in children and adults as well as their possible overlap and effects throughout the life course.

Epigenetic research is a rapidly growing field. In this thesis we show that large numbers of CpGs were identified using EWAS. However, in both studies, additional analyses showed that for none or only a handful of these CpGs evidence for a causal relationship was observed. Additional research should further examine the causality of the CpGs found associated with maternal BMI and birthweight, and, in case of the CpGs associated with birthweight, whether differences in methylation at these CpGs are causally associated with later-life disease. Given that DNA methylation is variable over time, monitoring methylation sites at multiple time points in life and the use of narrow age-ranges may be of great value for the understanding of the phenotype of interest. Also, even larger sample sizes would be necessary to be able to identify additional CpGs with sufficient power. For the studies presented in this thesis we used blood samples. The choice of target tissue may also need further investigation. As in some studies already available, for future data collection the more stable buccal samples may be obtained and compared to methylation analysis in

blood samples at the same time point. Buccal samples have been suggested to be a more informative surrogate tissue for DNA methylation in other, less accessible, tissues than blood.[120] Further comparison of the results with DNA methylation in other tissues including fat, liver, brain and pancreas available in online databases should also be performed to conclude whether identified CpGs also play a role in tissues which cannot be examined in living subjects. Next to comparison of the extent of DNA methylation at a certain CpG, examining whether the CpGs relate similarly to the exposure or outcome as observed in blood or buccal samples is also of great interest. The use of a cord blood reference set including more than the currently available sets including a maximum of 17 subjects to control for cell type composition further enhances the accuracy of future EWAS.[116] In addition, it would be interesting to see which CpGs are found significantly associated with childhood BMI, whether these CpGs are causal, and how and if their effect varies throughout life.

Looking slightly beyond the scope of this thesis, more knowledge gained on the background of not solely this complex trait but also others, the better we will be able to predict, prevent, and treat these diseases. Future knowledge on the genetics and epigenetics of childhood and adult adiposity may help to predict with precision which people are at risk of becoming overweight. In addition, additional knowledge on adiposity may facilitate prediction of who will benefit more from certain medicine, surgeries, and lifestyle changes or be beneficial to the development of weight-regulating medicines. Although genetics may facilitate personalized medicine, the complex character of childhood adiposity, the fact that we can only clarify a minor part of childhood BMI with genetics thus far, as well as the accompanying ethical aspects should be considered carefully and limits the use of genetics for this purpose at the moment.

Conclusion

This thesis reveals new insights into the mechanisms and pathways underlying childhood adiposity. Multiple factors including BMI of the mother before and during pregnancy and specific genetic variants are associated with fat storage, regulation of energy homeostasis, and thus adiposity in children. We also found associations of maternal BMI and birthweight with DNA methylation. Causality of these associations remains to be further investigated. In addition, the effects are often small, but jointly they may help us to further unravel the environmental influences, genetic susceptibility and epigenetic mechanisms underlying childhood adiposity.

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Chapter 6

Summary
Samenvatting

Summary

In this thesis, we examined the genetic and epigenetic background of childhood adiposity. Little is known on the exact genetic variants, biological pathways, and mechanisms involved in the development of childhood overweight and fat deposition. Meanwhile, the prevalence of overweight and obesity is increasing, affecting both adults and children. A combination of environmental, lifestyle, and genetic factors may together contribute to the development of obesity. Some of these influences may already be present when the child still resides in the womb. Therefore, we examined whether maternal factors such as BMI, a measure for overweight (see Table 1), and weight gain during pregnancy are associated with specific sites of fat storage. In addition to the maternal factors, we tried to identify genetic variants associated with childhood BMI since no large scale GWAS had been performed on childhood BMI thus far. Identification of the genetic variants associated with childhood BMI could tell us more on the genetic background and if the identified variants showed overlap with those identified in adults. Subsequently, genetic variants previously identified for adiposity in adults and children were combined into genetic risk scores and tested for association with different child growth patterns, general adiposity measures, types of fat deposition, and eating behaviours. This approach showed if the presence of one additional risk allele for the exposure was associated with an increase or decrease in our outcome measure. Furthermore, we explored epigenetic mechanisms underlying childhood adiposity by performing genome-wide DNA methylation studies in relation to maternal BMI and birthweight. This thesis aimed to add knowledge on the mechanisms underlying childhood adiposity. This may contribute to the development of better prevention and treatment strategies for childhood adiposity and as such reduce the risk for cardio-metabolic disease in the future.

Chapter 1 provides the background and rationale of this thesis. The aims for all included studies are described and the outline of the thesis is given.

Chapter 2 describes the associations of maternal BMI during pregnancy and gestational weight gain with childhood adiposity at the age of 10 years. We observed associations for maternal pre-pregnancy BMI with body mass index, fat mass index, subcutaneous, visceral, pericardial, and liver fat (see Table 1 for definitions). Out of these, solely the associations for subcutaneous and visceral fat were independent of the child's BMI. Gestational weight gain was not found to be associated consistently with subcutaneous, visceral, pericardial and liver fat when tested independently of BMI. In conclusion, we found that a higher maternal BMI is associated with offspring abdominal fat accumulation independent of child BMI, and also with pericardial and liver fat, albeit dependent of child BMI.

Chapter 3 describes the genetics of childhood adiposity. In ***Chapter 3.1*** we performed a GWAS on childhood BMI. We identified 15 loci that reached genome-wide significance. Three of the identified loci were novel, including genetic variants located near *ELP*, *RAB27B*, and *ADAM23*. When combining the 15 SNPs into a genetic risk score it explained 2% of the genetic variance in childhood BMI. In conclusion, we showed the presence of a shared genetic background for childhood and adult BMI and identified three new childhood BMI associated loci. In ***Chapter 3.2*** we explored the associations of genetic risk scores based on known adult BMI and adult adiposity pathways with childhood growth and adiposity measures. The adult BMI risk score was associated with BMI at adiposity peak during infancy and with childhood BMI, total fat mass, android/gynoid fat ratio, and preperitoneal fat area at the age of 6 years. The risk scores based on the biological pathways were based on the 97 adult BMI SNPs that were previously identified by GWAS. We observed associations for the membrane proteins pathway risk score with infant peak weight velocity. Several biological pathway risk scores were associated with childhood adiposity measures, suggesting an important role for the central nervous system and hypothalamic related processes on body fat development from early life onwards. In ***Chapter 3.3*** we examined the associations of genetic risk scores based on SNPs identified for adult adiposity and childhood BMI with childhood subcutaneous (SAT), visceral (VAT), liver and pericardial fat. We found associations for the childhood BMI risk score with SAT and VAT. The adult BMI risk score was associated with SAT, VAT, and VAT/SAT ratio. The associations observed for the liver fat risk score and pericardial fat risk score with liver fat fraction and pericardial fat, respectively, suggest distinct biological pathways that underlie fat accumulation in these areas than for the more general adiposity measures. In addition, stratification on sex showed that the regulation of body fat distribution by adiposity-associated genetic variants may be different in boys and girls already before puberty. In ***Chapter 3.4*** we investigated the associations of genetic risk scores based on SNPs associated with childhood and adult BMI and ten individual appetite- and/or satiety-related SNPs with five categories of eating behaviour in children aged 4 years. No associations were observed for the childhood BMI risk score with any of the eating behaviours. The adult BMI risk score and two individual SNPs, one located in *BDNF* and one in *LMX1B*, were nominally associated with satiety responsiveness. Although no strong evidence was found showing that BMI-associated SNPs affect eating behaviour at this young age we observed a potential role for BMI SNPs in satiety responsiveness. In ***Chapter 3.5*** associations of adult alanine-aminotransferase (ALT) and non-alcoholic fatty liver disease (NAFLD) based risk scores with ALT concentrations in children aged 6 years were assessed. We found that the adult ALT risk score was associated with ALT concentrations in children. No association was observed for the adult NAFLD risk score with ALT in children. Our findings suggest that a genetic susceptibility for increased ALT concentrations already has effects in early life.

In conclusion, we identified 15 childhood BMI loci by GWAS including three newly identified genetic variants located near *ELP*, *RAB27B*, and *ADAM23*. Genetic risk scores based on childhood BMI and adult BMI SNPs showed that one or several BMI associated SNPs may play a role in BMI at adiposity peak during infancy and fat accumulation in the abdominal regions during childhood. We also suggested a possible role for BMI SNPs in satiety responsiveness. In addition, as for adult BMI, central nervous system related processes seem to be important for childhood BMI too. Pericardial and liver fat accumulation appear to be established via underlying pathways distinct from those underlying the more general adiposity measures. Associations found for the genetic risk score based on adult ALT SNPs suggested that one or multiple of these SNPs play a role in ALT concentrations already in early life.

Chapter 4 describes the identification of DNA-methylation sites associated with maternal BMI and birthweight. In ***Chapter 4.1*** we performed a meta-analyses on the association of pre-pregnancy maternal BMI and offspring DNA-methylation in cord blood. Maternal BMI was found associated with offspring DNA methylation at 104 sites after adjusting for estimated cell proportions. Out of 104 sites, 86 sites were found robustly associated, of which 72 showed the same direction of effect in adolescence. Evidence for a causal intrauterine effect was observed for only 8 of the 86 sites, suggesting most of the findings may be explained by genetic or lifestyle factors instead of a causal intrauterine effect. In ***Chapter 4.2*** we meta-analyzed the association of cord blood DNA methylation with birthweight across 19 different cohorts. We also tried to identify links with intrauterine exposures and later-life health. We identified 955 DNA methylation sites associated with birthweight. Analysis of these 955 DNA methylation sites in a relatively small sample of 7,278 participants found none to persist into adulthood. An exploratory Mendelian Randomization analyses did not suggest a causal role for the 955 sites in relation to birthweight, although a possible role for methylation at three methylation sites in relation to cardiovascular disease was observed. The association of DNA methylation with birthweight possibly links intrauterine exposures with birthweight and potentially with health outcomes later in life.

In conclusion, we found that maternal pre-pregnancy BMI is associated with variations in DNA-methylation at 86 different sites. Evidence for a causal intrauterine effect was present for only 8 of these methylation sites. We also found 955 methylation sites that were associated with birthweight, but for none of these we were able to infer causality in relation to birthweight. Exploratory Mendelian Randomization analysis did suggest a possible role for methylation at three of the birthweight associated methylation sites in relation to cardiovascular disease.

Finally, in **Chapter 5** a general discussion of all studies included in this thesis is provided. We also present methodological issues and suggestions and thoughts for future research.

In conclusion, findings from this thesis suggest that genetic susceptibility of childhood adiposity already has an effect in the early stages of life. Epigenetic mechanisms may play a role in mediating associations of intrauterine exposures and childhood adiposity outcomes, but more studies need to be done before strong conclusions can be drawn. The observed associations may form the basis of a better understanding of the underlying mechanisms of childhood adiposity.

Table 1. Definitions fat measurements

Term	Definition	Formula
Body mass index	Measurement for body wieght in relation to body length	Total weight in kg/length in m^2
Fat mass index	Measurement for the amount of body fat in relation to body length	Fat mass in kg/length in m^2
Subcutaneous fat	Fat located directly underneath the skin	-
Visceral fat	Fat located around the organs	-
Pericardial fat	Fat located around the heart	-
Android fat	Fat located around the belly	-
Gynoid fat	Fat located around the hips	-

SAMENVATTING

In dit proefschrift hebben we de genetische en epigenetische achtergrond van overgewicht bij kinderen onderzocht. Overgewicht en obesitas zijn belangrijke problemen, maar er is maar weinig bekend over de exacte genetische achtergrond en de biologische mechanismen die betrokken zijn bij de ontwikkeling van overgewicht en vetverdeling bij kinderen. Een combinatie van omgevingsfactoren, leefstijl en genetica draagt bij aan de ontwikkeling van obesitas. Sommige van deze factoren kunnen zelfs al een rol spelen als het kind nog in de baarmoeder zit. Daarom hebben wij onderzocht of factoren zoals body mass index ((BMI), een maat voor overgewicht (zie Tabel 1)), van de moeder en haar gewichtstoename tijdens de zwangerschap geassocieerd zijn met vetopslag op specifieke plekken in het lichaam van het kind. Daarnaast hebben we ook onderzocht welke genetische varianten (single nucleotide polymorphisms, SNPs) geassocieerd zijn met de BMI van kinderen, aangezien er nog geen grote genoom-brede associatie studie (GWAS) op BMI bij kinderen was uitgevoerd. De identificatie van genetische varianten die geassocieerd zijn met BMI bij kinderen kan ons meer vertellen over de mechanismen die leiden tot overgewicht. Wij hebben associaties onderzocht van genetische risicoscores, gebaseerd op de nieuw geïdentificeerde en reeds bekende genetische varianten voor overgewicht bij kinderen en volwassenen, met groeipatronen, overgewicht en obesitas, vetverdeling, en eetpatronen van kinderen. Tevens hebben we onderzocht of verschillen in DNA-methylatie mogelijk gerelateerd zijn aan BMI van de moeder en geboortegewicht. Dit proefschrift heeft als doel bij te dragen aan de kennis over de mechanismen die ten grondslag liggen aan overgewicht bij kinderen. Dit zou kunnen helpen bij de ontwikkeling van betere preventie- en behandelingsstrategieën voor overgewicht bij kinderen en zou zo in de toekomst het risico op cardio-metabole ziekten kunnen verlagen.

In **hoofdstuk 1** wordt de achtergrond van dit proefschrift besproken en de redenen om het onderzoek uit te voeren. De onderzoeksvragen voor alle geïncludeerde studies staan hier beschreven.

Hoofdstuk 2 beschrijft de associaties van BMI van de moeder en haar gewichtstoename tijdens de zwangerschap met overgewicht en vetverdeling bij kinderen op de leeftijd van 10 jaar. We vonden associaties van BMI van de moeder voor de zwangerschap met BMI en vetmassa-index, met subcutaan (SAT), visceraal (VAT) en pericardiaal vet en met levervet in kinderen (zie Tabel 1 voor definities). Van deze associaties waren enkel de associaties met subcutaan en visceraal vet onafhankelijk van de BMI van het kind. De gevonden associaties voor gewichtstoename tijdens de zwangerschap met subcutaan, visceraal, pericardiaal en levervet verdwenen na correctie voor BMI van het kind. Samenvattend hebben we in dit hoofdstuk laten zien, dat een hogere maternale BMI geassocieerd is met de hoeveelheid

buikvet bij het kind onafhankelijk van de BMI van het kind. Daarnaast is de BMI van de moeder ook geassocieerd met pericardiaal en levervet, maar deze associaties zijn wel afhankelijk van de BMI van het kind.

Hoofdstuk 3 gaat over de genetische achtergrond van overgewicht bij kinderen. In ***Hoofdstuk 3.1*** hebben we een GWAS op BMI bij kinderen uitgevoerd. We identificeerden 15 genoom-breed geassocieerde genetische loci. Drie van deze 15 loci, in de buurt van de genen *ELP*, *RAB27B* en *ADAM23*, waren nieuw. Samen verklaarden de 15 genetische varianten 2% van de variatie in BMI bij kinderen. Samenvattend laten we in dit hoofdstuk zien dat BMI bij kinderen en volwassenen een grotendeels overlappende genetische achtergrond heeft. Wel hebben we drie nieuwe loci geidentificeerd die geassocieerd zijn met BMI bij kinderen. Deze drie loci zijn mogelijk specifiek voor BMI bij kinderen of het effect van deze SNPs op BMI is groter voor kinderen dan voor volwassenen waardoor ze enkel voor kinderen en (tot op heden) niet voor volwassenen zijn geïdentificeerd. In ***Hoofdstuk 3.2*** hebben we de associaties onderzocht van genetische risicoscores gebaseerd op 97 SNPs die geassocieerd zijn met BMI en overgewicht bij volwassenen, met groei en overgewicht bij kinderen. De risicoscore gebaseerd op SNPs voor BMI bij volwassenen was geassocieerd met BMI tijdens de BMI-piek in de kindertijd en met BMI, vetmassa, de ratio van android (rond de buik) en gynoid (rond de heupen) vet, en preperitoneaal vet op de leeftijd van 6 jaar. Naast de risicoscore gebaseerd op alle 97 SNPs hebben we ook risicoscores gemaakt die gebaseerd waren op SNPs met een mogelijke rol in specifieke biologische mechanismen. We vonden een associatie van de risicoscore gebaseerd op SNPs met een rol in het "membraaneiwit-mechanisme" met de hoogste groeisnelheid in de kindertijd. Meerdere andere biologische mechanismen waren geassocieerd met overgewicht bij kinderen. Met name de processen in het centrale zenuwstelsel en de hypothalamus lijken al vanaf het vroege leven een belangrijke rol spelen bij de ontwikkeling van lichaamsvet. In ***Hoofdstuk 3.3*** hebben we de associaties onderzocht van genetische risicoscores gebaseerd op SNPs die een relatie hadden met overgewicht bij volwassenen of kinderen met SAT, VAT, lever en pericardiaal vet bij kinderen. We vonden associaties voor de risicoscore gebaseerd op SNPs voor BMI bij kinderen met SAT en VAT. De volwassen BMI risicoscore was geassocieerd met SAT, VAT en VAT/SAT-ratio. De levervet-risicoscore en de pericardiaalvet-risicoscore waren geassocieerd met respectievelijk levervetfractie en pericardiaal vet. Dit suggereert dat de biologische mechanismen die een rol spelen bij vetophoping rond de lever en het hart anders zijn dan de mechanismen die een rol spelen bij de meer algemene vetmaten zoals BMI, SAT en VAT. Toen we bij jongens en meisjes apart keken, zagen we dat mechanismen onderliggend aan de verdeling van lichaamsvet mogelijk al voor de puberteit verschillend is in jongens en meisjes. In ***Hoofdstuk 3.4*** onderzochten we de associaties van genetische risicoscores gebaseerd op SNPs die gerelateerd zijn aan BMI bij volwassenen en kinderen en van tien individuele SNPs gerelateerd aan eetlust en/

of verzadiging met vijf types eetgedrag bij kinderen van 4 jaar. Er werden geen associaties gevonden voor de risicoscore gebaseerd op BMI bij kinderen met deze eetgewoonten. De risicoscore gebaseerd op BMI bij volwassenen en twee individuele SNPs, een in het gen *BDNF* en een in het gen *LMX1B*, waren nominaal geassocieerd met verzadigingsrespons bij de kinderen. In ***Hoofdstuk 3.5*** onderzochten we de associaties van genetische risicoscores gebaseerd op SNPs die gerelateerd zijn aan volwassen alanine-aminotransferase (ALT) en non-alcoholic fatty liver disease (NAFLD) met ALT concentraties in kinderen. We zagen dat de volwassen ALT risicoscore geassocieerd was met ALT concentraties bij kinderen. We vonden geen associaties voor de volwassen NAFLD risicoscore met ALT concentraties bij kinderen. Onze bevindingen laten zien dat de aanwezigheid van een of meerdere risico verhogende genetische varianten gerelateerd aan ALT concentraties in volwassenen ook tijdens de vroege levensjaren kan leiden tot verhoogde ALT concentraties.

Samenvattend hebben we met behulp van GWAS 15 genetische loci geïdentificeerd die geassocieerd zijn met BMI bij kinderen, waarvan er 3, gelegen in de buurt van *ELP*, *RAB27B* en *ADAM27*, nog niet eerder waren beschreven. Analyses met genetische risicoscores gebaseerd op SNPs voor BMI bij kinderen en volwassenen lieten zien dat een of meer van deze SNPs mogelijk een rol spelen bij BMI tijdens de BMI-piek en bij vetopslag in de buik op de kinderleeftijd. We zagen ook een mogelijke rol voor BMI SNPs in verzadigingsrespons bij kinderen op de leeftijd van 4 jaar. Daarnaast lijkt het erop dat, net als bij volwassenen, bij kinderen processen gerelateerd aan het centrale zenuwstelsel ook een belangrijke rol spelen in de ontwikkeling van overgewicht bij kinderen. Pericardiaal- en levervetophoping lijken via andere onderliggende biologische mechanismen te ontstaan dan de meer algemene vetmaten zoals BMI en buikvet. De associatie voor de genetische risicoscore gebaseerd op volwassen ALT SNPs suggereerde dat een of meer van deze SNPs al vanaf de vroege levensjaren een rol spelen bij ALT concentraties.

Hoofdstuk 4 beschrijft de identificatie van sites in het genoom waar methylering geassocieerd was met BMI van de moeder en met geboortegewicht. In ***Hoofdstuk 4.1*** beschrijven we een meta-analyse waarin gekeken is naar de associatie van BMI van moeders voor de zwangerschap en DNA methylatie in navelstrengbloed van hun kinderen. Na correctie voor de geschatte witte bloedcelproporties in het bloed was BMI van de moeder geassocieerd met DNA methylatie op 104 verschillende sites in het genoom. Van de 104 sites waren er 86 robuust geassocieerd. 72 van deze 86 sites toonden dezelfde richting van het effect in jong volwassenen. Voor slechts 8 van de 86 sites vonden we bewijs voor een direct causaal intra-uterien effect, wat suggereert dat het gros van de gevonden associaties wellicht beter verklaard kan worden door genetica of leefstijl die gedeeld worden door moeder en kind. In ***Hoofdstuk 4.2*** meta-analyseerden we de associatie van DNA methylatie in navelstrengbloed met geboortegewicht in 19 verschillende cohorten. We probeerden

tevens om verbindingen aan te tonen tussen methylatie en intra-uteriene blootstellingen en tussen methylatie en gezondheid later in het leven. We identificeerden 955 sites in het genoom waar methylatie geassocieerd was met geboortegewicht. Verdere analyse van deze 955 DNA methylatie sites in een relatief klein sample van 7,278 deelnemers wees uit dat geen van deze sites tevens geassocieerd waren in volwassenen. In een verkennende Mendelian Randomization analyse vonden we geen causaal effect voor de 955 sites in relatie tot geboortegewicht. Wel was er een mogelijke rol voor methylatie op drie methylatie sites in relatie tot cardiovasculaire ziekten te zien, maar die bevinding moet nog in meer detail onderzocht worden. We zagen ook dat geboortegewicht geassocieerde methylatie sites overlapten met methylatie sites welke eerder geassocieerd bleken met roken van de moeder tijdens de zwangerschap en met BMI van de moeder tijdens de zwangerschap. De associatie van DNA methylatie met geboortegewicht is een mogelijke verklaring voor de link van intra-uteriene blootstellingen met geboortegewicht en met gezondheidsuitkomsten later in het leven.

Samenvattend vonden we dat BMI van de moeder voor de zwangerschap geassocieerd is met DNA methylatie op 86 verschillende sites. We zagen bewijs voor een causaal intra-uterien effect voor slechts 8 van deze methylatie sites. We vonden tevens 955 sites in het genoom waar methylering geassocieerd was met geboortegewicht, maar we hebben voor geen van deze sites een causaal verband in relatie tot geboortegewicht aan kunnen tonen.

Tot slot wordt in **Hoofdstuk 5** een algemene discussie over alle studies in dit proefschrift gepresenteerd. Ook behandelen we hier de methodologische aspecten en suggesties voor verder onderzoek.

Op basis van de bevindingen in dit proefschrift kunnen we stellen dat genetische varianten geassocieerd met overgewicht bij volwassenen en/of kinderen al op jonge leeftijd een rol spelen. Mogelijk spelen ook epigenetische mechanismen een rol bij het mediëren van de associaties van intra-uteriene blootstellingen en overgewicht bij kinderen. Desondanks moeten er meer studies worden uitgevoerd voordat er sterke conclusies getrokken kunnen worden op dit gebied. De geobserveerde associaties kunnen echter wel een basis vormen voor een beter begrip van de mechanismen die betrokken zijn bij overgewicht in kinderen.

Tabel 1. Definities vetmetingen

Term	Definitie	Formule
Body mass index	Maat om het gewicht in relatie tot lichaamslengte weer te geven	Lichaamsgewicht in kg/lengte in m^2
Vet massa index	Maat om de hoeveelheid lichaamsvet in relatie tot lichaamslengte weer te geven	Vetmassa in kg/lengte in m^2
Subcutaan vet	Vet dat is opgeslagen direct onder de huid	-
Visceraal vet	Vet dat is opgeslagen rond de organen	-
Pericardiaal vet	Vet dat is opgeslagen rond het hart	-
Android vet	Vet rond de buik	-
Gynoid vet	Vet rond de heupen	-

Chapter 7

Abbreviations
Publication list
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PhD portfolio
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Generation R