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Influence of Maternal BMI on Offspring BMI from In-utero to Adolescence and offspring BMI trajectories via Epigenetic Changes

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INFLUENCE OF MATERNAL BMI ON OFFSPRING BMI FROM IN-UTERO TO ADOLESCENCE AND OFFSPRING BMI TRAJECTORIES VIA EPIGENETIC CHANGES

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Abstract

The impact of maternal body mass index (BMI) on offspring health outcomes such as obesity has been widely investigated, with evidence suggesting that in-utero conditions may influence DNA methylation (DNAm) and BMI developmental trajectories. However, it is unclear whether differential DNAm on sites with a cytosine followed by a guanine linked by phosphate dinucleotide (CpG) related to gestational BMI are linked to BMI changes in offspring. Using data from the Isle of Wight birth cohort, UK, this study aims to investigate BMI trajectories and address the role of DNAm.

Using trajectory analysis four distinct BMI developmental trajectories - 'normal' (n= 1042), 'early persistent obesity' (EPO, n=61), 'early transient overweight' (ETO, n=185), and 'delayed overweight' (DOW, n=149) - that spanned first 26 year of life were identified. The trajectories were found to be influenced by gestational BMI.

To identify CpGs related to gestational BMI academic database were explored and 1,090 CpGs were found in the IOW cohort data as candidate CpGs for specific aims 2 and 3 out of 1,773 differentially methylated CpGs reported in prior investigations. Fourteen of these candidate CpGs were found to be significantly associated with BMI trajectories, two survived multiple testing. Higher methylation of cg23089913 (*NANOS1* gene) was associated with decreased odds of being in the EPO trajectory with an odds ratio of 0.84 (95%CI: 0.76-0.93). In contrast, increased methylation of cg13217064 (*SOX14*) was associated with a 1.4 times higher odds (95%CI: 1.13-1.67) of being in the DOW compared to the 'normal' trajectory.

Finally, associations between candidate CpGs and repeated BMI measurements from infancy to 26 years of age were investigated.Five CpGs - cg00488692 (*SP3*), cg14434213 (*RNF5P1*), cg23089913 (*NANOS1*), cg26862527 (*BAI3*), and cg17812850 (*TMEM184C*) were found to be statistically significantly linked with BMI. Female participants exposed to prenatal

paternal smoking and mixed feeding during infancy had a higher BMI, while male participants with lower birth weight had 0.4 kg/m^2 higher BMI.

The study identified candidate CpGs on genes critical to metabolic disorders and provides a basis for further investigations to understand the biological role of DNAm sites in BMI development.

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List of Abbreviations

CpG: Cytosine-phosphate-Guanine

DNAm: DNA methylation

IOW: Isle of Wight

IOT: International Obesity Taskforce

EWAS: Epigenome-wide association studies

NT: Normal Trajectory

EPO: Early Persistent Obesity

ETO: Early Transient Obesity

DOW: Delayed Overweight Trajectory

SOX14: sex-determining region on Y box gene

BAI3: Brain-Specific Angiogenesis Inhibitor 3 gene

RNF5P1: Ring Finger Protein 5 Pseudogene 1 gene

ADGRB3: Adhesion G Protein Couples Receptor B3 gene

GIPR: Glucose-dependent insulinotropic polypeptide receptor gene

TMEM184C: Transmembrane protein 184c gene

SP3*:* Specificity Protein transcription factor gene

NANOS1: Nanos C2HC-Type Zinc Finger 1 gene

Chapter 1

1. Introduction

Over the last two decades, obesity and being overweight have become a major public health crisis, with a global increase in childhood obesity [1]. The prevalence of obesity has risen to 23.8% and 22.6% in boys and girls, respectively, in developed countries, and 12.9% and 13.4% in boys and girls, respectively, in developing countries [2]. In children, the United States is one of the 34 countries with the highest prevalence of overweight (25.1%) and obesity (6.8%) while Lithuania showed the lowest prevalence of overweight and obesity in school-aged children [3].The prevalence of obesity varied with age, gender, race/ethnicity, and region [1-4].

Children who are overweight in childhood tend to be obese in their adulthood [5]. In children, obesity was also found to be associated with chronic comorbidities such as elevated cholesterol levels which may lead to adverse cardiac health outcomes and elevated blood pressure [6], increased risk of diabetes and a certain type of cancers in adults [7]. There is a 10-fold increased risk of Type II diabetes (non-insulin dependent diabetes mellitus) in obese children aged 5–10 years [8]. Obesity does not only impact the individual's health but also increases the economic burden for individuals, families, and healthcare systems. Of a countries' health care cost, 0.7% to 2.8% is attributable to obesity-related health issues. Individual's medical costs of obese individuals are approximately 30% higher compared to normal individuals [9]. It has been speculated that the medical cost associated with obesity will increase to $44 - 66 billion/year and £1.9 – 2 billion/year in US and UK by 2030 [10]. Given the impact of childhood obesity on obesity-related health outcomes in adulthood and economic burden on individual families and health care system, identifying risk factors that are related to obesity in early childhood is critical. These risk factors may assist to identify the children who are at higher risk of becoming obese adults and develop health promotion strategies.

Intrauterine, perinatal, and early childhood risk factors

Maternal smoking during pregnancy, maternal pre-pregnancy body mass index (BMI), birth weight, and formula feeding are a few known major risk factors for childhood and adolescence obesity [11, 12]. For a population-based birth-cohort in Brisbane, Australia, it was reported that the prevalence of obesity at the age of 14 years was higher in offspring's of the mothers who smoked during pregnancy [13]. Similar effects were observed with maternal pre-pregnancy BMI and offspring BMI. Maternal pre-pregnancy BMI is an independent risk factor for offspring obesity and obese mothers often delivered infants with high birth weight [14, 15]. A prospective study by Riley et al [11] and Pirkola et al [16] showed that maternal pre-pregnancy BMI was positively associated with the offspring BMI in early childhood (7 years of age) and at age 16 years suggesting the long-term influence of maternal pre-pregnancy BMI. Epidemiological studies to date have demonstrated a direct association between the maternal pre-pregnancy BMI and offspring BMI, but the underlying mechanism is unknown. Hence, it is important to investigate links between the maternal pre-pregnancy BMI and offspring BMI in next generation.

Trajectories of childhood obesity

Cross-sectional and/or longitudinal studies often have focused on the specific period of life. Only recently, the interests on individual development pattern of BMI, called trajectories, has increased. Analyses of trajectories of BMI will help to identify whether individual trends in BMI leading to obesity later in life are related to early life risk factors. For instances, early life unhealthy eating may predict obesity in later life [17]. Hence, modifying eating habits in early childhood may modify the obesity risk in later life. A longitudinal study from rural upstate New York [18] demonstrated that early childhood poverty influences BMI trajectories, i.e., children who experienced poverty in early childhood are more likely to gain weight and tend to be obese in their adolescence [18]. Interestingly, BMI trajectories can also predict later life mortality; and

the effect of these associations seems not to be confounded by the race or sex [19]. In addition, Huang et al reported that developmental trajectories of childhood obesity in children aged 6 to 18 are not influenced by adolescence behaviors such as smoking, alcohol consumption etc. [20]. Hence, these findings based on longitudinal studies suggest that the BMI trajectories are set early, e.g., before the age of 6 years, and may not be influenced by race and/or behavioral changes at a later age. In conclusion, identifying the age at which the BMI trajectories are initiated and associated with modifiable risk factors (intrauterine or early childhood) will provide a unique opportunity to modify these risk factors and implement health promotion strategies very early in life.

BMI trajectories from infancy to 18 years of age had been investigated in population birth cohorts and the long-term effects of prenatal exposures such as maternal smoking during pregnancy on these trajectories were demonstrated [21]. In the Isle of Wight birth cohort study, four BMI trajectories (normal, early persistent obesity, delayed overweight and early transient overweight) spanning age 1 to 18 years were identified [21]. These findings suggest that children who were obese (early persistent obesity group) or normal, respectively, by the age of four tend to be obese or normal at ages 10 and 18 years while the other two groups remained transient. However, it is yet to be understood if these BMI trajectories can be extended to adulthood until age of 26 years.

Findings from the Isle of Wight birth cohort also demonstrated that offspring's BMI trajectories were influenced by maternal smoking during pregnancy, explaining the critical role of intrauterine exposure that tunes the offspring obesity risk from birth to adolescence [21]. As highlighted above [11, 16], maternal pre-pregnancy BMI is associated with the offspring BMI in early childhood (age 7 years) and adolescence (16 years of age). However, the influence of maternal pre-pregnancy BMI on the offspring BMI trajectories from infancy to adolescence has not been studied yet. It is important to investigate the relationship between pre-pregnancy

maternal BMI and offspring BMI trajectories and to better understand the intrauterine and long-term effects of maternal pre-pregnancy BMI on offspring BMI.

Intrauterine exposures may initiate epigenetic modification in offspring

Molecular mechanisms such as, but not limited to epigenetic processes-DNA methylation (DNAm), histone modifications, and gene expression are known to play a critical role in the individual's health [22, 23]. These markers can be altered by environmental exposures [24, 25] and establish a memory of past exposures [26]. DNAm, the addition of a methyl group to a cytosine followed by a guanine linked by phosphate dinucleotide (CpGs) [27], is one of the epigenetic processes that has been extensively studied. DNAm programming can be initiated *in utero* and the offspring may carry a memory of DNAm markers related to intrauterine exposures. For instances, maternal smoking during pregnancy influences differential methylation at birth (cord blood) in offspring [28] and such effects can continue into later childhood [29]. A large prospective birth cohort in South West England showed persistent differential methylation patterns at some CpGs, observed at birth, age seven, and 17 years, that were associated with maternal smoking during pregnancy [30].

Lawlor et al [31] reported that maternal obesity is linked to higher BMI in offspring, and suggested this connection may be explained by epigenetic changes. A systematic review yielded comparable results, indicating that epigenetic memories developed in the fetal and early life environment can impact BMI in later life. [32]. These findings emphasize the significance of epigenetic mechanisms, which could provide the connections between maternal pre-pregnancy factors and BMI in offspring. In 2009, Gemma et al found a positive relationship between methylation of the promoter of the peroxisome proliferator-activated receptor γ coactivator 1 α gene (PPARGC1A) in umbilical cord blood cells and maternal BMI [33]. An epigenome-wide association study (EWAS) in 2014 using a 27K bead chip identified 20 CpGs that were differentially methylated in offspring of obese mothers compared to those of normal-weight

mothers [34]. In further studies, the epigenome-wide assessments covered more CpGs: Illumina Infinium 450k bead chip covers ~1.6% of the genome with provided information on > 450,000 CpGs. More recently, using the Illumina Infinium 450k bead chip, Sharp et al [35, 36] identified various epigenetic markers (1,649) associated with maternal pre-pregnancy BMI in offspring DNAm at birth by assessing over 450,000 CpGs, which represents about 1.6% of the genome. Authors replicated the findings from Gemma et al [33] and Liu et al [34] and observed a consistent direction of association between maternal BMI and the levels of differential methylation in offsprings [35].

The results mentioned above were reinforced by a recent meta-analysis of data from the 450k bead chip conducted by the PACE consortium (pregnancy and childhood epigenetics) across 19 cohorts. This study provided information on maternal BMI at the beginning of pregnancy and DNAm in umbilical cord blood, and it found that 104 CpGs were differentially methylated in offspring cord blood and influenced by maternal BMI at the beginning of pregnancy [36]. It is worth noting that, despite the findings of the meta-analysis, there was no overlap between the CpGs identified in the analysis and those found in previous studies. The author of the meta-analysis suggested that the inconsistency in results could be due to false positive findings and limited statistical power of the small EWAS.

Although several studies have shown a link between maternal pre-pregnancy BMI and DNAm levels, none of these studies have investigated the potential impact of these changes on offspring BMI and offspring BMI trajectories from infancy to adolescence. For instance, the question: “Can differentially methylated CpGs, which are associated with maternal BMI, predict offspring BMI developmental pattern?” has not been answered yet.

To provide insights into extension of BMI development developmental trajectories from 18 years of age to 26 years, association between epigenetic memories and offspring development of BMI

and BMI measurements covering first 26 years of life, this dissertation will test the following hypotheses:

H1: Offspring BMI trajectories between age 1 and 18 years based on weight and height measurements taken at ages 1, 4, 10, and 18 years, can be extended to cover up to 26 years of age. Furthermore, these BMI development trajectories are impacted by maternal pre-pregnancy BMI (**Phenotypic association hypothesis**).

H2: Epigenetic alterations at birth, which are linked to maternal pre-pregnancy BMI, may predict BMI trajectories (1-26 years) in offspring based on height and weight measured at 1, 4, 10, 18, and 26 years, demonstrating in early initiation of the BMI development (**Early initiation hypothesis**).

H3: Epigenetic changes at birth, that are related to maternal pre-pregnancy BMI, are associated with BMI of the offspring at different stages of development - age1, 4, 10, 18, and 26 years, demonstrating long-lasting effects on the health outcomes of offspring. (**Development origin of Health and Disease hypothesis**).

Data from the Isle of Wight birth cohort study (n= 1,456) initiated in 1989 on the Isle of Wight, will be used to test the above-mentioned hypotheses H1 to H3. In this birth cohort, participants were enrolled at birth and followed prospectively at ages 1, 4, 10, 18 years, during pregnancy (F1), and at 26 years.

The dissertation has the following specific aims:

SA1: Phenotypic association: To determine if the BMI developmental trajectories identified in the first 18 years life can be extended to 26 years of age. To investigate whether maternal pre-pregnancy BMI is associated with extended BMI trajectories in offspring.

SA2: Epigenetic link to BMI trajectories: To test whether epigenetic signatures in F1 (DNA methylation at birth) related to maternal pre-pregnancy BMI (F0) is related to offspring BMI trajectories from age 1 to 26 years based on the weight and height measures at ages1, 4, 10, 18, and 26 years.

SA3: Epigenetic link to BMI at different ages of offspring: To determine whether differential DNAm at birth related to maternal pre-pregnancy BMI (F0) is associated with repeated measurements of offspring BMI measures at ages 1, 4, 10, 18, and 26 years.

Chapter 2

2. Developmental BMI trajectories of first 26 years of life and influence of maternal prenatal BMI – a birth cohort study

Introduction

Obesity is a prevalent and complex health issue that has become a major public health concern globally. The prevalence of obesity has been steadily increasing over the past few decades and has been associated with numerous health risks such as diabetes, cardiovascular disease, and various cancers. [2, 3, 6, 7, 8, 10]. Understanding the dynamics of obesity and overweight from children to young adults will provide information on critical ages for prevention.

BMI trajectories, which describe the patterns of weight gain and loss over time, have been shown to be an important predictor of obesity-related health outcomes. A study conducted in rural upstate New York on a longitudinal basis found that experiencing poverty in early childhood can influence BMI trajectories, resulting in children gaining more weight and being more likely to become obese during adolescence. [18]. It is worth noting that BMI trajectories can serve as an indicator of mortality risk in later life, and these associations are not affected by factors such as race or sex [19]. Understanding the factors that contribute to BMI development and its trajectories may aid in the development of effective interventions to modify the risk of overweight and obesity in children.

In the Isle of Wight birth cohort (IOWBC) study, researchers investigated BMI trajectories from infancy to 18 years of age and identified four distinct patterns of BMI changes, including normal, early persistent obesity, delayed overweight, and early transient overweight [21]. The study found that children who became obese or remained normal could be detected by the age of four, while the other two groups (delayed overweight and early transient overweight) remained transient. Using this cohort, we will examine whether BMI trajectories change between

18 and 26 years of age or whether the information obtained in early life remains valuable until the age of 26.

It has been established that both maternal smoking and maternal pre-pregnancy body mass index (BMI) increase the likelihood of offspring obesity during the prenatal period. Studies have shown that infants born to obese mothers tend to have a high birth weight, which is a factor that increases the risk of childhood obesity [14, 15]. Research by Riley et al [11] and Pirkola et al [16] found that maternal pre-pregnancy BMI has a lasting impact on offspring BMI, positively influencing it during early childhood (at 7 years old) and at age 16. This highlights the need to determine if this influence extends to offspring during adolescence and beyond. The IOW birth cohort study also found that offspring's BMI trajectory is impacted by maternal pre-pregnancy BMI and smoking during pregnancy, emphasizing the significant role of prenatal exposure in determining the risk of offspring obesity from birth to adolescence [21]. However, it remains to be seen if gestational BMI continues to influence offspring BMI trajectory into adulthood.

Considering these gaps in the literature, this study aims to investigate (1) whether existing BMI trajectories reported by Ziyab et al., [21] from birth to age 18 years can be extended to adulthood, i.e., age 26 years or if their patterns change; (2) whether offspring BMI trajectories extended to 26 year are still influenced by maternal pre-pregnancy BMI. We will use the data from IOWBC, established in 1989 IOW, UK, with provided information on the BMI from infancy to adulthood and gestational BMI.

Methods

Study population

The IOWBC was established to study the natural etiology of allergic diseases on Isle of Wight, UK in January 1989. Parents of 1,536 children born between January 1, 1989, and February 28, 1990, were invited to enroll their children into the study. Of these 1,536 children, after written consent, 1,456 newborns were enrolled at birth and followed at ages 1, 2, 4, 10, 18, and 26 years of age. Detailed questionnaires were obtained from the parents of these children at 1, 2, 4, and 10 years of age; self-administered questionnaires were obtained from the participants themselves at ages 18 and 26 years. The birth cohort has been explained in detail elsewhere [37, 38]. The study population is 99% Caucasian. The investigation was approved by the local ethics committee –National Research Ethics Service, NRES Committee South Central – Hampshire B, U.K. and by the University of Memphis Institutional Review Board in Memphis, U.S (#2423). Written informed consents were obtained from the parents or participants at each follow-up.

Phenotypes

BMI measurement from infancy to 26 years of age

Height and weight of the children were ascertained at each follow-up visits at ages 1, 4, 10, 18, and 26 years. BMI is calculated as the weight (kilogram) to height (meter^2) ratio and BMI information was used for the analyses. For descriptive purposes, BMI was converted to z-BMI scores to increase the comparability across the ages for research purpose [39]. Children are considered as obese or overweight with respect to the age-specific BMI thresholds as specified by International Obesity Taskforce (IOTF) [40] for 4, 8, 18, and 26 years of age while for age 1 years World Health Organization (WHO) [41] standards were considered.

Exposure of interest

Maternal pre-pregnancy BMI is considered as the main exposure of interest. Maternal BMI at 14 weeks of pregnancy was used for our analysis. The height and weight of the mother were obtained from hospital record provided during prenatal visits. Prenatal maternal BMI is considered as a continuous variable in our analyses.

Covariates

Potential confounders were identified from prior literature based on their plausible associations with the outcome of interest. Information on the gestational age at birth (weeks), birth weight (kilograms), maternal age at delivery (years), and gender were collected soon after the birth from hospital records. Duration of breastfeeding (weeks), birth order, and age when the formula was introduced (weeks) were collected at the 1- and 2-years follow-up visits. Second-hand smoke exposure at ages 1,2, 4, and 10 years, were obtained from the follow-up questionnaires completed at respective ages. Personal smoking history at age 18 and 26 years were obtained from the self-administered questionnaires.

Statistical analysis

Developmental BMI trajectories from infancy to age 26 years

A group-based trajectory analyses using the procedure PROC TRAJ macro [42] in SAS (9.4) was implemented to identify the different BMI trajectories. BMI z-scores were used for trajectory analysis and individuals with no BMI information at all ages (infancy to 26 years of age) were excluded from the trajectory analyses. Groups of sizes 2 to 4 were tested. Standard recommendations were followed for the best model selections: A maximum likelihood approach with censored normal model were used to estimate the trajectory parameters. Bayesian Information criteria values were used to select the best model as it summarizes the distinctive features of the trajectories with minimal overlap in the confidence intervals among the adjacent

trajectories. Models with smallest Bayesian Information criteria value and individuals group membership probabilities of at least 0.7 for each individual [43] in each group were considered as the final model. Following the identification of the final model, individuals were categorized into trajectories based on their highest estimated membership probabilities. The trajectories were then used in further analytical steps. We tested the agreement between the trajectory groups formed from infancy to age 18 in IOW reported by Ziyab et al [21] and trajectory groups from infancy to 26 years using Cohen's Kappa coefficient.

Association analysis

To investigate the association between maternal pre-pregnancy BMI and BMI trajectories from infancy to 18 years and 26 years, a multinomial logistic regression analysis (PROC GLIMMIX, SAS) was conducted. Odds ratios and 95% confidence intervals (CI) were calculated. BMI trajectories were outcomes and maternal pre-pregnancy BMI was the exposure of interest. Covariates that changed the estimates by 10% were included in the final model. An association with a p-value ≤ 0.05 is considered statistically significant. All statistical analyses were performed in SAS, version 9.4 (SAS Institute, Cary, NC, USA).

Results

Descriptive characteristics of the cohort are shown in table 1. The mean maternal prenatal BMI at 14 (±4) weeks of pregnancy was 24.43 and maternal age at delivery was 28 years. Average gestational age and birth weight are 40 weeks and 3.39 kilograms respectively. 48% of the participants are female, 25% and 37% were exposed to maternal and paternal smoking during pregnancy respectively. BMI measurements were available at age 1, 4, 10, 18, and 26 years for 1076 (74%), 1053 (72%), 1043 (72%), 964 (66%), and 557 (38%) participants respectively (Table 2).

Table 1 Characteristics of the participants enrolled into the study

Variable		Mean (n)	Standard deviation
Maternal early pregnancy BMI (kg/m^2)		24.4 (1124)	4.2
Maternal age at delivery (Years)		27.7 (1197)	5.3
Gestational age (Weeks)		39.9 (1437)	1.5
Birth weight (Kg)		3.4 (1432)	0.5
Formula feeding (Weeks)		10.2 (1336)	11.6
Duration of Breast feeding (Weeks)		14.3 (1319)	14.8
		N	**Percentage (%)**
Gender	Male	735	50.5
	Female	721	49.5
Maternal smoking during pregnancy	No	1098	75.4
	Yes	357	24.5
	Missing	1	0.07
Paternal smoking during pregnancy	No	885	60.8
	Yes	542	37.2
	Missing	29	2
Birth order	1	507	34.8
	2	412	28.3
	3	281	19.3
	Missing	256	17.6
Secondhand smoke exposure at age one	No	778	53.4
	Yes	557	38.3
	Missing	121	8.3
Secondhand smoke exposure at 4 years of age	No	738	50.7
	Yes	468	32.1
	Missing	250	17.2
Secondhand smoke exposure at 10 years of age	No	763	52.4
	Yes	556	38.2
	Missing	137	9.4
Smoking at 18 years of age	No	899	61.7
	Yes	368	24.5
	Missing	258	13.8
Smoking at 26 years of age	No	699	48
	Yes	314	21.6
	Missing	443	30.4

BMI developmental trajectories

From the trajectory analysis, four distinct developmental courses of BMI z-scores across the first 26 years of life were identified (Figure 1).

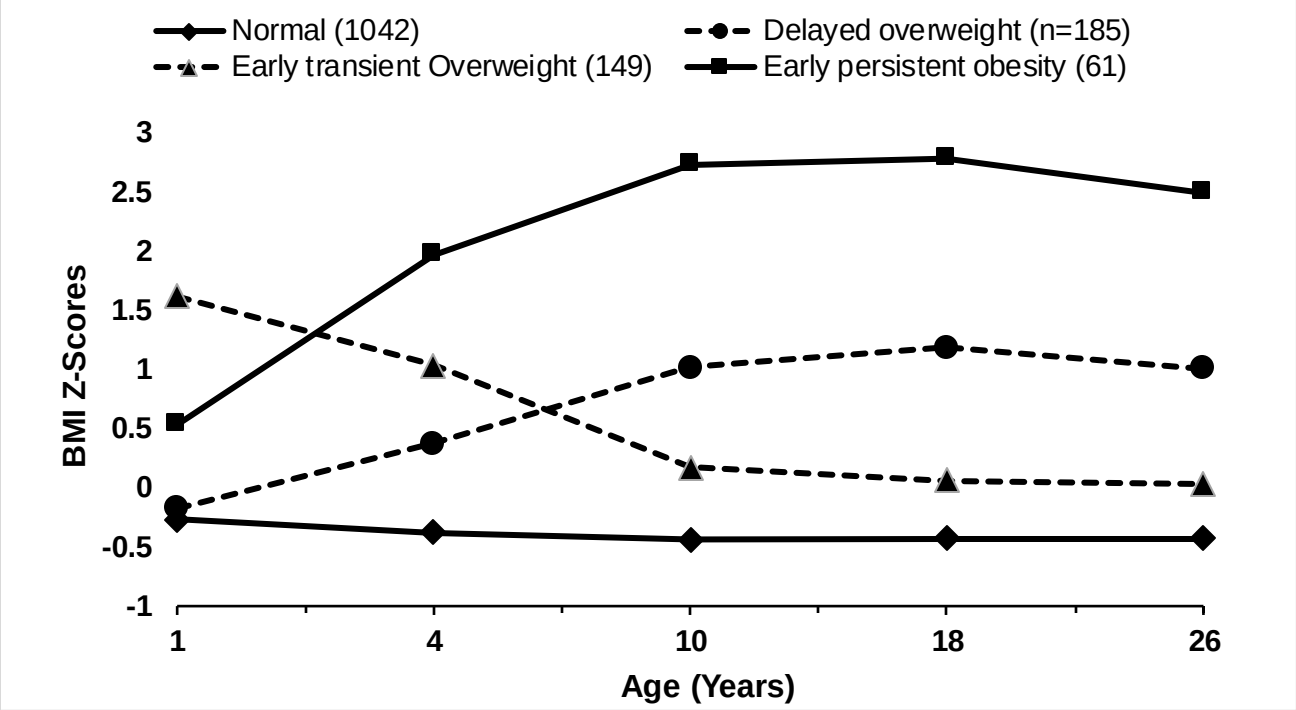

Figure 1 Developmental Body Mass Index z-score trajectories age 1 to 26 year of age representing the latent growth patterns of body mass index 1 to 26 years of age.

The four trajectories are labeled as 'normal' (n= 1042), 'early persistent obesity' (EPO, n=61), 'early transient overweight' (ETO, n=185), and 'delayed overweight' (DOW, n=149) respectively. The average BMI of the EPO trajectory crossed the age-specific threshold at ages 4, 10, 18, and 26 years as specified by the IOTF. The mean BMI of DOW trajectory exceeded recommended BMI threshold at age 26 years while the ETO trajectory was higher than the age-specific overweight cut-off as suggested by the WHO [41]. Descriptive characteristics of the trajectories are shown in table 2.

Table 2 Descriptive characteristics of the BMI and BMI Z-score of BMI developmental trajectories

BMI Z-score trajectories	Normal (n=1042) Mean (95% CI)	Early Persistent obesity (n=61) Mean (95% CI)	Early transient overweight (n=185) Mean (95% CI)	Delayed Overweight (n=149) Mean (95% CI)
Age 1 year				
BMI	16.94 (16.85 to 17.04)	18.27 (17.78 to 18.7 7)	20.08 (19.8 to 20.36)	17.09 (16.9 to 17.29)
BMI Z-score	-0.26 (-0.32 to -0.21)	0.53 (0.24 to 0.83)	1.62 (1.45 to 1.79)	-0.18 (-0.29 to -0.06)
Age 4 year				
BMI	16.67 (16.45 to 16.89)	18.38 (17.48 to 19.28)	18.53 (17.36 to 19.69)	16.98 (16.4 to 17.56)
BMI Z-score	-0.38 (-0.43 to -0.32)	1.98 (1.61 to 2.35)	1.03 (0.9 to 1.17)	0.38 (0.26 to 0.5)
Age 10 year				
BMI	15.62 (15.55 to 15.7)	19.04 (18.5 to 19.57)	17.67 (17.48 to 17.86)	16.72 (16.54 to 16.89)
BMI Z-score	-0.44 (-0.48 to -0.4)	2.74 (2.44 to 3.03)	0.18 (0.05 to 0.3)	1.02 (0.91 to 1.12)
Age 18 year				
BMI	16.8 (16.69 to 16.93	26.25 (25.37 to 27.14)	18.63 (18.25 to 19.01)	21.14 (20.83 to 21.45)
BMI Z-score	-0.43 (-0.47 to -0.39)	2.78 (2.48 to 3.09)	0.06 (-0.04 to 0.16)	1.19 (1.08 to 1.3)
Age 26 year				
BMI	21.34 (21.17 to 21.52)	35.24 (33.92 to 36.56)	23.45 (23 to 23.9)	28.34 (27.86 to 28.81)
BMI Z-score	-0.43 (-0.48 to -0.37)	2.5 (2.02 to 2.98)	0.03 (-0.12 to 0.18)	1.01 (0.84 to 1.18)

Mean and 95% CI of BMI and BMI Z-scores of the four trajectories
BMI - Body Mass Index

The BMI developmental trajectories across 26 years of life are in agreement with the BMI trajectories from infancy to 18 years of age identified by Ziyab et al [21]. The BMI trajectories from infancy to age 18 years and BMI trajectories from infancy to 26 years of age will be referred as '1-18 year-trajectories' and '1-26 years-trajectories' respectively. Further some, the agreement between the '1-18 year- and '1-26 years' -trajectories was tested using Cohen's Kappa coefficient. A significant statistical agreement between these groups, with a

Kappa coefficient of 0.8.and an overlap of 98% (n=853) of normal, 74% (n=130) of the DOW, 97% (n=139) of ETO, and 85 % (n=46) of the EPO trajectory groups was observed (Table 3).

Table 3 Overlap of trajectory groups between 'infancy to 18 years of age' and 'infancy to 26 years of age.

BMI Trajectories		**Infancy to 18 years of age**			
		Normal (n=886)	Delayed overweight (n=143)	Early Transient Overweight (n=163)	Early persistent Overweight(n=48)
Infancy to 26 years of age (n, %)	Normal (n=868)	853 (98.3)	4 (0.5)	11 (1.27)	0
	Delayed Overweight (n=175)	32(18.3)	130 (74.3)	11 (6.3)	2 (1.1)
	Early Transient Overweight (n=143)	1 (0.7)	3 (2.1)	139 (97.2)	0
	Early persistent Overweight(n=54)	0	6 (11.1)	2 (3.7)	46 (85.2)

Maternal pre-pregnancy BMI as predictor of BMI trajectories and offspring BMI

This study examined the association between risk factors and BMI trajectories over two different time periods - '1-18 year-trajectories' and 1-26 year-trajectories' (Table 4). The 'normal' group serves as reference. A statistically significant association of maternal pre-pregnancy BMI with DOW and EPO trajectories was observed. A unit increase in maternal BMI is associated with 18% and 24% increased odds of being in DOW and EPO trajectories respectively. Maternal age at delivery was inversely associated with the EPO trajectories in both '1-18 year- 'and '1-26 year-trajectories. If the mother smoked during pregnancy, the odds of being in EPO increased by three times in the '1-18 year-trajectories' while odds of being in DOW doubled in the '1-26 year-'trajectories'; however, the latter was not statistically significant. No such effect of paternal smoking and birth order of the child were observed. Shorter gestational age, higher birth weight, age of initiation of formula feeding, duration of breastfeeding, and gender showed a statistically significant association with the EPO and/or DOW trajectory groups in both trajectory solutions

(Table 4). For instance, increased birth weight doubled the odds of being in ETO and EPO trajectories in ‘1-18 year-trajectories’ while it showed an impact on only early transient overweight group in ‘1-26 year-trajectories’.

Table 4 Association of maternal pre-pregnancy BMI and BMI trajectories from infancy to 18 years and infancy to 26 years of age

Risk Factors of Interest	Trajectory Groups ‡	Association of trajectories from infancy to 18 years (N=1240)				Association of BMI trajectories from infancy to 26 years (N = 1437)			
		Parameter estimate	Standard Error	p-Value	Odds Ratio (95% CI)	Parameter estimate	Standard Error	p-Value	Odds Ratio (95% CI)
Maternal Pre-pregnancy BMI	DOW	0.16	0.02	<0.0001	1.18 (1.12 - 1.23)	0.16	0.02	<.0001	1.17 (1.11 - 1.23)
	ETO	0.04	0.03	0.21	1.04 (0.98 - 1.1)	0.05	0.03	0.08	1.05 (0.99 - 1.11)
	EPO	0.22	0.04	<0.0001	1.24 (1.16 - 1.34)	0.21	0.04	<.0001	1.23 (1.13 - 1.33)
Maternal age at delivery	DOW	0.01	0.02	0.58	1.01 (0.97 - 1.06)	0.00	0.03	0.92	1 (0.95 - 1.05)
	ETO	0.04	0.03	0.104	1.04 (0.99 - 1.1)	0.03	0.02	0.17	1.03 (0.99 - 1.09)
	EPO	-0.11	0.05	0.02	0.89 (0.81 - 0.98)	-0.11	0.05	0.03	0.89 (0.8 - 0.99)
Maternal smoking during pregnancy	DOW	0.41	0.28	0.14	1.51 (0.87 - 2.59)	0.70	0.29	0.02	2.01 (1.13 - 3.57)
	ETO	0.32	0.30	0.29	1.38 (0.76 - 2.51)	0.29	0.29	0.31	1.34 (0.76 - 2.36)
	EPO	1.12	0.43	0.01	3.06 (1.31 - 7.13)	0.82	0.49	0.1	2.27 (0.86 - 5.98)
Paternal smoking during pregnancy	DOW	-0.01	0.24	0.95	0.99 (0.62 - 1.57)	0.01	0.26	0.96	1.01 (0.61 - 1.68)
	ETO	0.37	0.26	0.16	1.44 (0.87 - 2.39)	0.42	0.24	0.08	1.53 (0.95 - 2.46)
	EPO	0.26	0.41	0.52	1.3 (0.59 - 2.88)	0.44	0.45	0.33	1.56 (0.64 - 3.79)
Birth order (1)#	DOW	0.14	0.29	0.63	1.15 (0.66 - 2)	0.15	0.31	0.61	1.17 (0.64 - 2.13)
	ETO	0.34	0.33	0.30	1.41 (0.74 - 2.69)	0.15	0.31	0.64	1.16 (0.63 - 2.12)
	EPO	0.11	0.52	0.83	1.12 (0.4 - 3.1)	0.22	0.61	0.72	1.25 (0.38 - 4.12)
Birth order (2)#	DOW	-0.29	0.28	0.29	0.75 (0.43 - 1.29)	-0.41	0.31	0.18	0.66 (0.36 - 1.21)
	ETO	-0.01	0.32	0.97	0.99 (0.53 - 1.83)	-0.08	0.29	0.78	0.92 (0.52 - 1.64)
	EPO	-0.74	0.57	0.19	0.48 (0.16 - 1.45)	-0.14	0.61	0.82	0.87 (0.26 - 2.88)
Gestational age	DOW	-0.12	0.08	0.14	0.89 (0.76 - 1.04)	-0.22	0.09	0.01	0.8 (0.68 - 0.95)
	ETO	-0.14	0.10	0.17	0.87 (0.71 - 1.06)	-0.02	0.10	0.82	0.98 (0.79 - 1.2)
	EPO	-0.28	0.14	0.06	0.76 (0.57 - 1.01)	-0.33	0.16	0.04	0.72 (0.53 - 0.99)
Birthweight¥ (Kg)	DOW	0.15	0.24	0.52	1.17 (0.73 - 1.87)	0.41	0.26	0.12	1.5 (0.9 - 2.51)
	ETO	1.09	0.27	<0.0001	2.99 (1.76 - 5.07)	1.03	0.26	<.0001	2.8 (1.69 - 4.62)
	EPO	0.88	0.45	0.049	2.41 (1.01 - 5.78)	0.82	0.49	0.1	2.26 (0.86 - 5.94)
	DOW	0.00	0.01	0.927	1 (0.97 - 1.02)	-0.01	0.01	0.71	0.99 (0.97 - 1.02)

Risk Factors of Interest	Trajectory Groups ǂ	Association of trajectories from infancy to 18 years (N=1240)				Association of BMI trajectories from infancy to 26 years (N = 1437)			
Age at the initiation of formula feeding	ETO	0.01	0.02	0.658	1.01 (0.97 - 1.04)	0.01	0.02	0.57	1.01 (0.98 - 1.05)
	EPO	-0.05	0.02	0.051	0.95 (0.91 - 1)	-0.06	0.03	0.025	0.94 (0.89 - 0.99)
Duration of breast feeding	DOW	0.01	0.01	0.301	1.01 (0.99 - 1.03)	0.02	0.01	0.12	1.02 (1 - 1.04)
	ETO	-0.02	0.01	0.133	0.98 (0.95 - 1.01)	-0.02	0.01	0.08	0.98 (0.95 - 1)
	EPO	0.03	0.02	0.044	1.03 (1 - 1.07)	0.05	0.02	0.01	1.05 (1.01 - 1.08)
Gender (Female)*	DOW	0.60	0.21	0.004	1.83 (1.22 - 2.76)	0.70	0.23	0.003	2.02 (1.28 - 3.17)
	ETO	-0.27	0.24	0.257	0.77 (0.48 - 1.21)	-0.08	0.22	0.70	0.92 (0.6 - 1.41)
	EPO	1.24	0.42	0.003	3.44 (1.52 - 7.79)	1.39	0.49	0.004	4.02 (1.55 - 10.47)

ǂ corresponds to specific trajectory: DOW– Delayed overweight; ETO - Early transient overweight; EPO - Early persistent obesity, NDT – Normal developmental trajectory is the reference group (not shown in the table); * Male gender is used as reference group; # Highest birth order (3) is considered as the reference group; ¥ Birthweight -measured in kilograms (Kg)

Associations between repeated measurement analyses of offspring BMI from 1 to 26 years of age and risk factors are presented in table 5. Intrauterine and early life risk factors such as maternal pre-pregnancy BMI, birth order, maternal smoking during pregnancy, gestational age, birth weight, age of the offspring, and gender are statistically significantly associated with repeated measurements of offspring BMI. Every unit increase in the maternal gestational BMI was associated with 5% increase in the offspring BMI weight gain which may lead to later life overweight or obesity. Maternal smoking during pregnancy increased the risk of offspring weight gain by 28%. Children with higher birth weight/kg were at 43% elevated risk of being overweight. Being a female also increase the risk by 12% compared to males. Gestational age is inversely associated with offspring BMI and lower birth order showed a higher risk of offspring BMI.

Table 5 Association of maternal pre-pregnancy BMI, early life risk factors and offspring BMI

Effect		Parameter Estimate	Standard Error	p-Value	Risk Ratio (95% CI)
Maternal BMI		0.05	0.01	<0.0001	1.05 (1.04 - 1.06)
Age (years)	1	-0.1	0.05	0.04	0.91 (0.82 - 0.99)
	4	-0.08	0.05	0.12	0.93 (0.84 - 1.02)
	10	-0.07	0.05	0.19	0.94 (0.85 - 1.03)
	18	-0.02	0.05	0.68	0.98 (0.88 - 1.08)
	26	**Ref**			
Age of the mother		0.002	0.01	0.79	1 (0.99 - 1.01)
Maternal smoking during pregnancy	**Yes**	0.24	0.07	0.0004	1.28 (1.12 - 1.46)
	No	**Ref**			
Paternal smoking during pregnancy	**Yes**	0.08	0.06	0.15	1.09 (0.97 - 1.22)
	No	**Ref**			
Birth Order	**1**	0.15	0.07	0.03	1.17 (1.01 - 1.34)
	2	-0.02	0.07	0.79	0.98 (0.86 - 1.12)
	3	**Ref**			
Gestational Age (weeks)		-0.07	0.02	0.0003	0.93 (0.89 - 0.97)
Birth weight (Kg)		0.36	0.06	<0.0001	1.43 (1.28 - 1.61)
Age when the formula was introduced (weeks)		-0.01	0.003	0.12	1 (0.99 - 1)
Duration of Breast feeding (weeks)		0.003	0.003	0.141	1 (1 - 1.01)
Gender	**Female**	0.11	0.05	0.024	1.12 (1.02 - 1.23)
	Male	**Ref**			

Discussion

Four distinct BMI developmental trajectories that covered the first 26 years of life were identified in this longitudinal study. These trajectories agree (overlap of about 74% to 98% individual trajectories) with the BMI trajectories that were identified in the IOW for the age-spanning between infancy and 18 years of age. Individual BMI trajectories of EPO and normal trajectory groups were set by age four and remained consistent through adolescence (26 years age) highlighting the critical timeline in early childhood that needs preventive attention. Unlike EPO and normal trajectories, ETO and DOW trajectories were set between age four and 10 years. Gestational BMI was found to be associated with increased odds of being in EPO and

DOW trajectories illustrating the impact of the maternal prenatal BMI on '1-18 year-trajectories' and '1-26 year-trajectories' that covered the first 18 and 26 years of life respectively. Similar pattern of associations of prenatal maternal smoking and BMI trajectories were observed.

To date, population-based studies attempted to study the course of BMI development from childhood to adolescence [20, 44-46]. A systematic review conducted by Ho et al [47] suggested that, lifestyle changes between ages 5 to 18-years have a significant impact on the childhood overweight supporting our findings in regard to the two transient BMI trajectories that changed their course of directions. A systemic review by Mattsson et al identified 14 studies that studied BMI trajectories from birth to 15 years of age. Three to four BMI trajectories with similar patterns including but not limited to rapid weight gain trajectories and stable high trajectory were identified across all studies [48]. Similar developmental trajectories- early persistent obesity, early transient overweight and delayed overweight' were identified in IOW underlining the validity and generalizability of our findings. Unlike the prior studies that were restricted from birth to -infancy, early childhood and/or adolescence (15 years age of age), the BMI trajectories identified in the IOWBC has added advantage of covering the first 26 years of life since birth with multiple BMI measurements (1, 4, 10, 18, and 26 years of age). This prospective birth cohort study identified a critical time window of life where the BMI trends of individuals who are at high risk of later being overweight and/or obesity can be identified. We suggest that any lifestyle interventions focused on childhood obesity/overweight should target this time window where the BMI trajectories are yet to be set and the individuals who are likely to be in the high-risk trajectories.

This study replicated the association of the intrauterine risk factors-gestational BMI, smoking, and BMI developmental trajectories reported in the literature [21]. We also observed a consistent and statistically significant association of early risk factors -gestational age, birth weight, age of initiation of formula feeding, duration of breastfeeding, and gender with EPO

and/or DOW trajectories in '1-18 year-trajectories'- and/or '1-26 year-trajectories'. Female offspring are more likely be at higher odds being in DOW (twice) and EPO trajectories (4 times higher odds) compared to males in '1-26 year-trajectories'. Furthermore, our findings complement the work by Elrashidi et al [49] , who demonstrated four distinct BMI trajectories and also concluded that female offspring are more likely to be obese compared to males. Interesting, a population-based study demonstrated that female offspring have greater impact of gestational BMI compared to male offspring [50].

Though the literature reported that trajectories were not influenced by the offspring behavioral changes such as smoking status, alcohol consumption in adolescence [20], BMI trajectories are influenced by modifiable intrauterine risk factors such as maternal-pregnancy BMI and prenatal maternal smoking that have long-term consequences of offspring overweight and obesity (Table 4). Gestational BMI is positively correlated with offspring birth weight which in turn increases the risk of offspring being in EPO and DOW trajectories (Table 4).

The underlying biological explanation for the aforementioned associations includes an interplay between genetic, epigenetic, and other behavioral changes. A meta-analysis that included 18 studies conducted by Tyrrell et al [51] suggested a 'causal relation' between maternal prenatal BMI and offspring BMI, though no specific gene/SNP was reported. Epigenetic epidemiology recently has provided a new insight on the associations of transgenerational effects. Khot et al [52] has demonstrated that an altered one-carbon metabolism influences the epigenetic programming in the offspring and increased the risk of adult diseases in offspring. Similarly, Morales et al [53], Gemma et al [33] and a recent meta-analysis by PACE consortium [36] that included 19 cohorts identified epigenetic signatures of gestational BMI in the cord blood of their offspring's providing a plausible explanation for the relation between gestational BMI and offspring BMI. However, further studies are necessary to

test whether epigenetic modifications linked with maternal BMI are related to the development of offspring BMI.

This study has some limitations. Only five measurements of BMI (1 and 2 years, 4, 10, 18, and 26 years of age) were available for the trajectory analysis. However, these repeated BMI measurements prospectively covered the first 26 years of life in contrast to the other studies that are restricted to a specific time periods of childhood and/or adolescence. The BMI z-score calculated is standardized by the standard deviations within IOW but not as per the CDC recommendations. Use of internal standardized BMI z-scores for trajectories and association analysis could be a limitation. However, it has been suggested that z-BMI derived as per CDC is a poor predictor of adiposity changes [39, 54]. Early pregnancy BMI was used as main predictor of offspring BMI trajectories is a plausible limitation in our study. Harris et al [55] suggested that the weight gain in first trimester is less compared to the second or third trimesters and prenatal maternal BMI reliable measure that represent the pre-pregnancy BMI. Ziyab et al utilized participants with a minimum of two BMI measurements, while the present study only utilized participants with one BMI measurement, which may be a drawback when comparing the BMI trajectories between the ages of 1 to 18 years and 1 to 26 years. Initial analysis indicated that the BMI trajectories produced using either one or two BMI measurements displayed similar qualities and the connections with early life risk factors remained unchanged. No distinct BMI trajectory groups based on gender were identified ruling out the need of gender stratified analysis.

This study identified four different BMI developmental paths over the first 26 years of life that are established in early childhood and strongly align with BMI trajectories from infancy to 18 years. Prenatal maternal BMI is linked with increased odds of falling into the Extremely Prevalent Overweight (EPO) and Developmental Overweight (DOW) trajectories. Early life risk factors such as gestational age, birth weight, breastfeeding duration, and gender are associated

with an increased risk of offspring being in the EPO and/or DOW trajectories. Given the long-lasting impact of maternal BMI, it is recommended that women of childbearing age and expectant mothers be informed about the dangers of pre-pregnancy BMI and its effects on their offspring's BMI. Future interventions aimed at preventing childhood overweight and obesity should focus on promoting healthy weight in women of childbearing age and encouraging lifestyle changes in their offspring during early childhood, before the age of 10.

Chapter 3

3. Offspring epigenetic markers at birth related to gestational BMI predict offspring BMI-trajectories from infancy to 26 years

Introduction

Epidemiological studies to date have suggested a direct association between maternal pre-pregnancy body mass index (BMI) and offspring BMI suggesting an early programming of adverse health outcomes in the offspring [56, 57]. These associations can be explained by molecular mechanisms such as DNA methylation (DNAm) and histone modification altering gene expression. DNAm, the addition of a methyl group on sites with a cytosine followed by a guanine linked by phosphate dinucleotide (CpGs) [58], is one of the epigenetic processes that has been extensively studied. DNAm programming can be initiated in utero and the offspring may carry a memory of DNAm markers related to intrauterine exposures.

Lawlor et al [59] reported that maternal obesity is positively associated with offspring BMI and suggested that these effects are mediated by epigenetic changes. In a candidate gene approach in 2009, Gemma et al. showed a positive correlation between the peroxisome proliferator-activated receptor (γ co-activator 1 α gene, PPARGC1A) promoter methylation in umbilical cord blood cells and maternal BMI [33]. Later in 2014, an epigenome-wide association study (EWAS) that used 27K bead chip reported 20 CpGs to be differentially methylated in the offspring of mothers who are obese compared to normal weight mothers [57]. In further studies, the epigenome-wide assessments covered more CpGs: Illumina Infinium 450k bead chip covers ~1.6% of the genome providing information on > 450,000 CpGs. Using 450k CpGs, Sharp et al [35, 60] detected several epigenetic markers related to maternal pre-pregnancy BMI in offspring DNAm at birth.

These findings were further supported by a meta-analysis conducted by a PACE consortium (pregnancy and childhood epigenetics) across 19 cohorts, which provided

information on maternal BMI at the start of pregnancy and associated DNAm sites in umbilical cord blood. The meta-analysis found a robust association between maternal pre-pregnancy BMI and variations in newborn DNA methylation [60]. Interestingly, no overlap between the CpGs reported in this meta-analysis and CpGs reported in earlier studies was observed. The lack of consistent results in the meta-analysis could be due to low sample sizes of individual studies resulting in false negative findings in some EWAS. In addition, the effect of maternally BMI-induced DNAm on CpG sites at birth on the development of offspring BMI are not yet known.

BMI trajectories have been used to characterize typical patterns of individual BMI developments during childhood and adolescence. Such BMI trajectories from infancy to 18 years of age were determined in a prior study in the Isle of Weight birth cohort (IOWBC) [21]. Four distinct trajectories were identified: normal, early persistent obesity (EPO), delayed overweight (DOW), and early transient overweight (ETO). The findings suggest that trajectories of EPO and normal trajectories can be detected as early as age 4 years. Findings from the IOWBC also demonstrated that offspring's BMI trajectories were influenced by gestational BMI [21].

Although a variety of studies have reported associations, first, between maternal pre-pregnancy BMI and BMI trajectories [61, 62], and second, between maternal pre-pregnancy BMI and differential DNAm sites [33, 35, 57, 60], these studies did not yet investigated effects of epigenetic changes induced by maternal BMI on offspring BMI trajectories. Considering these gaps in the literature, this study aims to determine whether CpG at birth related to maternal pre-pregnancy BMI in prior studies, are also associated with offspring BMI trajectories. This study hypothesizes that CpGs related to gestational BMI, identified at birth, are also associated with trajectories of offspring BMI covering the age span from infancy to 26 years.

Method

Study population

The Isle of Wight birth cohort was established to study the natural etiology of allergic diseases on Isle of Wight (IOW), UK in January 1989. Parents of 1,536 children born between January 1, 1989, and February 28, 1990, were invited to enroll their children into the study. Of 1,536 children, after exclusion of birth failure and missing written consent, 1,456 newborns were enrolled at birth. These children were referred as the F1-generation. Parents of the F1 generation are the F0-generation. Dried blood spots (Guthrie cards) were collected in the first 5 days after delivery and peripheral blood samples were taken at ages 10 and 18 years in the F1-generation. The F1 participants were assessed by detailed physical examinations and questionnaires at ages 1, 2, 4, 10, 18, and 26 years. The birth cohort has been described in detail elsewhere [38, 63]. F1-children with DNAm at birth and with characterized BMI trajectories were used in the investigation. The study population is 99% Caucasian.

The investigation was approved by the local ethics committee –National Research Ethics Service, NRES Committee South Central – Hampshire B, U.K. and by the University of Memphis Institutional Review Board in Memphis, U.S (#2423). Written informed consents were obtained from the parents at each assessment through age 10 and from the participants at 18 and 26 years.

DNA methylation

DNA from the Guthrie cards (dried blood spots; n = 796) at birth was extracted using QIAamp DNA Investigator Kits (Qiagen, Germantown, MD, USA) according to manufacturer's instructions. At age 10 and 18 years, DNAm was assessed using the Illumina 450k platform. DNAm from selected peripheral blood samples at age 10 (n= 330) and 18 (n= 476) were extracted via a standard salting out procedure [64]. For Guthrie cards, measurements of genome wide DNAm were performed using the Illumina Methylation EPIC 850K platform

(Illumina, Inc., CA, USA) which interrogates > 850,000 CpGs associated with over 24,000 genes. Arrays were processed on the respective platform using standard protocol as described elsewhere [65].

Methylation data were preprocessed and batch-effect removed using Bioconductor packages IMA [66] and ComBat [67]. After quantile normalization and background corrections, beta values for the each queried CpG sites were generated. The beta values, which represent the proportion of the methylated (M) sites over the sum of the methylated (M) and unmethylated (U) sites = (β =M/(c+M+U), with c as a constant to preventing dividing by zero were used to estimate DNA methylation levels. CpGs - with low quality, that are missing at random, and on sex-chromosomes were not taken into account. CpG sites that have a potential single nucleotide polymorphism (SNP) within 10 base pairs (a probe SNP) and with a minor allele frequency of greater than 0.007 since these probe-SNPs may interfere with the DNAm measurement were excluded. After excluding the CpGs on the sex chromosomes and probe SNPs - for EPIC methylation array data, 551,711 CpGs were retained from the Guthrie's cards collected at birth. At ages 10 and 18 years, 349,456 CpGs were kept. Samples that were processed on 450k and 850K platforms, CpGs overlapping between two methylation arrays were taken. To estimate associations between DNAm at birth and BMI trajectories, we estimated odds related to the percent methylation by multiplying beta-value with 100.

Cell proportions

The cell proportions of the Guthrie cards were estimated using the DNAm of Guthrie cards with blood as a reference. Minfi, an R-package, was used to estimate seven cell proportions: B cells, CD4T and CD8T cells, granulocytes, monocytes, neutrophil, and eosinophils [68, 69]. Since Guthrie cards were collected between day 3-5 after delivery, there was no need to additionally adjust the DNAm for nRBCs, as on postnatal day 3 -5 nRBCs are no longer seen in the blood circulation of the newborn.

Exposure of interest

Differentially methylated DNAm that is associated with maternal pre-pregnancy BMI at a birth is the exposure of interest. To identify CpGs related to maternal BMI, academic databases - PUBMED, GOOGLE SCHOLAR, & EMBASE were explored using search terms such as "Maternal BMI", "Maternal pre-pregnancy BMI", "Pregestational BMI", "Preconception BMI", and "Maternal obesity" along with "Cord blood DNA methylation", "DNA methylation", and "Epigenome-wide association" within a 10 years' time span of 2007 -2017. Prior investigations reported 1,773 differentially methylated CpGs at birth related to maternal pre-pregnancy BMI [35, 57, 60]. Among the CpGs of the F1-generation at birth that were kept after quality control in the IOW birth cohort, 1,090 were identified and these CpGs constitute the exposure of interest.

Outcome of interest

BMI developmental trajectories from infancy to 18 years of age were determined in a prior study in the Isle of Weight birth cohort, BMI trajectories [21]. To identify BMI trajectories from birth to 26 years, this study repeated the approach described by Ziyab et al [21]. for infancy to 18 years and added information on body mass at 26 years. Four distinct BMI trajectories- normal, early persistent obesity (EPO), delayed overweight (DOW), and early transient overweight (ETO) were identified (Figure 2). These four BMI developmental trajectories from infancy to 26 years are outcome of interest in this study. The detailed methodology of BMI trajectory development from infancy to 26 years and the definition of the BMI trajectories are explained in chapter 2.3. Figure 2.1 illustrates the development of the BMI trajectories.

Covariates

The following potential confounder were considered: maternal age, socio-economic status, low birth weight (≤2,500 g), infant feeding method (exclusive breast feeding, exclusive formula, and mixed feeding), paternal smoking during pregnancy, and second-hand smoke exposure at ages 1, and 4 years. Gestational age, maternal smoking during pregnancy, and

maternal BMI were not considered as covariates in this study. Gestational age was correlated with birth weight and since the effects of these two variables can substitute one another, low birth weight was chosen to be controlled as confounder. Maternal smoking during pregnancy [70] and also maternal BMI [35] were known to alter newborn DNAm. Taking these two variables as confounder, would turn newborn DNAm into a mediator, which then would not provide an appropriate assessment of the role of newborn DNAm. Since DNAm based on blood cells was the exposure, estimated blood cell proportions were also taken into account to estimate the net effect of DNAm independent of varying cell-composition found in newborn blood.

Statistical analysis

When analyzing the IOW birth cohort, the analytical sample with BMI trajectories from infancy to 26 years of age (dependent variable) and DNAm data (independent variable) was assessed for [71] its representability of the total cohort. Statistical assessment to test the proposed hypothesis was conducted in five steps (Figure 2). Differentially methylated DNAm sites that were associated with maternal pre-pregnancy BMI at birth (as described under "Exposure of interest") were gathered from literature. In total, 1,090 candidate CpGs collected from literature and present in the IOW cohort were considered as potential predictors of BMI trajectories in their offspring.

An R screening computing package, ttScreening, was used to filter informative from non-informative CpGs potentially associated with the four BMI trajectories [71]. This computing package facilitates a screening process where each CpG was tested through 100 iterations of training and testing. In each iteration, 2/3rd of the sample was used as training dataset while 1/3rd was used as testing dataset. In these robust regressions, DNAm was considered as dependent variable and BMI trajectories as independent variable with cell proportions in blood

as potential covariates. CpGs that showed a statistical significance in at least 50% of the training and testing samples with p-value <0.05 were considered as informative.

The informative CpGs were then tested for their association with BMI trajectories by additionally adjusting for potential confounders. Multinomial logistic regression models were used (PROC GLIMMIX, SAS) to study associations between CpGs and BMI trajectories. The normal trajectory was the reference group. Risk factors that did not change the odds ratio by 10% were excluded from the model using backward elimination in the association analysis. Associations with a p-value ≤0.05 were considered statistically significant. Correction for multiple testing was performed using the false discovery rate (FDR) [72]. Odds ratios and 95% confidence intervals (CI) for the statistically significant CpGs were calculated.

To illustrate the odds of being in a trajectory with level of methylation, each CpG that passed the multiple testing was further categorized into four groups by diving the range of methylation into four quartiles. Multinominal logistic regression models were applied to study the association between the BMI trajectories as dependent variable and the categorized CpG groups as predictors.

Finally, the descriptive characteristics of the DNAm at birth, age 10, and 18 years in different BMI trajectories were illustrated. Mean methylation value of the CpGs of the associated BMI trajectories were plotted to understand changes in the methylation levels at different time periods (age 10 and 18 years). All statistical analyses were performed in SAS, version 9.4 (SAS Institute, Cary, NC, USA) and R.

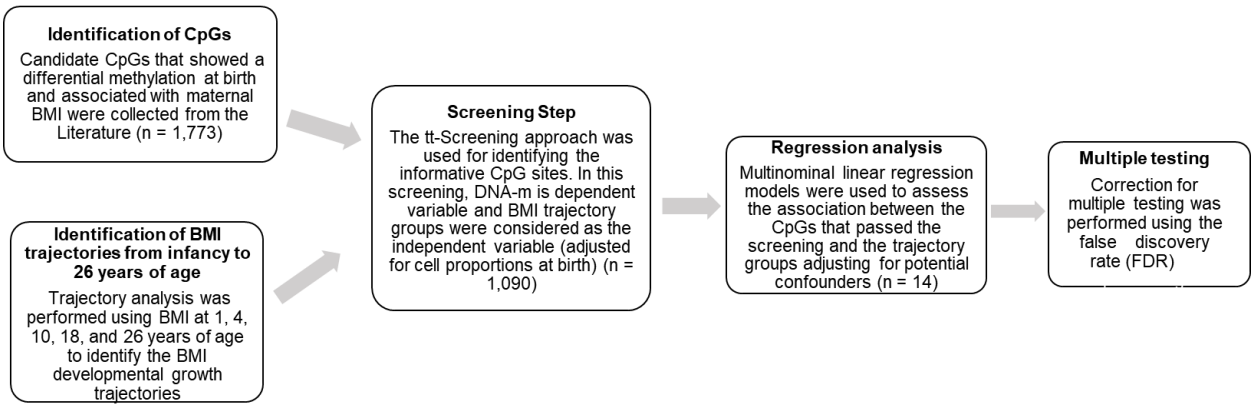

Figure 2 Study flowchart of statistical analysis of CpGs associated with maternal pre-pregnancy BMI

Results

Participant characteristics

In the analytical sample (n=794), 51% of the sample population were female, 95% were born with normal weight (Table 6) and 11% of the individuals were exposed to secondhand smoke 4 years of age. 19% of the individuals were breastfed while 9% were exclusively formula fed. No statistically significant difference was observed between the study population (n= 1,456) and the analytical sample. The descriptive characteristics of the participant by each trajectory were illustrated in table 7.

Table 6 Descriptive characteristics of the study population and the population analyzed

Factors		Study Population n= 1,456 (% (n))	Analytical Sample n=794 (% (n))	P- Value
Sex	Male	50.5 (735)	50.4 (401)	0.9
	Female	49.5 (721)	50.6 (395)	
Low Birth Weight	< 2.5 Kg*	3.7 (54)	3.8 (30)	0.9
	>= 2.5kg*	94.6 (1378)	95 (755)	
	Missing	1.7 (24)	1.4 (11)	
Infant feeding method	Breast feeding Only	16.5 (368)	19 (151)	0.4
	Formula feeding only	8.2 (119)	8.7 (69)	
	Mixed feeding	66.6 (969)	65.1 (518)	
	Missing	8.8 (128)	7.3 (58)	
Paternal smoking during pregnancy	Yes	37.2 (542)	35.6 (283)	0.5
	No	60.8 (885)	62 (492)	
	Missing	2 (29)	2.4 (19)	
Socio-economic Status	Low	14 (204)	14 (109)	0.7
	Medium	70.5 (1026)	76.7 (609)	
	High	7.6 (110)	7.8 (62)	
	Missing	8 (116)	1.8 (14)	
Secondhand Smoking at 1 years of age	Yes	38.3 (557)	36.5 (290)	0.2
	No	53.4 (778)	57.2 (454)	
	Missing	8.3 (121)	6.3 (50)	
Secondhand Smoking at 4 years of age	Yes	32.1 (468)	11.1 (270)	0.8
	No	50.7 (738)	55 (438)	
	Missing	17.2 (318)	11 (88)	
		Mean (95% Confidence Intervals)		
Maternal age (years)		29.2 (29 – 30)	29.3 (29 – 30)	

*Kg Kilograms

Table 7 Descriptive characteristics of the individuals with information on BMI trajectories, DNAm, and confounders

Factors		BMI Trajectory Group			
		Normal trajectory (n = 562) %(n)	**Delayed Overweight (n = 118) %(n)**	**Early Transient Overweight (n = 77) %(n)**	**Early Persistent Overweight (n = 37) %(n)**
Sex	Male	52.5 (295)	36.4 (43)	36.4 (43)	29.7 (11)
	Female	47.5 (267)	63.6 (75)	63.6 (75)	70.1 (26)
Low Birth Weight	< 2.5 Kg*	94.9 (533)	94.1 (111)	96.1 (74)	94.6 (35)
	>= 2.5kg*	4 (22)	4.2 (5)	2.6 (2)	2.7 (1)
	Missing	1.3 (7)	2 (2)	1 (1)	2.7 (1)
Infant feeding method	Breast feeding only	20 (112)	22.9 (27)	14.3 (11)	2.7 (1)
	Formula Feeding Only	8.4 (47)	12.7 (12.7)	6.5 (5)	5.4 (2)
	Mixed feeding	64.1 (360)	61.9 (73)	71.4 (55)	81.1 (30)
	Missing	5.4 (30)	2.5 (3)	6 (6)	10.8 (4)
Paternal smoking during pregnancy	Yes	25.3 (201)	5 (37)	3.7 (29)	2 (16)
	No	44 (349)	10 (78)	5.5 (44)	2.6 (21)
	Missing	1.5 (12)	0.4 (3)	0.5 (4)	-
Socio-economic Status	Low	10.8 (86)	1.4 (11)	0.8 (6)	0.8 (6)
	Medium	52.8 (419)	12.1 (96)	8.2 (65)	3.7 (26)
	High	5.8 (46)	1.4 (11)	0.5 (4)	0.1 (1)
	Missing	1.4 (11)	-	0.3 (2)	0.1 (1)
Secondhand Smoking at 1 year of age	Yes	24.2 (192)	6.2 (49)	0.5 94)	2.3 (18)
	No	41.2 (192)	8.3 (66)	5.3 (42)	1.8 (14)
	Missing	4.8 (38)	0.4 (3)	3.9 (31)	0.6 (5)
Secondhand Smoking at 4 years of age	Yes	31.7 (178)	38.9 (46)	37.6 (29)	46 (17)
	No	55.9 (314)	55.9 (66)	53.25 (41)	43.2 (16)
	Missing	12.5 (70)	5.1 (6)	9.1 (7)	10.8 (4)

Table 7 (Continued)		BMI Trajectory Group			
Factors		**Normal trajectory (n = 562) %(n)**	**Delayed Overweight (n = 118) %(n)**	**Early Transient Overweight (n = 77) %(n)**	**Early Persistent Overweight (n = 37) %(n)**
Cell proportions		**Mean (95% Confidence Intervals)**			
	B cells	0.08 (0.03 - 0.13)	0.08 (0.04 - 0.14)	0.08 (0.03 - 0.13)	0.08 (0.03 - 0.13)
	CD8T	0.11 (0.02 - 0.19)	0.11 (0.02 - 0.19)	0.11 (0.02 - 0.19)	0.10 (0.02 - 0.19)
	CD4T	0.3 (0.2 - 0.39)	0.29 (0.17 - 0.38)	0.29 (0.21 - 0.39)	0.3 (0.2 - 0.41)
	Monocytes	0.13 (0.08 - 0.19)	0.13 (0.08 - 0.2)	0.13 (0.07 - 0.19)	0.13 (0.07 - 0.24)
	NK Cells	0.01 (0 - 0.07)	0.02 (0 - 0.07)	0.02 (0 - 0.06)	0.01 (0 - 0.07)
	Eosinophils	0.01 (0 - 0.05)	0.01 (0 - 0.05)	0.01 (0 - 0.05)	0.01 (0 - 0.11)
	Maternal age (years)	29.4 (29 - 30)	29.3 (29 – 30)	29.4 (29 - 30)	29.1 (29 - 30)

*Kg Kilograms

Identification of candidate CpGs associated with BMI trajectories from infancy to 26 years of age

Of the 1,773 CpGs identified in the literature, 1,090 were found in the cord blood DNAm of IOW birth cohort. Based on ttScreening, 14 CpGs showed a statistically significant association with BMI trajectories and were considered informative (Table 8 & 9).

Table 8 CpGs that passed tt-screening and considered as informative

		Delayed Overweight		Early Transient Obesity		Early Persistent Obesity	
CpG Name	**Selection Proportion**	**Parameter Estimate**	**Pvalue**	**Parameter Estimate**	**Pvalue**	**Parameter Estimate**	**Pvalue**
cg23089913	96	-0.10	0.002	-0.21	0.71	0.01	0.00004
cg12076012	87	0.00	0.95	-0.26	0.0001	-0.22	0.001
cg11015251	74	0.01	0.81	-0.41	0.45	-0.05	0.00002
cg08102602	72	0.02	0.6	0.21	0.56	-0.03	0.0005
cg14434213	70	0.06	0.08	-0.08	0.001	-0.14	0.2
cg12737392	66	0.02	0.54	-0.16	0.22	-0.04	0.001
cg13344237	64	0.10	0.001	-0.003	0.59	0.02	0.95
cg07654559	62	0.05	0.125	0.17	0.54	-0.02	0.001
cg08486961	61	0.00	0.99	-0.21	0.28	0.05	0.001
cg17812850	58	-0.12	0.001	-0.06	0.84	-0.01	0.29
cg13217064	54	0.14	0.002	0.07	0.89	-0.01	0.36
cg23490166	54	0.13	0.005	-0.01	0.04	0.11	0.92
cg16645202	53	0.03	0.32	0.15	0.79	-0.01	0.001
cg01106145	52	-0.08	0.004	-0.09	0.04	-0.07	0.04

Table 9 Gene names and location of the CpGs that passed the screening

CpG Name	Gene Name	Chromosome	MAP INFO	Coordinate	Strand	Location on the gene	Relation to UCSC CpG Island
cg23089913	*NANOS1*	10	120788366	120778356	F	TSS1500	
cg12076012	*ZFYVE1*	14	73494092	72563845	F	TSS1500	S Shore
cg11015251	*HOXA4*	7	27170554	27137079	F	TSS200	Island
cg08102602	*PTMS*	12	6875642	6745903	F	1stExon; 5'UTR	Island
cg14434213		8	38508780	38627937	F		S Shore
cg12737392	*TRAPPC9*	8	140945843	141015025	F	Body	
cg13344237	*DNMT3A*	2	25565575	25419079	F	TSS1500; TSS200	Island
cg07654559	*TTC31; CCDC142*	2	74710618	74564126	R	Body; TSS1500	S Shore
cg08486961	*DNAJB12*	10	74114051	73784057	F	Body	Island
cg17812850	*TMEM184C*	4	148538527	148757977	R	TSS200	Island
cg13217064	*SOX14*	3	137483099	138965789	F	TSS1500	Island
cg23490166	*RAB1B*	11	66035951	65792527	R	TSS200	Island
cg16645202	*SLCO3A1*	15	92397488	90198492	R	Body	Island
cg01106145	*ANO10*	3	43663861	43638865	R	TSS1500	Island

After adjusting for the potential confounder such sex, second-hand smoke exposure, birthweight, infant feeding method, and cell types, two CpGs remained statistically significant after taking false discovery due to multiple testing into account. DNAm sites cg23089913 (*NANOS1*) and cg13217064 (*SOX14*) were associated with EPO and DOW trajectories, respectively (Table 10). Increased methylation of cg23089913 was associated with decreased odds of being in EPO trajectory (OR: 0.84, 95% CI: 0.8-0.9) while higher methylation of cg13217064 resulted in increased odds of being in DOW trajectory (OR: 1.4, 95% CI: 1.1-1.7) when compared to the Normal trajectory.

Table 10 Association of DNAm and BMI trajectories from infancy to 26 years**.**

CpG	BMI trajectory group¥	Parameter estimate	p-Value	FDR p-Value	Odds ratio (95% CI*)
Cg23089913 (*NANOS1*)	DOW	-0.05	0.06	0.1	0.94 (0.9 - 0.99)
	ETO	0.0008	0.98	0.97	1 (0.9 - 1.06)
	EPO	**-0.18**	**0.0006**	**0.02**	**0.84 (0.76 - 0.93)**
	DOW	**0.32**	**0.001**	**0.02**	**1.38 (1.13 - 1.67**)
Cg13217064 (*SOX14*)	ETO	-0.04	0.75	0.87	0.95 (0.73 - 1.23)
	EPO	-0.01	0.9	0.96	0.97 (0.67 - 1.42)

¥ corresponds to specific trajectory: DOW– Delayed overweight (n=108); ETO - Early transient overweight (n=64); EPO - Early persistent obesity (n=28), NT – Normal trajectory is the reference group (not shown in the table, n=456); CI- Confidence Interval. Regression models were adjusted for maternal age, socioeconomic status, gender, secondhand smoking at age 4, birth weight, type of feeding., and cell proportions.

When analyzing these two CpGs, it was noted that the odds of being in the ETO trajectory was higher for participants who were exposed to mixed feeding method (formula & breast feeding) during infancy (data not shown). It is also noteworthy that the odds of being in DOW and EPO for female participants increased by 2- and 3- times respectively when compared to male participants (data not shown).

Given the association on of the female participants with the BMI trajectories, the interaction between the methylation and gender were tested. A statistically significant interaction was observed between the DANm and gender (data not shown). The association between BMI trajectories and the DNAm was further stratified by gender to investigate if the association varied in each gender. Table 11 illustrates the odds ratios and 95% confidence intervals of stratified analysis. Cg23089913 showed a statistically significant association in both, male and female participants, and higher methylation levels of this CpGs were related to lower odds of developing EPO. However, cg13217064 suggested a statistically significant association with DOW trajectory in females but not in males. The odds of developing into DOW trajectory were 1.8 times higher when compared to the participants in the normal trajectory group in females.

Table 11 Odds ratios and 95% confidence intervals for the CpGs associated with BMI trajectories stratified by gender

		Male			Female		
	Trajectory Group	**Parameter Estimate**	**p-value**	**Odds Ratio (95% Confidence Intervals)**	**Parameter Estimate**	**p-value**	**Odds Ratio (95% Confidence Intervals)**
Cg23089913 (*NANOS1*)	DOW	-0.06	0.16	0.94 (0.86-1.02)	-0.04	0.2	0.95 (0.89-1.01)
	ETO	-0.01	0.74	0.97 (0.9-1.06)	0.004	0.94	1.02 (0.91-1.13)
	EPO	**-0.42**	**0.01**	**0.71 (0.54-0.92)**	**-0.15**	**0.02**	**0.86 (0.76-0.98)**
Cg13217064 (*SOX14*)	DOW	-0.02	0.89	1 (0.72-1.36)	**0.55**	**<0.0001**	**1.8 (1.36-2.27)**
	ETO	-0.12	0.48	0.87 (0.63-1.24)	0.004	0.98	1.02 (0.63-1.6)
	EPO	-0.03	0.94	0.95 (0.39-2.38)	0.14	0.54	1.1 (0.73-1.83)

¥ corresponds to specific trajectory: DOW– Delayed overweight ETO (Male (n) = 39; female(n) = 69)- Early transient overweight (Male (n) = 40; female(n) = 24); EPO (Male (n) = 7; female(n) = 21) - Early persistent obesity, NT (Male (n) = 240; female(n) = 216)– Normal trajectory is the reference group (not shown in the table); Regression models were adjusted for maternal age, socioeconomic status, secondhand smoking at age 4, birth weight, type of feeding, and cell proportions.

Association of BMI trajectories and different methylation levels of CpGs: cg23089913 and cg13217064 stratified by gender

Sex-specific DNAm quartiles were identified by categorizing cg23089913 and cg13217064 into four groups (first, second, third, and fourth quartiles of methylation separate for male and female participants. The odds of being in a trajectory was evaluated using multinomial logistic regression analysis (Table 12). For cg23089913, when compared to the fourth quartile (methylation level (β) between 0.27 – 0.8), male participants in the first quartile (β-value: 0.08 - 0.21) had a 3- and females had 5-times higher odds of being in DOW and EPO respectively. This suggests that participants with lower methylation of cg23089913 have a higher odd of being in obese trajectory groups.

For cg13217064, first quartile (β-value: 0.04 - 0.026) was considered as the reference group. Females showed a statistically significant association of developing a DOW trajectory while no such association was observed in male participants. Interestingly, the odds of being in DOW increased from 3.7 to 5.7 times as methylation value increased from 0.026 to 0.094 (Table 3.7).

Table 12 Odds ratios and 95% confidence intervals for the CpGs associated with BMI trajectories**.**

CpG Name	Trajectory group[@]	Proportion of methylation ranges	Males				Females			
			n	Odds Ratio	95% CI[#]	pvalue	n	Odds Ratio	95% CI[#]	pvalue
Cg23089913 (*NANOS1*)	**DOW**	0.08 - 0.21	86	**2.94**	**1.04 - 8.3**	**0.04**	94	1.51	0.67 - 3.44	0.32
	ETO			1.35	0.52 – 3.53	0.54		0.92	0.26 – 3.56	0.89
	EPO¥			9512.86	0 - 1.589E+39	0.89		**6.55**	**1.2 – 35.29**	**0.02**
	DOW	0.21 - 0.24	97	0.62	0.2 - 2.32	0.44	115	1.2	0.54 - 2.7	0.66
	ETO			0.62	0.25 - 1.87	0.36		0.38	0.07 - 1.63	0.19
	EPO¥			3834.14	0 - 6.382E+38	0.84		4.21	0.78 – 22.80	0.1
	DOW	0.24 - 0.27	123	1.2	0.43 - 3.24	0.74	99	0.53	0.22 - 1.30	0.17
	ETO			0.51	0.19 - 1.36	0.18		0.94	0.3 - 2.95	0.92
	EPO¥			1512.62	0 - 2.557E+38	0.86		1.29	0.19 – 8.60	0.79
	DOW	**0.27 - 0.8**	93	*Reference group*			87	*Reference group*		
	ETO									
	EPO									
Cg13217064 (*SOX14*)	DOW	**0.004 - 0.026**	89	*Reference group*			104	*Reference group*		
	ETO									
	EPO									
	DOW	0.026 - 0.032	97	0.63	0.33 - 2.66	0.32	90	**3.32**	**1.24 – 8.86**	**0.02**
	ETO			0.92	0.58 - 4.05	0.86		3.1	0.83 - 11.53	0.09
	EPO			2.33	0.29 - 42.36	0.5		0.95	0.25 – 3.64	0.93
	DOW	0.032 - 0.039	104	1.01	0.27 - 2.18	0.9	106	**3.46**	**1.36 – 8.76**	**0.01**
	ETO			0.49	0.23 - 1.97	0.39		0.92	0.22 – 3.89	0.91
	EPO¥			0.00	0 – 5.11e70	0.95		1.14	0.35 - 3.69	0.83
	DOW	0.039 - 0.094	109	1.01	0.39 – 2.61	0.98	94	**6.55**	**2.44 - 17.61**	**0.0002**
	ETO			0.78	0.26 - 2.04	0.55		2.35	0.57 – 9.67	0.24
	EPO			3.12	0.27 – 71.11	0.3		0.98	0.21 – 4.44	0.98

@ Corresponds to specific trajectory: DOW– Delayed overweight; ETO - Early transient overweight; EPO - Early persistent obesity, Normal trajectory (NT) is the reference group; [#]CI- Confidence Interval; * Reference group. Regression models were adjusted for maternal age, socioeconomic status, secondhand smoking at age 4, birth weight, and type of feeding. ¥ the odds ratios, and confidence intervals showed large values for these methylation ranges which could be attributed to the small sample sized in these subsections.

Descriptive characteristics of the developmental pattern of CpGs associated with BMI measurements

To illustrate the developmental pattern of these CpGs over time the mean methylation of cg23089913 and cg13217064 at birth, and ages 10 and 18 years for BMI trajectories which they were associated with as well as normal trajectory in males and females was examined. For cg23089913, a parallel development of DNAm was observed in males for EPO and NT (normal trajectory). Male participants in EPO showed a lower DNAm from birth to 26 years of age (Figure 3). Though a marginally higher DNAm was observed in the NT participants at birth and 10 years of age, no difference in DNAm was observed at 18 years of age. In females, similar pattern of DNAm development were noted in NT and EPO groups. However, a cross-over of DNAm at 10 years of age was noted.

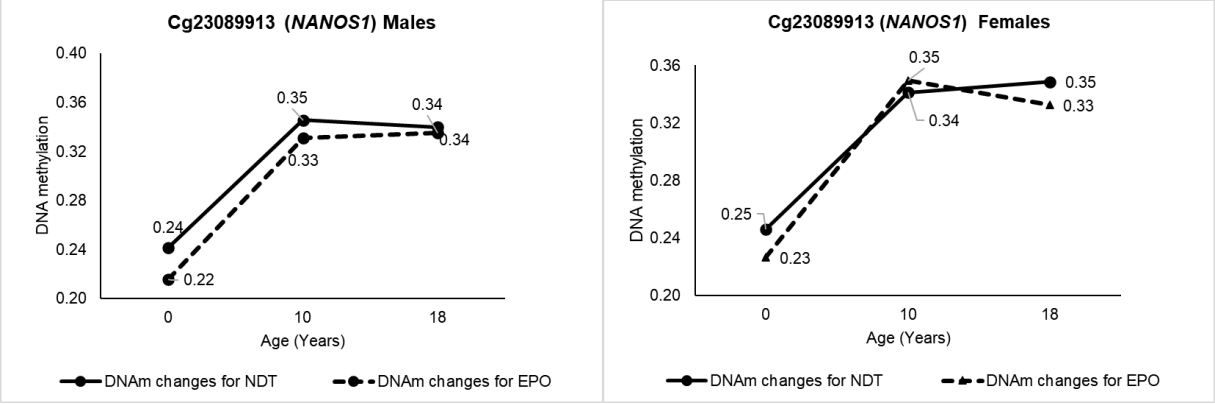

Figure 3 Distribution of mean methylation of cg23089913 across BMI trajectory group at different stages of life- at birth, 10, and 18 years of age for male and female participants.

Early persistent obesity (EPO) trajectory showed a parallel development of BMI and DNAm over time from birth to age 18 years in males while a cross over for the DNAm at age 10 was observed for females. NT- Normal trajectory

For cg13217064 this study detected that higher methylation was associated with higher odds of developing a DOW trajectory (Table 12). Females in the DOW group showed a unique DNAm development compared to the participants in NT group. Male participants in the DOW trajectory displayed higher methylation levels at a birth and 18 years while lower methylation at

10 years of age was noted. Males in both the NT and DOW groups exhibited parallel DNAm development at birth and 10 years (Figure 4).

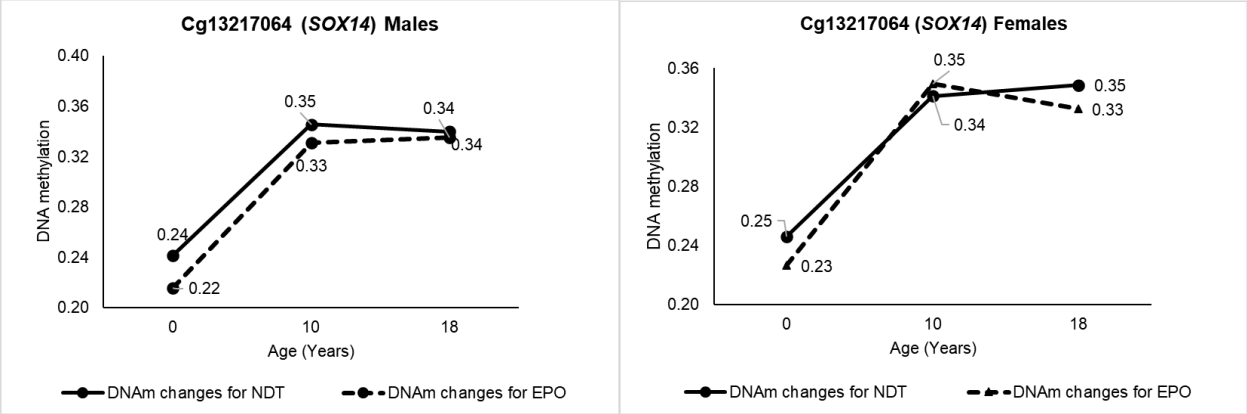

Figure 4 Distribution of mean methylation of cg13217064 across BMI trajectory group at different stages of life- at birth, 10, and 18 years of age for male and female participants.

A parallel development of DNAm and BMI was observed with methylation cross over at age 18 years. However, the DNAm for females showed an inverted pattern of DNAm for delayed overweight (DOW) trajectory when compared to the normal trajectory group (NT).

Discussion

Of 1,773 differentially methylated CpGs at birth that were reported in at least one prior study to be associated with maternal pre-pregnancy BMI, in this investigation 1,090 CpGs could be evaluated for their odds of developing a specific BMI trajectory in offspring from infancy to age 26 years (Table 10). With the normal trajectory as reference group, high or low levels of DNAm at CpG sites cg23089913 (*NANOS1*) and cg13217064 (*SOX14*) were statistically significantly associated with the EPO and DOW trajectories after adjusting for multiple testing. Surprisingly, cg23089913 (*NANOS1*) and cg13217064 (*SOX14*) identified by Sharp et al., did not survive a later meta-analyses by Sharp et al. (2017); however, lower sample sizes of studies included in meta-analyses may explain this limitation. Participant with higher methylation levels of cg23089913 (OR: 0.84) at birth showed a low risk of being in EPO while increased methylation of cg13217064 (OR: 1.4) showed a positive association concluding higher odds of progressing into DOW trajectory compared to the normal trajectory. In a gender stratified

analysis, the odds of developing into delayed overweight developmental trajectory was 1.8 times high in female participants for cg13217064 while not such association was detected in males.

A lower gestational BMI was known to be associated with higher methylation of cg23089913 (*NANOS1*) at birth [35] and the current investigation additionally demonstrated low odds of being in the EPO trajectory with increased methylation. Cg23089913 is located on the transcription start site of the gene *NANOS1* on chromosome 10. A multiphase dietary intervention by Boltman et al reported that *NANOS1* is highly expressed in obese individuals suggesting an association with obesity [73]. When comparing the odds, this study also observed that participants with lower methylation levels of this CpG were associated with higher odds of developing into a EPO trajectory (Table 3.5). Cg23089913 may be considered potential predictor of BMI growth trajectories, given the modified risk of being in different BMI trajectories with differential methylation of this CpG.

Cg13217064 is located on transcription start site of the gene *SOX14* on chromosome 3. *SOX14* is a member of *SOX* (sex-determining region on Y box) family transcription factors that play critical role in cell fate determination as well as tissue development including pancreas [74]. Li et al reported that *SOX14* promotes the growth of cervical cancer cells by activation of WNT/β-catenin signaling pathway [75]. This pathway is known to be involved in organ development, and physiological processes such as cell proliferation, migration, apoptosis, and invasion in metabolic diseases (24). Animal studies illustrated high fat diet induced hypertrophy and hyperplasia in visceral as well as subcutaneous white adipose tissues via Wnt/β-catenin activation [76]. Also, *SOX* family transcription factors are known modulators of canonical Wnt/β-catenin signaling in disease development and cell growth [77]. Despite the lack of direct association between *SOX14* and obesity, existing evidence suggests that *SOX14* has a critical

role in cell proliferation via Wnt/β-catenin activation which in turn is involved in fat deposition in adipose tissues [76].

SOX14 was found to be differentially methylated in siblings born before and after maternal bariatric surgery and substantial weight loss [78]. *SOX14* transcription factor was found in abundance in omentum of diabetic compared to non-diabetic patients [79]. Sharp et al illustrated that children born to mothers with lower BMI (underweight) showed higher methylation levels of the CpG site cg13217064 on *SOX14* at birth [35]. This investigation additionally showed that the children with higher methylation levels of cg13217064 showed higher odds of progressing into DOW trajectory. To our knowledge, this is the first study that demonstrated a link between differential methylation of cg13217064 on SOX14 and excess bodyweight. Future studies should focus on understanding the association between *SOX14* and obesity as DNAm of cg13217064 which could be a potential predictor of BMI growth trajectories from infancy to 26 years of age.

To illustrate differential developments of DNAm at birth, age 10 and 18 years for specific trajectories compared to the normal growth trajectory, selected CpGs that passed multiple testing were examined (Figure 3 & 4). Concurrent changes in the DNAm normal and EPO trajectory were observed for cg23089913 (Figure 3). Participants in the EPO showed a lower methylation when compared to normal trajectory. This reflects the higher odds ratio for lower DNAm (Table 10). Cg13217064 showed a parallel change in DNAm in males. However, females exhibited a distinct pattern of DNAm changes with a cross-over at 10 years of age (Figure 4). It would be interesting to test whether the risk of obesity changes with age and the differential methylation of these CpGs in a repeated measurement analysis.

This study suggests that the differential DNAm at birth associated with maternal pre-pregnancy BMI constitute a risk for the offspring being in EPO or DOW trajectory. Further

replications of these associations in independent cohorts are needed. However, currently no other study has data on BMI trajectories and DNA methylation. Gene expression levels based on Guthrie card (F1) were not available to understand the biological relevance of the identified CpGs. The study participants are Caucasians only, thus future studies should consider racial diversity. Nevertheless, this study identified two novel CpGs of obesity-associated factors that were known to be influenced by environmental exposures or lifestyle changes. Interventions altering the methylation of cg23089913, and cg13217064 in newborns may change the risk of developing overweight in childhood and adolescence.

Conclusion

The results from this study suggest that gestational BMI-related epigenetic markers identified at birth were associated with offspring BMI trajectories from birth to 26 years of age. Variations in the methylation levels of these CpGs imposed various risk levels of obesity in offspring. Additional studies are needed to investigate the potential conditions that can change the DNAm of these two CpGs further modifying the risk of obesity.

Chapter 4

4. Methylation at birth associated with maternal pre-pregnancy BMI may predict offspring BMI from infancy to adulthood in mixed linear models with repeated measurements

Introduction

Epigenetics changes, the mitotically heritable alternations that occurred due to the influence of exposures but not due to changes in the DNA sequence were studied to delineate the link between the intrauterine exposures and offspring health outcomes [80]. Epigenetic modification such as DNA methylation (DNAm) - the addition or removal of a methyl group to a cytosine followed by a guanine linked by phosphate dinucleotide (CpG) - has documented the role of intrauterine exposures such as maternal smoking during pregnancy and maternal body mass index (BMI) during gestation [81, 82].

Maternal pre-pregnancy BMI has shown to have prominent influence on the offspring BMI from childhood to adolescence and was considered as major risk factor [83]. Such an association is considered to be mediated via epigenetic modifications such as DNAm [84].To date, epigenome wide association studies (EWAS) identified several differentially methylated CpGs in offspring at birth with respect to the maternal gestational BMI. The number of CpGs identified by different studies varies. An EWAS by Liu et al who compared the DNAm of offspring born to obese vs normal mothers using 27K bead identified 20 CpGs [84] while Sharp et al using the 450K Illumina BeadChip identified 1,649 differential methylated CpGs at birth [85]. The PACE (pregnancy and childhood epigenetics) consortium conducted a meta-analysis using maternal BMI information and variation in newborn umbilical cord blood DNAm from 19 cohorts and identified 104 differentially methylated CpGs [36]. Among the CpGs identified by these studies, there was no overlap, which can be attributed to false positive results and small sample sizes of some EWAS. Despite these inconsistencies, it has nevertheless, been suggested that epigenetic marker of maternal pre-pregnancy BMI can be identified in the offspring at birth.

Sharp et al who studied associations between maternal pre-pregnancy BMI and DNAm at birth, also tested the predictability of CpGs identified at birth for adiposity in childhood and adolescence. No specific CpGs were reported with such associations after multiple statistical correction [85]. The lack of significant results may be due to many non-informative CpGs which eliminated informative CpG when adjusting for multiple testings. Also, potential post-partum confounder such as duration and mode of feeding during infancy (breast feeding, formula feeding and mixed feeding- breast), second-hand smoke exposure, and active smoking of offspring were not considered when associations were studied. Exclusive breast feeding was known to prevent the development of overweight and obesity [86, 87] whereas early introduction of formula feeding elevated the risk of overweight in later infancy [88]. Similarly, children who were exposed second smoke in childhood and/or adolescence have higher odds of obesity compared to children who were not exposed [89].

For the current study, the study hypothesis is that the differentially methylated DNAm related to maternal BMI identified in offspring at birth are associated with the BMI development from infancy to adolescence. Hence, we aim to identify CpGs known to be associated with maternal pre-pregnancy BMI at birth that may 'predict' BMI from infancy to adolescence. We try to overcome prior limitations by (i) focusing on informative 1,773 CpG related to gestational BMI in prior studies and not all CpGs, (ii) excluding non-informative CpGs that were not associated with either BMI measurements at different ages (1, 4, 10, 18, 26 years); (iii) including potential covariates such as mode of infant feeding, second-hand smoking, and cell proportions; in addition to other potential confounder; and (iv) further exploring the stability of DNAm at age 10 and 18 years with BMI overtime.

Methods

Study population

Parents of the children born between January 1, 1989, and February 28, 1990 (n=1,536) in the Isle of Wight (IOW), UK were invited to participate in a longitudinal study. After exclusion of adaptions, perinatal deaths and missing written consent, 1,456 (94.8%) of the parents enrolled their newborns. The birth cohort has been described in detail elsewhere [38, 63]. The study population is 99% Caucasian. The enrolled newborns will be refereed as the F1 generation. The F1 participants were followed up from infancy into adulthood (birth, age 1, 4,10, 18, and 26 years). The investigation was approved by the local ethics committee –National Research Ethics Service, NRES Committee South Central – Hampshire B, U.K. and by the University of Memphis Institutional Review Board in Memphis, U.S (#2423). Written informed consents were obtained from the parents until age 10 and at 18 and 26 years from the participants at each follow-up.

Variables

Maternal and paternal smoking during pregnancy, birthweight, gender, infant feeding method (breast milk only, formula only, breast milk plus formula) during infancy, secondhand smoke exposure, and active smoking of participants at age 18 and 26 years are considered as potential confounding variables. At birth, maternal and paternal history of smoking during pregnancy were gathered while birth weight and gender of the child were collected from the birth records. At the 1-year and 2-year follow-up, information on type of feeding (formula and/or breast milk), length and weight of the child, exposure to secondhand smoking were collected. Information on secondhand smoke exposure at ages 4 and 10 years was collected from the questionnaires completed by parents during follow-ups. The smoking history of the participants at ages 18 and 26 years were acquired from the self-administered questionnaires. Height (length) and weight were measured at 1, 2, 4, 10, 18, and 26 years of age.

BMI measurements from infancy to 26 years of age

BMI at each time point (1, 4, 10, 18, and 26 years) is calculated as weight (kg) to height (m^2) ratio. BMI was standardized as Z-BMI scores, which were calculated by dividing BMI by its the standard deviations within IOW.

DNA methylation measurements

DNA was extracted from the Guthrie cards (dried blood spots collected at birth) of the F1 participants (n=796), peripheral blood samples at age 10 (n = 330) and 18 years (n = 476) using a standard procedure as described by Bryan et al [90]. The Illumina Infinium Methylation EPIC BeadChips (Illumina, Inc., San Diego, CA, USA) was used to obtain the methylation levels following the manufacturer's protocol for samples obtained from Guthrie card. The CPACOR pipeline [91] was used for pre-processing and QC of the samples. Batch effects were corrected using Bioconductor package IMA [92] and COMBAT [93].

Peripheral blood samples at age 10 (n = 330) and 18 years (n = 476) of F1 participants was extracted using a standard salting out procedure [94]. DNA concentration was determined using the Qubit quantification. Following the manufacturers protocol of EZ 96-DNA methylation kit (Zymo Research, CA, USA), up to 1 µg of DNA extracted from the samples was bisulfite-treated for cytosine to thymine conversion. DNA methylation (DNAm) was assessed using the Illumina Infinium HumanMethylation450 Beadchip (Illumina, Inc., CA, USA) [95]. Arrays were used to process the samples following standard protocol. Assay variability was assessed by assigning multiple identical controls to each bisulfide conversion batch. Batch effect was controlled by random distribution of samples in the arrays. Each beadchip was scanned using a BeadStation. The methylation level of each queried CpG locus was calculated using Methylation module of BeadStudio software.

The beta values represent the proportion of the methylated (M) sites over the sum of the methylated (M) and unmethylated (U) sites = (β =M/(c+M+U), with c as a constant to preventing dividing by zero were used to estimate DNA methylation levels. During pre-processing, (1) CpGs that are on sex-specific chromosomes, (2) CpGs with signals that were not distinguishable against background noise and (3) CpGs with a potential single nucleotide polymorphic (SNP) within 10 base pairs and with a minor allele frequency of greater than 0.007 since these probe-SNPs may interfere with the DNAm measurement were excluded. After pre-processing, controlling for batch effect, and quality control 551,711 CpGs identified from Guthrie cards were considered reliable.

Cell proportions

The Minfi R-package with estimateCellcounts() function and adult reference panel of the respective blood samples. Seven cell types: B cells, CD4T and CD8T cells, monocytes, natural killer cells, neutrophil, and eosinophils were assessed using cell-specific CpGs, default settings in minfi and the method by Housemen et al [96] and Aryee et al [68]. nRBCs were not estimated as the Guthrie cards are collected between 3-5 days after delivery and nRBC are no longer seen in the blood circulation of the newborn after few days.

Exposure of interest - CpGs previously identified to be associated with maternal BMI at birth

A literature review was performed to identify studies that performed epigenome wide association (EWAS) between maternal pre-pregnancy BMI and the offspring DNAm at birth. Academic databases - PUBMED, GOOGLE SCHOLAR, & EMBASE were queried using search terms such as “Maternal BMI”, “Maternal pre-pregnancy BMI”, “Pregestational BMI”, “Preconception BMI”, and “Maternal obesity” along with “Cord blood DNA methylation”, “DNA methylation”, and “Epigenome-wide association” within a 10 years’ time span of 2007 -2017 to

capture all past studies that reported such results. Studies with similar exposure of interest (maternal pre-pregnancy BMI) and confounders (sex of the child, age of the mother, cell proportions etc.) were considered. 1,773 candidate CpGs from three EWAS studies with maternal pre-pregnancy BMI as exposure and DNAm of the offspring at birth were discovered. Of these 1,773 CpGs, 1,090 CpGs were also identified among the 551,711 CpGs from F1 Guthrie cards in the IOW birth cohort and constitute the exposure of interest.

Statistical analysis

To test whether the analytical sample represents the cohort, one-sample proportion tests were used. For normal distributed continuous variables such as BMI, the one-sample Wilcoxon signed test was applied. Data available on participants' BMI at ages 1, 4, 10, 18, and 26 years and 1,090 candidate CpGs known to be associated with gestational BMI and present in IOW birth cohort data were used to test the hypothesis of DNAm at birth to be associated with repeated BMI measurements from infancy to age 26 years.

1,090 candidate CpGs were tested for their associations with offspring BMI at each age 1, 4, 10, 18, and/or 26 years using ttScreening [71], a R computing package that filters informative CpGs that were potentially associated with the BMI at different time periods. In total, five screenings were performed with BMI at age 1, 4, 10, 18, and 26 years as independent variables and DNAm at birth as outcome of interest respectively. In the screenings, cell proportions were included as potential confounder in the robust regression but no other potential confounder. ttScreening facilities a screening process, where each CpG was tested through 100 iterations of training and testing. 2/3rd of the sample was used as training dataset while 1/3rd was used as training dataset for each iteration. A CpG with at least 50% of selection probability in training and testing samples and statistically significantly associated with BMI at each respective age was considered informative.

CpGs identified in at least one ttScreening were considered informative and were further tested using mixed linear models for association with repeated measurements of BMI at age 1, 4,10, 18, and 26 years adjusting for potential confounders such as paternal smoking during pregnancy, low birth weight, feeding pattern (breast feeding only, formula feeding only, and both), secondhand smoking at age 1, 4, and 10 years and personal smoking at ages 18 and 26 years of age in addition cell proportions. Linear mixed models were used to study the overall effect and to account for the repeated measurements (age) of BMI in each subject. Within-subject variation and the random time/age effect were included in the models. In these linear mixed models, covariates that did not change the parameter estimate by at least 10% were considered not to confound the association and were excluded. Associations with a p-value ≤ 0.05 were considered statistically significant. Results were corrected for multiple testing using false discovery rate methods (FDR) [72]. For the CpG sites, M-values were used to study the association between BMI and DNAm that passed screening. For CpGs that passed multiple testing, 95% confidence intervals were calculated. Additionally, the CpGs that survived the FDR were further stratified by sex to test if the associations were distinct in male and female participants.

Lastly, to understand variations in methylation at birth, age 10, and age 18 years, and BMI at ages 1, 4, 10, 18, and 26 years, the mean methylation value of the corresponding CpGs and the BMI z-scores were inspected in male and female participants. All statistical analyses were performed in R-4.1 and SAS, version 9.4 (SAS Institute, Cary, NC, USA).

Results

Participant's characteristics

The characteristics of the analytical sample (n=796) and the total cohort (n=1,459) are presented in table 13. No statistically significant differences in the characteristics were observed

concluding the analytical sample is representative of total population enrolled in the study. The majority of the participants have normal birth weight (94.9%) and had a mixed pattern of feeding (breast and formula feeding-64%). The sample consisted of equal proportions of male and female members. Participant exposed to paternal smoking during pregnancy, secondhand smoking or personal smoking varied between 24% to 37%.

Table 13 Characteristics of the study population and the analyzed sample

Variable		Study population n = 1456 % (n)	Analytical Sample n = 796 % (n)	p Value
Sex	Male	50.5 (735)	50.4 (401)	0.72
	Female	49.5(721)	49.6 (395)	
Paternal smoking during pregnancy	No	60.8 (885)	62.1 (494)	0.35
	Yes	37.2 (542)	35.6 (283)	
	Missing	2 (29)	2.4 (19)	
Low birth weight	< 2.5 Kg*	3.7 (54)	94.9 (755)	0.63
	>= 2.5kg*	94.6 (1378)	3.8 (30)	
	Missing	1.7 (24)	1.4 (11)	
Infant feeding method	Formula feeding only	16.5 (368)	8.7 (69)	0.34
	Brest feeding only	8.2 (119)	19 (151)	
	Mixed feeding	66.6 (969)	65.1 (518)	
	Missing	8.8 (128)	7.3 (58)	
Secondhand smoking at age 1	No	53.4 (778)	57 (454)	0.16
	Yes	38.3 (557)	36.4 (290)	
	Missing	8.3 (121)	6.5 (52)	
Secondhand smoking at age 4	No	50.7 (738)	55 (438)	0.76
	Yes	32.1 (468)	34 (270)	
	Missing	17.2 (250)	11.1 (88)	
Secondhand smoking at age 10	No	52.4 (763)	56.4 (449)	0.57
	Yes	38.2 (556)	39 (310)	
	Missing	9.4 (137)	4.7 (37)	
Smoking at age 18 years	No	61.7 (899)	67.2 (535)	0.15
	Yes	24.5 (356)	23.4 (186)	
	Missing	13.8 (201)	9.4 (75)	
Smoking at age 26 years	No	48 (699)	51.1 (407)	0.53
	Yes	21.6 (314)	25 (198)	
	Missing	30.4 (443)	24 (191)	
BMI (kilograms/meter^2) **(n=Study population/analytical sample)**		**Mean (95% Confidence Intervals)**		
Age 1 (n = 1,054/584)		17 (15 - 20)	17 (15 - 20)	0.75
Age 4 (n= 1,043/635)		16 (14 - 19)	16 (14 - 19)	0.98
Age 10 (n = 1,043/717)		18 (15 - 24)	18 (15 - 24)	0.99
Age 18 (n = 949/617)		23 (18 - 32)	23 (18 - 33)	0.92
Age 26 (n = 549/358)		26 (19 - 39)	26 (19 - 40)	0.93

*Kg Kilograms

Identification of candidate CpGs that were associated with BMI from age 1, 4, 10, 18, to 26 years of age

Of the 1,090 candidate CpGs identified from the literature, by using the ttScreening approach four CpGs at age 1; six CpGs at age 4; nine CpGs at age 10; two CpGs at age 18; and one CpG at age 26 (total 20 unique CpGs) were found to be statistically significantly associated with BMI at the respective ages (Table 14). Two CpGs - cg23089913 (*NANOS1*) and cg13422881 (*ERCC3*) were associated with BMI at both, age 10 and 18 years.

Table 14 CpGs that were informative for BMI at each age with at least 50% of selection proportion

Time Period	CpG Name	Selection Proportion	Parameter Estimate	p-value	Gene name(s)	Chromosome
Age 1 (n = 584)	cg00488692	85	-0.04	2.40E-05	unknown	2
	cg14434213	81	-0.04	8.10E-06	unknown	8
	cg10182317	73	0.03	1.70E-04	CLVS2	6
	cg22038796	57	0.03	4.80E-04	PSTPIP2	18
Age 4 (n = 635)	cg12076012	79	-0.05	5.70E-05	ZFYVE1	14
	cg07428439	77	-0.03	9.50E-05	LOC284023	17
	cg15852879	63	-0.05	2.20E-04	ASB6	9
	cg04891917	54	-0.04	5.70E-04	KBTBD3;AASDH	11
	cg00522555	52	-0.05	1.70E-03	KEAP1	19
	cg26864230	52	-0.03	5.50E-04	unknown	1
Age 10 (n = 717)	**cg23089913**	**92**	**-0.02**	**3.20E-06**	**NANOS1**	10
	cg24471459	73	-0.01	6.70E-05	UBXN8	8
	cg26862527	73	-0.02	1.60E-04	BAI3	6
	cg10592690	71	-0.01	3.90E-04	LRRC41;UQCRH	1
	cg07654559	69	0.02	5.60E-05	CCDC142;TTC31	2
	cg26884261	66	-0.02	3.50E-04	UBTD1;MMS19	10
	cg17812850	55	-0.02	6.20E-04	TMEM184C	4
	cg13422881	**51**	**-0.02**	**6.40E-04**	**ERCC3**	**2**
Age 18 (n = 617)	cg05667158	50	0.02	4.40E-04	CCND2	12
	cg23089913	**74**	**-0.01**	**3.10E-05**	**NANOS1**	**4**
	cg13422881	**68**	**-0.02**	**2.50E-04**	**ERCC3**	**2**
Age 26 (n = 358)	cg05925497	61	-0.01	1.00E-03	FLJ32810	11

Regression models were adjusted for cell proportions.

Plausible predictors of BMI from infancy to 26 years

This set of 20 unique CpG sites were then tested for their associations with BMI at different ages using mixed linear models with repeated measurements of BMI and adjusting for potential confounders. Of the 20 CpGs, eight CpGs showed a statistical significance with repeated BMI measurements but only five CpGs survived multiple testing. The estimated associations and their 95% confidence intervals of the CpGs that passed the multiple testing are presented in table 15. All CpG sites [cg00488692 (*SP3-nearest gene*), cg14434213 (*RNF5P1-nearest gene*), cg23089913 (*NANOS1*), cg26862527 (*BAI3*), and cg17812850 (*TMEM184C*)] were inversely associated with BMI Z-scores from infancy to 26 years of age. For cg14434213 the effect of repeated measurements showed a reduction of BMI of -0.28 kg/m2, for cg00488692 and cg23089913 the estimated effect is a reduction of BMI by -0.21 kg/m2, for cg26862527 and cg17812850 the reduction is -0.14 and -0.17, respectively. Of interest is also that participants with low birthweights had BMI that was 0.3 kg/m^2 larger than those of participants with normal birthweights (data not shown).

Table 15 Parameter estimates and 95% confidence intervals for five CpG sites that are associated with BMI after adjusting for confounders and multiple testing (n = 2,483)

CpGs (Exposure)	Annotated genes	CpG location relative to gene	Parameter Estimate	Standard error	95% Confidence Intervals	Raw *P*value	FDR-adjusted *P* value
cg14434213 (Intragenic)	*RNF5P1*		-0.28	0.08	-0.44 to -0.12	0.0008	0.16
cg00488692 (Intragenic)	*SP3*		-0.21	0.07	-0.35 to -0.06	0.005	0.045
cg23089913	*NANOS1*	TSS1500	-0.21	0.08	-0.37 to -0.05	0.008	0.047
cg26862527	*BAI3*	5'UTR; TSS1500	-0.14	0.06	-0.25 to -0.03	0.01	0.047
cg17812850	*TMEM184C*		-0.17	0.07	-0.3 to -0.04	0.01	0.047

Regression models were adjusted for cell proportions, gender, paternal smoking during pregnancy, secondhand smoking at age 4, 10, 18, and 26 years, birth weight, and feeding pattern.

A gender stratified analysis was performed to illustrate the associations of the CpGs that passed the FDR and showed a statistically significant association in the repeated measurement analysis (Table 16). In male participants, cg00488692, cg14434213, and cg23089913 displayed a significant association with 0.2kg/m^2 decrease of BMI per unit increase in DNAm. In female participants cg00488692 and cg14434213 showed a statistically significant association with 0.3kg/m^2 and 0.4kg/m^2 decrease in BMI with every unit increase in the methylation levels of these CpGs respectively. Interestingly, no significant associations were found for cg23089913 and cg17812850 in male or female participants. It is noteworthy that female participants who are exposed to paternal smoking during pregnancy are at 20% higher risk of overweight while those who are exposed to mixed mode of feeding (formula + breast feeding) were at 30% higher risk of overweight (data not shown).

Table 16 Parameter estimates and 95% confidence intervals for male and female participants

CpGs	Male (n = 352)				Female (n = 359)			
	Parameter Estimate	**Standard error**	**95% Confidence Intervals**	***P* value**	**Parameter Estimate**	**Standard error**	**95% Confidence Intervals**	***P* value**
cg14434213 (*RNF5P1*)	**-0.19**	**0.08**	**-0.34 to -0.03**	**0.02**	**-0.37**	**0.13**	**-0.61 to -0.12**	**0.005**
cg00488692 (*SP3*)	**-0.15**	**0.07**	**-0.29 to -0.02**	**0.03**	**-0.28**	**0.11**	**-0.51 to -0.06**	**0.01**
cg23089913 (*NANOS1)*	**-0.2**	**0.1**	**-0.39 to -0.01**	**0.04**	-0.22	0.12	-0.46 to 0.02	0.07
cg26862527 (*BAI3)*	-0.14	0.09	-0.31 to 0.03	0.1	-0.15	0.11	-0.35 to 0.06	0.17
cg17812850 (*TMEM184C)*	-0.15	0.1	-0.35 to 0.04	0.12	-0.11	0.09	-0.28 to 0.06	0.19

Regression models were adjusted for cell proportions, paternal smoking during pregnancy, secondhand smoking at age 4, 10, 18, and 26 years, birth weight, and feeding pattern.

Descriptive characteristics of the developmental pattern of CpG sites in male and female participants that are associated with BMI measurements

Participants with DNAm measurements available at birth, age 10, and age 18 years (n = 218) were used to inspect methylation patterns and BMI measurements at different ages in males and female. Male participants showed a decline in BMI Z scores from age 1 to 10 years, followed by gradual increment until 26 years of age. Females showed different patterns. A gradual increase in BMI Z scores from age 1 to 10 years of age was observed that remained more stable afterwards.

The CpG sites cg00488692, cg14434213, cg23089913 displayed similar developmental patterns in male and female participants (figures 5-7). These CpGs showed lower methylation level with gradual increase in methylation to age 10 years and stayed consistent thereafter (figures 5 & 6). A similar development of BMI and DNAm for cg00488692, cg14434213, cg23089913 was observed in females.

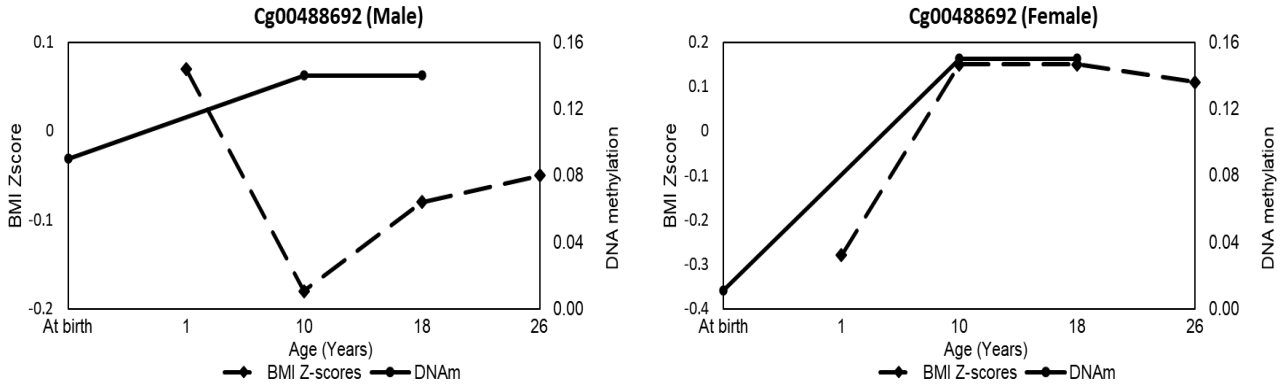

Figure 5 DNAm pattern of cg00488692 (*SP3*-nearest gene) and BMI Z scores from birth to adolescence in male and female participants

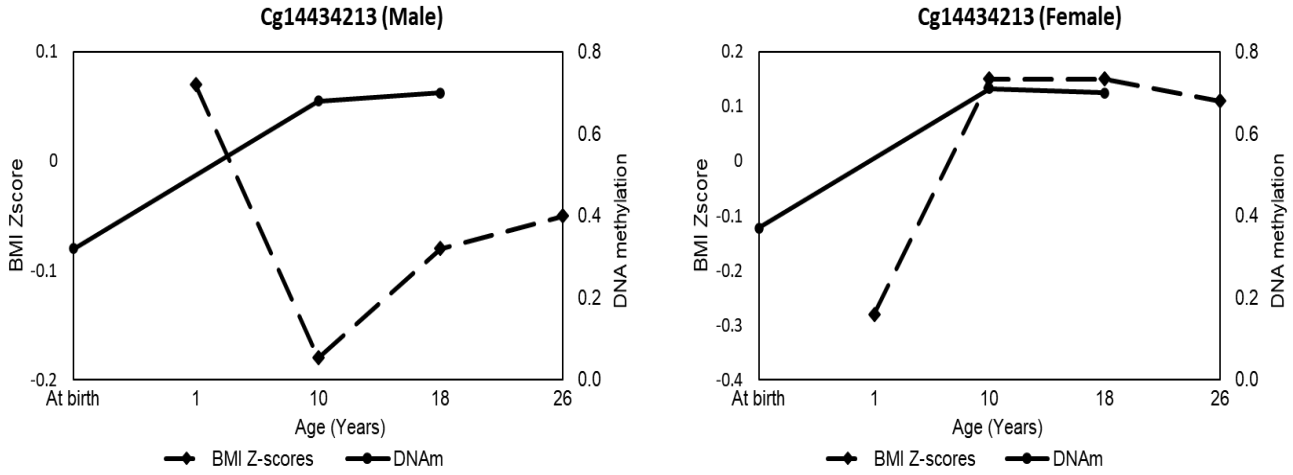

Figure 6 DNAm pattern of cg14434213 (*RNF5P1-nearest gene*) and BMI Z scores from birth to adolescence in male and female participants

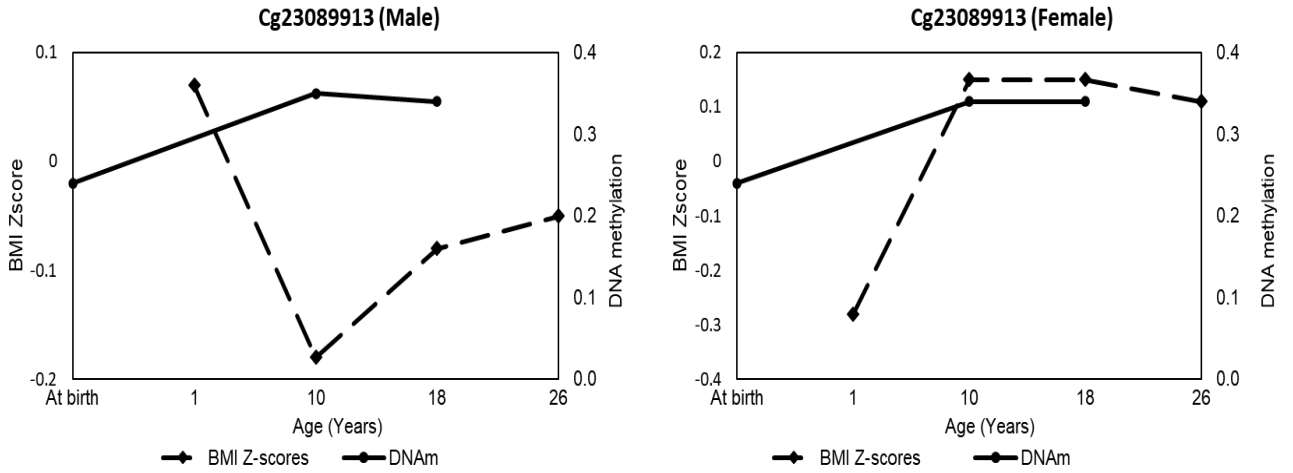

Figure 7 DNAm pattern of cg23089913 (*NANOS1*) and BMI Z scores from birth to adolescence in male and female participants

5. Discussion

Among the 1,090 epigenetic markers of maternal pre-pregnancy identified at birth in the offspring, 20 unique CpGs were found to be associated with BMI at age 1, 4, 10, 18, and/or 26 years of age. Five of the 20 informative CpGs that were identified in the study (cg23089913, cg12076012, cg14434213, cg07654559, and cg17812850) were already known to be linked with BMI growth trajectories replicating the study findings. A higher methylation of these CpGs was associated with lower odds of being in high risk growth trajectories. Of these 20 CpGs, five sites namely cg00488692, cg14434213, cg23089913, cg26862527, and cg17812850 showed a statistically significance in mixed linear models with repeated measurement analysis of BMI controlling for FDR. Individuals with higher methylation levels at these CgGs have lower BMI (Table 15). This study also noted that BMI increased by 0.3kg/m^2 for participants with low birthweight vs. normal birthweight. In a gender stratified analysis, female participants who were exposed to paternal smoking and mixed method of feeding were at a greater risk of developing higher BMI.

Cg00488692 is located on chromosome 2 and the nearest upstream gene is *SP3*. *SP3* transcription factor belongs to the *SP1* family transcription factors that regulate transcription of several genes by binding to consensus GC- and GT-box regulatory element in target genes and cell-cycle regulation [97]. *SP3* in conjugation with *SP1* stimulates the *GIP* expression via *GIPR* (Glucose-dependent insulinotropic polypeptide receptor) [98]. Animal studied shows that overnutrition and high food intake induced obesity can be explained by abnormal expression of *GIPR* followed by absorption of fat and glucose. This series of events demonstrates that SP3 may play a critical role in food induced obesity via GIPR expression and glucose uptake in obese individuals. Interventions focusing on modifying children's diet may alter the cascade of diet induced *GIPR* glucose absorption followed by reduced risk of obesity.

Cg14434213 is an intragenic CpG located between the genes *RNF5P1* (Ring Finger Protein 5 Pseudogene 1) and *TACC1* with *RNF5P1* as closest upstream gene on chromosome 8. Cg14434213 near *RNF5P1* was known to show lower methylation in children born to mother with high prenatal BMI. When the pre-/post gene expression profile of adipose tissue in men with six-weeks exercise illustrated 18% increase in *RNF5P1* expression. They also concluded that study participants showed reduced waist circumference and low waist/hip ratios implicating the role of this gene in weight management [99]. A higher methylation level of this CpGs was also association of lower odds of being in early transient overweight and early persistent obesity trajectories (data not shown).

In a meta-analysis by PACE Consortium, the CpG sites cg00488692 and cg14434213 showed an inverse association with prenatal BMI. Children born to mothers with higher BMI displayed lower methylation levels at these CpG sites. Mulder et al also demonstrated that these CpGs showed a change from birth to 6 and 9 years and were stable thereafter. This study observed that a higher methylation of these CpGs to be associated with lower BMI ($0.2kg/m^2$/unit change in methylation), which is in agreement with the findings where offspring's of mothers with higher BMI showed lower methylation which may constitute an adverse setting.

Sharp et al has reported that higher maternal pre-pregnancy BMI is associated with lower methylation of cg23089913 of the *NANOS1* gene at birth [85] which in our study is associated with higher BMI of F1 offspring , suggesting that maternal BMI may be mediated to higher offspring BMI by cg23089913. Cg23089913 was also associated with BMI growth trajectories from infancy to 26 years of age [100]. Participants with higher methylation levels of this CpGs showed a higher risk of being in delayed overweight trajectory group. Cg23089913 is located on the transcription start site of the gene *NANOS1* on chromosome 10. In a multiphase dietary intervention study *NANOS1* was found to be highly expressed in obese individuals compared to normal weight individuals suggesting its relationship with obesity [101]. *NANOS1* is

differentially expressed in obese high insulin resistant individuals compare to non-obese individuals. We only identified one transcript that is related to *NANOS1 [102]*.

Cg26862527 belong to the promoter region (TSS1500) of the gene *BAI3*. Children born to mothers with lower prenatal BMI tend to have higher methylation levels of cg26862527 compared to normal weight mother [85]. Findings from our study demonstrated that higher methylation of this CpGs was associated with decreased BMI of 0.1k/m^2. BIA3 (Brain-Specific Inhibitor 3) also known as Adhesion G Protein Couples Receptor B3 (*ADGRB3*) is associated with G protein-couples receptor activity and transmembrane signaling receptor activity. BIA3 is the cell surface receptor and the sole mediator of the inhibitory effects of C1ql3. Signaling protein- compliment 1q-like-3 (C1ql3) is a metabolic regulator of insulin secretion from pancreatic β-cell in response to glucose levels. Alterations in the C1ql3/BIA3 signaling pathway were suggested to alter insulin secretion and may contribute to the type 2 diabetes in obese individuals [103]. BIA3 was also known to be significantly differentially methylated in obese women with systemic insulin resistance [104].

Cg17812850 is located upstream of the transcriptional start site of *TMEM184C* (also known as *TMEM34*/*FLJ10846*) on chromosome 4. *TMEM184C* is a protein encoding a gene that plays a crucial role in cell growth and is highly expressed in thyroid tissue https://www.proteinatlas.org/ENSG00000164168-TMEM184C. It has been demonstrated that FLJ10846 is highly expressed in individual with Prader-Will syndrome [105]; children with this syndrome in early childhood tend to consume more food if not monitored strictly and then are at higher risk of overweight and obesity [106]. From EWAS-based analysis, Sharp et al reported that the cg17812850 is inversely associated with maternal pre-pregnancy BMI [36]. A higher maternal pre-pregnancy BMI was associated with reduced methylation of cg17812850, which, in turn, in our study was connected with increased BMI. Hence, if we could modify the methylation of cg17812850, we might be able to minimize the risk of overweight in offspring.

This study has some limitations. The study population comprises of Caucasians only. Future studies should be conducted with more racial diversity and the identified associations should be replicated in independent cohorts. Using internal standardized BMI z-scores could be a limitation. BMI z-scores used in our analysis were calculated and standardized by the standard deviations within the IOW but not as per the CDC recommendations. BMI z scores derived as per CDC guidelines were criticized as poor measures of obesity and degree of adiposity in children younger than 9 years of age. Our sample consisted of participants from infancy to young adulthood (age 1 to 26 years) and BMI z-scores are yet considered a valid measure of BMI across different time periods and as a screening tool for obesity [107] concluding that the study results are acceptable.

Conclusion

The study showed that certain CpG sites, located near/on *SP3*, *RNF5P1*, *NANOS1*, *BAI3*, and *TMEM184C* genes, were differentially methylated and may serve as potential markers for identifying children at birth who are at risk of developing a higher BMI. These genes were known to be influenced by lifestyle factors, such as diet and exercise. Future studies should focus on interventions that modify these CpG sites, as well as identify conditions that may modify the association between the CpG sites and BMI, which may modify the risk of overweight in childhood and adolescence.

Chapter 5

5. Conclusion

It is well established that intrauterine risk factors, such as the prenatal body mass index (BMI) of the mother, have an effect on the offspring's BMI growth trajectories and BMI from childhood into adulthood. BMI trajectories were developed and studied to understand the dynamics and developmental patterns of BMI. In specific aim 1 (SA1) of this dissertation we aimed to study if the BMI trajectories established in early childhood can be extended to adulthood and are influenced by prenatal maternal BMI. Using the trajectory analysis and the data from IOW birth cohort, F1-generation, established in 1989 IOW, UK, which provided information on BMI from infancy to adulthood and gestational maternal BMI, SA1 investigated (1) whether existing BMI trajectories reported by Ziyab et al., [21] from infancy to age 18 years can be extended 26 years or alternatively if their patterns change; (2) whether BMI trajectories extended to 26 year are influenced by intrauterine risk factors- prenatal BMI and smoking.

After excluding participants with no BMI information at all ages (1, 4, 10, 18, and 26 years) and utilizing the group-based trajectory analyses four distinct developmental trajectories that covered the first 26 years of life were identified. These BMI trajectories were labelled as 'normal', 'early persistent obesity' (EPO), 'early transient overweight' (ETO), and 'delayed overweight' (DOW) trajectories. These trajectories agree (overlap of about 74% to 98% individual trajectories) with the BMI trajectories that were identified in the IOW for the age-span between infancy and 18 years. Individual BMI trajectories of EPO and the 'normal' trajectory group were set as early at age four years and remained consistent through adolescence (26 years age) highlighting the critical timeline in the early childhood that need preventive attention. Unlike the EPO and 'normal' trajectories, ETO and DOW trajectories were set later between age 4 and 10 years of age.

Prenatal maternal BMI was found to be associated with increased odds of being in EPO and DOW trajectories illustrating the impact of the maternal prenatal BMI on the -trajectories of their offspring from age 1-26 years. We also observed a consistent and statistically significant association of early risk factors such as gestational age, birth weight, age of initiation of formula feeding, duration of breastfeeding, and gender with EPO and/or DOW trajectories.

The underlying biological explanations for the aforementioned associations between intrauterine risk factors and offspring BMI may include an interplay of genetic, epigenetic, and other behavioral changes during and after pregnancy. Maternal pre-pregnancy BMI has been shown to have prominent influence on the offspring BMI from childhood to adolescence and was suggested to be mediated via epigenetic alterations such as DNAm. In specific aims 2 and 3 of this dissertation, we studied if epigenetic markers of prenatal BMI identified at birth in offspring predict offspring BMI trajectories and BMI from infancy to adulthood.

A literature review was performed to identify studies that performed epigenome wide associations (EWAS) between maternal pre-pregnancy BMI and the offspring DNAm at birth. Academic databases - PUBMED, GOOGLE SCHOLAR, & EMBASE were queried using search terms such as “Maternal BMI”, “Maternal pre-pregnancy BMI”, “Pregestational BMI”, “Preconception BMI”, and “Maternal obesity” along with “Cord blood DNA methylation”, “DNA methylation”, and “Epigenome-wide association” within a 10 years’ time span of 2007 -2017 to capture all past studies that reported results on maternal BMI and DNAm. Studies with similar exposure of interest (maternal pre-pregnancy BMI) and confounders (sex of the child, age of the mother, cell proportions etc.) were considered. The query identified 1,773 candidate CpGs from three EWAS studies with maternal pre-pregnancy BMI as exposure and DNAm of the offspring at birth.

Of 1,773 differentially methylated CpGs at birth that were reported in at least one prior study to be associated with maternal pre-pregnancy BMI, 1,090 CpGs were also found in the F1 generation data set and thus could be evaluated for their odds of developing a specific BMI trajectory in offspring from infancy to age 26 years. Most other CpGs not found in the F1 generation dataset were eliminated since they did not pass quality control. The CpG excluded via quality control includes- i) CpGs with low quality; ii) CpGs that are missing at random; iii) CpGs located on sex-chromosomes; and iv) CpG sites that have a potential single nucleotide polymorphism (SNP) within 10 base pairs (a probe SNP) and with a minor allele frequency of greater than 0.007 since these probe-SNPs may interfere with the DNAm measurement.

After adjusting for multiple testing, with the normal trajectory as reference group, CpG sites cg23089913 (*NANOS1*) and cg13217064 (*SOX14*) were statistically significantly associated with EPO and DOW trajectories, respectively. Higher methylation levels of cg23089913 (OR: 0.84, 95%CI: 0.76-0.93) at birth displayed lower odds of being in EPO while increased methylation of cg13217064 (OR: 1.4, 95%CI:1.13-1.67) showed positive association concluding higher odds of progressing into DOW trajectory compared to the normal trajectory. In a gender-stratified analysis, the odds of developing into delayed overweight developmental trajectory was 1.8 times (95%CI:1.36-2.27) higher per methylation level of cg13217064 in female participants while no such association was detected in males.

In aim 3, epigenetic markers of maternal pre-pregnancy identified at birth in the offspring and available in IOW were further evaluated for their association with BMI from infancy to 26 years using repeated measurement of BMI in linear mixed models. Of the 1,090 CpGs tested, 20 unique CpGs were found to be associated with BMI at age 1, 4, 10, 18, and/or 26 years of age. Of these 20 CpGs, five sites cg14434213 (*RNF5P1*; *TACC1*), cg00488692 (*SP3-nearest gene*), cg23089913 (*NANOS1*), cg26862527 (*BAI3*), and cg17812850 (*TMEM184C*) showed a statistical significance after controlling for the false discovery rate (FDR). For every unit

decrease in the methylation level of these CpGs, the BMI increased by 0.1 to 0.3 kg/m^2. In a gender stratified analysis, cg14434213 and cg00488692 showed a statistically significant association in males and females while cg23089913 displayed significant relation with BMI in male participants only. However, for cg26862527 (*BAI3*) and cg17812850 (*TMEM184C*), no such associations were observed in either gender. In addition to these finding focused on epigenetics, female participants who were exposed to paternal smoking during pregnancy and mixed infant feeding during infancy (breast milk and formula) were at higher risk of weight gain or becoming obese. It was also observed that the male participant with lower birthweight showed 0.3 kg/m^2 higher BMI than those with normal birthweight while no significant associations were observed in females.

The outcome of BMI in the first 26 years of life was addressed with three different approaches in this dissertation. First, identification of trajectories of BMI from infancy to 26 years of age, which were classified into normal, overweight, and obesity; second, investigating the association of these trajectories with differential DNAm related to gestational BMI (candidate CpGs) at birth using multinominal logistic regression models; third, examining the relation between BMI and differential DNAm (candidate CpGs) with generalized linear regression models using the repeated measurements of BMI from age 1 to 26 years. Observations from these approaches demonstrate that the developmental pattern of BMI were set as early as the age of four years and were associated with candidate CpGs (differential DNAm at birth related to maternal BMI during pregnancy). Of the seven candidate CpGs that showed a statically significant associations, cg23089913 (*NANOS1*) was linked with BMI developmental trajectories as well as repeated measurements of the BMI from infancy to 26 years of age. The odds of developing in the EPO decreased with higher methylation levels of cg23089913. Also, every unit increase in methylation of this CpG was associated with 0.2 kg/m^2 decrease in BMI. These findings suggest that methylation of this specific CpG (cg23089913) is linked to BMI

developmental trajectories and may play a role in the development of obesity. The candidate CpGs identified in this work were also known be located on the genes that play critical role in metabolic disorders such as overweight/obesity, type 2 diabetes and physiological process like cell growth, differentiation, proliferation, and fat deposition. These findings constitute reliable base for further investigations that understand the biological role of the identified DNAm sites and BMI developmental patterns and BMI development with age.

Limitations and Strengths

1. Five measurements of BMI at age 1, 4, 10, 18, and 26 years were available for the trajectory analysis. Developments in the intervals cannot be assessed. However, these repeated BMI measurements prospectively cover the first 26 years of life in contrast to the other studies that are restricted to a specific time periods of childhood and/or adolescence.
2. The BMI z-score calculated are standardized by the standard deviations within IOW but not as per the CDC recommendations. Use of internal standardized BMI z-scores for trajectories and association analysis could be a limitation. BMI z scores derived as per CDC guidelines were criticized as poor measures of obesity and degree of adiposity in children younger than 9 years of age. Our sample consisted of participants from infancy to young adulthood (age 1 to 26 years) and BMI z-scores are yet considered a valid measure of BMI across different time periods and as a screening tool for obesity [107] concluding that the study results are acceptable.
3. Early pregnancy BMI was used as main predictor of offspring BMI trajectories is a plausible limitation in our study. Harris et al [55] suggested that the weight gain in first trimester is less compared to the second or third trimesters and prenatal maternal BMI reliable measure that represent the pre-pregnancy BMI.

4. Gene expression levels based on Guthrie card (F1) were not available to additionally explore the biological relevance of the identified CpGs. Therefore, additional research should be carried out to investigate the biological significance of these CpGs.
5. The study participants are only Caucasians. Future studies should consider the racial diversity. Nevertheless, the CpGs that showed an association in this study were located on genes that were recognized as potential biomarkers for obesity and were known to be influence by environmental exposures or lifestyle changes.
6. Although these investigations focused on DNAm sites, that were previously linked to the exposure of maternal BMI, this study did not test whether the identified CpGs act as mediators between maternal BMI and offspring BMI, neither for trajectories nor for the repeated BMI assessments. It was investigated whether differential DNAm shown to be associated with maternal BMI also was linked to offspring BMI and BMI developmental pattern from infancy to adulthood. It is likely that epigenome-wide analyses may detect other CpGs not linked with maternal BMI but associated with offspring BMI for other reasons. However, all the CpGs that showed an association with trajectories and/or repeated BMI assessments showed a lower methylation at birth. A higher gestational BMI was associated with lower DNAm at these CpG in offspring's at birth in IOW which is in agreement with the direction of association reported in literature.
7. This study focused solely on examining the association between DNAm and maternal BMI, BMI trajectories, and repeated BMI measurements, while intrauterine and early life risk factors such as maternal smoking during pregnancy, early formula feeding, and birth weight were not fully investigated. This limits our understanding of the potential influence of these risk factors on BMI. To gain a more comprehensive understanding of the relationship between modifiable risk factors, DNAm, and the risk of overweight/obesity, future studies should explore the effects of other risk factors on DNAm.

In conclusion, the analysis from SA1 identified four distinct developmental BMI trajectories across the first 26 years of life are set in early childhood that show strong agreement with BMI trajectories from infancy to 18 years identified in a prior investigation. Among the offspring trajectories, prenatal maternal BMI seems to be associated with increased odds of developing two specific trajectories, namely early persistent obesity' (EPO) and 'delayed overweight' (DOW), but not 'early transient overweight' (ETO). Early life risk factors such as gestational age, birth weight, duration of breastfeeding and gender are related to increased risk of offspring being in EPO and/or DOW trajectories.

In trajectory modeling, various subgroups of individuals with distinct patterns of change are identified. When performing association analysis with trajectories, the focus is on exploring the relationship between the predictor variable and the changes in the outcome variable pattern of interest. During SA2 (trajectory analysis), the association between differentially methylated CpGs collected from existing literature and BMI developmental trajectories identified in SA1 was examined. Two differentially methylated CpGs that might predict BMI pattern changes over time (in the first 26 years of life) were identified by SA2. However, it's important to note that these associations may not fully account for the correlation between repeated measurements and within-subject variations in the same individual.

On other hand, repeated measurement examines the association between the outcome and exposure accounting for within-subject changes of the outcome overtime while accounting for the correlation between the repeated measurements on the same individuals. SA3 (repeated measurement analyses ignoring trajectories) of this dissertation identified two and six distinct CpGs that may predict BMI growth trajectories and BMI measurement from infancy to adolescence respectively, which were known to be associated prenatal BMI.

This dissertation tested CpGs reported in the literature with the aforementioned approaches and identified CpGs that were linked to the changes in BMI patterns and/or repeated BMI measurements over time. The associations of the CpGs reported in this dissertation as well as functional roles of the genes on which there CpGs are outlined as below.

1. Of the two CpGs that presented an association with BMI developmental trajectories and repeated measurement of BMI from infancy to 26 years respectively, cg23089913 (*NANOS1 gene*) was identified to be statistically significant in both association models- namely using BMI trajectories and repeated measurements of BMI. SA2 demonstrated that lower methylation of this CpGs was associated with increased odds of developing into developing into EPO. In the repeated measurement analysis (SA3), the BMI increased by 0.2kg/m^2 with every unit decrease in the methylation of this CpG. Cg23089913 is located on the transcription start site of the NANOS*1* gene on chromosome 10. *NANOS1* is known to be differentially expressed in obese individuals with high insulin resistance completed to non-obese individuals [102]. A multiphase dietary intervention concluded that NANOS1 is highly expressed in obese individuals compared to normal weigh individuals' suggestion its connection with altered BMI [101].

2. The cg13217064 belongs to promoter region of *SOX14* gene on chromosome 3, a member of *SOX* (sex-determining region on Y box) family transcription factors that play critical role in cell fate determination as well as tissue development including pancreas. An EWAS demonstrated that children born to underweight mother displayed higher methylation of this CpGs at birth and our demonstrated that a higher methylation of this CpG increased the risk of developing into DOW trajectory. Existing evidence suggests that *SOX14* plays a crucial role in cell proliferation via Wnt/-catenin activation, which in turn was implicated in fat deposition in adipose tissues although not direct association of *SOX14* and obesity was reported [76]. *SOX14* was shown to be methylated differently in siblings born before and after bariatric surgery and substantial weight loss in the mother [78]. Also, when compared to non-diabetic patients,

diabetic patients omentum had an excess of the transcription factor *SOX14* [79].This evidence emphasizes the role *SOX14* in fat deposition and obesity related health ailments.

3. In SA3 (repeated measurement analyses) we observed that BMI increased by 0.2kg/m^2 per unit change in methylation of cg00488692. This CpG site is located in the intragenic region of the genes *SP3* and *OLA1* with close proximity to *SP3* on chromosome 2. *SP3* transcription factor belongs to the *SP1* family transcription factors that regulate transcription of several genes by binding to consensus GC- and GT-box regulatory element in target genes and cell-cycle regulation. Glucose-dependent Insulinotropic Polypeptide (GIP) has shown to potentiate insulin release in β-cells of the pancreatic islets and appears to play a vital role in maintaining glucose homeostasis. *SP3* in conjugation with SP1 stimulates the *GIP* expression via *GIPR* (Glucose-dependent insulinotropic polypeptide receptor) [98]. Animal studies suggested that overnutrition and high food intake induced obesity can be explained by abnormal expression of *GIPR* expression followed by absorption of fat and glucose [108]. This series of events reveals that *SP3* plays a critical role in food induced obesity via GIPR expression and glucose uptake in obese individuals. Interventions focusing on modifying children's diet may alter the cascade of diet induced *GIPR* glucose absorption followed by reduced risk of obesity.

4. Also in SA3, cg14434213 was identified. It is an intragenic CpG located between the genes *RNF5P1* (Ring Finger Protein 5 Pseudogene 1) and *TCCA1* with *RNF5P1* as closest upstream gene on chromosome 8. Cg14434213 near *RNF5P1* was known to show lower methylation in children born to mother with high prenatal BMI. When the pre-/post gene expression profile of adipose tissue in men with six-weeks exercise illustrated 18% increase in *RNF5P1* expression. They also concluded that study participants showed reduced waist circumference and low waist/hip ratios implicating the role of this gene in weight management [99].

5. In addition, in SA3, the repeated measurement analyses, identified cg26862527 on the gene *BIA3* (Brain-Specific Inhibitor 3) also identified as Adhesion G Protein Couples Receptor B3

(*ADGRB3*) to be associated with G protein-couples receptor activity and transmembrane signaling receptor activity. Signaling protein- compliment 1q-like-3 (C1ql3) is a metabolic regulator of insulin secretion from pancreatic β-cell in response to glucose levels. BIA3 is a cell surface receptor and the sole mediator of the inhibitory effects of C1ql3. Gupta et al. outlined that impairment in the C1ql3/BIA3 signaling pathway may effect insulin secretion followed by contributing to type 2 diabetes advancement in obese individuals [103]. *BAI3* is equally expressed in brain and islets of pancreas compared to other tissues. Cg26862527 is located on the to the promoter region (TSS1500) of the *BAI3 gene*. Findings from our study demonstrated that higher methylation of this CpGs to be associated with decreased BMI of 0.1k/m^2. *BIA3* was also known to be significantly differentially methylated in obese women with systemic insulin resistance [104].

6. Lastly, the repeated measurement analyses, found that among CpGs previously related to maternal BMI, cg17812850 seem to play a role. This CpG is on the *TMEM184C* gene encoding a protein that regulates cell proliferation and is highly expressed in thyroid tissue. Cg17812850, which is situated upstream of the promoter of *TMEM184C* (also known as TMEM34/FLJ10846). *FLJ10846* is significantly expressed in individuals with Prader-Will syndrome [105], where young children prefer to consume more food if not closely controlled and are at a greater risk of overweight and obesity [106]. The existing evidence is in agreement with findings of SA3, where every unit decrease in the methylation of cg17812850 increased BMI by 0.2kg/m^2 showing an increased risk of overweight.

This dissertation concludes that maternal pre-pregnancy epigenetic markers identified at birth could predict the BMI development from infancy to adolescence. SA2 and SA3 identified differentially methylated CpGs that could be used as potential biomarkers for identifying children who are at risk of being overweight. Future studies are warranted to further test the biological role of these genes and emphasize conditions that may modify these CpGs in early childhood and test whether respective modification also change the risk of overweight/obesity in offspring. Also, this

dissertation illustrates that maternal pre-pregnancy markers have long term impact on offspring. Population strategies should be developed to bring awareness in women of childbearing age about the impact of prenatal maternal BMI on offspring's BMI development.

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PRO-FY2022-68 - Admin Withdrawal: Not Human Subject Research
2 messages

do-not-reply@cayuse.com <do-not-reply@cayuse.com> 10 September 2021 at 08:57
To: karmaus1@memphis.edu, vjnjanam@memphis.edu

Institutional Review Board
Division of Research and Innovation
Office of Research Compliance
University of Memphis
315 Admin Bldg
Memphis, TN 38152-3370

September 10, 2021

PI Name: Vimala-Devi Janjanam
Co-Investigators:
Advisor and/or Co-PI: Wilfried Karmaus
Submission Type: Admin Withdrawal
Title: Developmental BMI Trajectories of first 26 years of life and Influence of Maternal prenatal BMI-A Birth Cohort Study
IRB ID: PRO-FY2022-68

From the information provided on your determination review request for "Developmental BMI Trajectories of first 26 years of life and Influence of Maternal prenatal BMI-A Birth Cohort Study", the IRB has determined that your activity does not meet the Office of Human Subjects Research Protections definition of human subjects research and 45 CFR part 46 does not apply.

This study does not require IRB approval nor review. Your determination will be administratively withdrawn from Cayuse IRB and you will receive an email similar to this correspondence from irb@memphis.edu. This submission will be archived in Cayuse IRB.

Thanks,

IRB Administrator
Division of Research and Innovation
Office of Research Compliance
315 Administration Building
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P: 901.678.2705
F: 901.678.4409

PRO-FY2022-92 - Admin Withdrawal: Not Human Subject Research

2 messages

do-not-reply@cayuse.com <do-not-reply@cayuse.com> 10 September 2021 at 09:01
To: karmaus1@memphis.edu, vjnjanam@memphis.edu

Institutional Review Board
Division of Research and Innovation
Office of Research Compliance
University of Memphis
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Memphis, TN 38152-3370

September 10, 2021

PI Name: Vimala-Devi Janjanam
Co-Investigators:
Advisor and/or Co-PI: Wilfried Karmaus
Submission Type: Admin Withdrawal
Title: Differential methylation at birth associated with maternal pre-pregnancy BMI may predict offspring BMI trajectories from Infancy to adulthood.
IRB ID: PRO-FY2022-92

From the information provided on your determination review request for "Differential methylation at birth associated with maternal pre-pregnancy BMI may predict offspring BMI trajectories from Infancy to adulthood.", the IRB has determined that your activity does not meet the Office of Human Subjects Research Protections definition of human subjects research and 45 CFR part 46 does not apply.

This study does not require IRB approval nor review. Your determination will be administratively withdrawn from Cayuse IRB and you will receive an email similar to this correspondence from irb@memphis.edu. This submission will be archived in Cayuse IRB.

Thanks,

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From: do-not-reply@cayuse.com <do-not-reply@cayuse.com>
Sent: Friday, September 10, 2021 9:04 AM
To: Wilfried Joachim Jurgen Karmaus (karmaus1) <karmaus1@memphis.edu>; Vimala-Devi Janjanam (vjnjanam) <vjnjanam@memphis.edu>
Subject: PRO-FY2022-69 - Admin Withdrawal: Not Human Subject Research

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September 10, 2021

PI Name: Vimala-Devi Janjanam
Co-Investigators:
Advisor and/or Co-PI: Wilfried Karmaus
Submission Type: Admin Withdrawal
Title: Epigenetic markers of maternal pre-pregnancy BMI at birth may predict offspring BMI-trajectories from Infancy to 18 years
IRB ID: PRO-FY2022-69

From the information provided on your determination review request for "Epigenetic markers of maternal pre-pregnancy BMI at birth may predict offspring BMI-trajectories from Infancy to 18 years ", the IRB has determined that your activity does not meet the Office of Human Subjects Research Protections definition of human subjects research and 45 CFR part 46 does not apply.

This study does not require IRB approval nor review. Your determination will be administratively withdrawn from Cayuse IRB and you will receive an email similar to this correspondence from irb@memphis.edu. This submission will be archived in Cayuse IRB.

Thanks,

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