Gene-environment interactions in obesity: results from the multi-ethnic cohort EpiDREAM

by

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Title: Gene-environment interactions in obesity: results from the multi-ethnic cohort EpiDREAM

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# Abstract

Background: Obesity is now considered to be a global epidemic and gene-environment interaction studies are crucial to understanding the genetic architecture of this disease. The objectives of this research were to (1) review the current evidence of gene-environment interactions in the field of obesity, (2) examine the interactions between obesity predisposing gene variants and physical activity using precise physical activity data and (3) analyze a novel gene-environment interaction between obesity predisposing gene variants and multiple pregnancies.

Methods: The data for the gene-environment interaction analyses were collected from the EpiDREAM study: a prospective cohort including participants of six ethnic backgrounds from 21 countries worldwide. A subset of 17 423 participants with complete genotype and phenotype information was included in the analysis. Obesity predisposing single nucleotide polymorphisms were analyzed independently and as a genetic risk score. General linear models were used to analyze all main effects and interactions.

Results: Physical activity interacted with *FTO* rs9939609 to modulate BMI (Pinteraction=0.032) and BAI (Pinteraction=3.26 x 10-4). Increased physical activity attenuated the impact of *FTO* on obesity. Four SNPs displayed significant associations with physical activity: *NTRK2* rs1211166 (P=0.015), *BDNF* rs6265 (P=0.007), *BDNF* rs1401635 P=0.003) and *NPC1* rs1805081 (P=3.52 x 10-4). Multiple pregnancies was significantly associated with BMI (Pinteraction=1.17 x 10-5) BAI (Pinteraction=3.47 x 10-7)and also interacted with *FTO* rs9939609 to modulate BMI (Pinteraction=0.014). The impact of *FTO* on BMI was accentuated by multiple pregnancies in the EpiDREAM cohort.

Discussion: Both physical activity and parity have a significant impact on obesity measures and these effects appear to be relevant on a global scale. Our results confirm the physical activity x *FTO* interaction in a multi-ethnic context and indicate that parity may also interact with *FTO* polymorphisms*.*

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# List of Abbreviations

BAI: body adiposity index

BMI: body mass index

BPA: basic physical activity score

FSH: follicle stimulating hormone

GEWIS: gene-environment-wide interaction studies

GLM: general linear models

GS: genetic predisposition score

GWAS: genome wide association study

HC: hip circumference

IGT: impaired glucose tolerance

METS: metabolic equivalent score

NGT: normal glucose tolerance

PA: physical activity

SNP: single nucleotide polymorphism

T2D: type 2 diabetes

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# Chapter 1-Introduction

The prevalence of obesity has doubled over the past three decades and is now considered to be a global epidemic[1](#_ENREF_1). Obesity is a known risk factor for a number of adverse health outcomes, including cardiovascular disease, type 2 diabetes, osteoarthritis, psychological disturbance, cancer and ultimately 8-13 years shorter life expectancy in its more severe forms[2](#_ENREF_2),[3](#_ENREF_3). Consequences of this epidemic include an important economic burden that was estimated to be 147 billion dollars in the United States in 2008, corresponding to 9.1% of the total annual health care expenditures[4](#_ENREF_4). Over nutrition and declines in physical activity are the two main environment causes of this epidemic, yet additional factors such as sleep debt, endocrine disruptors and multiple pregnancies have emerged as significant contributors to the escalating prevalence of obesity[5](#_ENREF_5),[6](#_ENREF_6).

Despite the global impact of these environmental changes, obesity appears to manifest preferentially in genetically predisposed subgroups, and a high level of inter-individual variation has been observed among exposed populations[7](#_ENREF_7). Early indications of the shared influence of genes and environment in modulating obesity originated from twin and family studies, which suggested that 40-80% of the inter-individual variation in obesity-related traits observed in the population can be attributed to genetic differences[8](#_ENREF_8). However, identifying the source of this variation has proven difficult. Single gene mutations that cause obesity autonomously are extremely rare[9](#_ENREF_9) and most cases of obesity appear to be influenced by the combined effect of many common gene variants with more modest effects on weight gain[10](#_ENREF_10),[11](#_ENREF_11). Genome wide association studies (GWAS) have revolutionized the search for these common gene variants associated with obesity, but a large portion of this heritability remains unexplained[8](#_ENREF_8). To date, over 70 polygenic obesity loci have been identified and in aggregate, only account for 2-4% of the variability that can be attributed to genetics[12](#_ENREF_12),[13](#_ENREF_13). This ‘missing heritability’ may be explained by genetic variants that have yet to be discovered, and perhaps hundreds of these variants are needed to account for more of this variability[14](#_ENREF_14). Other possible sources of this missing heritability include overestimation of heritability, rare variants with larger effects, structural variation[15](#_ENREF_15) and epistasis (interactions between two or more genetic loci)[16](#_ENREF_16). An alternative explanation is that the interaction between gene variants and environmental exposures may influence the development of obesity[17](#_ENREF_17). Obesity predisposing gene variants in the *FTO* gene have been found to interact with physical activity[18](#_ENREF_18) and diet patterns[19](#_ENREF_19) in populations of European ancestry, and these findings have been successfully replicated[20](#_ENREF_20),[21](#_ENREF_21).

In response to these findings, our research group undertook this project to further understand how obesity predisposing gene variants interact with different environmental exposures. The specific goals of this thesis were to (1) review the current evidence of gene-environment interactions in the field of obesity, (2) examine the interaction between obesity predisposing gene variants and physical activity using precise physical activity data among a multi-ethnic cohort and (3) analyze a novel gene-environment interaction between obesity predisposing gene variants and multiple pregnancies.

# Chapter 2-Literature Review

The year 2000 was the first time in history that the number of overfed people in the world (1.1 billion) equaled the number who were underfed[22](#_ENREF_22). By 2008, 1.4 billion adults were overweight and over 500 million were obese[23](#_ENREF_23). Current projections estimate that the prevalence of obesity will continue to increase and could exceed 40% by 2030[24](#_ENREF_24).

The classification of obesity in epidemiological studies is typically based on the body mass index (BMI) guidelines established by the world health organization (WHO)[25](#_ENREF_25). BMI is calculated as weight in kilograms (kg) divided by height in meters (m) squared, and those with a BMI greater than 30 are classified into one of the three obesity categories: obese class 1 BMI= 30.00-34.99, obese class 2 BMI= 35.00-39.99, obese class 3 BMI ≥ 40.00[23](#_ENREF_23). BMI is closely correlated with body fat and obesity related disease[25](#_ENREF_25),[26](#_ENREF_26). Specifically, increased BMI is an established risk factor for stroke[27](#_ENREF_27), ischaemic heart disease[28](#_ENREF_28) and cancers of the large intestine, kidney and endometrium[29](#_ENREF_29),[30](#_ENREF_30). Above a BMI of 25kg/m2,each 5kg/m2 increase is associated with a 30% higher risk of all-cause mortality[31](#_ENREF_31). Average BMI is increasing by a few percent per decade in many populations[32](#_ENREF_32), and no specific cause of death is inversely associated with BMI[31](#_ENREF_31). Of particular concern is the projection of a 33% increase in obesity prevalence and a 133% increase in severe obesity prevalence (obese class 3) over the next 20 years[24](#_ENREF_24). Those with a BMI above 40kg/m2 are at a much greater risk for diabetes and other adverse medical conditions than those with a BMI in the range of 30-35[32](#_ENREF_32). These individuals also have a much shorter life expectancy and incur greater lifetime medical costs[33](#_ENREF_33).

This recent epidemic cannot be entirely explained by changes in the human genome and has been mainly attributed to lifestyle modifications[34](#_ENREF_34). Despite the many environmental exposures that have been identified as obesity risk factors[35](#_ENREF_35), some individuals are more susceptible than others to weight gain in an obesity-prone environment[36](#_ENREF_36). Obesity is a multifactorial disorder that requires environmental influences to manifest, and who becomes obese at the individual level is largely determined by genetic factors[37](#_ENREF_37). Technological and methodological breakthroughs in the last fifteen years, such as GWAS, have led to important progress in the elucidation of the genetic architecture of obesity[38](#_ENREF_38). The first two genes (*LEP* and *MKKS*) associated with a mendelian non-syndromic or syndromic form of obesity were identified in 1997 and 2000[39](#_ENREF_39),[40](#_ENREF_40). Seven years later, the first common variant (located in the intron 1 of the *FTO* gene) reproducibly associated with polygenic obesity was identified[41](#_ENREF_41),[42](#_ENREF_42). *FTO* has also been identified as a nucleic acid demethylase and is associated with different methylation profiles and BMI variance[43](#_ENREF_43),[44](#_ENREF_44). Currently, 28 monogenic obesity loci (with or without syndromic features) and over 70 polygenic obesity loci have been described, and this list is destined to grow over the coming years[8](#_ENREF_8). In parallel with successful gene identification efforts, the number of studies on gene-environment interactions has logically grown rapidly[45](#_ENREF_45). Following the identification of *FTO* intronic variation as a major contributor to polygenic obesity[41](#_ENREF_41),[46](#_ENREF_46), several studies have investigated whether polymorphisms in *FTO* may interact with specific lifestyle factors to modulate the risk of obesity. In addition to the gene-environment interactions identified between *FTO*, diet habits[19](#_ENREF_19), and physical activity[18](#_ENREF_18), further exposures such as education level[47](#_ENREF_47) and depression status[48](#_ENREF_48) were found to mediate the risk of obesity associated with *FTO*. Other studies have built a genetic predisposition score combining the information of multiple associated SNPs to reflect the genetic predisposition to obesity. These analyses have evidenced significant interaction between obesity gene scores and physical activity[49](#_ENREF_49), television watching[50](#_ENREF_50) and consumption of sugar-sweetened beverages[51](#_ENREF_51) in the variation of BMI. Given the continuing increase in gene-environment interaction studies, a comprehensive review of these analyses is needed to provide an up to date summary of the literature. In this review, we summarize the growing body of evidence supporting gene-environment interaction in obesity and discuss noteworthy perspectives in this fast-moving field of research.

## Definitions

Obesity is a complex disorder that is caused by both genetic and environmental risk factors[52](#_ENREF_52). The genetic etiology of obesity can be classified into three categories. First, Mendelian obesity describes individuals who carry a rare gene variant with a dramatic impact on adiposity[9](#_ENREF_9). These variants are associated with a high lifetime risk of disease and exhibit a near one-to-one relationship between genotype and phenotype[11](#_ENREF_11),[53](#_ENREF_53),[54](#_ENREF_54). Second, other cases of obesity can be attributed to the concerted presence of DNA variation in multiple genes, known as polygenic obesity. Any collection of alleles at gene loci that regulate the inheritance of a quantitative trait or modulate the expression of a quantitative trait, are termed polygenic variants[11](#_ENREF_11). With respect to body weight regulation, it is estimated that the total number of genes with a small effect exceeds 100, and the lower limit of effect sizes is likely below 100g[10](#_ENREF_10). If an individual carries several polygenic variants that augment body weight, the synergistic effect of these variants may result in obesity[11](#_ENREF_11). Third, syndromic obesity refers to obesity that co-occurs with a distinct set of clinical phenotypes, such as mental retardation, dysmorphic features and organ-specific developmental abnormalities[55](#_ENREF_55). Syndromic forms of obesity result from chromosomal abnormalities or distinct genetic defects, which can be autosomal or X-linked disorders[56](#_ENREF_56)..

The concept of gene-environment interaction in the context of human diseases is not recent and has been discussed since proposed by J.B. Haldane in 1946 [57](#_ENREF_57). The definition of gene-environment interaction differs slightly depending on the field of study. The statistical definition of an interaction between two or more risk factors is simply the coefficient of the product term of the risk factors, also known as effect modification or effect modulation. Interaction is thus measured in terms of departure from a multiplicative or an additive model [58](#_ENREF_58), although the majority of common variants follow an additive model[59](#_ENREF_59). Alternatively, biological interaction between two factors is defined as their co-participation in the same causal mechanism to disease development[60](#_ENREF_60). As an example, gene-environment interactions are observed when a behavioural factor, such as physical activity or diet, mediates the association between a genetic variant and a phenotypic trait. For the purposes of this review, we adopt the epidemiological definitions of an interaction, also termed effect modification/effect modulation. With respect to binary outcomes, the combined risk of genetic and environmental factors must be significantly greater or less than would be expected if their effects were additive for an interaction to exist. In order to constitute an interaction for quantitative traits, the genetic effect estimate must vary significantly across a range of environmental exposures[16](#_ENREF_16). For statistical evidence of gene × environment interaction to be convincing, it is typically necessary to replicate the findings in additional samples or support the evidence with plausible underlying biological mechanisms [58](#_ENREF_58). Early indications of the shared influence of genetics and the environment in shaping obesity originated from heritability studies involving environmental exposures among twins.

## Heritability estimates are influenced by environmental exposure

Heritability is the proportion of total phenotypic variability caused by genetic variance in a population. Large pedigree, twin and adoption studies allow the calculation of heritability and they all evidence a strong genetic component in human obesity[61-63](#_ENREF_61). Before the first obesity gene identification reports, scientists considered the possibility that heritability, a global estimate of genetic predisposition to obesity, may be modulated by specific environments[64](#_ENREF_64). Specific environmental exposures known to mediate heritability estimates include prenatal conditions[65](#_ENREF_65), basic demographic features[66](#_ENREF_66), socio-structural factors[67](#_ENREF_67) and lifestyle behaviours[68](#_ENREF_68).

*In utero* factors have been proposed to modulate offspring’s future risk for obesity[69](#_ENREF_69). The higher estimates of heritability for BMI observed in mother-offspring pairs in comparison with father-offspring pairs suggest a possible modification effect of maternal *in utero* environment on the offspring’s genetic predisposition to obesity[70](#_ENREF_70). Maternal weight gain during pregnancy may interact with genetic factors to render the offspring more susceptible to develop obesity in young adulthood[71](#_ENREF_71).

Genetic influence on BMI may also interact with demographic factors such as gender and age. Sex-specific genetic effects on BMI have been observed in adolescents as well as in adults[72](#_ENREF_72),[73](#_ENREF_73). Heritability of obesity strongly varies with age, and a recent study with over 12,000 twin pairs reported a heritability estimate of 4-9% for BMI at birth, which increased to more than 50% at 5 months of age[66](#_ENREF_66). Heritability estimates increase from infancy to childhood[74](#_ENREF_74), from childhood to pre-adolescence[75](#_ENREF_75), from preadolescence to adolescence[76](#_ENREF_76), and reach a plateau during adolescence and adulthood, and then slightly decrease in late adulthood[77](#_ENREF_77). Longitudinal BMI change from adolescence to young adulthood and from young adulthood to adulthood is a heritable trait, but genetic variants for change in BMI partially overlap with those affecting the level of BMI[78](#_ENREF_78),[79](#_ENREF_79). Lastly, heritability estimates increase with the severity of obesity[80](#_ENREF_80).

The investigation of socio-structural factors and lifestyle behaviours has revealed many additional conditions that impact heritability estimates. One may naively think that the emergence of a society characterized by food abundance and physical inactivity may increase the impact of environment (and therefore decrease the impact of genes) in the determination of the obese phenotypes. Counter intuitively perhaps, the transition to an obesity-prone environment did not decrease the impact on the genetic predisposition to obesity, with values of heritability for BMI close to 80% in childhood and young adulthood reported in the post-obesity epidemic period[81](#_ENREF_81),[82](#_ENREF_82). These results are congruent with the seminal work by Claude Bouchard and colleagues showing that the BMI response to long-term overfeeding in young adult male twins is mainly influenced by genetic factors[64](#_ENREF_64). Twin studies have shown that a high level of physical activity can substantially reduce the influence of genetic factors on BMI in both young and older adults[83](#_ENREF_83),[84](#_ENREF_84). In a recent article, PT. Williams studied the parental contribution to offspring’s BMI in 47,691 adult runners and showed that a vigorous physical activity (running distance ≥ 9 km/day) decreased the parental contribution to BMI, by 48-58 %, in comparison with runners with moderate physical activity (running distance < 3 km/day)[85](#_ENREF_85). Socio-structural research indicates that educational status is negatively associated with obesity[86](#_ENREF_86), but heritability estimates for BMI in late childhood/adolescence are positively correlated with the level of education of parents[87](#_ENREF_87). Sleep duration is negatively associated with obesity[88](#_ENREF_88). In a twin study, the heritability of BMI (h² = 70%) in short-sleepers (< 7 hours/day) was more than twice the heritability of BMI (h² = 32%) when sleep duration was longer (≥ 9 h/day)[89](#_ENREF_89). Weight gain is a well-known adverse effect of antipsychotic medication[90](#_ENREF_90), but a considerable degree of inter-individual variability has been described in literature[91](#_ENREF_91). Two pilot twin/sibs comparison studies have reported heritability estimates of 60-80% for body weight gain in response to antipsychotics in adolescents and adults[92](#_ENREF_92),[93](#_ENREF_93). Weight loss in response to vigorous exercise, diet restriction or bariatric surgery is highly variable and may also interact with genetic factors[94-96](#_ENREF_94).

## Obesity predisposing gene variants interact with the environment

Although heritability studies provided early evidence for the genetic contribution to obesity, recent efforts have focused on the identification of specific genes, and their interactions, that impact obesity risk. Our knowledge about the genetic architecture of mendelian (syndromic and non-syndromic) and polygenic forms of obesity has greatly expanded in the last 15 years[38](#_ENREF_38). It is noteworthy that even the more severe forms of mendelian (syndromic and non syndromic) obesity display an incompletely penetrant and heterogeneous obese phenotype[97-100](#_ENREF_97). This variability in obesity phenotype can be attributed not only to genetic heterogeneity or gene × gene interactions[101](#_ENREF_101),[102](#_ENREF_102), but interactions with environmental factors should be considered as a potential means to close the “missing heritability” gap. Since the rapid increase in obesity prevalence over the last few decades indicates a strong environmental influence on BMI (e.g. physical activity, diet, educational status, age, gender)[35](#_ENREF_35),[68](#_ENREF_68), many researchers have worked on the identification of specific environmental factors that interact with syndromic, monogenic and polygenic obesity predisposing genes. The existing evidence regarding the study of obesity indicates that lifestyle factors can significantly modify the manifestation of obesity predisposing gene variants.

## Obesity predisposing gene variants interact with physical activity

Recent data suggest that genetic predisposition to obesity may be blunted in part through physical activity. Fifteen independent studies reported an interaction between the *FTO* obesity risk genotype and physical activity on BMI variation or obesity in children, adolescents and adults[18](#_ENREF_18),[20](#_ENREF_20),[85](#_ENREF_85),[103-107](#_ENREF_103). An interaction between *FTO* intron 1 variant and the level of physical activity on obesity was recently confirmed in a meta-analysis of 218,166 adults where physical activity attenuated the odds of obesity by 27% conferred by the variant[108](#_ENREF_108). No such interaction was found in 19,268 children and adolescents[108](#_ENREF_108). Similar results were obtained for a genetic predisposition score combining the information of 12 obesity-associated SNPs, and a high level of physical activity was associated with a 40% reduction in the genetic predisposition to obesity in adults, as well as for BMI level and BMI change across time[109](#_ENREF_109). Significant gene × physical activity interactions on obesity have also been found in 6 obesity-predisposing SNPs in Chinese children and adolescents[110](#_ENREF_110). Although the sufficient sample size of the meta-analytic approach was able to corroborate the impact of the *FTO*polymorphism (rs9939609), the 6 SNPs identified by Xi et al.[110](#_ENREF_110) require further replication to confirm their effects.

## Obesity predisposing gene variants interact with diet

There is growing evidence that dietary habits interact with gene variants to increase obesity predisposition. Five studies, including independent samples of Caucasians and Latin Americans, suggest that a high daily energy intake, high fat intake or high saturated fat intake can amplify the effect of the *FTO* genotype on obesity risk in children, adolescents and adults[111-115](#_ENREF_111). The Apolipoprotein A-II (*APOA2*) -265T>C promoter functional polymorphism also interacts with high-saturated fat to increase BMI and obesity risk in five independent populations (Mediterranean, Asian, Caucasian, Hispanic and Carribean)[116](#_ENREF_116),[117](#_ENREF_117). High saturated fat intake was associated with significant increases in the genetic risk of obesity across populations[118](#_ENREF_118). Specifically, C allele homozygotes with high saturated fat intake displayed a 1.84 (95% CI, 1.38-2.47) odds of obesity compared to a 0.81 (95% CI, 0.59-1.11) odds in those with low saturated fat intake[118](#_ENREF_118). Rouskas et al. recently reported that the penetrance of *MC4R* loss-of-function heterozygous mutations on obesity is exceptionally low (6.3 %) in the Greek population, in comparison with those observed in other European countries (60-100%)[119](#_ENREF_119). A possible explanation of this ‘Greek paradox’ may be a protective effect of the Mediterranean diet against MC4R deficiency-induced obesity[119](#_ENREF_119). Although the generalizability of dietary interactions involving *FTO* and *APOA2* have been substantiated through multi-ethnic replication, the findings by Rouskas et al. require further study given that 979 participants were included in the sample.

In contrast to the robust gene-environment interactions involving physical activity and diet, the following interactions offer preliminary findings that will be more convincing after further replication in independent samples.

## Obesity predisposing gene variants interact with sex

Females are generally more likely to develop morbid obesity than males[120](#_ENREF_120) and these discrepancies may be explained in part by sex-specific genetic effects. In line with this hypothesis, pathogenic monogenic mutations in *MC4R* have an effect on BMI as about twice strong in females as in males[121](#_ENREF_121),[122](#_ENREF_122). Seven out of 14 loci convincingly associated with waist to hip ratio displayed sexual dimorphism, all with a stronger effect on the phenotype in women than in men[123](#_ENREF_123),[124](#_ENREF_124).

## Obesity predisposing gene variants interact with age

The syndrome of Prader-Willi has two distinct phenotypic stages. In infancy, it is characterized by poor suck, feeding problems and failure to thrive, followed by hyperphagia in later childhood that leads to excessive weight gain[125](#_ENREF_125). Rare deletions in the region p11.2 of the chromosome 16 have been associated with a highly penetrant mendelian form of obesity with additional developmental features[126](#_ENREF_126). These individuals generally have early feeding and growth difficulties, and start to gain excessive weight around 5-6 years of age. As a result, an incomplete penetrance for childhood obesity but a complete penetrance for adult obesity has been observed for the carriers of the chromosome 16p11.2 deletion[126](#_ENREF_126),[127](#_ENREF_127). The longitudinal study of adult *MC4R* mutation carriers show an increasing age-dependent penetrance (37% at 20 years versus 60% at >40 years)[122](#_ENREF_122). The life-course analysis of the intronic *FTO* gene variant and BMI in longitudinal studies indicates that the obesity-predisposing variant is negatively associated with BMI during infancy (age: 0-2.5 years) but positively associated with BMI from the age of 4 years, with an age-dependent increase during childhood, adolescence and young adulthood[75](#_ENREF_75),[128-131](#_ENREF_128). Most of the effect of the *FTO* intron 1 SNP on BMI gain occurs during this period, and no appreciable effect of *FTO* on BMI increase is observed during adulthood and agedness[130](#_ENREF_130),[132-135](#_ENREF_132). Studying the association of a obesity gene score from multiple markers in longitudinal cohorts gave similar results: the genetic predisposition score was slightly positively associated with birth weight, and more strongly associated with BMI gain during early infancy and childhood, but no association with BMI change during adulthood was observed[136-138](#_ENREF_136).

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## Obesity predisposing gene variants interact with pregnancy and *in utero* factors

Pre-pregnancy maternal obesity and excessive weight gain during pregnancy are both associated with increased birth weight, higher rate of macrosomia in the offspring[139](#_ENREF_139),[140](#_ENREF_140) and with higher risk of adiposity in offspring during childhood, adolescence and adulthood[69](#_ENREF_69),[141](#_ENREF_141),[142](#_ENREF_142). Recently, a morbidly obese female patient with a rare homozygous LEPR mutation was reported to gain 110 lbs during pregnancy, far beyond the 11-40 lbs gestational weight gain range recommended by the Institute of Medicine, and gave birth to a baby with macrosomia[143](#_ENREF_143" \o "Nizard, 2012 #2249). These data suggest that a mendelian predisposition for obesity increases gestational weight gain and offspring’s birth weight. However, no such effect on gestational weight gain was observed for a polygenic gene score composed of four common obesity-predisposing common variants in or near *FTO*, *MC4R*, *TMEM18* and *GNPDA2*[144](#_ENREF_144). Studies with gene scores including more SNPs are needed to further investigate this hypothesis.

## Obesity predisposing gene variants interact with lifestyle modifications

A strict, fat-reduced, and carbohydrate-modified diet leads to a long-term marked weight reduction in adolescents with Prader-Willi syndrome who are already overweight[145](#_ENREF_145). Importantly, if diagnosis is made at an early age and intensive diet management starts early, reasonable weight control is achieved in non-obese patients with Prader-Willi syndrome[146](#_ENREF_146),[147](#_ENREF_147). Regular exercise training has beneficial effects on body composition and weight loss in Prader-Willi syndrome patients[148](#_ENREF_148),[149](#_ENREF_149), especially as they tend to be less physically active than obese non-syndromic subjects[150](#_ENREF_150). *MC4R* or *POMC* monogenic patients respond as well as non-monogenic obese patients to hypocaloric dietary or multidisciplinary (exercise, behavior, nutrition therapy) interventions[151](#_ENREF_151),[152](#_ENREF_152) but *MC4R* monogenic patients fail to maintain weight loss after intervention[152](#_ENREF_152). The obesity risk variant rs9939609, an allele in *FTO,* does not modify the weight loss response to lifestyle intervention[153-155](#_ENREF_153) or caloric restriction[156](#_ENREF_156),[157](#_ENREF_157), but is associated with lower additional weight loss and higher risk of weight regain during the weight maintenance phase that follows the caloric restriction program[157](#_ENREF_157). Carriers of the *FTO* intron 1 obesity risk variant experience a higher rate of dropout when they are submitted to a high-fat/low carbohydrate (in comparison with a low-fat/high carbohydrate) hypocaloric diet[158](#_ENREF_158), but they achieve better weight maintenance than wild-type individuals during a 3-year intervention with a Mediterranean diet[159](#_ENREF_159). They also lose less weight in response to exercise training[160](#_ENREF_160),[161](#_ENREF_161). Among five childhood obesity susceptibility loci identified in a French-German GWAS meta-analysis[162](#_ENREF_162), only one (*SDCCAG8*) was associated with differential weight loss after lifestyle intervention in 401 children and adolescents[163](#_ENREF_163). Eight out of 15 obesity predisposing gene variants recently identified by GWAS showed trends of association with weight loss or weight regain during lifestyle intervention in 3,356 adults of the Diabetes Prevention Program[164](#_ENREF_164).

## Obesity predisposing gene variants interact with an obesity-prone environment

The promotion and globalization of societal changes leading to an imbalance between calorie intake and calorie expense partly explain the current obesity epidemic, but interactions between the genes and this obesity-prone environment may further clarify the development of obesity. Dudley et al. reported a significant cohort effect on the prevalence of obesity in Prader-Willi syndrome[99](#_ENREF_99). Prevalence of obesity was higher in patients born after 1990 than before[99](#_ENREF_99). A generation-dependent penetrance of *MC4R* pathogenic monogenic mutations on obesity was also found in multigenerational pedigrees, the effect of mutations on the obesity phenotype being amplified by the emergence of an "obesogenic" environment[122](#_ENREF_122),[165](#_ENREF_165). The *FTO* intron 1 variant is weakly associated with BMI in South Asian Indian populations, but its effect on weight is stronger in urban compared to rural dwellers (*P*=0.03)[166](#_ENREF_166),[167](#_ENREF_167). A lack of association of *FTO* with obesity-related traits was also observed in a Gambian rural population[168](#_ENREF_168). Altogether, these data suggest a stronger influence of genetic factors on obesity in obesity-prone environments.

## Obesity predisposing gene variants interact with educational status

Epidemiological studies have shown an association between a low level of education and higher risk of overweight and obesity[86](#_ENREF_86). A significant negative association between BMI and educational status was found in *MC4R* non-mutation carriers but not in *MC4R* loss-of function mutation carriers issued from the same pedigrees[122](#_ENREF_122). These results show that a high level of education has no protective effect on obesity risk in presence of *MC4R* pathogenic mutations. On the contrary, a significant gene x education interaction has been found in the intron 1 variant in *FTO*, the significant effect of the SNP on BMI and obesity risk restricted to subjects with no university education[169](#_ENREF_169). However, each of these results will require further analyses to verify these findings.

## Obesity predisposing gene variants interact with sleep duration

A pilot study in Spanish children indicates that the well-established negative correlation between sleep duration and BMI is restricted to subjects carrying the obesity-predisposing alleles in or near the *FTO*, *TMEM18* and *NRXN3* genes[170](#_ENREF_170).

## Obesity predisposing gene variants interact with specific health conditions

Recent work by Beyerlein and colleagues suggests that pre-existent overweight may double the effect of an obesity genetic predisposition score on body fat mass in children[171](#_ENREF_171). If true, it signifies that obesity predisposing genes may have an even more detrimental effect on adiposity once overweight is established. Depression predicts subsequent development of obesity[172](#_ENREF_172) and depression status has been shown to amplify the effect of *FTO* SNPs on BMI[173](#_ENREF_173). Obesity predisposing gene variants in *FTO* and *MC4R* are associated with more weight gain in response to antipsychotic treatments[174](#_ENREF_174),[175](#_ENREF_175). Moreover, obesity has an important role in the etiology of polycystic ovary syndrome[176](#_ENREF_176) and *FTO* intronic SNP has larger effects on BMI in patients with polycystic ovary syndrome than in subject from the general population[177](#_ENREF_177),[178](#_ENREF_178).

## Obesity predisposing gene variants interact with therapeutic treatment

As most obese persons are resistant to the weight-reducing effects of leptin, administration of recombinant leptin to obese subjects does not generally result in significant weight loss[179](#_ENREF_179). However, patients with congenital leptin deficiency markedly reverse obesity and associated phenotypic abnormalities when they are treated with daily injections of recombinant human leptin[180](#_ENREF_180" \o "Farooqi, 2002 #810). Leptin administration reduces energy intake, fat mass, hyperinsulinemia, and hyperlipidemia, restores normal pubertal development, endocrine and immune function and improves neurocognitive performances[181](#_ENREF_181). Patients with complete leptin deficiency are extremely rare (33 patients reported so far worldwide) but leptin supplementation may help a far more numerically significant group of obese patients with partial leptin deficiency (heterozygous for a loss-of-function mutation in the *LEP* gene) to lose weight, based on the observation that leptin therapy induces more significant weight loss in subjects with low leptin levels[182](#_ENREF_182).

The guanine nucleotide binding protein beta polypeptide 3 (*GNB3*) geneC825T functional gene variant predicts that obese individuals will benefit more from the anti-obesity drug sibutramine treatment. Three independent studies showed that the carriers of the 825 T allele lose more weight in response to sibutramine administration[183-185](#_ENREF_183).

## Obesity predisposing gene variants interact with bariatric surgery

Bariatric surgery is the most effective long-term treatment for severe obesity[186](#_ENREF_186). Bariatric surgery procedures are however not recommended for morbidly obese patients with Prader-Willi syndrome due to their inability to understand the necessary operative and follow-up procedures, altered pain threshold, inability to vomit and potential for the development of gastric dilation/necrosis, therefore, an alternative approach including the use of supervised reduced-energy diets with vitamin/mineral supplementation, restricted access to food, and a daily exercise regimen may be more adequate[187](#_ENREF_187). Laparoscopic adjustable gastric banding did not result in a long-term weight reduction in a 18-years old patient with complete MC4R deficiency[188](#_ENREF_188), and was associated with an high risk of conversion to bypass operations in individuals with partial MC4R deficiency[189](#_ENREF_189). On the contrary, two recent studies confirmed that Roux-en-Y gastric bypass surgery was an efficient strategy to lose weight in *MC4R* mutation carriers[190](#_ENREF_190),[191](#_ENREF_191). These results suggest that diversionary operations, which are more invasive but efficiently improve the neuro-hormonal control of satiety than gastric banding procedures, be recommended in the context of non-syndromic monogenic forms of hyperphagic obesity.

The *FTO* allele carriers lose less weight than common allele homozygous individuals after banding surgery[192](#_ENREF_192), but experience similar level or more weight reduction after gastric bypass surgery[192](#_ENREF_192),[193](#_ENREF_193). A genetic risk score combining the genetic information of four obesity-associated SNPs was found to be associated with poorer postoperative weight loss trajectories in a study with 1,001 severely obese subjects who underwent gastric bypass surgery[194](#_ENREF_194).

## Conclusion

Heritability, syndromic, monogenic and polygenic obesity studies provide converging evidence that obesity predisposing genetic factors strongly interact with environment, from birth to agedness and in a wide range of situations (summarized in Table 1). A prolific period of discovery is foreseen in this fast-moving field, especially with methodological innovations like GEWIS and variance prioritization that will address the “missing heritability” of obesity. A comprehensive understanding of gene-environment interactions in obesity may lead to tremendous applications in the emerging field of personalized medicine and individualized lifestyle recommendations, as it implies that specific subgroups of individuals may have an increased risk to develop obesity in specific environments but may also benefit more from a lifestyle intervention, a treatment or a surgical procedure [195](#_ENREF_195). This information will definitely help determine if population-wide or personalized subgroup interventions are the best suited to fight the worldwide obesity epidemic[196](#_ENREF_196),[197](#_ENREF_197).

# Chapter 3-Increased Energy expenditure attenuates the effect of *FTO* rs9939609 polymorphism on BMI: Results from the multi-ethnic longitudinal study EpiDREAM

## Introduction

The current trends of the obesity epidemic are exacerbated by the limited success of the treatment options for obesity. Traditional non-invasive treatments for obesity, such as lifestyle modifications or pharmaceuticals have shown a little to modest impact on durable weight loss[198](#_ENREF_198),[199](#_ENREF_199). Bariatric surgery has been identified as an effective treatment for severe obesity in the long-term[200-202](#_ENREF_200), yet this procedure is not applicable to all obese cases on a national scale[203](#_ENREF_203). Furthermore, perioperative mortality rates of 1-2% have been reported among bariatric surgical cases[204](#_ENREF_204). Since the rising prevalence of obesity has been attributed primarily to changes in environmental exposures, such as excessive energy intake, sedentary lifestyle and sleep debt, among others[5](#_ENREF_5), recent research has focused on preventative strategies to curb the obesity epidemic[205](#_ENREF_205).

As previously mentioned, physical activity and diet represent the most robust examples of environmental factors that can mediate genetic predisposition to obesity[18-21](#_ENREF_18). With respect to physical activity, significant gene-environment interactions between *FTO* intron 1 variation and physical activity (PA) have been consistently found in 16 independent cross-sectional and intervention studies performed with children and adult populations of European, East Asian and African ancestry[18](#_ENREF_18),[105](#_ENREF_105),[206](#_ENREF_206). A recent large-scale meta-analysis in subjects of European ancestry confirmed the PA x *FTO* interaction in 218 166 adults but not in 19 268 children and adolescents[20](#_ENREF_20). A significant interaction between PA and a genetic predisposition score (GS) combining the information of 12 obesity-associated SNPs was observed in 20,430 European subjects from the U.K.[49](#_ENREF_49). A high level of physical activity was associated with a 40% reduction in the genetic predisposition to common obesity in this study[49](#_ENREF_49). This result was confirmed in two U.S. prospective studies including 12 304 white Europeans and using a GS based on the information of 32 body mass index (BMI)-associated SNPs[50](#_ENREF_50). A recent meta-analysis in 111 421 subjects of European ancestry confirmed a significant PA x GS interaction using 12 obesity predisposing SNPs and showed that this interaction was more apparent in subjects living in North America[207](#_ENREF_207).

Although these data provide convincing evidence of an interaction between genetic predisposition to obesityand physical activity, we identified several important limitations of the existing literature. First, despite the fact that longitudinal prospective cohorts are better suited to study the consequences of a specific environmental exposure on subsequent adiposity, almost all current gene x PA interaction studies in the obesity field have been conducted using cross sectional designs. Second, since large sample studies are necessary to identify GEI with adequate power, most GEI analyses of physical activity used imprecise self-report measurements of physical activity due to concerns regarding cost, feasibility and participant burden[208](#_ENREF_208). As a result, most GEI investigations have analyzed physical activity as a binary or categorical variable at the cost of statistical power, and no study to date has compared the value of using raw or precise quantitative measurements of physical activity in GEI studies[18](#_ENREF_18),[49](#_ENREF_49),[104](#_ENREF_104). Simulations of GEI have shown that a sample of about 2000 participants with precisely measured environmental exposure and outcome data are needed to detect a gene-environment interaction of large magnitude (a doubling of the genetic risk estimate in the exposed group compared to the unexposed group) with reasonable power (95% power, critical α P = 1x10-4)[209](#_ENREF_209). With less precise measurement of environmental exposure, the sample size requirement can increase to 100,000 participants to detect the same interaction with comparable power[16](#_ENREF_16),[209](#_ENREF_209). Third, nearly all GEI studies of obesity have used BMI as the outcome measure of obesity, although the validity of the BMI can be compromised by different body compositions (e.g. lean vs. fat mass)[210](#_ENREF_210). Fourth, GEI studies have mainly been performed in European populations, but the transferability of the conclusions of these studies to other ethnic backgrounds remains unclear[20](#_ENREF_20),[49](#_ENREF_49). This prompted us to assess the association between 14 obesity predisposing variants (analyzed independently and as a GS) and obesity-related traits in interaction with physical activity in the multi-ethnic prospective cohort EpiDREAM. We used an accurate quantitative measure of energy expenditure (Metabolic Equivalent score, METS)[211](#_ENREF_211) and the recently validated body adiposity index (BAI), which is more strongly correlated to body fat content than BMI [212](#_ENREF_212).

## Methods

### Study Participants

The data for this investigation were collected through a prospective cohort of participants at risk for type 2 diabetes, which has been described in detail previously[213](#_ENREF_213),[214](#_ENREF_214" \o "Anand, 2012 #2155). Briefly, EpiDREAM enrolled a total of 24 872 individuals recruited from 21 countries who were screened for eligibility to enter the DREAM clinical trial[213](#_ENREF_213). Individuals who were identified as at risk for type 2 diabetes based on family history, ethnicity and abdominal adiposity were screened using a 75-gram oral glucose tolerance test (OGTT). All participants were between the ages of 18-85 years and were screened between July 2001 and August 2003. We focused on 17 423 subjects from six ethnic groups (South Asian, East Asian, European, African, Latin American, Native North American) having both phenotypic and 50K gene-centric array information in the EpiDREAM study (Supplementary Figure 1). Self-reported ethnicity has been validated in the 17,423 individuals using the eigensoft software (<http://genepath.med.harvard.edu/~reich/Software.htm>). Of these 17 423 individuals, 9 224 have been prospectively followed for a median of 3.3 years. Samples that failed to cluster with individuals of the same self-reported ethnicity were removed. The EpiDREAM study has been approved by local ethics committee and informed consent was obtained from each subject before participating in the study, in accordance with the Declaration of Helsinki.

### Genotyping

Buffy coats for DNA extraction have been collected in 19 197 participants of the EpiDREAM study (Supplementary Figure 1). DNA has been extracted by the Gentra System. Genotyping was performed using the Illumina CVD bead chip microarray ITMAT Broad Care (IBC) array [215](#_ENREF_215). Genotyping was performed at the McGill University and Genome Quebec Innovation Centre using the Illumina Bead Studio genotyping module, version 3.2. We selected 14 SNPs that displayed genome-wide significant association (*P*<5×10-8) for BMI and/or obesity in the literature and were genotyped on the versions 1 and 2 of the IBC 50K SNP array (Supplementary Table 1): rs9939609, rs7203521 in *FTO*, rs1514176 in *TNNI3K*, rs6265 and rs1401635 in *BDNF*, rs1805081 in *NPC1*, rs6232, rs6235 in *PCSK1*, rs2206734 in *CDKAL1*, rs2075650 in *TOMM40/APOE/APOC1*, rs2272903 in *TFAP2B*, rs997295 in *MAP2K5*, rs1211166 in *NTRK2*, rs11671664 in *GIPR*. The SNPs selected showed no deviation from Hardy-Weinberg Equilibrium in the six ethnic groups (P>1 x 10-6)[216](#_ENREF_216). The call rate for each of the 14 SNPs was comprised between 99.8-100% (Supplementary Table 1).

### Phenotyping

In addition to the OGTT, participants completed a questionnaire that collects information including demographic data, medical history, physical activity behaviors and diet patterns at baseline and after follow-up. Anthropometric measurements including height, weight, waist and hip circumference and electrocardiogram were performed using a standardized protocol[213](#_ENREF_213). Height (m), weight (kg) and hip circumference (HC) (cm) were measured by trained medical staff. Standing height was measured to the nearest 0.1 cm and weight was measured to the nearest 0.1 kg in light clothing. Waist circumference was measured in duplicate using a non-flexible tape measure with an attached spring balance with a mass of 750g. Waist circumference was assessed at the smallest diameter between the costal margin and iliac crest. Averages of the two measures were used in all analyses. Body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in meters (m) squared. We used Body Adiposity Index (BAI), which estimates body adiposity percentage directly based on height and hip circumference[212](#_ENREF_212). Specifically, BAI=[(hip circumference)/((height)(1.5))-18], with hip circumference and height being expressed in centimeters and meters, respectively[212](#_ENREF_212).

The 2003 ADA criteria were used to classify participants as having normal glucose tolerant (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or T2D at baseline, as confirmed by an oral glucose tolerance test. Normoglycemia was defined as a fasting plasma glucose < 5.6 mmol/L, IFG was defined as a fasting plasma glucose of 5.6 to 6.9 mmol/L, IGT was defined as a fasting plasma glucose below 7.0 mmol/L and a 2-h glucose between 7.8 and 11.0 mmol/L, and diabetes was defined if either the fasting plasma glucose was ≥ 7.0 mmol/L or the 2-h glucose was ≥ 11.1 mmol/L [217](#_ENREF_217).

Physical activity measures were based on self-reported time of participation in 39 different physical activities (Supplementary Table 2). A quantitative measure of energy expenditure (Metabolic Equivalent score, METS) was derived using this information and the updated compendium of physical activities[211](#_ENREF_211). A MET score was assigned to each activity based on the energy cost of that activity. The energy expended on each activity was estimated by multiplying the hours/week of participation by the corresponding MET value. These measures were summed across all activities to provide an overall estimate of energy expenditure for each participant. This quantitative measure was compared to a brief, self-reported, categorical measure based on physical activity participation during leisure time and at work. Participants rated their physical activity level at work or during leisure on a scale of one to five (1=sedentary, 2=somewhat active, 3=moderately active, 4=very active, 5=extremely active). The results from leisure time activity and employment related activity were combined to form a composite score of one to three to represent an estimate of overall physical activity (1=inactive, 2=moderately active, 3=active). Given that measuring the MET score required participants to report participation in 39 different physical activities, it is not surprising that far fewer subjects reported this information (n=11,015, 63.2%) compared to the basic physical activity score (n=17,407, 99.9%).

### Statistical Analyses

Statistical analyses were performed using SPSS (version 20). Single SNPs analyses were performed under the additive model, and the previously identified obesity risk alleles for each of the 14 SNPs were used as the risk allele for the analyses. General linear models (GLM) were used to examine (1) the association between energy expenditure (METS) / basic physical activity score and BMI/BAI at baseline and follow-up (2) the association between 14 obesity predisposing gene variants (analyzed independently and as a GS) and METS/basic physical activity score and (3) the association between 14 obesity predisposing gene variants (analyzed independently and as a GS) and BMI/BAI at baseline and follow-up. These tests were adjusted for covariates including, sex, age, ethnicity and glucose homeostasis status. If any of the SNPs or GS are significantly associated with METS/basic physical activity score, the effect of the SNPs/GS on BMI/BAI will also be adjusted for METS/PA in addition to the previously mentioned covariates. Significant associations from these main effect analyses will be carried forward to test for interactions. General linear models were used, with or without the inclusion of a SNP x physical activity or a gene score x physical activity interaction term. These tests were also adjusted for covariates including, sex, age, ethnicity and glucose homeostasis status. Ordinal logistic regression was used to analyze the impact of the 14 obesity predisposing SNPs on the basic physical activity score. This analysis was first performed while controlling for sex, age, ethnicity and glucose homeostasis status, and a second time controlling for sex, age, ethnicity, glucose homeostasis status and BMI. The genetic predisposition score was calculated by summing the alleles of the 14 obesity predisposing SNPs so that the score ranged from 0 to 28. Since weighting has been shown to have no major impact on the overall GS,[218](#_ENREF_218) an un-weighted GS was used for these analyses. We performed imputations for the missing genotypic values as previously described[219](#_ENREF_219). The imputation was performed for each locus using the mean number of the predisposing obesity alleles successfully genotyped for all individuals. This procedure was performed separately in each ethnic group. Individuals with more than one out of 14 missing genotype were not included in the gene score calculation. Two-tailed P-values are presented in this manuscript and *P*<0.05 were considered as nominally significant. After applying a Bonferroni’s correction for multiple testing, a *P* <0.00033 (0.05/162) was considered as significant. All SNPs reaching a nominal level of significance were followed up for interaction analysis.

## Results

### Descriptive Statistics

The clinical and anthropometric characteristics of the EpiDREAM study are summarized in Table 1. The mean age of participants was 52.7 years with a mean BMI of 30.2 (SD=6.22) kg/m2 and a mean BAI of 33.0 (SD=7.51). At baseline, the mean energy expenditure was 320.54 MET-minutes/week (SD=409.20) and the ethnic distribution of the cohort was 53.9% European, 18.9% Latino, 15.8% South Asian, 7.2% African, 2.9% Native American, 1.3% East Asian. After follow-up the mean BMI and BAI were 30.32 (SD=5.80) and 26.23 (SD=6.37), respectively. The mean energy expenditure appeared to decrease slightly to 301.38 MET-minutes/week (SD=367.92), although this sample only included 9228 of the original 17423 participants.

### Effect of Physical Activity on BMI/BAI

At baseline, the quantitative MET score was negatively associated with both BMI (β= -0.001, 95% Confidence interval [CI]= -0.001 to -5.89 x 10-4, P=3.27 x 10-10) and BAI (β= -0.001, 95% CI= -1.50 x 10-3 to -9.20 x 10-4, P= 3.13 x 10-16) (Table 2). Congruently, the basic physical activity score (low – medium – high physical activity) was significantly associated with lower baseline BMI (β= -1.561, 95% CI= -1.710 to -1.412, P=9.43 x 10-9) and BAI (β= -1.877, 95% CI= -2.040 to -1.715, P=1.60 x 10-15). Similar associations were found between the MET score at baseline and BMI and BAI at follow-up (BMI: β= -0.001, 95% CI= -1.23 x 10-3 to -5.01 x 10-4, P=3.48 x 10-13; BAI: β= -0.001, 95% CI= -1.24 x 10-3 to -4.98 x 10-4, P=8.09 x 10-14). The baseline basic physical activity score was also negatively associated with BMI (β= -1.332, 95% CI= -1.456 to -0.962, P=1.20 x 10-21) and BAI (β= -1.403, 95% CI= -1.613 to -1.193, P=2.30 x 10-23) at follow-up. The baseline MET score was negatively associated with BMI change (β= -1.13 x 10-4, 95% CI= -0.001 to -2.48 x 10-4, P=1.84 x 10-6), and BAI change (β= -0.001, 95% CI= -0.001 to 1.61 x 10-4, P= 5.45 x 10-6). The basic physical activity score was negatively associated with BMI change (β= -0.224, 95% CI= -0.333 to -0.115, P= 5.55 x 10-5) and BAI change (β= -0.383, 95% CI= -0.570 to -0.196, P= 6.01 x 10-5) over follow-up. These analyses were adjusted for sex, age, ethnicity and glucose homeostasis status.

### Effect of SNPs/GS on Physical Activity

We first investigated the association of 14 obesity predisposing SNPs and corresponding GS on basic physical activity score, controlling for sex, age, ethnicity and glucose homeostasis status. Of the 14 SNPs analyzed, the obesity risk alleles of three of them were associated with increased basic physical activity score: *NTRK2* rs1211166 (OR=1.06, 95% CI= 1.008 to 1.119, P=0.023), *BDNF* rs6265 (OR=1.07, 95% CI= 1.013 to 1.113, P=0.016), *BDNF* rs1401635 (OR=1.09, 95% CI= 1.035 to 1.138, P=0.001).In contrast, we observed a negative association between *PCSK1* rs6235 (OR=0.95, 95% CI= 0.904 to 0.998, P=0.040), *FTO* rs9939609 (OR=0.95, 95% CI= 0.907 to 0.990, P=0.016), *NPC1* rs1805081 (OR=0.92, 95% CI= 0.877 to 0.963, P=0.001) and the basic physical activity score (Table 3). The obesity risk GS was not associated with the basic physical activity score (OR=1.00, 95% CI= 0.989 to 1.016, P=0.708). After controlling for BMI, in addition to sex, age, ethnicity and glucose homeostasis status, only four of these SNPs remained significantly associated with the basic physical activity score: *NTRK2* rs1211166 (OR=1.07, 95% CI= 1.013 to 1.124, P=0.015), *BDNF* rs6265 (OR=1.08, 95% CI= 1.021 to 1.143, P=0.007), *BDNF* rs1401635 (OR=1.09, 95% CI= 1.043 to 1.147, P=0.003) and *NPC1* rs1805081 (OR=0.92, 95% CI= 0.879 to 0.966, P=3.52 x 10-4). Again, the association between the obesity risk GS and the basic physical activity score was not significant (OR=1.01, 95% CI= 0.996 to 1.023, P=0.161).

None of the 14 obesity predisposing SNPs or the GS were significantly associated with the MET score when controlling for sex, age, ethnicity and homeostasis status (Table 3). Yet, the obesity risk alleles of two SNPs were positively associated with the MET score after adjusting for sex, age, ethnicity, glucose homeostasis status and BMI: *BDNF* rs6265 (β=14.272, 95% CI= 0.432 to 28.112, P=0.043) and *BDNF* rs1401635 (β=12.536, 95% CI=0.846 to 24.227, P=0.036). The association between the obesity risk GS and the MET remained not significant after further adjustment for BMI (β=1.384, 95% CI= -1.838 to 4.606, P=0.40).

Of the 14 SNPs analyzed, only one (*NTRK2* rs1211166) showed a significant association with change in the basic physical activity score (OR=0.88, 95% CI= 0.809 to 0.962, P=0.005). None of the 14 SNPs displayed a significant association with a change in the MET score. The obesity risk GS was not associated with change in the basic physical activity score (OR=1.00, 95% CI= 0.975 to 1.018, P=0.748) or change in the MET score (β=-0.017, 95% CI=-7.470 to 1.732, P=0.222) (Supplementary Table 3).

### Effect of SNPs/GS on BMI/BAI

At baseline, the obesity risk alleles of four SNPs displayed a positive association with BMI and BAI: *FTO* rs9939609 (BMI: β= 0.451, 95% CI= 0.326 to 0.576, P= 1.50 x 10-12; BAI: β= 0.394, 95% CI= 0.258 to 0.531, P= 1.53 x 10-8), *TNNl3K* rs1514176 (BMI: β= 0.196, 95% CI= 0.074 to 0.319, P= 0.002; BAI: β= 0.148, 95% CI= 0.013 to 0.282, P= 0.031), *GIPR* rs11671664 (BMI: β= 0.301, 95% CI= 0.105 to 0.498, P= 0.003; BAI: β= 0.225, 95% CI= 0.101 to 0.440, P= 0.040) and *CDKAL1* rs2206734 (BMI: β= 0.294, 95% CI= 0.143 to 0.444, P= 1.33 x 10-4; BAI: β= 0.319, 95% CI= 0.155 to 0.484, P= 1.44 x 10-4) (Table 4). At baseline, the GS was significantly associated with greater BMI (β= 0.128, 95% CI= 0.091 to 0.165, P= 1.43 x 10-11) and BAI (β= 0.106, 95% CI= 0.065 to 0.146, P= 3.05 x 10-7).

After follow-up, only *FTO* rs9939609 (BMI: β= 0.408, 95% CI= 0.247 to 0.570, P= 7.50 x 10-7; BAI: β= 0.438, 95% CI= 0.263 to 0.614, P= 1.01 x 10-6) and *TNNl3K*, rs1514176 (BMI: β= 0.191, 95% CI= 0.035 to 0.348, P= 0.017; BAI: β= 0.193, 95% CI= 0.023 to 0.362, P= 0.026) were significantly associated with both increased BMI and BAI. At follow-up, *GIPR* 11671664 showed borderline positive association with BMI (β= 0.253, 95% CI= -0.002 to 0.507, P= 0.052), and was associated with greater BAI (β= 0.430, 95% CI= 0.154 to 0.706, P= 0.002). *CDKAL1* rs2206734 was not associated with BMI or BAI at follow-up (BMI: β= 0.004, 95% CI= -0.191 to 0.200, P= 0.965; BAI: β= -0.025, 95% CI= -0.238 to 0.187, P= 0.814). However, *CDKAL1* rs2206734 was negatively associated with BMI and BAI change (BMI: β= -0.113, 95% CI= -0.205 to -0.020, P= 0.017; BAI: β= -0.272, 95% CI= -0.433 to -0.112, P= 0.001)]. *TFA2PB* rs2272903 was negatively associated with BAI change (β= -0.214, 95% CI= -0.401 to -0.027, P= 0.025) but not with BMI change (β= -0.048, 95% CI= -0.155 to 0.058, P= 0.375). No other SNPs showed evidence of association with BMI or BAI change (Table 4).

Associations were observed between the obesity risk GS and BMI/BAI after follow-up (BMI: β= 0.093, 95% CI= 0.045 to 0.141, P= 1.36 x 10-4; BAI: β= 0.059, 95% CI= 0.007 to 0.111, P= 0.026). However, the GS was not associated with BMI change (β= -0.012, 95% CI= -0.035 to 0.010, P=0.281), yet was negatively associated with BAI change (β= -0.047, 95% CI= -0.087 to -0.008, P= 0.019). These analyses were adjusted for sex, age, ethnicity and glucose homeostasis status. Interaction tests with physical activity were restricted to the subset of SNPs / GS displaying a nominal or significant association with BMI/BAI at baseline and / or at follow-up.

### Interaction Analyses

At baseline, the MET score significantly modified the effect of the *FTO* risk allele on BMI (Pinteraction=0.032) and BAI (Pinteraction=3.26 x 10-4; Table 5). Each additional *FTO* risk allele (A) was associated with a (1) BMI increase of 0.504 kg/m2 (95% CI=0.218 to 0.789, P=0.001), BAI increase of 0.484(95% CI=0.181 to 0.787, P=0.002) in the lowest MET score tertile, (2) BMI increase of 0.399 kg/m2 (95% CI=0.141 to 0.656, P=0.002), BAI increase of 0.363(95% CI=0.079 to 0.647, P=0.012) in the middle MET score tertile and (3) BMI increase of 0.306 kg/m2 (95% CI=0.052 to 0.559, P=0.018), BAI increase of 0.242(95% CI=-0.032 to 0.515, P=0.083) in the highest MET score tertile. This indicates that the effect of *FTO* rs9939609 on obesity can be reduced by 15-20% through physical activity. The MET score did not moderate the effect of *TNNl3K* rs1514176 (BMI: Pinteraction=0.449; BAI: Pinteraction=0.719), *CDKAL1* rs2206734 (BMI: Pinteraction=0.599; BAI: Pinteraction=0.407), *GIPR* rs11671664 (BMI: Pinteraction=0.467; BAI: Pinteraction=0.921) or the obesity risk GS (BMI: Pinteraction=0.178; BAI: Pinteraction=0.164) at baseline.

The basic physical score also modified the effect of the *FTO* risk allele on BMI (Pinteraction=0.004) and BAI (Pinteraction=0.005) at baseline. Each additional obesity risk allele (A) was associated with a (1) BMI increase of 0.746 kg/m2 (95% CI=0.490 to 1.002, P=1.14 x 10-8), BAI increase of 0.665(95% CI=0.385 to 0.945, P=3.38 x 10-6) in the inactive group, (2) BMI increase of 0.311 kg/m2 (95% CI=0.155 to 0.467, P=9.48 x 10-5), BAI increase of 0.227(95% CI=0.057 to 0.398, P=0.009) in the moderately active group, and (3) BMI increase of 0.302 kg/m2 (95% CI=-0.004 to 0.608, P=0.053), BAI increase of 0.372(95% CI=0.057 to 0.688, P=0.021) in the active group. This indicates that physical activity is associated with a 33-36% decrease in the effect of *FTO* rs9939609 on obesity. However, the basic physical activity score did not modulate the effect of the remaining three SNPs on BMI or BAI at baseline: *TNNl3K* rs1514176 (BMI: Pinteraction=0.372; BAI: Pinteraction=0.183), *CDKAL1* rs2206734 (BMI: Pinteraction=0.527; BAI: Pinteraction=0.625) or *GIPR* rs11671664 (BMI: Pinteraction=0.780; BAI Pinteraction=0.989). Similar to the MET score, the basic physical activity score did not moderate the impact of the obesity risk GS on BMI (Pinteraction=0.204) or BAI (Pinteraction=0.223) at baseline.

After follow-up, only *TNNl3K* rs1514176 showed a significant interaction with the MET score in modulating BMI (Pinteraction=0.037), yet the interaction did not significantly moderate BAI (Pinteraction=0.152). Each additional *TNNl3K* risk allele (G) was associated with a (1) BMI increase of 0.455 kg/m2 (95% CI=0.111 to 0.799, P=0.010) in the lowest MET score tertile, (2) BMI increase of 0.240 kg/m2 (95% CI=-0.080 to 0.560, P=0.141) in the middle MET score tertile and (3) BMI increase of 0.159 kg/m2 (95% CI=-0.172 to 0.491, P=0.346) in the highest MET score tertile. This corresponds to a 34% decrease in the effect of *TNNl3K* rs1514176 on obesity associated with physical activity. The interaction between the MET score and adiposity indexes at follow-up was not significant for *FTO* rs9939609 (BMI: Pinteraction=0.723; BAI: Pinteraction=0.928) or *GIPR* rs11671664 (BMI: Pinteraction=0.983; BAI: Pinteraction=0.831). The MET score did not modulate the effect of the obesity risk GS on BMI (Pinteraction=0.441) or BAI at follow-up (Pinteraction=0.827).

The basic physical activity score significantly interacted with *FTO* rs9939609 in modulating BMI (Pinteraction=0.036) but not BAI (Pinteraction=0.410) after follow-up. Each additional obesity risk allele (A) was associated with a (1) BMI increase of 0.722 kg/m2 (95% CI=0.408 to 1.036, P=7.03 x 10-6) in the inactive group, (2) BMI increase of 0.252 kg/m2 (95% CI=0.047 to 0.456, P=0.016) in the moderately active group, and (3) BMI increase of 0.204 kg/m2 (95% CI=-0.232 to 0.639, P=0.359) in the active group. This corresponds to a 50% decrease in the effect of *FTO* rs9939609 on obesity. The interaction between the basic physical activity score and adiposity indexes at follow-up was not significant for *TNNl3K* rs1514176 (BMI: Pinteraction=0.450; BAI: Pinteraction=0.958), *GIPR* rs11671664 (BMI: Pinteraction=0.663, BAI: Pinteraction=0.575) or the obesity risk GS (BMI: Pinteraction=0.864; BAI: Pinteraction=0.467).

*CDKAL1* rs2206734 was the only SNP significantly associated with both BMI and BAI change (BMI: β= -0.113, 95% CI= -0.205 to -0.020, P= 0.017, BAI: β= -0.272, 95% CI= -0.433 to -0.112, P= 0.001). The MET score did not moderate the effect of *CDKAL1* rs2206734 on BMI change (Pinteraction=0.480) or BAI change (Pinteraction=0.220). The basic physical activity score did not moderate the effect of *CDKAL1* rs2206734 on BMI change (Pinteraction=0.412) or BAI change (Pinteraction=0.300). The interaction between the baseline basic physical activity score and the obesity risk GS was not significant for BAI change either (Pinteraction=0.925), similar to the interaction between the baseline MET score and GS for BAI change (Pinteraction=0.628).

In summary, we found significant GEI between i) *FTO* rs9939609, the MET score or the basic physical activity score in modulating BMI / BAI at baseline, ii) the basic physical activity score mediated the effect of *FTO* rs9939609 on BMI at follow-up and iii) the MET score mediated the effect of *TNNl3K* rs1514176 on BMI at follow-up.

## Discussion

For the first time, we observed significant GEI between *FTO* rs9939609 and physical activity at baseline and at follow-up in an international multiethnic population using both basic and precise assessment of physical activity. Our data are supported by ample evidence in literature. First, the power to detect GEI at the nominal level of association (P<0.05) is adequate, although our power estimations for the Bonferroni corrected threshold are moderate (see Figures 2-4). Second, twin studies have shown that a high level of physical activity can substantially reduce the influence of genetic factors on BMI in both young and older adults[68](#_ENREF_68),[85](#_ENREF_85),[220](#_ENREF_220). Third, the interaction between *FTO* rs9939609 and physical activity has been demonstrated in several cross-sectional studies, and currently represents the most robust example of GEI in the field of metabolic disorders[18](#_ENREF_18),[20](#_ENREF_20),[105](#_ENREF_105),[206](#_ENREF_206),[207](#_ENREF_207),[221](#_ENREF_221). Fourth, there is plausible underlying biological process to justify this association. *FTO* is a nucleic acid demethylase and *FTO* is associated with different methylation profiles and BMI variance[43](#_ENREF_43),[222](#_ENREF_222),[223](#_ENREF_223). Since methylation is sensitive to environmental changes (e.g. physical activity and diet) there is a strong biological rationale to identify GEI with the rs9939609 in *FTO* as previously reported[18](#_ENREF_18),[19](#_ENREF_19). Two recent studies show that physical activity can change the methylation and mRNA expression pattern of many genes, including *FTO*, in both muscle and adipose tissue[223](#_ENREF_223),[224](#_ENREF_224). Based on this evidence, there is a strong biological rationale for physical activity to modify the genetic predisposition to obesity by changing methylation patterns. The biological mechanisms underlying this association provide support for a causal relationship between physical activity and genetic predisposition to obesity[225](#_ENREF_225).

Although this association has been demonstrated previously[105](#_ENREF_105),[206](#_ENREF_206), this study addressed several important limitations of the existing literature. This is the first time that GEI between physical activity and obesity predisposing genes has been performed in a multi-ethnic context that is representative on a global scale (six ethnicities from 21 countries). Current meta-analyses have been performed among Europeans[20](#_ENREF_20),[207](#_ENREF_207), while other individual studies have been performed in East Asian[206](#_ENREF_206),[226](#_ENREF_226),[227](#_ENREF_227) or South Asian[228](#_ENREF_228) samples. Three existing studies have been performed with multi-ethnic samples (Europeans, African Americans and / or Hispanics), but these samples were restricted to people living in the U.S.A[103](#_ENREF_103),[105](#_ENREF_105),[161](#_ENREF_161). We were able to confirm the *FTO* rs9939609x physical activity interaction in a multi-ethnic sample (six ethnic groups) recruited in 21 countries. These findings support a more global generalization of the interaction between *FTO* and physical activity, and suggest that obesity prevention programs emphasizing vigorous physical activity may be a valuable solution to fight obesity globally. Since genetic predisposition can be blunted by physical activity (likely through methylation mechanisms) reinforces the view that the delivery and efficiency of obesity prevention programs can be improved by targeting genetically at risk subgroups of the population. In conjunction with existing data collected in children and adults, we argue that obesity prevention programs focused on physical activity should begin early in life and target high-risk subsets of the population. For instance, physical activity programs targeting youth with a family history of obesity may be an appropriate strategy to curb obesity. Our conclusions and the molecular mechanisms implicated in GEI contribute to the widespread controversy regarding the value of physical activity in obesity prevention[229](#_ENREF_229),[230](#_ENREF_230).

Another strength of this investigation is the comparison of an energy expenditure score (MET score) with a traditional basic measurement of physical activity. Given the recent consensus that food intake may be the main driver of the obesity epidemic[231](#_ENREF_231), it is important to note that both measures displayed significant associations with both adiposity measures at baseline and at follow-up. This indicates that physical activity can influence obesity, despite the broad range of lifestyles among the participants. The value of physical activity for managing obesity has been recognized in a meta-analysis of over 130 000 children from 34 countries[232](#_ENREF_232). In addition, a recent analysis of the National Health and Nutrition Examination Survey (NHANES) cohort from 1988-2010 found that physical activity had a larger impact on BMI and waist circumference trends than calorie intake[233](#_ENREF_233). Our results indicate that one hour of jogging or swimming (8.0 MET activities) per week was associated with approximately a 0.5 kg/m2 decrease in BMI. Together, these data challenge the recent reports attributing the obesity epidemic mainly to excessive caloric intake[231](#_ENREF_231) and support the universal value of physical activity to maintain a healthy body weight[233](#_ENREF_233).

Although the MET score provides a more comprehensive assessment of physical activity participation by considering exercise intensity as well as duration, this measurement has disadvantages. The MET score requires much more time to complete and recalling participation in over 30 different activities creates a burden, as well as a potential for recall bias on behalf of the participants. The basic physical activity score only required participants to rate their leisure time and employment related activity on a scale of one to five, which produced a less burdensome but also far less precise measure for the participants. Consistent with this limitation, the genetic effect of *FTO* rs9939609 on adiposity-related traits is reduced by 30-40% using the basic physical activity score, comparable to previous reports[108](#_ENREF_108),[234](#_ENREF_234). In comparison, only a 20-25% decrease of the genetic effect of *FTO* rs9939609 on BMI and BAI was found using the more precise MET score. The advantages of each method were reflected by the data. At baseline, only 11 015 (63%) participants completed the assessment of the MET score, compared to the 17 407 (99%) participants who completed the basic physical activity score. Despite this difference, we were able to identify GEI using each of these measures (both physical activity measures showed significant interaction with *FTO* rs9939609 on BMI and BAI at baseline). The loss of power induced by the smaller sample size may have been compensated by the added precision of the MET score. Similar findings have been reported by statistical power simulations to detect GEI between genetic factors and a continuous environmental exposure on a continuously distributed outcome variable[235](#_ENREF_235). Given that we were able to detect more GEI using the basic physical activity score, it appears that the lack of precision was not as detrimental to the statistical power in comparison to the loss of sample size. Using a short physical activity questionnaire may be the best compromise to balance the sample size requirements with the need for sufficient precision, as recently suggested by Peters and colleagues[236](#_ENREF_236).

Although BMI is the classical measure of adiposity in GEI of obesity, we used the recently developed BAI in addition to the BMI. The BAI provides a direct estimate of body fat percentage and unlike BMI, the estimate of body fat percentage can be applied to both males and females without statistical correction[212](#_ENREF_212). Calculating BAI only requires measures of height and hip circumference and the applicability of the BAI has been demonstrated in three ethnic groups: Mexican Americans, African Americans and Europeans[212](#_ENREF_212),[237](#_ENREF_237). Given the large sample size requirements of GEI studies, measuring body fat content data is rarely feasible. Only two current GEI studies have used direct body fat content measures (DEXA and underwater weighing) and these studies involved limited samples (N<800)[238](#_ENREF_238),[239](#_ENREF_239). To our knowledge, this is the first large-scale study to report an interaction between physical activity and *FTO* rs9939609 using an adiposity index that provides a more direct estimate of body fat content than BMI. The interaction between *FTO* rs9939609 and physical activity measures at baseline yielded similar results when using BMI or BAI as the dependent variable. Given that the BAI provides a direct estimate of body fat composition and the BMI does not distinguish between lean and fat mass, it is possible that the BAI may be more sensitive to subtle changes in body fat composition induced by physical activity. Since it is financially prohibitive to assess body fat content in large-scale studies, the BAI may be an acceptable method to assess adiposity in GEI studies of obesity.

Despite being regarded as the gold standard in observational methodology[240](#_ENREF_240), prospective cohorts have only been employed in two current physical activity x gene interaction studies[50](#_ENREF_50),[239](#_ENREF_239). Two additional physical activity intervention studies have also supported the decrease in genetic risk associated with engagement in physical activity[238](#_ENREF_238),[241](#_ENREF_241). Advantages of the prospective design include the opportunity to assess the temporality of relationships and by extension, identify potentially causal relationships[240](#_ENREF_240). However, the power reduction from dropout/loss to follow-up[242](#_ENREF_242) and the high cost of this design explain the high prevalence of cross-sectional studies in the GEI literature. The prospective design of our study permitted the comparison of results between baseline and follow-up. At baseline, four SNPs (*FTO* rs9939609, *TNNl3K* rs1514176, *CDKAL1* rs2206734 and *GIPR* rs11671664) displayed significant associations with BMI/BAI compared to only two SNPs at follow-up (*FTO* rs9939609, *TNNl3K* rs1514176). This discrepancy is not surprising given the reduced power at follow-up (N=9 228) compared to baseline (N=17 423).

Another notable finding was that *CDKAL1* rs2206734 and the obesity risk GS were negatively associated with BMI/BAI change. Given that *CDKAL1* is associated with increased risk for obesity and type 2 diabetes[243](#_ENREF_243),[244](#_ENREF_244" \o "Li, 2013 #2466), participants harboring these polymorphisms may have been more likely to receive lifestyle interventions during the EpiDREAM study. As a result, these people may have gained less weight over follow-up, which would account for the negative association between *CDKAL1* rs2206734 and BMI change. Alternatively, the genetic architecture of BMI change may differ from the obesity predisposing genes associated with BMI level in cross-sectional studies, as suggested by two independent heritability studies[79](#_ENREF_79),[245](#_ENREF_245). Further analyses, ideally through a GWAS for BMI change, are needed to determine which SNPs are major contributors to weight change.

Assessing the impact of the 14 SNPs on physical activity identified some noteworthy associations. Several SNPs displayed significant associations at the nominal level, yet the associations between *BDNF* rs1401635 and *NPC1* rs1805081 with the basic physical activity score were the only SNPs to survive the Bonferroni correction. Despite being an obesity predisposing gene, *BDNF* appears to be related to increased physical activity levels in our cohort. While these relationships appear contradictory, the loss of one functional copy of *BDNF* gene has been associated with Mendelian obesity, cognitive impairment and hyperactivity in both humans[246](#_ENREF_246) and rodents[247](#_ENREF_247). The interpretation of these findings is unclear but it has been proposed that the increased physical activity may be a behavioral response to compensate for their proclivity to gain weight[248](#_ENREF_248). Alternatively, obesity predisposing SNPs that are also associated with increased physical activity have been hypothesized to be a remnant of our hunter-gatherer past. These SNPs may have been favored by natural selection because they promote an “active-foraging” phenotype that induces a preference for energy-dense foods and the physical disposition to attain those foods[248](#_ENREF_248). In contrast, *NPC1* is an obesity predisposing SNP that appears to contribute to sedentary behavior. We speculate that genes associated with inactivity may complement the thrifty genotype hypothesis[249](#_ENREF_249). It seems plausible that genetic variants increasing food intake and decreasing physical activity may have been positively selected based on their parallel effects on energy balance. The contrasting effects of these obesity predisposing SNPs on physical activity may account for the challenges in substantiating the thrifty genotype hypothesis[250](#_ENREF_250). Our findings and previous studies of *BDNF*[246](#_ENREF_246),[247](#_ENREF_247)and *FTO*[251](#_ENREF_251), suggest that the thrifty genotype hypothesis may be complicated by the conflicting effects of these SNPs on physical activity behaviors. SNPs such as *NPC1* are congruent with the thrifty genotype hypothesis while *BDNF* may reflect a more “hyperactive” genotype. Additional studies are necessary to clarify the impact of additional obesity predisposing genes on physical activity behaviors.

We acknowledge that the present study has several limitations. The multi-ethnic composition of the EpiDREAM cohort may have added significant heterogeneity in the analyses, especially as important physical activity differences are observed in different ethnic backgrounds[252](#_ENREF_252). This issue was likely more pronounced in the assessment of the basic physical activity score since this measure is more vulnerable to interpretation on behalf of the participants. Fortunately, the MET score was calculated with more objective criteria of physical activity participation. It should also be noted that both physical activity variables were measured by self-report, and the reliability and validity of this method is inferior to more objective techniques[253](#_ENREF_253). Since most of the obesity predisposing SNPs selected in the study were originally identified in European populations, they may not be ideal proxies for the causal SNP in the six ethnic groups represented in EpiDREAM. We are also aware that the selection of 14 SNPs only represent a subset of the current list of validated obesity predisposing SNPs. Lastly, we appreciate that the EpiDREAM population (participants identified as at risk for type 2 diabetes) is not representative of the general population.

In summary, we identified an interaction between *FTO* rs9939609 and physical activity in a prospective cohort of six ethnic groups from 21 countries. Comparing the use of a basic physical activity score and a precise measure of energy expenditure showed that each yield similar results. We also demonstrate that the recently developed BAI may be a relevant measure of obesity in addition to BMI in GEI studies. Analyzing the impact of obesity predisposing SNPs on physical activity revealed novel associations, although further study is needed to confirm these effects.

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| Table 1. Baseline characteristics by physical activity status in EpiDREAM study | | | | | |
|  | **Category** | **PA-Low** | **PA-Moderate** | **PA-High** | **All** |
| Total at baseline  N(%) |  | 4727 (27.1%) | 10529 (60.4%) | 2151 (12.3%) | 17407 (99.9%) |
| Gender  N(%) | Male | 1498 (31.6%) | 4088 (38.8%) | 1213 (56.4%) | 6799 (39.1%) |
| Female | 3229 (68.3%) | 6441 (61.2%) | 938 (45.6%) | 10608 (60.9%) |
| aAge  (years) |  | 51.24 ±12.13 | 53.17 ± 11.05 | 53.25 ± 10.97 | 52.66 ± 11.38 |
| aGHS  N (%) | Normal  IFG  Diabetes | 2138 (45.2%)  1821 (38.5%)  768 (16.2%) | 4384 (41.6%)  4614 (43.8%)  1531 (14.5%) | 921 (42.8%)  961 (44.7%)  269 (12.5%) | 7446 (42.7%)  7408 (42.5%)  2569 (14.7%) |
| aBMI at baseline  (kg/m2) |  | 30.90 ± 7.07 (4723) | 30.09 ± 5.96 (10527) | 28.89 ± 5.17 (2151) | 30.16 ± 6.22 (17417) |
| aBMI at follow-up  (kg/m2) |  | 30.82 ± 6.24 (2434) | 30.35 ± 5.68 (5719) | 29.02 ± 5.10 (1072) | 30.32 ± 5.80 (9228) |
| aBMI Change  (kg/m2) |  | 0.15 ± 2.87  (2428) | 0.25 ± 2.54  (5731) | 0.25 ± 2.28  (1042) | 0.23 ± 2.60  (9224) |
| aBAI at baseline |  | 35.18 ± 8.09  (4717) | 32.64 ± 7.23  (10504) | 29.91 ± 6.02  (2138) | 32.99 ± 7.51  (17375) |
| aBAI at follow-up |  | 27.47 ± 7.28  (2327) | 26.15 ± 6.04  (5511) | 23.87 ± 4.75  (1034) | 26.23 ± 6.27  (8875) |
| aBAI Change |  | -7.94 ± 5.41  (2324) | -6.97 ± 4.39  (5507) | -6.09 ± 3.54  (1033) | -7.12 ± 4.63  (8867) |
| Ethnic groups N(%) | South Asian | 1527 (32.3%) | 1122 (10.7%) | 111 (5.2%) | 2762 (15.9%) |
| East Asian | 34 (0.7%) | 154 (1.5%) | 37 (1.7%) | 225 (1.3%) |
| European | 1689 (35.7%) | 6091 (57.8%) | 1601 (74.4%) | 9395 (53.9%) |
| African | 362 (7.7%) | 793 (7.5%) | 94 (4.4%) | 1249 (7.2%) |
| Latino American | 1002 (21.2%) | 2057 (19.5%) | 233 (10.8%) | 3292 (18.9%) |
| Native-North American | 113 (2.4%) | 312 (3.0 %) | 75 (3.5%) | 500 (2.9%) |
| GHS: glucose homeostasis status, BMI: body mass index, BAI: body adiposity index, SD = standard deviation; N = sample size  aData are presented as mean ± S.D. (N). | | | | | |

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| Table 2: Effect of physical activity on obesity outcomes. | | | | |
| Effect of MET score on BMI/BAI (adjusted for gender, age, ethnicity and glucose homeostasis status) | | | | |
| **MET Score** | **Outcome** | **β** | **95% CI** | **P-value** |
| Baseline | BMI | -0.001 | -0.001 to -5.89 x 10-4 | 3.27 x 10-10 |
|  | BAI | -0.001 | -1.50 x 10-3 to -9.20 x 10-4 | 3.13 x 10-16 |
|  |  |  |  |  |
| Follow-up | BMI | -0.001 | -1.23 x 10-3 to -5.01 x 10-4 | 3.48 x 10-13 |
|  | BAI | -0.001 | -1.24 x 10-3 to -4.98 x 10-4 | 8.09 x 10-14 |
|  |  |  |  |  |
| Change | BMI | -1.13 x 10-4 | -0.001 to -2.48 x 10-4 | 1.84 x 10-6 |
|  | BAI | -0.001 | -0.001 to 1.61 x 10-4 | 5.45 x 10-6 |
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| Effect of basic physical activity score on BMI/BAI (adjusted for gender, age, ethnicity and glucose homeostasis status) | | | | |
| **Basic PA Score** | **Outcome** | **β** | **95% CI** | **P-value** |
| Baseline | BMI | -1.561 | -1.710 to -1.412 | 9.43 x 10-9 |
|  | BAI | -1.877 | -2.040 to -1.715 | 1.60 x 10-15 |
|  |  |  |  |  |
| Follow-up | BMI | -1.332 | -1.527 to -1.138 | 8.29 x 10-21 |
|  | BAI | -1.403 | -1.613 to -1.193 | 2.30 x 10-23 |
|  |  |  |  |  |
| Change | BMI | -0.224 | -0.333 to -0.115 | 5.55 x 10-5 |
|  | BAI | -0.383 | -0.570 to -0.196 | 6.01 x 10-5 |
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| Table 3: Effect of SNPs/GS on physical activity measures. | | | | |
| Effect of SNPs/GS on basic physical activity score (adjusted for gender, age, ethnicity and glucose homeostasis status) | | | | |
| **rs** | **Gene** | **OR** | **95% CI** | **P-value** |
| rs1514176 | *TNNI3K* | 1.01 | 0.965 to 1.052 | 0.735 |
| rs6235 | *PCSK1* | 0.95 | 0.904 to 0.998 | 0.040 |
| rs6232 | *PCSK* | 0.99 | 0.887 to 1.096 | 0.079 |
| rs2206734 | *CDKAL1* | 0.98 | 0.930 to 1.034 | 0.468 |
| rs2272903 | *TFAP2B* | 1.02 | 0.961 to 1.085 | 0.510 |
| rs1211166 | *NTRK2* | 1.06 | 1.008 to 1.119 | 0.023 |
| rs6265 | *BDNF* | 1.07 | 1.013 to 1.133 | 0.016 |
| rs1401635 | *BDNF* | 1.09 | 1.035 to 1.138 | 0.001 |
| rs997295 | *MAP2K5* | 1.04 | 0.993 to 1.082 | 0.099 |
| rs7203521 | *FTO* | 1.01 | 0.971 to 1.059 | 0.533 |
| rs9939609 | *FTO* | 0.95 | 0.907 to 0.990 | 0.016 |
| rs1805081 | *NPC1* | 0.92 | 0.877 to 0.963 | 0.001 |
| rs2075650 | *APOE* | 0.96 | 0.897 to 1.018 | 0.160 |
| rs11671664 | *GIPR* | 1.03 | 0.958 to 1.100 | 0.458 |
|  | Gene Score | 1.00 | 0.989 to 1.016 | 0.708 |
| Effect of SNPs/GS on basic physical activity score (adjusted for gender, age, ethnicity, glucose homeostasis status and BMI) | | | | |
| **rs** | **Gene** | **OR** | **95% CI** | **P-value** |
| rs1514176 | *TNNI3K* | 1.02 | 0.975 to 1.064 | 0.402 |
| rs6235 | *PCSK1* | 0.95 | 0.904 to 1.039 | 0.440 |
| rs6232 | *PCSK* | 0.98 | 0.833 to 1.093 | 0.747 |
| rs2206734 | *CDKAL1* | 1.00 | 0.944 to 1.049 | 0.858 |
| rs2272903 | *TFAP2B* | 1.03 | 0.967 to 1.093 | 0.374 |
| rs1211166 | *NTRK2* | 1.07 | 1.013 to 1.124 | 0.015 |
| rs6265 | *BDNF* | 1.08 | 1.021 to 1.143 | 0.007 |
| rs1401635 | *BDNF* | 1.09 | 1.043 to 1.147 | 0.003 |
| rs997295 | *MAP2K5* | 1.04 | 0.996 to 1.085 | 0.077 |
| rs7203521 | *FTO* | 1.02 | 0.972 to 1.061 | 0.490 |
| rs9939609 | *FTO* | 0.97 | 0.928 to 1.013 | 0.169 |
| rs1805081 | *NPC1* | 0.92 | 0.879 to 0.966 | 3.52 x 10-4 |
| rs2075650 | *APOE* | 0.96 | 0.899 to 1.021 | 0.187 |
| rs11671664 | *GIPR* | 1.04 | 0.975 to 1.121 | 0.210 |
|  | Gene Score | 1.01 | 0.996 to 1.023 | 0.161 |
| Effect of SNPs/GS on MET score (adjusted for gender, age, ethnicity, glucose homeostasis status) | | | | |
| **rs** | **Gene** | **β** | **95% CI** | **P-value** |
| rs1514176 | *TNNI3K* | -6.436 | -17.087 to 4.215 | 0.236 |
| rs6235 | *PCSK1* | 3.795 | -8.290 to 15.880 | 0.538 |
| rs6232 | *PCSK* | -3.699 | -30.120 to 22.723 | 0.784 |
| rs2206734 | *CDKAL1* | -3.870 | -16.972 to 9.233 | 0.563 |
| rs2272903 | *TFAP2B* | 2.056 | -13.478 to 17.589 | 0.795 |
| rs1211166 | *NTRK2* | 7.589 | -5.400 to 20.578 | 0.252 |
| rs6265 | *BDNF* | 13.191 | -0.665 to 27.048 | 0.062 |
| rs1401635 | *BDNF* | 11.624 | -0.081 to 23.328 | 0.052 |
| rs997295 | *MAP2K5* | -0.945 | -11.564 to 9.675 | 0.862 |
| rs7203521 | *FTO* | -1.771 | -12.438 to 8.896 | 0.745 |
| rs9939609 | *FTO* | 0.992 | -9.809 to 11.794 | 0.857 |
| rs1805081 | *NPC1* | -1.954 | -13.230 to 9.322 | 0.734 |
| rs2075650 | *APOE* | -10.080 | -25.648 to 5.489 | 0.204 |
| rs11671664 | *GIPR* | -1.400 | -18.327 to 15.527 | 0.871 |
|  | Gene Score | 0.738 | -2.483 to 3.959 | 0.653 |
| Effect of SNPs/GS on MET score (adjusted for gender, age, ethnicity, glucose homeostasis status and BMI) | | | | |
| **rs** | **Gene** | **β** | **95% CI** | **P-value** |
| rs1514176 | *TNNI3K* | -5.354 | -15.995 to 5.287 | 0.324 |
| rs6235 | *PCSK1* | 3.971 | -8.097 to 16.038 | 0.519 |
| rs6232 | *PCSK* | -2.972 | -29.365 to 23.422 | 0.825 |
| rs2206734 | *CDKAL1* | -2.827 | -15.920 to 10.266 | 0.672 |
| rs2272903 | *TFAP2B* | 2.969 | -12.544 to 18.482 | 0.708 |
| rs1211166 | *NTRK2* | 7.600 | -5.370 to 20.570 | 0.251 |
| rs6265 | *BDNF* | 14.272 | 0.432 to 28.112 | 0.043 |
| rs1401635 | *BDNF* | 12.536 | 0.846 to 24.227 | 0.036 |
| rs997295 | *MAP2K5* | -0.627 | -11.231 to 9.978 | 0.908 |
| rs7203521 | *FTO* | -1.373 | -12.028 to 9.282 | 0.801 |
| rs9939609 | *FTO* | 2.756 | -8.043 to 13.555 | 0.617 |
| rs1805081 | *NPC1* | -1.770 | -13.033 to 9.492 | 0.758 |
| rs2075650 | *APOE* | -10.304 | -25.857 to 5.249 | 0.194 |
| rs11671664 | *GIPR* | 0.277 | -16.634 to 17.189 | 0.974 |
|  | Gene Score | 1.384 | -1.838 to 4.606 | 0.400 |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table 4: Effect of SNPs/GS on obesity measures. | | | | | | | | |
| Effect of SNPs/GS on BMI/BAI at baseline (adjusted for gender, age, ethnicity, physical activity and glucose homeostasis status) | | | | | | | | |
|  | | | **BMI Baseline** | | | **BAI Baseline** | | |
| rs | gene | | **β** | **95% CI** | **P-value** | **β** | **95% CI** | **P-value** |
| rs1514176 | *TNNI3K* | | 0.196 | 0.074 to 0.319 | 0.002 | 0.148 | 0.013 to 0.282 | 0.031 |
| rs6235 | *PCSK1* | | 0.028 | -0.112 to 0.169 | 0.692 | 0.008 | -0.145 to 0.161 | 0.916 |
| rs6232 | *PCSK* | | 0.025 | -0.276 to 0.326 | 0.870 | 0.070 | -0.259 to 0.398 | 0.678 |
| rs2206734 | *CDKAL1* | | 0.294 | 0.143 to 0.444 | 1.33x10-4 | 0.319 | 0.155 to 0.484 | 1.44x10-4 |
| rs2272903 | *TFAP2B* | | 0.133 | -0.041 to 0.307 | 0.134 | 0.175 | -0.015 to 0.366 | 0.071 |
| rs1211166 | *NTRK2* | | 0.058 | -0.091 to 0.206 | 0.446 | 0.030 | -0.131 to 0.192 | 0.713 |
| rs6265 | *BDNF* | | 0.140 | -0.019 to 0.300 | 0.085 | 0.102 | -0.073 to 0.276 | 0.254 |
| rs1401635 | *BDNF* | | 0.155 | 0.022 to 0.289 | 0.223 | 0.070 | -0.075 to 0.216 | 0.344 |
| rs997295 | *MAP2K5* | | 0.033 | -0.090 to 0.156 | 0.597 | -0.068 | -0.202 to 0.066 | 0.317 |
| rs7203521 | *FTO* | | 0.027 | -0.096 to 0.150 | 0.666 | 0.027 | -0.108 to 0.161 | 0.697 |
| rs9939609 | *FTO* | | 0.451 | 0.326 to 0.576 | 1.50x10-12 | 0.394 | 0.258 to 0.531 | 1.53x10-8 |
| rs1805081 | *NPC1* | | 0.015 | -0.116 to 0.146 | 0.820 | 0.095 | -0.048 to 0.237 | 0.193 |
| rs2075650 | *APOE* | | 0.069 | -0.113 to 0.250 | 0.459 | 0.114 | -0.084 to 0.312 | 0.258 |
| rs11671664 | *GIPR* | | 0.301 | 0.105 to 0.498 | 0.003 | 0.225 | 0.101 to 0.440 | 0.040 |
|  | Gene Score | | 0.128 | 0.091 to 0.165 | 1.43x10-11 | 0.106 | 0.065 to 0.146 | 3.05x10-7 |
|  |  | |  |  |  |  |  |  |
| Effect of SNPs/GS on BMI/BAI at follow-up (adjusted for sex, age, ethnicity, physical activity and glucose homeostasis status) | | | | | | | | |
|  | | | **BMI at Follow-up** | | | **BAI at Follow-up** | | |
| rs | | gene | **β** | **95% CI** | **P-value** | **β** | **95% CI** | **P-value** |
| rs1514176 | | *TNNI3K* | 0.191 | 0.035 to 0.348 | 0.017 | 0.193 | 0.023 to 0.362 | 0.026 |
| rs6235 | | *PCSK1* | -0.007 | -0.186 to 0.173 | 0.941 | 0.070 | -0.124 to 0.265 | 0.479 |
| rs6232 | | *PCSK* | 0.071 | -0.320 to 0.463 | 0.722 | 0.118 | -0.311 to 0.548 | 0.590 |
| rs2206734 | | *CDKAL1* | 0.004 | -0.191 to 0.200 | 0.965 | -0.025 | -0.238 to 0.187 | 0.814 |
| rs2272903 | | *TFAP2B* | 0.169 | -0.057 to 0.395 | 0.144 | 2.08x10-5 | -0.247 to 0.247 | 0.999 |
| rs1211166 | | *NTRK2* | 0.072 | -0.121 to 0.265 | 0.465 | -0.142 | -0.352 to 0.067 | 0.182 |
| rs6265 | | *BDNF* | 0.056 | -0.148 to 0.261 | 0.590 | 0.010 | -0.212 to 0.232 | 0.932 |
| rs1401635 | | *BDNF* | 0.165 | -0.009 to 0.339 | 0.064 | 0.130 | -0.042 to 0.302 | 0.139 |
| rs997295 | | *MAP2K5* | -0.015 | -0.172 to 0.142 | 0.853 | -0.154 | -0.323 to 0.016 | 0.076 |
| rs7203521 | | *FTO* | 0.007 | -0.151 to 0.164 | 0.934 | -0.010 | -0.181 to 0.162 | 0.912 |
| rs9939609 | | *FTO* | 0.408 | 0.247 to 0.570 | 7.50x10-7 | 0.438 | 0.263 to 0.614 | 1.01x10-6 |
| rs1805081 | | *NPC1* | 0.031 | -0.136 to 0.191 | 0.713 | -0.098 | -0.262 to 0.066 | 0.243 |
| rs2075650 | | *APOE* | -0.036 | -0.271 to 0.200 | 0.767 | 0.031 | -0.224 to 0.286 | 0.809 |
| rs11671664 | | *GIPR* | 0.253 | -0.002 to 0.507 | 0.052 | 0.430 | 0.154 to 0.706 | 0.002 |
|  | | Gene Score | 0.093 | 0.045 to 0.141 | 1.36x10-4 | 0.059 | 0.007 to 0.111 | 0.026 |
|  | |  |  |  |  |  |  |  |
| Effect of SNPs/GS on BMI/BAI change (adjusted for sex, age, ethnicity, physical activity and glucose homeostasis status) | | | | | | | | |
|  | | | **BMI Change** | | | **BAI Change** | | |
| rs | | gene | **β** | **95% CI** | **P-value** | **β** | **95% CI** | **P-value** |
| rs1514176 | | *TNNI3K* | -0.016 | -0.090 to 0.057 | 0.662 | -0.103 | -0.232 to 0.025 | 0.114 |
| rs6235 | | *PCSK1* | -0.012 | -0.097 to 0.073 | 0.780 | -0.007 | -0.154 to 0.141 | 0.930 |
| rs6232 | | *PCSK* | -0.087 | -0.272 to 0.099 | 0.360 | -0.126 | -0.451 to 0.199 | 0.447 |
| rs2206734 | | *CDKAL1* | -0.113 | -0.205 to -0.020 | 0.017 | -0.272 | -0.433 to -0.112 | 0.001 |
| rs2272903 | | *TFAP2B* | -0.048 | -0.155 to 0.058 | 0.375 | -0.214 | -0.401 to -0.027 | 0.025 |
| rs1211166 | | *NTRK2* | 0.014 | -0.077 to 0.104 | 0.770 | -0.084 | -0.242 to 0.075 | 0.301 |
| rs6265 | | *BDNF* | 0.039 | -0.057 to 0.135 | 0.429 | -0.110 | -0.279 to 0.058 | 0.198 |
| rs1401635 | | *BDNF* | 0.028 | -0.055 to 0.111 | 0.506 | 0.029 | -0.098 to 0.157 | 0.651 |
| rs997295 | | *MAP2K5* | -0.018 | -0.092 to 0.056 | 0.639 | -0.026 | -0.155 to 0.103 | 0.692 |
| rs7203521 | | *FTO* | 0.004 | -0.070 to 0.079 | 0.913 | 0.022 | -0.107 to 0.152 | 0.736 |
| rs9939609 | | *FTO* | -0.023 | -0.099 to 0.053 | 0.556 | -0.035 | -0.169 to 0.098 | 0.602 |
| rs1805081 | | *NPC1* | -0.001 | -0.081 to 0.079 | 0.984 | -0.043 | -0.164 to 0.078 | 0.489 |
| rs2075650 | | *APOE* | -0.034 | -0.145 to 0.077 | 0.552 | 0.014 | -0.179 to 0.207 | 0.887 |
| rs11671664 | | *GIPR* | 4.98x10-5 | -0.120 to 0.120 | 0.999 | 0.193 | -0.016 to 0.403 | 0.070 |
|  | | Gene Score | -0.012 | -0.035 to 0.010 | 0.281 | -0.047 | -0.087 to -0.008 | 0.019 |

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| --- | --- | --- | --- | --- | --- | --- |
| Table 5: Interaction analysis of SNP/GS associated with obesity measures. | | | | | | |
| **Baseline Interaction Tests** | | | | | | |
| **Interaction terms** | **Outcome Variable** | | **Impact of Risk Allele** | | | |
|  |  | |  | **β** | **95% CI** | **P** |
| *FTO* rs9939609 x  MET score | BMI-Baseline (Pinteraction=0.032) | | MET Tertile  Low  Medium  High | 0.504 kg/m2  0.399 kg/m2  0.306 kg/m2 | 0.218-0.789  0.141-0.656  0.052-0.559 | 0.001  0.002  0.018 |
| *FTO* rs9939609 x  MET score | BAI-Baseline (Pinteraction=3.26 x 10-4) | | MET Tertile  Low  Medium  High | 0.484  0.363  0.242 | 0.181-0.787  0.079-0.647  -0.032-0.515 | 0.002  0.012  0.083 |
| *TNNl3K* rs1514176 x MET score | BMI-Baseline  (Pinteraction=0.449) | |  |  |  |  |
| *TNNl3K* rs1514176 x MET score | BAI-Baseline  (Pinteraction=0.719) | |  |  |  |  |
| *CDKAL1* rs2206734 x MET score | BMI-Baseline  (Pinteraction=0.599) | |  |  |  |  |
| *CDKAL1* rs2206734 x MET score | BAI-Baseline  (Pinteraction=0.407) | |  |  |  |  |
| *GIPR* rs11671664 x  MET score | BMI-Baseline  (Pinteraction=0.467) | |  |  |  |  |
| *GIPR* rs11671664 x MET score | BAI-Baseline  (Pinteraction=0.921) | |  |  |  |  |
| GS x MET score | BMI-Baseline  (Pinteraction=0.178) | |  |  |  |  |
| GS x MET score | BAI-Baseline  (Pinteraction=0.164) | |  |  |  |  |
| *FTO* rs9939609 x  BPA score | BMI-Baseline  (Pinteraction=0.004) | | BPA score  Low  Medium  High | 0.746 kg/m2  0.311 kg/m2  0.302 kg/m2 | 0.490-1.002  0.155-0.467  -0.004-0.608 | 1.14 x 10-8  9.48 x 10-5  0.053 |
| *FTO* rs9939609 x  BPA score | BAI-Baseline  (Pinteraction=0.005) | | BPA score  Low  Medium  High | 0.665  0.227  0.372 | 0.385-0.945  0.057-0.398  0.057-0.688 | 3.38 x 10-6  0.009  0.021 |
| *TNNl3K* rs1514176 x BPA score | BMI-Baseline  (Pinteraction=0.372) | |  |  |  |  |
| *TNNl3K* rs1514176 x BPA score | BAI-Baseline  (Pinteraction=0.183) | |  |  |  |  |
| *CDKAL1* rs2206734 x BPA score | BMI-Baseline  (Pinteraction=0.527) | |  |  |  |  |
| *CDKAL1* rs2206734 x BPA score | BAI-Baseline  (Pinteraction=0.625) | |  |  |  |  |
| *GIPR* rs11671664 x  BPA score | BMI-Baseline  (Pinteraction=0.780) | |  |  |  |  |
| *GIPR* rs11671664 x BPA score | BAI-Baseline  (Pinteraction=0.989) | |  |  |  |  |
| GS x BPA score | BMI-Baseline  (Pinteraction=0.204) | |  |  |  |  |
| GS x BPA score | BAI-Baseline  (Pinteraction=0.223) | |  |  |  |  |
| **Follow-up Interaction Tests** | | | | | | |
| **Interaction terms** | | **Outcome Variable** | **Impact of Risk Allele** | | | |
|  | |  |  | **β** | **95% CI** | **P** |
| *FTO* rs9939609 x  MET score | | BMI-Follow-up (Pinteraction=0.723) |  |  |  |  |
| *FTO* rs9939609 x  MET score | | BAI-Follow-up (Pinteraction=0.928) |  |  |  |  |
| *TNNl3K* rs1514176 x MET score | | BMI-Follow-up  (Pinteraction=0.037) | MET Tertile  Low  Medium  High | 0.455 kg/m2  0.240 kg/m2  0.159 kg/m2 | 0.111-0.799  -0.080-0.560  -0.172-0.491 | 0.010  0.141  0.346 |
| *TNNl3K* rs1514176 x MET score | | BAI-Follow-up  (Pinteraction=0.152) |  |  |  |  |
| *GIPR* rs11671664 x  MET score | | BMI- Follow-up  (Pinteraction=0.983) |  |  |  |  |
| *GIPR* rs11671664 x MET score | | BAI- Follow-up  (Pinteraction=0.831) |  |  |  |  |
| GS x MET score | | BMI- Follow-up  (Pinteraction=0.441) |  |  |  |  |
| GS x MET score | | BAI- Follow-up  (Pinteraction=0.827) |  |  |  |  |
| *FTO* rs9939609 x  BPA score | | BMI- Follow-up  (Pinteraction=0.036) | BPA score  Low  Medium  High | 0.722 kg/m2  0.252 kg/m2  0.204 kg/m2 | 0.408-1.036  0.047-0.456  -0.232-0.639 | 7.03 x 10-6  0.016  0.359 |
| *FTO* rs9939609 x  BPA score | | BAI- Follow-up  (Pinteraction=0.410) |  |  |  |  |
| *TNNl3K* rs1514176 x BPA score | | BMI- Follow-up  (Pinteraction=0.450) |  |  |  |  |
| *TNNl3K* rs1514176 x BPA score | | BAI- Follow-up  (Pinteraction=0.958) |  |  |  |  |
| *GIPR* rs11671664 x  BPA score | | BMI- Follow-up  (Pinteraction=0.663) |  |  |  |  |
| *GIPR* rs11671664 x BPA score | | BAI- Follow-up  (Pinteraction=0.575) |  |  |  |  |
| GS x BPA score | | BMI- Follow-up  (Pinteraction=0.864) |  |  |  |  |
| GS x BPA score | | BAI- Follow-up  (Pinteraction=0.467) |  |  |  |  |
| **Interactions Tests with Obesity Change** | | | | | | |
| **Interaction terms** | | **Outcome Variable** | **Impact of Risk Allele** | | | |
|  | |  |  | **β** | **95% CI** | **P** |
| *CDKAL1* rs2206734 x MET score | | BMI- Change  (Pinteraction=0.480) |  |  |  |  |
| *CDKAL1* rs2206734 x MET score | | BAI- Change  (Pinteraction=0.220) |  |  |  |  |
| *CDKAL1* rs2206734 x BPA score | | BMI- Change  (Pinteraction=0.412) |  |  |  |  |
| *CDKAL1* rs2206734 x BPA score | | BAI- Change  (Pinteraction=0.300) |  |  |  |  |
| GS x MET score | | BAI- Change  (Pinteraction=0.628) |  |  |  |  |
| GS x BPA score | | BAI- Change  (Pinteraction=0.925) |  |  |  |  |
| Notes:  BPA score=basic physical activity score  GS=Gene Score | | | | | | |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Supplementary Table 1: Genetic information from the EpiDREAM study. | | | | | | | | | | | | | | | | | | | |
| **rs** | 1514176 | 6235 | 6232 | | 2206734 | | 2272903 | 1211166 | 6265 | 1401635 | | 997295 | | 7203521 | 9939609 | 1805081 | 2075650 | 11671664 | |
| Gene | *TNN13k* | *PCSK1* | *PCSK1* | | *CDKAL1* | | *TFA2B* | *NTRK2* | *BDNF* | *BDNF* | | *MAP2K5* | | *FTO* | *FTO* | *NPC1* | *APOE* | *GIPR* | |
| Risk Allele | G | C | G | | C | | G | A | G | C | | T | | A | A | A | A | G | |
| Major Allele | A | G | A | | C | | G | A | G | G | | T | | A | T | A | A | A | |
| Minor Allele | G | C | G | | T | | A | G | A | C | | G | | G | A | G | G | A | |
| **Frequency of Risk Allele** | | | | | | | | | | | | | | | | | | | |
| **rs** | 1514176 | 6235 | 6232 | | 2206734 | | 2272903 | 1211166 | 6265 | 1401635 | | 997295 | | 7203521 | 9939609 | 1805081 | 2075650 | 11671664 | |
| **Gene** | *TNN13k* | *PCSK1* | *PCSK1* | | *CDKAL1* | | *TFA2B* | *NTRK2* | *BDNF* | *BDNF* | | *MAP2K5* | | *FTO* | *FTO* | *NPC1* | *APOE* | *GIPR* | |
| European | 0.419 | 0.265 | 0.049 | | 0.802 | | 0.893 | 0.807 | 0.814 | 0.291 | | 0.590 | | 0.610 | 0.419 | 0.612 | 0.139 | 0.107 | |
| North  American | 0.560 | 0.243 | 0.029 | | 0.781 | | 0.892 | 0.869 | 0.844 | 0.225 | | 0.441 | | 0.390 | 0.231 | 0.680 | 0.106 | 0.108 | |
| Latin American | 0.518 | 0.213 | 0.029 | | 0.799 | | 0.859 | 0.798 | 0.841 | 0.218 | | 0.457 | | 0.469 | 0.338 | 0.692 | 0.112 | 0.089 | |
| East Asian | 0.689 | 0.296 | 0.004 | | 0.662 | | 0.793 | 0.811 | 0.536 | 0.080 | | 0.189 | | 0.258 | 0.176 | 0.762 | 0.126 | 0.380 | |
| African American | 0.669 | 0.153 | 0.007 | | 0.757 | | 0.708 | 0.672 | 0.967 | 0.253 | | 0.543 | | 0.632 | 0.491 | 0.932 | 0.121 | 0.114 | |
| South Asian | 0.549 | 0.294 | 0.062 | | 0.766 | | 0.781 | 0.722 | 0.774 | 0.381 | | 0.455 | | 0.429 | 0.332 | 0.768 | 0.130 | 0.106 | |
| **Genotype Counts** | | | | | | | | | | | | | | | | | | | |
| **rs** | 1514176 | 6235 | | 6232 | | 2206734 | 2272903 | 1211166 | 6265 | | 1401635 | | 997295 | 7203521 | 9939609 | 1805081 | 2075650 | | 11671664 |
| **Gene** | *TNN13k* | *PCSK1* | | *PCSK1* | | *CDKAL1* | *TFA2B* | *NTRK2* | *BDNF* | | *BDNF* | | *MAP2K5* | *FTO* | *FTO* | *NPC1* | *APOE* | | *GIPR* |
| European | GG,1656  AG,4560  AA,3179 | GG,5092  CG,3617  CC,685 | | AA,8504  AG,868  GG,23 | | CC,6025  CT,3023  TT,346 | GG,7496  AG,1780  AA,119 | AA,6126  AG,2902  GG,363 | GG,6237  AG,2812  AA,346 | | GG,4763  CG,3805  CC,826 | | GG,1599  GT,4503  TT,3293 | GG,1474  AG,4379  AA,3542 | TT,3225  AT,4471  AA,1699 | AA,3555  AG,4396  GG,1444 | AA,6961  AG,2253  GG,181 | | AA,122  AG,1768  GG,7502 |
| North  American | GG,157  AG,46  AA,97 | GG,284  CG,189  CC,27 | | AA,471  AG,29  GG,0 | | CC,299  CT,183  TT,18 | GG,396  AG,100  AA,4 | AA,379  AG,111  GG,10 | GG, 358  AG,128  AA,14 | | GG,303  CG,169  CC,28 | | GG,168  GT,223  TT,109 | GG,203  AG,203  AA,93 | TT,301  AT,167  AA,32 | AA,224  AG,232  GG,44 | AA,399  AG,96  GG,5 | | AA,3  AG,102  GG,395 |
| Latin American | GG,921  AG,1565  AA,806 | GG,2049  CG,1086  CC,157 | | AA,3104  AG,185  GG,3 | | CC,2107  CT,1049  TT,136 | GG,2436  AG,784  AA,72 | AA,2117  AG,1021  GG,154 | GG, 2336  AG,862  AA,94 | | GG,2016  CG,1120  CC,156 | | GG,995  GT,1586  TT,710 | GG,972  AG,1550  AA,769 | TT,1458  AT,1442  AA,392 | AA,1591  AG,1373  GG,328 | AA,2598  AG,647  GG,47 | | AA,29  AG,532  GG,2730 |
| East Asian | GG,120  AG,70  AA,35 | GG,112  CG,93  CC,20 | | AA,223  AG,2  GG,0 | | CC,102  CT,94  TT,29 | GG,140  AG,77  AA,8 | AA,151  AG,63  GG,11 | GG, 62  AG,117  AA,46 | | GG,193  CG,28  CC,4 | | GG,151  GT,63  TT,11 | GG,124  AG,86  AA,15 | TT,151  AT,69  AA,5 | AA,133  AG,77  GG,15 | AA,173  AG,47  GG,5 | | AA,41  AG,89  GG,95 |
| African American | GG,560  AG,552  AA,137 | GG,893  CG,329  CC,27 | | AA,1231  AG,18  GG,0 | | CC,715  CT,462  TT,72 | GG,641  AG,487  AA,121 | AA,568  AG,541  GG,139 | GG,1171  AG,74  AA,4 | | GG,694  CG,478  CC,77 | | GG,262  GT,617  TT,370 | GG,175  AG,570  AA,504 | TT,315  AT,641  AA,293 | AA,1085  AG,158  GG,6 | AA,958  AG,278  GG,13 | | AA,15  AG,256  GG,978 |
| South Asian | GG,834  AG,1362  AA,566 | GG,1402  CG,1097  CC,262 | | AA,2437  AG,309  GG,16 | | CC,1628  CT,974  TT,160 | GG,1683  AG,947  AA,132 | AA,1440  AG,1106  GG,216 | GG,1660  AG,956  AA,146 | | GG,1080  CG,1257  CC,424 | | GG,838  GT,1337  TT,587 | GG,912  AG,1328  AA,521 | TT,1254  AT,1184  AA,324 | AA,1634  AG,973  GG,155 | AA,2090  AG,621  GG,51 | | AA,33  AG,524  GG,2205 |
| **SNP Call Rate (%)** | | | | | | | | | | | | | | | | | | | |
| **rs** | 1514176 | 6235 | | 6232 | | 2206734 | 2272903 | 1211166 | 6265 | | 1401635 | | 997295 | 7203521 | 9939609 | 1805081 | 2075650 | | 11671664 |
| **Gene** | *TNN13k* | *PCSK1* | | *PCSK1* | | *CDKAL1* | *TFA2B* | *NTRK2* | *BDNF* | | *BDNF* | | *MAP2K5* | *FTO* | *FTO* | *NPC1* | *APOE* | | *GIPR* |
| European | 100 | 99.989 | | 100 | | 100 | 100 | 99.979 | 100 | | 99.989 | | 100 | 100 | 100 | 100 | 100 | | 99.968 |
| North  American | 100 | 100 | | 100 | | 100 | 100 | 100 | 100 | | 100 | | 100 | 99.800 | 100 | 100 | 100 | | 100 |
| Latin American | 100 | 100 | | 100 | | 100 | 100 | 100 | 100 | | 100 | | 99.970 | 99.970 | 100 | 100 | 100 | | 99.970 |
| East Asian | 100 | 100 | | 100 | | 100 | 100 | 100 | 100 | | 100 | | 100 | 100 | 100 | 100 | 100 | | 100 |
| African American | 100 | 100 | | 100 | | 100 | 100 | 99.920 | 100 | | 100 | | 100 | 100 | 100 | 100 | 100 | | 100 |
| South Asian | 100 | 99.964 | | 100 | | 99.964 | 100 | 100 | 100 | | 99.964 | | 100 | 99.964 | 100 | 100 | 100 | | 100 |
| **Hardy-Weinberg Equilibrium Values** | | | | | | | | | | | | | | | | | | | |
| **rs** | 1514176 | 6235 | | 6232 | | 2206734 | 2272903 | 1211166 | 6265 | | 1401635 | | 997295 | 7203521 | 9939609 | 1805081 | 2075650 | | 11671664 |
| **Gene** | *TNN13k* | *PCSK1* | | *PCSK1* | | *CDKAL1* | *TFA2B* | *NTRK2* | *BDNF* | | *BDNF* | | *MAP2K5* | *FTO* | *FTO* | *NPC1* | *APOE* | | *GIPR* |
| European | 0.766 | 0.243 | | 0.823 | | 0.171 | 0.257 | 0.353 | 0.232 | | 0.083 | | 0.404 | 0.048 | 0.037 | 0.170 | 0.863 | | 0.144 |
| North  American | 0.978 | 0.627 | | 0.987 | | 0.113 | 0.492 | 0.553 | 0.496 | | 0.520 | | 0.037 | 0.002 | 0.167 | 0.151 | 0.989 | | 0.247 |
| Latin American | 0.009 | 0.375 | | 0.757 | | 0.785 | 0.347 | 0.064 | 0.172 | | 0.918 | | 0.138 | 0.002 | 0.211 | 0.218 | 0.334 | | 0.519 |
| East Asian | 7x10-5 | 0.874 | | 0.989 | | 0.371 | 0.684 | 0.194 | 0.592 | | 0.039 | | 0.194 | 0.995 | 0.492 | 0.461 | 0.368 | | 0.016 |
| African American | 0.997 | 0.742 | | 0.977 | | 0.817 | 0.033 | 0.519 | 0.035 | | 0.652 | | 0.954 | 0.542 | 0.334 | 0.820 | 0.184 | | 0.673 |
| South Asian | 0.818 | 0.028 | | 0.097 | | 0.367 | 0.956 | 0.850 | 0.587 | | 0.070 | | 0.205 | 0.332 | 0.079 | 0.557 | 0.615 | | 0.765 |

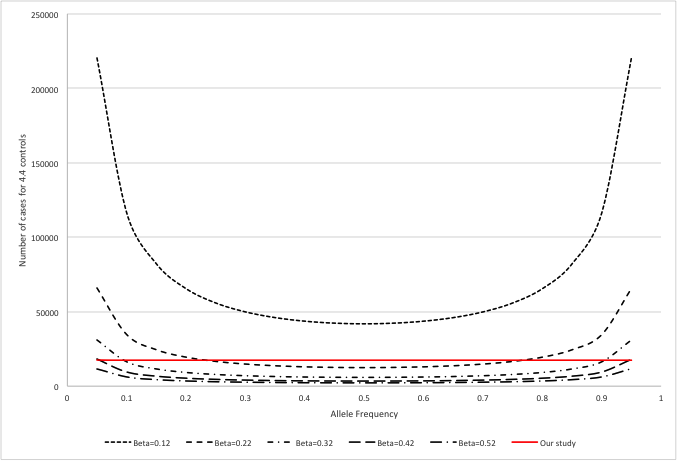
#### Supplementary Table 2: List of 39 self-reported physical activities.

1. Aerobics/Calisthenics
2. Badminton
3. Basketball
4. Bicycling
5. Bowling
6. Pilates
7. Dance
8. Fishing
9. Soccer
10. Yard work
11. Golfing
12. Hiking
13. Hockey
14. Horseback riding
15. Jogging
16. Jump rope
17. Martial arts
18. Squash
19. Mountain climbing
20. Rugby
21. Scuba diving
22. Skating
23. Snowshoeing
24. Downhill skiing
25. Cross country skiing
26. Softball
27. Stairs
28. Weightlifting
29. Swimming
30. Ping pong
31. Tai chi
32. Tennis
33. Volleyball
34. Walking
35. Aquatics exercise
36. Water skiing
37. Wood chopping
38. Yoga
39. Rowing

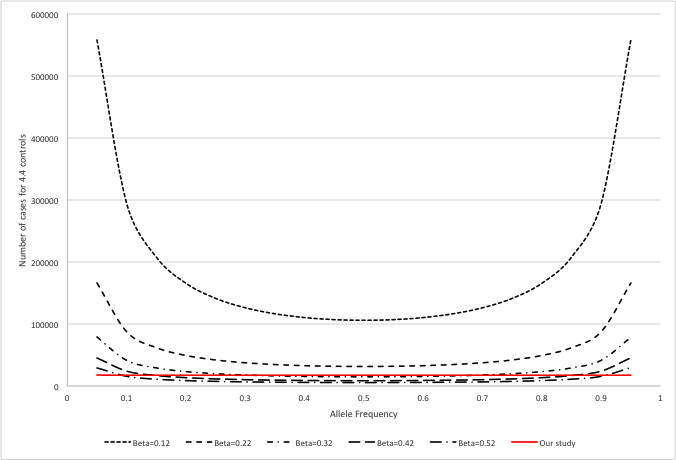
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Supplementary Table 3: Effect of SNPs/GS on change in physical activity measures. | | | | |
| Effect of SNPs/GS on change in basic physical activity score (adjusted for gender, age, ethnicity and glucose homeostasis status) | | | | |
| **rs** | **Gene** | **OR** | **95% CI** | **P-value** |
| rs1514176 | *TNNI3K* | 0.97 | 0.902 to 1.040 | 0.374 |
| rs6235 | *PCSK1* | 0.93 | 0.856 to 1.008 | 0.076 |
| rs6232 | *PCSK* | 1.03 | 0.863 to 1.226 | 0.753 |
| rs2206734 | *CDKAL1* | 1.06 | 0.972 to 1.161 | 0.182 |
| rs2272903 | *TFAP2B* | 0.99 | 0.897 to 1.094 | 0.859 |
| rs1211166 | *NTRK2* | 0.88 | 0.809 to 0.962 | 0.005 |
| rs6265 | *BDNF* | 0.98 | 0.893 to 1.073 | 0.658 |
| rs1401635 | *BDNF* | 0.95 | 0.875 to 1.024 | 0.170 |
| rs997295 | *MAP2K5* | 1.06 | 0.986 to 1.138 | 0.114 |
| rs7203521 | *FTO* | 0.99 | 0.920 to 1.062 | 0.747 |
| rs9939609 | *FTO* | 1.07 | 0.994 to 1.151 | 0.071 |
| rs1805081 | *NPC1* | 1.01 | 0.930 to 1.088 | 0.889 |
| rs2075650 | *APOE* | 1.01 | 0.908 to 1.125 | 0.846 |
| rs11671664 | *GIPR* | 1.10 | 0.978 to 1.235 | 0.112 |
|  | Gene Score | 1.00 | 0.975 to 1.018 | 0.748 |
| Effect of SNPs/GS on change in MET score (adjusted for gender, age, ethnicity, glucose homeostasis status) | | | | |
| **rs** | **Gene** | **β** | **95% CI** | **P-value** |
| rs1514176 | *TNNI3K* | -0.006 | -18.250 to 11.764 | 0.662 |
| rs6235 | *PCSK1* | -0.023 | -31.981 to 2.376 | 0.091 |
| rs6232 | *PCSK* | 0.007 | -28.363 to 48.331 | 0.610 |
| rs2206734 | *CDKAL1* | 0.010 | -11.724 to 25.728 | 0.464 |
| rs2272903 | *TFAP2B* | 0.020 | -6.073 to 38.416 | 0.154 |
| rs1211166 | *NTRK2* | 0.003 | -16.911 to 20.489 | 0.851 |
| rs6265 | *BDNF* | -1.56 x 10-4 | -19.760 to 19.538 | 0.991 |
| rs1401635 | *BDNF* | -0.015 | -26.135 to 7.389 | 0.273 |
| rs997295 | *MAP2K5* | 0.008 | -11.012 to 19.403 | 0.589 |
| rs7203521 | *FTO* | -0.013 | -22.352 to 8.393 | 0.373 |
| rs9939609 | *FTO* | -0.005 | -18.409 to 12.764 | 0.723 |
| rs1805081 | *NPC1* | -0.021 | -28.212 to 4.046 | 0.142 |
| rs2075650 | *APOE* | -0.005 | -26.199 to 18.252 | 0.726 |
| rs11671664 | *GIPR* | -0.016 | -38.794 to 10.069 | 0.249 |
|  | Gene Score | -0.017 | -7.470 to 1.732 | 0.222 |



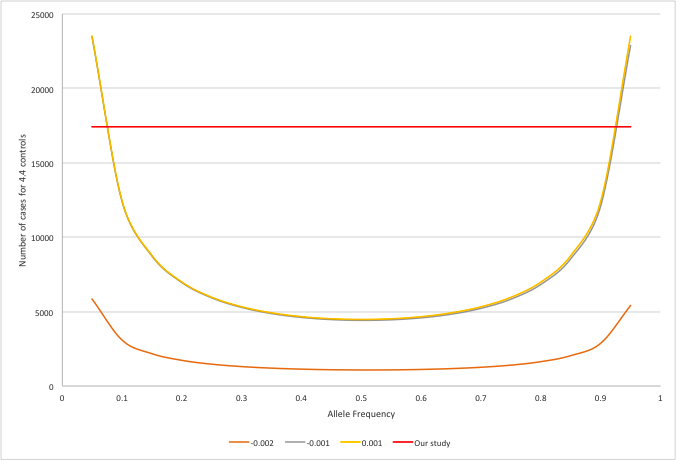
###### Figure 1: Flow chart of EpiDREAM study

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###### Figure 2: Power calculation for main effect of obesity predisposing SNPs for a P-value=0.05 (unadjusted for the multiple testing).

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###### Figure 3: Power calculation for main effect of obesity predisposing SNPs for a P-value=0.05 (adjusted for the multiple testing).

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###### Figure 4: Power calculation for interaction effect between obesity predisposing SNPs and physical activity for a P-value=0.05 (unadjusted for the multiple testing).

# Chapter 4- Multiparity interacts with *FTO* rs9939609 polymorphism to modulate BMI: Results from the multi-ethnic longitudinal study EpiDREAM

**Introduction**

The increased prevalence of overweight and obesity among adults is more prominent in women than men[254](#_ENREF_254) and this discrepancy has been attributed, in part, to the substantial weight changes associated with pregnancy[255](#_ENREF_255). This trend is particularly concerning given the adverse consequences of maternal obesity for the fetus, but also for the mother following gestation. Women of reproductive age (25-34 years) are at a greater risk of obesity than men of all ages[256](#_ENREF_256),[257](#_ENREF_257) and nearly half of all women of reproductive age are overweight or obese[258-260](#_ENREF_258). As a result, gestational weight gain and postpartum weight retention are highly germane to understanding the impact of pregnancy on obesity among adult women[256](#_ENREF_256).

Current guidelines from the Institute of Medicine recommend that gestational weight gain should range from 15-40 lbs and mothers with a lower pregravid BMI should gain relatively more gestational weight than mothers with a higher pregravid BMI[261](#_ENREF_261). Unfortunately, studies conducted between 1990 and 2007 indicate that only 30-40% of mothers in the United States complied with these recommendations[262](#_ENREF_262). Excess gestational weight gain has been identified as the strongest predictor of postpartum weight retention across multiple studies[263-267](#_ENREF_263). This residual weight retention is accompanied by an increased susceptibility to additional and excessive weight gain, which augments the risk of obesity development in the future[268](#_ENREF_268),[269](#_ENREF_269). These weight-related trends are compounded among women who have had multiple pregnancies (multiparous) compared to women who have not been pregnant (nulliparous) and women with only one child (primparous)[270](#_ENREF_270). The excess risk of obesity among multiparous women has been identified in over 30 countries[6](#_ENREF_6) and multiparity is now recognized as an independent predictor of obesity among women of reproductive age[271](#_ENREF_271). Lifestyle changes during the postpartum period typically involve a shift toward a more sedentary lifestyle[264](#_ENREF_264),[272](#_ENREF_272), which can result in cumulative weight gains from successive pregnancies[273-275](#_ENREF_273). Given the prevalence of excess gestational weight gain and the additional risk associated with multiparity, mothers giving birth to multiple children are at greater risk for obesity and the associated risk factors[2](#_ENREF_2),[271](#_ENREF_271).

Even though gestational weight gain and postpartum weight retention are common to most women, the magnitude of these changes is highly variable between individuals. Gestational weight gain was calculated in a cohort of women age 18-36 whose weight gains were in accordance with recommendations from the Institute of Medicine[276](#_ENREF_276). There was an inverse relationship between maternal BMI at 14 weeks gestation and gestational weight gain. Specifically, underweight, normal weight, overweight and obese women gained an average of 13.2 (standard deviation [SD]: 5.7), 8.4 (SD: 7.5), 7.7 (SD: 9.0) and -1.3 (SD: 10.1) lbs, respectively[276](#_ENREF_276). These findings indicate that gestational weight gain varies greatly between individuals, and this variance is more pronounced among overweight and obese women[277](#_ENREF_277). Postpartum weight retention appears to vary as well. Women with gestational weight gains within the Institute of Medicine’s guidelines only retained a median of 1.5 lbs six months after delivery compared to a median of 4.8 lbs among women whose weight gain exceeded these guidelines[278](#_ENREF_278). The variance in these trends may be partially explained by the biological processes associated with gestation. Leptin is a hormone secreted by adipose tissue and the obesity (*ob*) gene regulates leptin levels in the blood[277](#_ENREF_277). During pregnancy, leptin is also produced in the placenta[279](#_ENREF_279) and elevated leptin levels during pregnancy and postpartum are positively associated with gestational weight gain[280](#_ENREF_280). Additional support for the genetic influence of gestational weight gain came from analyses of obesity predisposing SNPs. A large prospective cohort of 6959 mothers demonstrated that the *FTO* (rs8050136) risk allele is associated with pre-pregnancy overweight and obesity during pregnancy[281](#_ENREF_281). A separate case-control study of 1501 women found that risk allele carriage in *PPARG* rs1801282, *KCNQ1* rs2237892 and *MC4R* rs17782313 was associated with greater gestational weight gain[282](#_ENREF_282). These findings indicate that patterns of gestational weight gain and postpartum weight retention may be related to an individual’s genetic susceptibility to gain fat mass during pregnancy.

In addition, recent studies suggest that *FTO* may influence weight gain by altering endocrine activity. The number of *FTO* risk alleles appears to be positively associated with plasma ghrelin levels and inversely related to levels of the satiety promoting hormone leptin[283](#_ENREF_283" \o "Benedict, 2014 #2582). In conjunction, the emerging evidence linking genetic variants to gestational changes in body weight and the biological changes associated with pregnancy, it is possible that these genetic variants may interact with certain environmental exposures to modulate BMI. Since multiparity is recognized as an independent predictor of maternal overweight and obesity[271](#_ENREF_271), this environmental exposure may have the potential to moderate the impact of genetic variants on obesity outcomes. To investigate this hypothesis, we analyzed gene x environment interactions between multiparity and 14 obesity predisposing SNPs in the multi-ethnic cohort EpiDREAM.

## Methods

The data for this investigation was also collected from the EpiDREAM study, which was described above. Methods of genotyping, phenotyping and statistical analysis were not changed for this analysis. For this investigation, we focused on 10 618 females with complete phenotypic and 50K gene-centric array information in the EpiDREAM study (Figure 5). Two-tailed P-values are presented in this manuscript and *P*<0.05 were considered as nominally significant. After applying a Bonferroni’s correction for multiple testing, a *P* <0.00056 (0.05/90) was considered as significant. All SNPs reaching a nominal level of significance were followed up for interaction analysis. In accordance with the Helsinki Declaration, all study participants provided informed consent and the EpiDREAM study has been approved by all local ethics committees.

## Results

### Descriptive Statistics

Table 7 describes the characteristics of the 10 618 female study participants. At baseline, the ethic distribution was 53.8% European, 20.7% Latino, 12.6% South Asian, 8.4% African, 3.3% Native American, 1.2% East Asian. The mean age of participants was 52.5 years with a mean BMI of 30.8 (SD=6.66) kg/m2 and a mean BAI of 36.1 (SD=7.23). The mean number of pregnancies among the participants was 2.3 (SD=1.63) with a range from 0-15. After follow-up the mean BMI and BAI were 30.8 (SD=6.10) and 28.2 (SD=6.50), respectively, although this sample only included 5628 of the original 10 618 participants.

### Effect of Multiple Pregnancies on Obesity Outcomes

At baseline, the number of pregnancies was positively associated with both BMI (β= 0.171, 95% Confidence interval [CI]= 0.095 to 0.248, P=1.17 x 10-5) and BAI (β= 0.048, 95% CI= 0.030 to 0.067, P= 3.47 x 10-7) (Table 8). The number of pregnancies was also nominally associated with BMI at follow-up (β= 0.040, 95% CI= 0.013 to 0.068, P=0.004) but was not significantly associated with BAI at follow-up (β= 0.025, 95% CI=-0.003 to 0.053, P=0.083). Multiple pregnancies were not associated with BMI change (β= 0.014, 95% CI=-0.014 to 0.043, P=0.328) or BAI change (β= -0.024, 95% CI=-0.053 to 0.005, P=0.101). These analyses were adjusted for sex, age, ethnicity and glucose homeostasis status.

### Effect of SNPs on Multiple Pregnancies

Three of the fourteen SNPs analyzed were nominally associated with the number of pregnancies (Table 9). *TNNI3K* rs1514176 was positively associated with number of pregnancies (β= 0.038, 95% CI=0.012 to 0.064, P=0.005) while *MAP2K5* rs997295 (β= -0.035, 95% CI=-0.060 to -0.009, P=0.009) and *FTO* rs7203521 (β= -0.042, 95% CI=-0.068 to -0.015, P=0.002) were negatively associated with number of pregnancies. These analyses were adjusted for sex, age, ethnicity and glucose homeostasis status. After controlling for BMI, in addition to the previous variables, the same three SNPs were nominally associated with number of pregnancies: *TNNI3K* rs1514176 (β= 0.036, 95% CI=0.010 to 0.062, P=0.008), *MAP2K5* rs997295 (β= -0.034, 95% CI=-0.060 to -0.008, P=0.010), *FTO* rs7203521 (β= -0.042, 95% CI=-0.068 to -0.016, P=0.002).

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### Effect of SNPs on BMI/BAI

At baseline, the obesity risk alleles of four SNPs displayed a positive association with BMI and BAI: *FTO* rs9939609 (BMI: β= 0.492, 95% CI= 0.318 to 0.666, P= 3.17 x 10-8; BAI: β= 0.438, 95% CI= 0.243 to 0.634, P= 1.12 x 10-5), *TNNl3K* rs1514176 (BMI: β= 0.328, 95% CI= 0.157 to 0.500, P= 1.73 x 10-4; BAI: β= 0.274, 95% CI= 0.082 to 0.466, P= 0.005), *GIPR* rs11671664 (BMI: β= 0.353, 95% CI= 0.080 to 0.626, P= 0.011; BAI: β= 0.346, 95% CI= 0.040 to 0.653, P= 0.027) and *CDKAL1* rs2206734 (BMI: β= 0.322, 95% CI= 0.109 to 0.534, P= 0.003; BAI: β= 0.323, 95% CI= 0.085 to 0.562, P= 0.008) (Table 10). However, *GIPR* rs11671664 and *CDKAL1* rs2206734 only reached a nominal level of significance. At baseline, the GS was significantly associated with greater BMI (β= 0.128, 95% CI= 0.076 to 0.180, P= 1.23 x 10-6) and BAI (β= 0.122, 95% CI= 0.064 to 0.180, P= 3.80 x 10-5).

After follow-up, only *FTO* rs9939609 (BMI: β= 0.473, 95% CI= 0.251 to 0.695, P= 3.08 x 10-5; BAI: β= 0.514, 95% CI= 0.268 to 0.761, P= 4.41 x 10-5) and *TNNl3K*, rs1514176 (BMI: β= 0.324, 95% CI= 0.109 to 0.539, P= 0.003; BAI: β= 0.305, 95% CI= 0.067 to 0.542, P= 0.012) were significantly associated with both increased BMI and BAI. *FTO* rs9939609 surpassed the Bonferroni corrected threshold of significance while *TNNl3K*, rs1514176 was only nominally associated. The obesity risk GS was nominally associated with BMI at follow-up (BMI: β= 0.076, 95% CI= 0.010 to 0.142, P= 0.023) but was not significantly associated with BAI at follow-up (BAI: β= 0.058, 95% CI= -0.016 to 0.131, P= 0.123). There were no significant associations observed between the 14 obesity predisposing SNPs/GS and change in BMI or change in BAI (Table 10).

### Interaction Analyses

At baseline, multiple pregnancies significantly modified the effect of the *FTO* risk allele on BMI (Pinteraction=0.014) but not BAI (Pinteraction=0.088; Table 11). Each additional *FTO* risk allele (A) was associated with a (1) BMI increase of 0.123 kg/m2 (95% CI=-0.304 to 0.549, P=0.574) in the lowest pregnancy tertile, (2) BMI increase of 0.477 kg/m2 (95% CI=0.253 to 0.700, P=2.95x10-5) in the middle pregnancy tertile and (3) BMI increase of 0.741 kg/m2 (95% CI=0.387 to 1.094, P=4.07x10-5) in the highest pregnancy tertile. This indicates that the effect of *FTO* rs9939609 on obesity can be increased by over 50% through multiple pregnancies. Multiple pregnancies did not moderate the effect of *TNNl3K* rs1514176 (BMI: Pinteraction=0.326; BAI: Pinteraction=0.325), *CDKAL1* rs2206734 (BMI: Pinteraction=0.428; BAI: Pinteraction=0.605), *GIPR* rs11671664 (BMI: Pinteraction=0.811; BAI: Pinteraction=0.625) or the obesity risk GS (BMI: Pinteraction=0.458; BAI: Pinteraction=0.545) at baseline. After follow-up, multiple pregnancies did not modify the effect of *FTO* rs9939609 (BMI: Pinteraction=0.928) or *TNNl3K* rs1514176 (BMI: Pinteraction=0.214).

## Discussion

Our results demonstrate that parity is a significant contributor to obesity among a large cohort of multi-ethnic women from 21 countries. Parity was significantly associated with measures of obesity at baseline and at follow-up. This relationship has long been established[284](#_ENREF_284) and is congruent with another multi-ethnic investigation that reported a positive association between parity and BMI in over 30 countries[6](#_ENREF_6). The mechanism proposed to explain this relationship is through cumulative cycles of pregnancy weight gain and postpartum weight retention[273](#_ENREF_273),[274](#_ENREF_274),[285](#_ENREF_285),[286](#_ENREF_286). Specifically, excessive weight gain during gestation has been reliably shown to increase postpartum weight retention in the short term[286-288](#_ENREF_286) and increase long-term weight gain[274](#_ENREF_274),[275](#_ENREF_275). Based on the susceptibility of multiparous women, future initiatives to combat the obesity epidemic should target this high-risk subgroup of the population.

The association between three obesity predisposing SNPs (*TNNI3K* rs1514176, *MAP2K5* rs997295, *FTO* rs7203521) and number of pregnancies represents a more novel finding. To our knowledge, this is the first study to report an association between these SNPs and parity. *TNNI3K* rs1514176 was positively associated with parity while *MAP2K5* rs997295 and *FTO* rs7203521 were negatively associated with number of pregnancies. This discrepancy may be explained by the relationship between body weight and fertility. A prior investigation of 12 073 young adults (aged 17-24 years) revealed that fertility was lower among underweight and obese individuals[289](#_ENREF_289). Being underweight may reduce fertility through increased levels of follicle-stimulating hormone (FSH)[290](#_ENREF_290), secondary amenorrhea[291](#_ENREF_291), and shortened luteal phase[291](#_ENREF_291). Overweight may adversely impact fertility through anovulation[292-294](#_ENREF_292) and altering biochemical levels in the pre-ovulatory follicular environment[295](#_ENREF_295). Moreover, lower levels of follicular-phase estradiol levels have been linked to both underweight and overweight[296](#_ENREF_296). This evidence demonstrates that there is an optimal body weight range to optimize fertility and obesity predisposing SNPs that displace individuals from this range may impact fecundity. The association of these SNPs with parity may also be related to the thrifty genotype hypothesis[249](#_ENREF_249). Given that a minimum fat storage is necessary to maintain reproductive ability (approximately 22%)[291](#_ENREF_291), obesity predisposing SNPs may have been positively selected during recurrent famines early in human evolutionary history[297](#_ENREF_297). The current ‘obesogenic’ environment of many cultures today is less amenable to the weight gain conferred by these SNPs, which contribute to obesity and possibly decreased fertility. However, it is important to note that these associations persisted even after adjustment for BMI, which suggests that these SNPs may have an impact on reproduction that is independent from their influence on body weight. Replication of this finding in independent samples is needed to validate these conclusions.

Although robust gene-environment interactions have been demonstrated between *FTO* rs9939609, physical activity[20](#_ENREF_20) and diet[21](#_ENREF_21),[118](#_ENREF_118), to our knowledge, this is the first study to show an interaction between *FTO* rs9939609 and multiple pregnancies. Multiple pregnancies accentuated the association between *FTO* rs9939609 and BMI at baseline. Specifically, our results indicate that the effect of *FTO* rs9939609 on BMI can be increased by approximately 50% among multiparous women. The magnitude of this estimate was likely influenced by the broad range of pregnancies (0-15) among the EpiDREAM cohort. The possible biological mechanisms underlying this association may involve the methylation properties of *FTO* and leptin regulation. *FTO* is a DNA demethylase and is associated with different methylation profiles and BMI variance[43](#_ENREF_43),[222](#_ENREF_222). Since environmental changes have been shown to influence methylation patterns[223](#_ENREF_223),[224](#_ENREF_224), it is plausible that multiple pregnancies could modify the methylation activity of *FTO*. The expression of *FTO* has also been linked to the satiety promoting hormone leptin[298](#_ENREF_298" \o "Bravard, 2014 #2682) and the elevated leptin levels during pregnancy and postpartum may influence *FTO* activity and weight gain during these periods. However, this is the first investigation to show an interaction between *FTO* rs9939609 and number of pregnancies, and replication of this finding is needed before these results can be applied in a public health context.

The limitations of this analysis resemble those mentioned in chapter three. The multi-ethnic composition of the cohort represents a substantial source of heterogeneity, and subgroup analyses of the interaction in each ethnic group were not possible due to power limitations. Second, SNPs included in the analysis were originally identified in ethnically homogeneous populations and may not be appropriate proxies for all six ethnicities included in EpiDREAM. These SNPs are also only a subset of the current obesity predisposing SNPs that have been identified. Lastly, the EpiDREAM sample is not representative of the general population since inclusion in the cohort was contingent on being at risk for type 2 diabetes.

In conclusion, our study validates the association between parity and BMI in a multi-ethnic cohort from 21 different countries throughout the world. Three of the obesity predisposing SNPs analyzed were significantly associated with parity after adjustment for BMI, yet the processes explaining this relationship are uncertain. We also identified a novel gene-environment interaction between *FTO* rs9939609 and multiple pregnancies on BMI. Further analysis of this interaction in independent studies is needed to confirm this association.

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| --- | --- | --- | --- | --- | --- |
| Table 7. Baseline characteristics stratified by number of pregnancies in the EpiDREAM study | | | | | |
|  | **Category** | **Nulliparous** | **Primiparous** | **Multiparous** | **All** |
| Total at baseline  N(%) |  | 1455 (13.7%) | 1593 (15.0%) | 7570 (71.3%) | 10 618 (100%) |
| aAge  (years) |  | 51.20 ±12.23 | 51.40 ± 11.63 | 52.96 ± 10.91 | 52.49 ± 11.23 |
| aGHS  N (%) | Normal  IFG  Diabetes | 739 (5.08%)  553 (38.0%)  163 (11.2%) | 740 (46.5%)  664 (41.7%)  189 (11.9%) | 3467 (45.8%)  3063 (40.5%)  1040 (13.7%) | 4946 (46.6%)  4280 (40.3%)  1392 (13.1%) |
| aBMI at baseline  (kg/m2) |  | 31.15 ± 7.76 (1454) | 30.46 ± 6.77 (1593) | 30.79 ± 6.40 (7566) | 30.79 ± 6.66 (10 613) |
| aBMI at follow-up  (kg/m2) |  | 30.87 ± 6.95 (745) | 30.92 ± 6.24 (784) | 30.79 ± 5.91 (4099) | 30.82 ± 6.10 (5628) |
| aBMI Change  (kg/m2) |  | 0.19 ± 2.95  (745) | 0.26 ± 2.83  (781) | 0.24 ± 2.71  (4037) | 0.24 ± 2.76  (5563) |
| aBAI at baseline |  | 36.12 ± 8.04  (1452) | 35.48 ± 7.14  (1587) | 36.19 ± 7.08  (7554) | 36.07 ± 7.23  (10 593) |
| aBAI at follow-up |  | 28.42 ± 6.76  (745) | 28.11 ± 6.90  (768) | 28.11 ± 6.37  (3952) | 28.15 ± 6.50  (5465) |
| aBAI Change |  | -8.11 ± 4.45  (744) | -8.05 ± 5.06  (767) | -8.26 ± 4.73  (3949) | -8.22 ± 4.74  (5460) |
| Ethnic groups N(%) |  |  |  |  |  |
| South Asian | 111 (7.6%) | 191 (12.0%) | 1034 (13.7%) | 1336 (12.6%) |
| East Asian | 25 (1.7%) | 23 (1.4%) | 81 (1.1%) | 129 (1.2%) |
| European | 884 (60.8%) | 910 (57.1%) | 3915 (51.7%) | 5709 (53.8%) |
| African | 106 (7.3%) | 148 (9.3%) | 643 (8.5%) | 897 (8.4%) |
| Latino American | 295 (20.3%) | 280 (17.6%) | 1624 (21.5%) | 2199 (20.7%) |
| Native-North American | 34 (2.3%) | 41 (2.6%) | 273 (3.6%) | 348 (3.3%) |
| aData are presented as mean ± S.D. (N).  GHS: glucose homeostasis status, BMI: body mass index, BAI: body adiposity index, SD = standard deviation; N = sample size. | | | | | |

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| Table 8: Effect of multiple pregnancies on obesity outcomes (adjusted for gender, age, ethnicity and glucose homeostasis status). | | | | |
|  | **Outcome** | β | **95% CI** | **P-value** |
| Baseline | BMI | 0.171 | 0.095 to 0.248 | 1.17 x 10-5 |
|  | BAI | 0.048 | 0.030 to 0.067 | 3.47 x 10-7 |
|  |  |  |  |  |
| Follow-up | BMI | 0.040 | 0.013 to 0.068 | 0.004 |
|  | BAI | 0.025 | -0.003 to 0.053 | 0.083 |
|  |  |  |  |  |
| Change | BMI | 0.014 | -0.014 to 0.043 | 0.328 |
|  | BAI | -0.024 | -0.053 to 0.005 | 0.101 |

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| Table 9: Effect of SNPs/GS on multiple pregnancies (adjusted for gender, age, ethnicity and glucose homeostasis status). | | | | |
|  | | | | |
| **rs** | **Gene** | **β** | **95% CI** | **P-value** |
| rs1514176 | *TNNI3K* | 0.038 | 0.012 to 0.064 | 0.005 |
| rs6235 | *PCSK1* | 0.003 | -0.027 to 0.033 | 0.851 |
| rs6232 | *PCSK* | -0.032 | -0.097 to 0.034 | 0.344 |
| rs2206734 | *CDKAL1* | 0.013 | -0.020 to 0.045 | 0.438 |
| rs2272903 | *TFAP2B* | -0.003 | -0.040 to 0.035 | 0.894 |
| rs1211166 | *NTRK2* | 3.11 x 10-3 | -0.031 to 0.032 | 0.984 |
| rs6265 | *BDNF* | 0.015 | -0.019 to 0.049 | 0.388 |
| rs1401635 | *BDNF* | -0.009 | -0.037 to 0.020 | 0.545 |
| rs997295 | *MAP2K5* | -0.035 | -0.060 to -0.009 | 0.009 |
| rs7203521 | *FTO* | -0.042 | -0.068 to -0.015 | 0.002 |
| rs9939609 | *FTO* | -0.014 | -0.040 to 0.013 | 0.307 |
| rs1805081 | *NPC1* | 0.030 | 0.002 to 0.059 | 0.034 |
| rs2075650 | *APOE* | 0.025 | -0.014 to 0.064 | 0.203 |
| rs11671664 | *GIPR* | 0.008 | -0.034 to 0.050 | 0.707 |
|  | Gene Score | -0.001 | -0.008 to 0.007 | 0.900 |
| Effect of SNPs/GS on multiple pregnancies (adjusted for gender, age, ethnicity, glucose homeostasis status and BMI) | | | | |
| **rs** | **Gene** | **β** | **95% CI** | **P-value** |
| rs1514176 | *TNNI3K* | 0.036 | 0.010 to 0.062 | 0.008 |
| rs6235 | *PCSK1* | 0.003 | -0.027 to 0.033 | 0.847 |
| rs6232 | *PCSK* | -0.028 | -0.094 to 0.037 | 0.397 |
| rs2206734 | *CDKAL1* | 0.010 | -0.022 to 0.043 | 0.535 |
| rs2272903 | *TFAP2B* | -0.003 | -0.040 to 0.034 | 0.857 |
| rs1211166 | *NTRK2* | 1.97 x 10-4 | -0.032 to 0.031 | 0.990 |
| rs6265 | *BDNF* | 0.015 | -0.020 to 0.049 | 0.401 |
| rs1401635 | *BDNF* | -0.009 | -0.038 to 0.019 | 0.521 |
| rs997295 | *MAP2K5* | -0.034 | -0.060 to -0.008 | 0.010 |
| rs7203521 | *FTO* | -0.042 | -0.068 to -0.016 | 0.002 |
| rs9939609 | *FTO* | -0.017 | -0.044 to 0.010 | 0.210 |
| rs1805081 | *NPC1* | 0.030 | 0.002 to 0.058 | 0.036 |
| rs2075650 | *APOE* | 0.024 | -0.015 to 0.063 | 0.225 |
| rs11671664 | *GIPR* | 0.006 | -0.036 to 0.048 | 0.781 |
|  | Gene Score | -0.001 | -0.009 to 0.007 | 0.729 |

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| Table 10: Effect of SNPs/GS on obesity measures. | | | | | | | | | | | | | | | | |
| Effect of SNPs/GS on BMI/BAI at baseline (adjusted for gender, age, ethnicity and glucose homeostasis status) | | | | | | | | | | | | | | | | |
|  | | | **BMI Baseline** | | | | | | **BAI Baseline** | | | | | | | |
| rs | gene | | **β** | **95% CI** | | **P-value** | | | **β** | | | **95% CI** | | | **P-value** | |
| rs1514176 | *TNNI3K* | | 0.328 | 0.157 to 0.500 | | 1.73x10-4 | | | 0.274 | | | 0.082 to 0.466 | | | 0.005 | |
| rs6235 | *PCSK1* | | 0.021 | -0.175 to 0.216 | | 0.836 | | | -0.015 | | | -0.235 to 0.205 | | | 0.894 | |
| rs6232 | *PCSK* | | 0.467 | 0.036 to 0.897 | | 0.034 | | | -0.296 | | | -0.778 to 0.187 | | | 0.230 | |
| rs2206734 | *CDKAL1* | | 0.322 | 0.109 to 0.534 | | 0.003 | | | 0.323 | | | 0.085 to 0.562 | | | 0.008 | |
| rs2272903 | *TFAP2B* | | 0.127 | -0.116 to 0.370 | | 0.306 | | | 0.273 | | | -0.007 to 0.546 | | | 0.050 | |
| rs1211166 | *NTRK2* | | 0.106 | -0.100 to 0.311 | | 0.313 | | | 0.107 | | | -0.123 to 0.338 | | | 0.362 | |
| rs6265 | *BDNF* | | 0.078 | -0.147 to 0.303 | | 0.499 | | | 0.121 | | | -0.132 to 0.373 | | | 0.348 | |
| rs1401635 | *BDNF* | | 0.096 | -0.092 to 0.284 | | 0.315 | | | 0.021 | | | -0.190 to 0.232 | | | 0.847 | |
| rs997295 | *MAP2K5* | | -0.054 | -0.224 to 0.116 | | 0.537 | | | -0.131 | | | -0.322 to 0.060 | | | 0.178 | |
| rs7203521 | *FTO* | | 0.044 | -0.127 to 0.215 | | 0.614 | | | 0.017 | | | -0.175 to 0.209 | | | 0.862 | |
| rs9939609 | *FTO* | | 0.492 | 0.318 to 0.666 | | 3.17x10-8 | | | 0.438 | | | 0.243 to 0.634 | | | 1.12x10-5 | |
| rs1805081 | *NPC1* | | 0.036 | -0.148 to 0.221 | | 0.698 | | | 0.128 | | | -0.079 to 0.335 | | | 0.225 | |
| rs2075650 | *APOE* | | 0.062 | -0.192 to 0.316 | | 0.459 | | | 0.132 | | | -0.153 to 0.418 | | | 0.363 | |
| rs11671664 | *GIPR* | | 0.353 | 0.080 to 0.626 | | 0.011 | | | 0.346 | | | 0.040 to 0.653 | | | 0.027 | |
|  | Gene Score | | 0.128 | 0.076 to 0.180 | | 1.23x10-6 | | | 0.122 | | | 0.064 to 0.180 | | | 3.80x10-5 | |
| Effect of SNPs/GS on BMI/BAI at follow-up (adjusted for sex, age, ethnicity and glucose homeostasis status) | | | | | | | | | | | | | | | | |
|  | | | **BMI at Follow-up** | | | | | | **BAI at Follow-up** | | | | | | | |
| rs | gene | | **β** | **95% CI** | | **P-value** | | | **β** | | | **95% CI** | | | **P-value** | |
| rs1514176 | *TNNI3K* | | 0.324 | 0.109 to 0.539 | | 0.003 | | | 0.305 | | | 0.067 to 0.542 | | | 0.012 | |
| rs6235 | *PCSK1* | | -0.026 | -0.272 to 0.220 | | 0.835 | | | 0.059 | | | -0.214 to 0.332 | | | 0.671 | |
| rs6232 | *PCSK* | | -0.111 | -0.664 to 0.442 | | 0.693 | | | 0.028 | | | -0.591 to 0.647 | | | 0.929 | |
| rs2206734 | *CDKAL1* | | -0.095 | -0.366 to 0.176 | | 0.494 | | | -0.021 | | | -0.322 to 0.281 | | | 0.893 | |
| rs2272903 | *TFAP2B* | | 0.064 | -0.248 to 0.376 | | 0.687 | | | 0.016 | | | -0.331 to 0.364 | | | 0.926 | |
| rs1211166 | *NTRK2* | | 0.093 | -0.171 to 0.358 | | 0.489 | | | -0.126 | | | -0.420 to 0.167 | | | 0.399 | |
| rs6265 | *BDNF* | | -0.033 | -0.318 to 0.251 | | 0.819 | | | 0.051 | | | -0.265 to 0.368 | | | 0.751 | |
| rs1401635 | *BDNF* | | 0.094 | -0.147 to 0.366 | | 0.444 | | | 0.071 | | | -0.199 to 0.340 | | | 0.607 | |
| rs997295 | *MAP2K5* | | -0.105 | -0.320 to 0.110 | | 0.337 | | | -0.233 | | | -0.460 to 0.015 | | | 0.066 | |
| rs7203521 | *FTO* | | -0.029 | -0.245 to 0.187 | | 0.793 | | | -0.062 | | | -0.302 to 0.178 | | | 0.614 | |
| rs9939609 | *FTO* | | 0.473 | 0.251 to 0.695 | | 3.08x10-5 | | | 0.514 | | | 0.268 to 0.761 | | | 4.41x10-5 | |
| rs1805081 | *NPC1* | | 0.017 | -0.213 to 0.248 | | 0.882 | | | -0.149 | | | -0.404 to 0.106 | | | 0.252 | |
| rs2075650 | *APOE* | | 0.040 | -0.286 to 0.367 | | 0.808 | | | 0.047 | | | -0.314 to 0.408 | | | 0.799 | |
| rs11671664 | *GIPR* | | 0.259 | -0.089 to 0.606 | | 0.145 | | | 0.462 | | | 0.075 to 0.849 | | | 0.019 | |
|  | Gene Score | | 0.076 | 0.010 to 0.142 | | 0.023 | | | 0.058 | | | -0.016 to 0.131 | | | 0.123 | |
| Effect of SNPs/GS on BMI/BAI change (adjusted for sex, age, ethnicity and glucose homeostasis status) | | | | | | | | | | | | | | | | |
|  | | | **BMI Change** | | | | | |  | | | **BAI Change** | | |  | |
| rs | gene | | **β** | **95% CI** | | **P-value** | | | **β** | | | **95% CI** | | | **P-value** | |
| rs1514176 | *TNNI3K* | | -0.053 | -0.153 to 0.047 | | 0.299 | | | -0.197 | | | -0.372 to -0.021 | | | 0.028 | |
| rs6235 | *PCSK1* | | 0.002 | -0.112 to 0.117 | | 0.966 | | | 0.020 | | | 0.181 to 0.222 | | | 0.844 | |
| rs6232 | *PCSK* | | -0.095 | -0.353 to 0.163 | | 0.470 | | | -0.010 | | | -0.468 to 0.448 | | | 0.966 | |
| rs2206734 | *CDKAL1* | | -0.121 | -0.247 to 0.006 | | 0.062 | | | -0.218 | | | -0.441 to 0.005 | | | 0.055 | |
| rs2272903 | *TFAP2B* | | -0.043 | -0.189 to 0.102 | | 0.558 | | | -0.226 | | | -0.483 to 0.031 | | | 0.085 | |
| rs1211166 | *NTRK2* | | 0.027 | -0.096 to 0.150 | | 0.668 | | | -0.127 | | | -0.344 to 0.090 | | | 0.250 | |
| rs6265 | *BDNF* | | 0.084 | -0.048 to 0.217 | | 0.212 | | | -0.100 | | | -0.334 to 0.134 | | | 0.402 | |
| rs1401635 | *BDNF* | | 0.005 | -0.108 to 0.117 | | 0.936 | | | -0.046 | | | -0.246 to 0.153 | | | 0.647 | |
| rs997295 | *MAP2K5* | | 0.001 | -0.099 to 0.101 | | 0.988 | | | -0.040 | | | -0.215 to 0.136 | | | 0.659 | |
| rs7203521 | *FTO* | | 0.003 | -0.097 to 0.104 | | 0.948 | | | -0.020 | | | -0.197 to 0.158 | | | 0.828 | |
| rs9939609 | *FTO* | | 0.009 | -0.094 to 0.113 | | 0.858 | | | -0.003 | | | -0.186 to 0.179 | | | 0.971 | |
| rs1805081 | *NPC1* | | 0.050 | -0.057 to 0.158 | | 0.359 | | | -0.056 | | | -0.245 to 0.133 | | | 0.561 | |
| rs2075650 | *APOE* | | -0.005 | -0.157 to 0.147 | | 0.948 | | | -0.006 | | | -0.273 to 0.261 | | | 0.967 | |
| rs11671664 | *GIPR* | | -0.132 | -0.293 to 0.030 | | 0.111 | | | 0.100 | | | -0.186 to 0.386 | | | 0.494 | |
|  | Gene Score | | -0.008 | -0.039 to 0.022 | | 0.597 | | | -0.064 | | | -0.118 to -0.010 | | | 0.020 | |
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| Table 11: Interaction analysis of SNP/GS associated with obesity measures. | | | | | | | | | | | | | | | |
| **Baseline Interaction Tests** | | | | | | | | | | | | | | | |
| **Interaction terms** | | **Outcome Variable** | | | **Impact of Risk Allele** | | | | | | | | | | |
|  | | | **β** | | | **95% CI** | | | **P** | |
| *TNNl3K* rs1514176 x  Multiple pregnancy | | BMI-Baseline  (Pinteraction=0.326) | | |  | | |  | | |  | | |  | |
| *TNNl3K* rs1514176 x Multiple pregnancy | | BAI-Baseline  (Pinteraction=0.325) | | |  | | |  | | |  | | |  | |
| *FTO* rs9939609 x  Multiple pregnancy | | BMI-Baseline  (Pinteraction=0.014) | | | Pregnancy Tertile  Low  Medium  High | | | 0.123  0.477  0.741 | | | -0.304 to 0.549  0.253 to 0.700  0.387 to 1.094 | | | 0.574  2.95x10-5  4.07x10-5 | |
| *FTO* rs9939609 x  Multiple pregnancy | | BAI-Baseline  (Pinteraction=0.088) | | |  | | |  | | |  | | |  | |
| *CDKAL1* rs2206734 x  Multiple pregnancy | | BMI-Baseline  (Pinteraction=0.428) | | |  | | |  | | |  | | |  | |
| *CDKAL1* rs2206734 x Multiple pregnancy | | BAI-Baseline  (Pinteraction=0.605) | | |  | | |  | | |  | | |  | |
| *GIPR* rs11671664 x  Multiple pregnancy | | BMI-Baseline  (Pinteraction=0.811) | | |  | | |  | | |  | | |  | |
| *GIPR* rs11671664 x  Multiple pregnancy | | BAI-Baseline  (Pinteraction=0.625) | | |  | | |  | | |  | | |  | |
| Gene Score x  Multiple pregnancy | | BMI-Baseline  (Pinteraction=0.458) | | |  | | |  | | |  | | |  | |
| Gene Score x  Multiple pregnancy | | BAI-Baseline  (Pinteraction=0.545) | | |  | | |  | | |  | | |  | |
| **Follow-up Interaction Tests** | | | | | | | | | | | | | | | |
| **Interaction terms** | | **Outcome Variable** | | | **Impact of Risk Allele** | | | | | | | | | | |
|  | | **β** | | | **95% CI** | | | **P** | | |
| *TNNl3K* rs1514176 x  Multiple pregnancy | | BMI-Follow-up  (Pinteraction=0.928) | | |  | | | | | | | | | | |
| *FTO* rs9939609 x  Multiple pregnancy | | BMI-Follow-up  (Pinteraction=0.214) | | |  | | | | | | | | | | |

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###### Figure 5: Flow chart of females in the EpiDREAM study

# Chapter 5- Current challenges and future directions on gene-environment interactions

## Current challenges and limitations of gene-environment interaction research

Although number of gene-environment interaction studies is growing rapidly[299](#_ENREF_299), there are a number of challenges that must be addressed in order to fully exploit the benefits of gene-environment interaction research. These challenges concern both the design and analysis of existing studies. Examples of study design challenges include inaccurate measurement of environmental exposures, selection bias and confounding, while sample size, biological assumptions and choice of statistical model persist as analytical concerns[16](#_ENREF_16),[300](#_ENREF_300),[301](#_ENREF_301).

Given the efficiency and feasibility of case-control study designs, they are often employed to explore gene-environment interactions in obesity research[300](#_ENREF_300). Case-control studies involve the collection of biomarkers after disease diagnoses and are therefore limited to retrospective assessment of environmental exposures[302](#_ENREF_302). Since genotypes are typically static, the retrospective nature of this design is usually not problematic for genotypic data. Unfortunately, relying on participant recall to measure environmental factors introduces potential recall bias. The measurement of biomarkers can, in part, attenuate this issue, yet biomarkers can rarely recreate past exposures and may be influenced by current disease status. Another issue inherent to case-control studies is the potential for selection bias[303](#_ENREF_303). A recent study indicates that it is possible for selection bias to impact gene-environment interaction analyses for certain genes and exposures if (1) population stratification is present; or (2) the alleles under study influence behaviour (e.g. aldehyde dehydrogenase exposure)[304](#_ENREF_304); or (3) alleles and exposure risk factors influence disease detection[300](#_ENREF_300). Other observational epidemiological designs are also vulnerable to confounding bias created by measured and unmeasured variables. For example, population stratification may create a genetic confounding bias in gene-environment interaction studies. In ethnically mixed populations, population stratification may result in misleading associations if allele frequency and baseline disease incidence differ by ethnicity[305](#_ENREF_305). Differentiating valid associations from population stratification bias can be accomplished by replicating findings in high-quality studies from different populations[306](#_ENREF_306). Alternatively, family-based studies have been suggested as a promising study design since they are not vulnerable to population stratification bias[307](#_ENREF_307),[308](#_ENREF_308). However, the application of this approach may be limited. Given the shared environments of family members and the retrospective assessment of variables, this design may be limited in the assessment of environmental exposures and create recall bias similar to case-control studies[16](#_ENREF_16),[307](#_ENREF_307).

In addition to concerns with study design, analysis issues also represent important challenges for gene-environment interaction research. Insufficient sample size and underpowered studies are frequently cited as current issues in interaction analyses of obesity predisposing genes[16](#_ENREF_16),[300](#_ENREF_300),[301](#_ENREF_301),[309](#_ENREF_309). These challenges are particularly concerning given the varying degree of precision in measuring environmental exposure within gene-environment interaction analyses, discussed previously. A previous study demonstrated that a sample of about 2000 participants with precisely measured environmental exposure and outcome data were needed to detect a gene-environment interaction of large magnitude (a doubling of the genetic risk estimate in the exposed group compared to the unexposed group) with reasonable power (95% power, critical α P = 1x10-4)[209](#_ENREF_209). With less precise measurement of environmental exposure, which is a common issue in epidemiological research, the sample size requirement can increase to 100 000 participants to detect the same interaction with comparable power[16](#_ENREF_16),[209](#_ENREF_209). Given that a doubling of genetic risk from an environmental exposure is at the upper limit of interaction effect estimates reported for common variants and exposures[310-312](#_ENREF_310), Franks et al.[16](#_ENREF_16) suggests that many of the current interaction studies are underpowered and report false-positive results.

In concert with issues of sample size and power, biological assumptions and statistical modelling also present challenges. It is well recognized that environmental factors vary over time, yet fluctuations in gene expression are seldom considered. Systematic expression and silencing of genes play an integral role in human development, which implies that gene-environment interactions are likely dependent upon the life stage of participants[300](#_ENREF_300). Instead of considering an exposure as having a single dose response curve, exposures appear to have varying dose response curves depending on the stage of development[313](#_ENREF_313). To apply this concept to statistical modelling, gene-environment interactions may need to be re-conceptualized with the time of exposure representing a third factor[313](#_ENREF_313). Considering time of exposure can also be accomplished by examining gene and time-varying-environment interactions and using lag effects[300](#_ENREF_300). Further discussion of statistical modelling for gene and time-varying-environment interactions is described in a comprehensive review by Liu et al[300](#_ENREF_300) and a recent article by Crossa[314](#_ENREF_314" \o "Crossa, 2012 #1681).

## Gene × environment interactions and obesity: future directions

Given that specific environments can hugely impact the magnitude of genetic predisposition for obesity, the systematic study of gene-environment interactions is an important field of investigation in addition to gene identification efforts. Gene-environment interaction studies in the context of various forms of obesity (syndromic, monogenic, polygenic) and in diverse experimental designs[58](#_ENREF_58) may lead to a better understanding of the protective or detrimental environmental exposures or medical interventions. Existing interactions need to be studied in additional obesity-prone (e.g. response to smoking cessation, response to insulin therapy in diabetic subjects) or obesity-protective (e.g. response to the anti-obesity drug orlistat administration) conditions. Gene-environment interaction studies are complementary to observational epidemiology, interventional study or clinical trial, and will certainly help to elaborate efficient strategies to reverse the obesity epidemic.

Currently, GWAS for obesity-related traits have focused on the marginal gene effect ignoring gene-environment interaction entirely[315](#_ENREF_315). Gene-environment interactions are nevertheless frequent in obesity, and statistical models that do not properly account for gene-environment interactions may attenuate the marginal effect size and reduce the power to detect true associations[316](#_ENREF_316). Applying a joint test for a main genotype effect and gene-environment interaction may increase the power to identify an individual SNP associated with a disease outcome[317](#_ENREF_317). As many completed GWAS for obesity have been conducted on samples with large amounts of existing environmental data, performing gene-environment-wide interaction studies (GEWIS) in these existing datasets is a cost-effective strategy to find additional obesity-associated gene variants that interact with specific environments but have been missed by initial GWAS[318](#_ENREF_318). As large sample sizes and meta-analytical approaches are required to reliably detect SNPs with subtle gene-environment interaction patterns[319](#_ENREF_319), GEWIS for obesity have been initiated in the context of large international obesity consortiums like GIANT(Genomewide Investigation of ANThropometric measures).

Hypothesis-free discovery of gene-environment interactions has also been proposed as an alternative to conventional GWAS experiments[16](#_ENREF_16). Franks et al.[16](#_ENREF_16) posits that the *P* value ranking system in GWAS may bias against the detection of prevalent variants that interact with environmental factors. For instance, the genetic effect estimate for a given loci must be relatively large and consistent in magnitude (within and between populations) to exceed the conservative genome-wide probability threshold used in most GWAS (*P*= 5 x 10-8)[16](#_ENREF_16). However, large gene-environment interactions are typically associated with greater variance of the main effects for the gene variant[320](#_ENREF_320). As a result, it is expected that gene variants implicated in gene-environment interactions rank poorly in GWAS meta-analyses[16](#_ENREF_16), although there are exceptions[20](#_ENREF_20),[321](#_ENREF_321). While hypothesis-free GEWIS have potential to identify gene variants that are less amenable with GWAS, there are limitations to this technique. These include the challenges of identifying adequately sized cohorts with appropriate genetic and phenotypic data, as well as issues with statistical power. As a novel alternative to these techniques, variance prioritization was developed as a method to model genetic associations with genetic variance, without requiring knowledge of the interacting variables[322](#_ENREF_322). As discusses previously, the main effects of gene variants involved in interactions are typically associated with a large degree of variance[322](#_ENREF_322). This strategy exploits this pattern to rank and prioritize variance estimates to identify gene variants that exert effects through interactions. Recent applications of this technique in a meta-analysis of 170,000 samples evidenced that the SNP rs7202116 at the *FTO* locus is associated with phenotypic variability of BMI[222](#_ENREF_222).

Recent GWAS for obesity have collected phenotypic information in individuals living in a broad range of environments. While successful, this approach may fail to identify potential gene variants associated with obesity-related traits in a context dependent manner. Gene identification efforts must therefore be targeted in populations that display homogeneous environment and lifestyle factors across time and across the community, as observed in the Plain people[323](#_ENREF_323). Performing genetic association studies for adiposity change in response to a standard major environment modification (antipsychotic drug use, smoking cessation, intensive caloric restriction, anti-obesity drug therapy, obesity surgery) is another valuable way to control the environmental exposure, to lower sources of heterogeneity and to provide a more comprehensive molecular basis for genetic predisposition to obesity.

# References

1 Finucane, M. M. *et al.* National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. *Lancet* **377**, 557-567, doi:10.1016/S0140-6736(10)62037-5 (2011).

2 Dixon, J. B. The effect of obesity on health outcomes. *Mol Cell Endocrinol* **316**, 104-108, doi:10.1016/j.mce.2009.07.008 (2010).

3 Fontaine, K. R., Redden, D. T., Wang, C., Westfall, A. O. & Allison, D. B. Years of life lost due to obesity. *Jama* **289**, 187-193 (2003).

4 Finkelstein, E. A., Trogdon, J. G., Cohen, J. W. & Dietz, W. Annual medical spending attributable to obesity: payer-and service-specific estimates. *Health Aff (Millwood)* **28**, w822-831, doi:10.1377/hlthaff.28.5.w822 (2009).

5 McAllister, E. J. *et al.* Ten putative contributors to the obesity epidemic. *Crit Rev Food Sci Nutr* **49**, 868-913, doi:10.1080/10408390903372599 (2009).

6 Kim, S. A., Stein, A. D. & Martorell, R. Country development and the association between parity and overweight. *Int J Obes (Lond)* **31**, 805-812, doi:10.1038/sj.ijo.0803478 (2007).

7 Wardle, J., Carnell, S., Haworth, C. M. & Plomin, R. Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment. *Am J Clin Nutr* **87**, 398-404 (2008).

8 Choquet, H. & Meyre, D. Molecular basis of obesity: current status and future prospects. *Curr Genomics* **12**, 154-168, doi:10.2174/138920211795677921 (2011).

9 Blakemore, A. I. & Froguel, P. Investigation of Mendelian forms of obesity holds out the prospect of personalized medicine. *Ann N Y Acad Sci* **1214**, 180-189, doi:10.1111/j.1749-6632.2010.05880.x (2010).

10 Hinney, A. & Hebebrand, J. Polygenic obesity in humans. *Obes Facts* **1**, 35-42, doi:10.1159/000113935 (2008).

11 Hinney, A., Vogel, C. I. & Hebebrand, J. From monogenic to polygenic obesity: recent advances. *Eur Child Adolesc Psychiatry* **19**, 297-310, doi:10.1007/s00787-010-0096-6 (2010).

12 Speliotes, E. K. *et al.* Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nat Genet* **42**, 937-948, doi:10.1038/ng.686 (2010).

13 Day, F. R. & Loos, R. J. Developments in obesity genetics in the era of genome-wide association studies. *J Nutrigenet Nutrigenomics* **4**, 222-238, doi:10.1159/000332158 (2011).

14 Willyard, C. Heritability: The family roots of obesity. *Nature* **508**, S58-60, doi:10.1038/508S58a (2014).

15 Manolio, T. A. *et al.* Finding the missing heritability of complex diseases. *Nature* **461**, 747-753, doi:10.1038/nature08494 (2009).

16 Franks, P. W., Pearson, E. & Florez, J. C. Gene-environment and gene-treatment interactions in type 2 diabetes: progress, pitfalls, and prospects. *Diabetes Care* **36**, 1413-1421, doi:10.2337/dc12-2211 (2013).

17 Franks, P. W. & Poveda, A. Gene-lifestyle and gene-pharmacotherapy interactions in obesity and its cardiovascular consequences. *Curr Vasc Pharmacol* **9**, 401-456 (2011).

18 Andreasen, C. H. *et al.* Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body fat accumulation. *Diabetes* **57**, 95-101 (2008).

19 Sonestedt, E. *et al.* Fat and carbohydrate intake modify the association between genetic variation in the FTO genotype and obesity. *Am J Clin Nutr* **90**, 1418-1425, doi:10.3945/ajcn.2009.27958 (2009).

20 Kilpelainen, T. O. *et al.* Physical activity attenuates the influence of FTO variants on obesity risk: a meta-analysis of 218,166 adults and 19,268 children. *PLoS Med* **8**, e1001116, doi:10.1371/journal.pmed.1001116 (2011).

21 Corella, D. *et al.* A high intake of saturated fatty acids strengthens the association between the fat mass and obesity-associated gene and BMI. *J Nutr* **141**, 2219-2225, doi:10.3945/jn.111.143826 (2011).

22 Gardner, G.; Halweil, B. Underfed and overfed: the global epidemic of malnutrition. Worldwatch Institute; 2000. Retrieved from <http://www.worldwatch.org/node/840>.

23 World Health Organization. Obesity and overweight: Fact sheet. In: WHO Media centre WHO. 2013. <http://www.who.int/mediacentre/> factsheets/fs311/en/. Accessed June 04 2014.

24 Finkelstein, E. A. *et al.* Obesity and severe obesity forecasts through 2030. *Am J Prev Med* **42**, 563-570, doi:10.1016/j.amepre.2011.10.026 (2012).

25 World Health Organization (WHO). Obesity: preventing and managing the global epidemic: Report of a WHO consultation. Geneva, Switzerland: World Health Organization, 2000. (Technical report series no. 894).

26 Wang, Y. Epidemiology of childhood obesity--methodological aspects and guidelines: what is new? *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* **28 Suppl 3**, S21-28, doi:10.1038/sj.ijo.0802801 (2004).

27 Song, Y. M., Sung, J., Davey Smith, G. & Ebrahim, S. Body mass index and ischemic and hemorrhagic stroke: a prospective study in Korean men. *Stroke; a journal of cerebral circulation* **35**, 831-836, doi:10.1161/01.STR.0000119386.22691.1C (2004).

28 Manson, J. E. *et al.* A prospective study of obesity and risk of coronary heart disease in women. *N Engl J Med* **322**, 882-889, doi:10.1056/NEJM199003293221303 (1990).

29 Calle, E. E., Rodriguez, C., Walker-Thurmond, K. & Thun, M. J. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* **348**, 1625-1638, doi:10.1056/NEJMoa021423 (2003).

30 Reeves, G. K. *et al.* Cancer incidence and mortality in relation to body mass index in the Million Women Study: cohort study. *Bmj* **335**, 1134, doi:10.1136/bmj.39367.495995.AE (2007).

31 Whitlock, G. *et al.* Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *Lancet* **373**, 1083-1096, doi:10.1016/S0140-6736(09)60318-4 (2009).

32 WHO Global InfoBase team. The SuRF Report 2. Surveillance of chronic disease risk factors: Country-level data and comparable estimates. Geneva: World Health Organization, 2005.

33 Finkelstein, E. A. *et al.* The lifetime medical cost burden of overweight and obesity: implications for obesity prevention. *Obesity (Silver Spring)* **16**, 1843-1848, doi:10.1038/oby.2008.290 (2008).

34 Haslam, D. W. & James, W. P. Obesity. *Lancet* **366**, 1197-1209 (2005).

35 McAllister, E. J. *et al.* Ten putative contributors to the obesity epidemic. *Crit Rev Food Sci Nutr* **49**, 868-913 (2009).

36 Levin, B. E. Factors promoting and ameliorating the development of obesity. *Physiol Behav* **86**, 633-639, doi:10.1016/j.physbeh.2005.08.054 (2005).

37 Levin, B. E. Factors promoting and ameliorating the development of obesity. *Physiol Behav* **86**, 633-639 (2005).

38 Choquet, H. & Meyre, D. Molecular Basis of Obesity: Current Status and Future Prospects. *Current Genomics* **vol.12 (3)**, 154-168 (2011).

39 Montague, C. T. *et al.* Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* **387**, 903-908. (1997).

40 Katsanis, N. *et al.* Mutations in MKKS cause obesity, retinal dystrophy and renal malformations associated with Bardet-Biedl syndrome. *Nat Genet* **26**, 67-70 (2000).

41 Frayling, T. M. *et al.* A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* **316**, 889-894, doi:10.1126/science.1141634 (2007).

42 Dina, C. *et al.* Variation in FTO contributes to childhood obesity and severe adult obesity. *Nat Genet* **39**, 724-726 (2007).

43 Bell, C. G. *et al.* Integrated genetic and epigenetic analysis identifies haplotype-specific methylation in the FTO type 2 diabetes and obesity susceptibility locus. *PLoS One* **5**, e14040, doi:10.1371/journal.pone.0014040 (2010).

44 Yang, J. *et al.* FTO genotype is associated with phenotypic variability of body mass index. *Nature* **490**, 267-+, doi:Doi 10.1038/Nature11401 (2012).

45 Khoury, M. J. & Wacholder, S. Invited commentary: from genome-wide association studies to gene-environment-wide interaction studies--challenges and opportunities. *Am J Epidemiol* **169**, 227-230; discussion 234-225 (2009).

46 Dina, C. *et al.* Variation in FTO contributes to childhood obesity and severe adult obesity. *Nat Genet* **39**, 724-726, doi:10.1038/ng2048 (2007).

47 Corella, D. *et al.* Education modulates the association of the FTO rs9939609 polymorphism with body mass index and obesity risk in the Mediterranean population. *Nutr Metab Cardiovasc Dis* **22**, 651-658, doi:10.1016/j.numecd.2010.10.006 (2012).

48 Rivera, M. *et al.* Depressive disorder moderates the effect of the FTO gene on body mass index. *Mol Psychiatry* **17**, 604-611, doi:10.1038/mp.2011.45 (2012).

49 Li, S. *et al.* Physical activity attenuates the genetic predisposition to obesity in 20,000 men and women from EPIC-Norfolk prospective population study. *PLoS Med* **7**, doi:10.1371/journal.pmed.1000332 (2010).

50 Qi, Q. *et al.* Television watching, leisure time physical activity, and the genetic predisposition in relation to body mass index in women and men. *Circulation* **126**, 1821-1827 (2012).

51 Qi, Q. *et al.* Sugar-sweetened beverages and genetic risk of obesity. *N Engl J Med* **367**, 1387-1396, doi:10.1056/NEJMoa1203039 (2012).

52 Walley, A. J., Asher, J. E. & Froguel, P. The genetic contribution to non-syndromic human obesity. *Nat Rev Genet* **10**, 431-442, doi:10.1038/nrg2594 (2009).

53 Khoury, M. J., Beaty, T. H. & Cohen, B. H. Scope and strategies of genetic epidemiology: analysis of articles published in Genetic Epidemiology, 1984-1991. *Genet Epidemiol* **10**, 321-329, doi:10.1002/gepi.1370100505 (1993).

54 Narod, S. A. Modifiers of risk of hereditary breast and ovarian cancer. *Nat Rev Cancer* **2**, 113-123, doi:10.1038/nrc726 (2002).

55 Ichihara, S. & Yamada, Y. Genetic factors for human obesity. *Cell Mol Life Sci* **65**, 1086-1098, doi:10.1007/s00018-007-7453-8 (2008).

56 Chung, W. K. & Leibel, R. L. Molecular physiology of syndromic obesities in humans. *Trends Endocrinol Metab* **16**, 267-272, doi:10.1016/j.tem.2005.06.009 (2005).

57 Haldane, J. B. The interaction of nature and nurture. *Ann Eugen* **13**, 197-205 (1946).

58 Hunter, D. J. Gene-environment interactions in human diseases. *Nat Rev Genet* **6**, 287-298 (2005).

59 Salanti, G. *et al.* Underlying genetic models of inheritance in established type 2 diabetes associations. *Am J Epidemiol* **170**, 537-545, doi:10.1093/aje/kwp145 (2009).

60 Rothman, K. J., Greenland, S. & Walker, A. M. Concepts of interaction. *Am J Epidemiol* **112**, 467-470 (1980).

61 Bayoumi, R. A. *et al.* Heritability of determinants of the metabolic syndrome among healthy Arabs of the Oman family study. *Obesity (Silver Spring)* **15**, 551-556 (2007).

62 Stunkard, A. J., Foch, T. T. & Hrubec, Z. A twin study of human obesity. *Jama* **256**, 51-54 (1986).

63 Stunkard, A. J. *et al.* An adoption study of human obesity. *N Engl J Med* **314**, 193-198 (1986).

64 Bouchard, C. *et al.* The response to long-term overfeeding in identical twins. *N Engl J Med* **322**, 1477-1482 (1990).

65 Ludwig, D. S. & Currie, J. The association between pregnancy weight gain and birthweight: a within-family comparison. *Lancet* **376**, 984-990, doi:10.1016/S0140-6736(10)60751-9 (2010).

66 Dubois, L. *et al.* Genetic and environmental contributions to weight, height, and BMI from birth to 19 years of age: an international study of over 12,000 twin pairs. *PLoS One* **7**, e30153, doi:10.1371/journal.pone.0030153 (2012).

67 Roskam, A. J. *et al.* Comparative appraisal of educational inequalities in overweight and obesity among adults in 19 European countries. *Int J Epidemiol* **39**, 392-404, doi:10.1093/ije/dyp329 (2010).

68 Mustelin, L., Silventoinen, K., Pietilainen, K., Rissanen, A. & Kaprio, J. Physical activity reduces the influence of genetic effects on BMI and waist circumference: a study in young adult twins. *Int J Obes (Lond)* **33**, 29-36, doi:10.1038/ijo.2008.258 (2009).

69 Whitaker, R. C. Predicting preschooler obesity at birth: the role of maternal obesity in early pregnancy. *Pediatrics* **114**, e29-36 (2004).

70 Murrin, C. M., Kelly, G. E., Tremblay, R. E. & Kelleher, C. C. Body mass index and height over three generations: evidence from the Lifeways cross-generational cohort study. *BMC Public Health* **12**, 81 (2012).

71 Lawlor, D. A., Lichtenstein, P., Fraser, A. & Langstrom, N. Does maternal weight gain in pregnancy have long-term effects on offspring adiposity? A sibling study in a prospective cohort of 146,894 men from 136,050 families. *Am J Clin Nutr* **94**, 142-148 (2011).

72 Pietilainen, K. H. *et al.* Distribution and heritability of BMI in Finnish adolescents aged 16y and 17y: a study of 4884 twins and 2509 singletons. *Int J Obes Relat Metab Disord* **23**, 107-115. (1999).

73 Schousboe, K. *et al.* Sex differences in heritability of BMI: a comparative study of results from twin studies in eight countries. *Twin Res* **6**, 409-421 (2003).

74 Demerath, E. W. *et al.* Genetic and environmental influences on infant weight and weight change: the Fels Longitudinal Study. *Am J Hum Biol* **19**, 692-702 (2007).

75 Haworth, C. M. *et al.* Increasing heritability of BMI and stronger associations with the FTO gene over childhood. *Obesity (Silver Spring)* **16**, 2663-2668 (2008).

76 Lajunen, H. R. *et al.* Genetic and environmental effects on body mass index during adolescence: a prospective study among Finnish twins. *Int J Obes (Lond)* **33**, 559-567 (2009).

77 Nan, C. *et al.* Heritability of body mass index in pre-adolescence, young adulthood and late adulthood. *Eur J Epidemiol* (2012).

78 North, K. E. *et al.* Genetic Epidemiology of BMI and Body Mass Change From Adolescence to Young Adulthood. *Obesity (Silver Spring)* (2009).

79 Hjelmborg, J. B. *et al.* Genetic influences on growth traits of BMI: a longitudinal study of adult twins. *Obesity (Silver Spring)* **16**, 847-852 (2008).

80 Allison, D. B., Faith, M. S. & Nathan, J. S. Risch's lambda values for human obesity. *Int J Obes Relat Metab Disord* **20**, 990-999. (1996).

81 Wardle, J., Carnell, S., Haworth, C. M. & Plomin, R. Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment. *Am J Clin Nutr* **87**, 398-404 (2008).

82 Rokholm, B. *et al.* Increasing genetic variance of body mass index during the Swedish obesity epidemic. *PLoS One* **6**, e27135 (2011).

83 Mustelin, L., Silventoinen, K., Pietilainen, K., Rissanen, A. & Kaprio, J. Physical activity reduces the influence of genetic effects on BMI and waist circumference: a study in young adult twins. *Int J Obes (Lond)* **33**, 29-36 (2009).

84 McCaffery, J. M., Papandonatos, G. D., Bond, D. S., Lyons, M. J. & Wing, R. R. Gene X environment interaction of vigorous exercise and body mass index among male Vietnam-era twins. *Am J Clin Nutr* **89**, 1011-1018 (2009).

85 Williams, P. T. Attenuating effect of vigorous physical activity on the risk for inherited obesity: a study of 47,691 runners. *PLoS One* **7**, e31436, doi:10.1371/journal.pone.0031436 (2012).

86 Roskam, A. J. *et al.* Comparative appraisal of educational inequalities in overweight and obesity among adults in 19 European countries. *Int J Epidemiol* **39**, 392-404 (2010).

87 Lajunen, H. R., Kaprio, J., Rose, R. J., Pulkkinen, L. & Silventoinen, K. Genetic and environmental influences on BMI from late childhood to adolescence are modified by parental education. *Obesity (Silver Spring)* **20**, 583-589 (2012).

88 Cappuccio, F. P. *et al.* Meta-analysis of short sleep duration and obesity in children and adults. *Sleep* **31**, 619-626 (2008).

89 Watson, N. F. *et al.* Sleep duration and body mass index in twins: a gene-environment interaction. *Sleep* **35**, 1-7 (2012).

90 Maher, A. R. *et al.* Efficacy and comparative effectiveness of atypical antipsychotic medications for off-label uses in adults: a systematic review and meta-analysis. *Jama* **306**, 1359-1369 (2011).

91 Correll, C. U. & Malhotra, A. K. Pharmacogenetics of antipsychotic-induced weight gain. *Psychopharmacology (Berl)* **174**, 477-489 (2004).

92 Theisen, F. M. *et al.* Clozapine-induced weight gain: a study in monozygotic twins and same-sex sib pairs. *Psychiatr Genet* **15**, 285-289 (2005).

93 Gebhardt, S. *et al.* Body weight gain induced by atypical antipsychotics: an extension of the monozygotic twin and sib pair study. *J Clin Pharm Ther* **35**, 207-211 (2010).

94 Hainer, V. *et al.* Intrapair resemblance in very low calorie diet-induced weight loss in female obese identical twins. *Int J Obes Relat Metab Disord* **24**, 1051-1057 (2000).

95 Bouchard, C. *et al.* The response to exercise with constant energy intake in identical twins. *Obes Res* **2**, 400-410 (1994).

96 Hatoum, I. J. *et al.* Heritability of the weight loss response to gastric bypass surgery. *J Clin Endocrinol Metab* **96**, E1630-1633 (2011).

97 Vaisse, C. *et al.* Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity. *J Clin Invest* **106**, 253-262. (2000).

98 Moore, S. J. *et al.* Clinical and genetic epidemiology of Bardet-Biedl syndrome in Newfoundland: a 22-year prospective, population-based, cohort study. *Am J Med Genet A* **132**, 352-360 (2005).

99 Dudley, O., McManus, B., Vogels, A., Whittington, J. & Muscatelli, F. Cross-cultural comparisons of obesity and growth in Prader-Willi syndrome. *J Intellect Disabil Res* **52**, 426-436 (2008).

100 Bachmann-Gagescu, R. *et al.* Recurrent 200-kb deletions of 16p11.2 that include the SH2B1 gene are associated with developmental delay and obesity. *Genet Med* **12**, 641-647 (2010).

101 Farooqi, I. S. *et al.* Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. *N Engl J Med* **348**, 1085-1095. (2003).

102 Carmi, R., Elbedour, K., Stone, E. M. & Sheffield, V. C. Phenotypic differences among patients with Bardet-Biedl syndrome linked to three different chromosome loci. *Am J Med Genet* **59**, 199-203 (1995).

103 Richardson, A. S. *et al.* Moderate to vigorous physical activity interactions with genetic variants and body mass index in a large US ethnically diverse cohort. *Pediatr Obes*, doi:10.1111/j.2047-6310.2013.00152.x (2013).

104 Vimaleswaran, K. S. *et al.* Physical activity attenuates the body mass index-increasing influence of genetic variation in the FTO gene. *Am J Clin Nutr* **90**, 425-428, doi:10.3945/ajcn.2009.27652 (2009).

105 Demerath, E. W. *et al.* Interaction of FTO and physical activity level on adiposity in African-American and European-American adults: the ARIC study. *Obesity (Silver Spring)* **19**, 1866-1872, doi:10.1038/oby.2011.131 (2011).

106 Sonestedt, E. *et al.* Association between fat intake, physical activity and mortality depending on genetic variation in FTO. *Int J Obes (Lond)* **35**, 1041-1049, doi:10.1038/ijo.2010.263 (2011).

107 Ruiz, J. R. *et al.* Attenuation of the effect of the FTO rs9939609 polymorphism on total and central body fat by physical activity in adolescents: the HELENA study. *Arch Pediatr Adolesc Med* **164**, 328-333, doi:10.1001/archpediatrics.2010.29 (2010).

108 Kilpelainen, T. O. *et al.* Physical activity attenuates the influence of FTO variants on obesity risk: a meta-analysis of 218,166 adults and 19,268 children. *PLoS Med* **8**, e1001116 (2011).

109 Ochnio, J. J., Patrick, D., Ho, M., Talling, D. N. & Dobson, S. R. Past infection with hepatitis A virus among Vancouver street youth, injection drug users and men who have sex with men: implications for vaccination programs. *Cmaj* **165**, 293-297 (2001).

110 Xi, B. *et al.* Influence of physical inactivity on associations between single nucleotide polymorphisms and genetic predisposition to childhood obesity. *Am J Epidemiol* **173**, 1256-1262 (2011).

111 Sonestedt, E. *et al.* Association between fat intake, physical activity and mortality depending on genetic variation in FTO. *Int J Obes (Lond)* (2011).

112 Ahmad, T. *et al.* Lifestyle interaction with fat mass and obesity-associated (FTO) genotype and risk of obesity in apparently healthy U.S. women. *Diabetes Care* **34**, 675-680, doi:10.2337/dc10-0948 (2011).

113 Corella, D. *et al.* A high intake of saturated fatty acids strengthens the association between the fat mass and obesity-associated gene and BMI. *J Nutr* **141**, 2219-2225 (2011).

114 Moleres, A. *et al.* Dietary fatty acid distribution modifies obesity risk linked to the rs9939609 polymorphism of the fat mass and obesity-associated gene in a Spanish case-control study of children. *Br J Nutr* **107**, 533-538 (2012).

115 Phillips, C. M. *et al.* High dietary saturated fat intake accentuates obesity risk associated with the fat mass and obesity-associated gene in adults. *J Nutr* **142**, 824-831 (2012).

116 Corella, D. *et al.* APOA2, dietary fat, and body mass index: replication of a gene-diet interaction in 3 independent populations. *Arch Intern Med* **169**, 1897-1906 (2009).

117 Corella, D. *et al.* Association between the APOA2 promoter polymorphism and body weight in Mediterranean and Asian populations: replication of a gene-saturated fat interaction. *Int J Obes (Lond)*.

118 Corella, D. *et al.* APOA2, dietary fat, and body mass index: replication of a gene-diet interaction in 3 independent populations. *Arch Intern Med* **169**, 1897-1906, doi:10.1001/archinternmed.2009.343 (2009).

119 Rouskas, K. *et al.* Loss-of-Function Mutations in MC4R Are Very Rare in the Greek Severely Obese Adult Population. *Obesity (Silver Spring)* (2012).

120 Ogden, C. L. *et al.* Prevalence of overweight and obesity in the United States, 1999-2004. *Jama* **295**, 1549-1555 (2006).

121 Dempfle, A. *et al.* Large quantitative effect of melanocortin-4 receptor gene mutations on body mass index. *J Med Genet* **41**, 795-800 (2004).

122 Stutzmann, F. *et al.* Prevalence of melanocortin-4 receptor deficiency in Europeans and their age-dependent penetrance in multigenerational pedigrees. *Diabetes* **57**, 2511-2518 (2008).

123 Lindgren, C. M. *et al.* Genome-wide association scan meta-analysis identifies three Loci influencing adiposity and fat distribution. *PLoS Genet* **5**, e1000508 (2009).

124 Heid, I. M. *et al.* Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. *Nat Genet* **42**, 949-960 (2010).

125 Butler, J. V., Whittington, J. E., Holland, A. J., McAllister, C. J. & Goldstone, A. P. The transition between the phenotypes of Prader-Willi syndrome during infancy and early childhood. *Dev Med Child Neurol* **52**, e88-93 (2010).

126 Walters, R. G. *et al.* A new highly penetrant form of obesity due to deletions on chromosome 16p11.2. *Nature* **463**, 671-675 (2010).

127 Yu, Y. *et al.* Age- and gender-dependent obesity in individuals with 16p11.2 deletion. *J Genet Genomics* **38**, 403-409 (2011).

128 Kaakinen, M. *et al.* Life-Course Analysis of a Fat Mass and Obesity-Associated (FTO) Gene Variant and Body Mass Index in the Northern Finland Birth Cohort 1966 Using Structural Equation Modeling. *Am J Epidemiol* (2010).

129 Sovio, U. *et al.* Association between Common Variation at the FTO Locus and Changes in Body Mass Index from Infancy to Late Childhood: The Complex Nature of Genetic Association through Growth and Development. *PLoS Genet* **7**, e1001307 (2011).

130 Hertel, J. K. *et al.* FTO, Type 2 Diabetes, and Weight Gain Throughout Adult Life: A Meta-Analysis of 41,504 Subjects From the Scandinavian HUNT, MDC, and MPP Studies. *Diabetes* (2011).

131 Rzehak, P. *et al.* Associations between BMI and the FTO gene are age dependent: results from the GINI and LISA birth cohort studies up to age 6 years. *Obes Facts* **3**, 173-180 (2010).

132 Qi, L. *et al.* Fat mass-and obesity-associated (FTO) gene variant is associated with obesity: longitudinal analyses in two cohort studies and functional test. *Diabetes* **57**, 3145-3151 (2008).

133 Wangensteen, T. *et al.* FTO genotype and weight gain in obese and normal weight adults from a Norwegian population based cohort (the HUNT study). *Exp Clin Endocrinol Diabetes* **118**, 649-652 (2010).

134 Vimaleswaran, K. S. *et al.* Association Between FTO Variant and Change in Body Weight and Its Interaction With Dietary Factors: The DiOGenes Study. *Obesity (Silver Spring)* (2012).

135 Jacobsson, J. A. *et al.* Detailed analysis of variants in FTO in association with body composition in a cohort of 70-year-olds suggests a weakened effect among elderly. *PLoS One* **6**, e20158 (2011).

136 Elks, C. E. *et al.* Genetic markers of adult obesity risk are associated with greater early infancy weight gain and growth. *PLoS Med* **7**, e1000284 (2010).

137 Kilpelainen, T. O. *et al.* Obesity-susceptibility loci have a limited influence on birth weight: a meta-analysis of up to 28,219 individuals. *Am J Clin Nutr* **93**, 851-860 (2011).

138 Elks, C. E. *et al.* Adult obesity susceptibility variants are associated with greater childhood weight gain and a faster tempo of growth: the 1946 British Birth Cohort Study. *Am J Clin Nutr* (2012).

139 Sebire, N. J. *et al.* Maternal obesity and pregnancy outcome: a study of 287,213 pregnancies in London. *Int J Obes Relat Metab Disord* **25**, 1175-1182 (2001).

140 Crozier, S. R. *et al.* Weight gain in pregnancy and childhood body composition: findings from the Southampton Women's Survey. *Am J Clin Nutr* **91**, 1745-1751 (2010).

141 Oken, E., Rifas-Shiman, S. L., Field, A. E., Frazier, A. L. & Gillman, M. W. Maternal gestational weight gain and offspring weight in adolescence. *Obstet Gynecol* **112**, 999-1006 (2008).

142 Reynolds, R. M., Osmond, C., Phillips, D. I. & Godfrey, K. M. Maternal BMI, Parity, and Pregnancy Weight Gain: Influences on Offspring Adiposity in Young Adulthood. *J Clin Endocrinol Metab* (2010).

143 Nizard, J., Dommergue, M. & Clement, K. Pregnancy in a woman with a leptin-receptor mutation. *N Engl J Med* **366**, 1064-1065 (2012).

144 Lawlor, D. A. *et al.* Maternal and offspring adiposity-related genetic variants and gestational weight gain. *Am J Clin Nutr* **94**, 149-155 (2011).

145 Bonfig, W., Dokoupil, K. & Schmidt, H. A special, strict, fat-reduced, and carbohydrate-modified diet leads to marked weight reduction even in overweight adolescents with Prader-Willi syndrome (PWS). *ScientificWorldJournal* **9**, 934-939 (2009).

146 Wigren, M. & Hansen, S. Prader-Willi syndrome: clinical picture, psychosocial support and current management. *Child Care Health Dev* **29**, 449-456 (2003).

147 Vogels, A. & Fryns, J. P. Age at diagnosis, body mass index and physical morbidity in children and adults with the Prader-Willi syndrome. *Genet Couns* **15**, 397-404 (2004).

148 Silverthorn, K. H. & Hornak, J. E. Beneficial effects of exercise on aerobic capacity and body composition in adults with Prader-Willi syndrome. *Am J Ment Retard* **97**, 654-658 (1993).

149 Eiholzer, U. *et al.* Improving body composition and physical activity in Prader-Willi Syndrome. *J Pediatr* **142**, 73-78 (2003).

150 Butler, M. G., Theodoro, M. F., Bittel, D. C. & Donnelly, J. E. Energy expenditure and physical activity in Prader-Willi syndrome: comparison with obese subjects. *Am J Med Genet A* **143**, 449-459 (2007).

151 Santoro, N. *et al.* Weight loss in obese children carrying the proopiomelanocortin R236G variant. *J Endocrinol Invest* **29**, 226-230 (2006).

152 Reinehr, T. *et al.* Lifestyle intervention in obese children with variations in the melanocortin 4 receptor gene. *Obesity (Silver Spring)* **17**, 382-389 (2009).

153 Muller, T. D. *et al.* 'Fat mass and obesity associated' gene (FTO): no significant association of variant rs9939609 with weight loss in a lifestyle intervention and lipid metabolism markers in German obese children and adolescents. *BMC Med Genet* **9**, 85 (2008).

154 Franks, P. W. *et al.* Assessing gene-treatment interactions at the FTO and INSIG2 loci on obesity-related traits in the Diabetes Prevention Program. *Diabetologia* **51**, 2214-2223 (2008).

155 Lappalainen, T. J. *et al.* The common variant in the FTO gene did not modify the effect of lifestyle changes on body weight: the Finnish Diabetes Prevention Study. *Obesity (Silver Spring)* **17**, 832-836 (2009).

156 Matsuo, T., Nakata, Y., Murotake, Y., Hotta, K. & Tanaka, K. Effects of FTO Genotype on Weight Loss and Metabolic Risk Factors in Response to Calorie Restriction Among Japanese Women. *Obesity (Silver Spring)* (2011).

157 Woehning, A. *et al.* The A-allele of the common FTO gene variant rs9939609 complicates weight maintenance in severe obese patients. *Int J Obes (Lond)* (2012).

158 Grau, K. *et al.* Macronutrient-specific effect of FTO rs9939609 in response to a 10-week randomized hypo-energetic diet among obese Europeans. *Int J Obes (Lond)* **33**, 1227-1234 (2009).

159 Razquin, C. *et al.* A 3-year intervention with a Mediterranean diet modified the association between the rs9939609 gene variant in FTO and body weight changes. *Int J Obes (Lond)* **34**, 266-272 (2010).

160 Mitchell, J. A. *et al.* FTO genotype and the weight loss benefits of moderate intensity exercise. *Obesity (Silver Spring)* **18**, 641-643.

161 Rankinen, T., Rice, T., Teran-Garcia, M., Rao, D. C. & Bouchard, C. FTO genotype is associated with exercise training-induced changes in body composition. *Obesity (Silver Spring)* **18**, 322-326 (2010).

162 Scherag, A. *et al.* Two new Loci for body-weight regulation identified in a joint analysis of genome-wide association studies for early-onset extreme obesity in French and german study groups. *PLoS Genet* **6**, e1000916 (2010).

163 Scherag, A. *et al.* SDCCAG8 obesity alleles and reduced weight loss after a lifestyle intervention in overweight children and adolescents. *Obesity (Silver Spring)* **20**, 466-470 (2012).

164 Delahanty, L. M. *et al.* Genetic predictors of weight loss and weight regain after intensive lifestyle modification, metformin treatment, or standard care in the Diabetes Prevention Program. *Diabetes Care* **35**, 363-366 (2012).

165 Wangensteen, T. *et al.* Mutations in the melanocortin 4 receptor (MC4R) gene in obese patients in Norway. *Exp Clin Endocrinol Diabetes* **117**, 266-273 (2009).

166 Taylor, A. E. *et al.* Associations of FTO and MC4R Variants with Obesity Traits in Indians and the Role of Rural/Urban Environment as a Possible Effect Modifier. *J Obes* **2011**, 307542 (2011).

167 Vasan, S. K. *et al.* Associations of Variants in FTO and Near MC4R With Obesity Traits in South Asian Indians. *Obesity (Silver Spring)* (2012).

168 Hennig, B. J. *et al.* FTO gene variation and measures of body mass in an African population. *BMC Med Genet* **10**, 21 (2009).

169 Corella, D. *et al.* Education modulates the association of the FTO rs9939609 polymorphism with body mass index and obesity risk in the Mediterranean population. *Nutr Metab Cardiovasc Dis*.

170 Prats-Puig, A. *et al.* Variations in the obesity genes FTO, TMEM18 and NRXN3 influence the vulnerability of children to weight gain induced by short sleep duration. *Int J Obes (Lond)* (2012).

171 Beyerlein, A., von Kries, R., Ness, A. R. & Ong, K. K. Genetic markers of obesity risk: stronger associations with body composition in overweight compared to normal-weight children. *PLoS One* **6**, e19057 (2011).

172 Luppino, F. S. *et al.* Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. *Arch Gen Psychiatry* **67**, 220-229 (2010).

173 Rivera, M. *et al.* Depressive disorder moderates the effect of the FTO gene on body mass index. *Mol Psychiatry* (2011).

174 Tiwari, H. K. *et al.* Association of allelic variation in genes mediating aspects of energy homeostasis with weight gain during administration of antipsychotic drugs (CATIE Study). *Front Genet* **2** (2011).

175 Chowdhury, N. I. *et al.* Genetic association study between antipsychotic-induced weight gain and the melanocortin-4 receptor gene. *Pharmacogenomics J* (2012).

176 Barber, T. M., McCarthy, M. I., Wass, J. A. & Franks, S. Obesity and polycystic ovary syndrome. *Clin Endocrinol (Oxf)* **65**, 137-145 (2006).

177 Kowalska, I. *et al.* The FTO gene modifies weight, fat mass and insulin sensitivity in women with polycystic ovary syndrome, where its role may be larger than in other phenotypes. *Diabetes Metab* **35**, 328-331 (2009).

178 Tan, S. *et al.* Large effects on body mass index and insulin resistance of fat mass and obesity associated gene (FTO) variants in patients with polycystic ovary syndrome (PCOS). *BMC Med Genet* **11**, 12 (2010).

179 Zelissen, P. M. *et al.* Effect of three treatment schedules of recombinant methionyl human leptin on body weight in obese adults: a randomized, placebo-controlled trial. *Diabetes Obes Metab* **7**, 755-761 (2005).

180 Farooqi, I. S. *et al.* Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J Clin Invest* **110**, 1093-1103 (2002).

181 Paz-Filho, G., Wong, M. L. & Licinio, J. Ten years of leptin replacement therapy. *Obes Rev*.

182 Heymsfield, S. B. *et al.* Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *Jama* **282**, 1568-1575 (1999).

183 Hauner, H., Meier, M., Jockel, K. H., Frey, U. H. & Siffert, W. Prediction of successful weight reduction under sibutramine therapy through genotyping of the G-protein beta3 subunit gene (GNB3) C825T polymorphism. *Pharmacogenetics* **13**, 453-459 (2003).

184 Hsiao, D. J., Wu, L. S., Huang, S. Y. & Lin, E. Weight loss and body fat reduction under sibutramine therapy in obesity with the C825T polymorphism in the GNB3 gene. *Pharmacogenet Genomics* **19**, 730-733 (2009).

185 Grudell, A. B. *et al.* A controlled pharmacogenetic trial of sibutramine on weight loss and body composition in obese or overweight adults. *Gastroenterology* **135**, 1142-1154 (2008).

186 Sjostrom, L. *et al.* Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *N Engl J Med* **351**, 2683-2693 (2004).

187 Scheimann, A. O., Butler, M. G., Gourash, L., Cuffari, C. & Klish, W. Critical analysis of bariatric procedures in Prader-Willi syndrome. *J Pediatr Gastroenterol Nutr* **46**, 80-83 (2008).

188 Aslan, I. R. *et al.* Bariatric surgery in a patient with complete MC4R deficiency. *Int J Obes (Lond)* **35**, 457-461 (2011).

189 Stutzmann, F. *et al.* Variability of the effect of bariatric surgery according to the genotype of the MC4R. *Diabetes Metab* **36**, A23-A24 (2010).

190 Aslan, I. R. *et al.* Weight Loss after Roux-en-Y Gastric Bypass in Obese Patients Heterozygous for MC4R Mutations. *Obes Surg* (2011).

191 Hatoum, I. J. *et al.* Melanocortin-4 Receptor Signaling Is Required for Weight Loss after Gastric Bypass Surgery. *J Clin Endocrinol Metab* (2012).

192 Sarzynski, M. A. *et al.* Associations of markers in 11 obesity candidate genes with maximal weight loss and weight regain in the SOS bariatric surgery cases. *Int J Obes (Lond)* **35**, 676-683 (2011).

193 Liou, T. H. *et al.* ESR1, FTO, and UCP2 genes interact with bariatric surgery affecting weight loss and glycemic control in severely obese patients. *Obes Surg* **21**, 1758-1765 (2011).

194 Still, C. D. *et al.* High allelic burden of four obesity SNPs is associated with poorer weight loss outcomes following gastric bypass surgery. *Obesity (Silver Spring)* **19**, 1676-1683 (2011).

195 Blakemore, A. I. & Froguel, P. Investigation of Mendelian forms of obesity holds out the prospect of personalized medicine. *Ann N Y Acad Sci* **1214**, 180-189 (2010).

196 Doyle, Y. G., Furey, A. & Flowers, J. Sick individuals and sick populations: 20 years later. *J Epidemiol Community Health* **60**, 396-398 (2006).

197 Bloss, C. S., Jeste, D. V. & Schork, N. J. Genomics for disease treatment and prevention. *Psychiatr Clin North Am* **34**, 147-166 (2011).

198 Rao, G. & Kirley, K. The future of obesity treatment: comment on "Integrating technology into standard weight loss treatment: a randomized controlled trial". *JAMA Intern Med* **173**, 111-112, doi:10.1001/jamainternmed.2013.1232 (2013).

199 Watts, G. The future of obesity treatment: what can drugs and surgery offer? *Bmj* **344**, e1011, doi:10.1136/bmj.e1011 (2012).

200 Christou, N. V. Impact of obesity and bariatric surgery on survival. *World J Surg* **33**, 2022-2027, doi:10.1007/s00268-009-0050-2 (2009).

201 Sjostrom, L. Bariatric surgery and reduction in morbidity and mortality: experiences from the SOS study. *Int J Obes (Lond)* **32 Suppl 7**, S93-97, doi:10.1038/ijo.2008.244 (2008).

202 Sjostrom, L. *et al.* Effects of bariatric surgery on mortality in Swedish obese subjects. *N Engl J Med* **357**, 741-752, doi:10.1056/NEJMoa066254 (2007).

203 Richards, N. G., Beekley, A. C. & Tichansky, D. S. The economic costs of obesity and the impact of bariatric surgery. *Surg Clin North Am* **91**, 1173-1180, vii-viii, doi:10.1016/j.suc.2011.08.010 (2011).

204 Kim, J. H. & Wolfe, B. Bariatric/metabolic surgery: short- and long-term safety. *Curr Atheroscler Rep* **14**, 597-605, doi:10.1007/s11883-012-0287-3 (2012).

205 Birch, L. L. & Ventura, A. K. Preventing childhood obesity: what works? *Int J Obes (Lond)* **33 Suppl 1**, S74-81, doi:10.1038/ijo.2009.22 (2009).

206 Xi, B. *et al.* [Impact on the risk of obesity due to interactions between fat mass- and obesity-associated gene rs9939609 variants and behavioral factors, in the Chinese school-aged children]. *Zhonghua Liu Xing Bing Xue Za Zhi* **31**, 737-741 (2010).

207 Ahmad, S. *et al.* Gene x physical activity interactions in obesity: combined analysis of 111,421 individuals of European ancestry. *PLoS Genet* **9**, e1003607, doi:10.1371/journal.pgen.1003607 (2013).

208 Hein, R., Beckmann, L. & Chang-Claude, J. Sample size requirements for indirect association studies of gene-environment interactions (G x E). *Genet Epidemiol* **32**, 235-245 (2008).

209 Wong, M. Y., Day, N. E., Luan, J. A., Chan, K. P. & Wareham, N. J. The detection of gene-environment interaction for continuous traits: should we deal with measurement error by bigger studies or better measurement? *Int J Epidemiol* **32**, 51-57 (2003).

210 Muller, M. J., Bosy-Westphal, A. & Krawczak, M. Genetic studies of common types of obesity: a critique of the current use of phenotypes. *Obes Rev* **11**, 612-618 (2010).

211 Ainsworth, B. E. *et al.* 2011 Compendium of Physical Activities: a second update of codes and MET values. *Med Sci Sports Exerc* **43**, 1575-1581, doi:10.1249/MSS.0b013e31821ece12 (2011).

212 Bergman, R. N. *et al.* A better index of body adiposity. *Obesity (Silver Spring)* **19**, 1083-1089, doi:10.1038/oby.2011.38 (2011).

213 Gerstein, H. C., Yusuf, S., Holman, R., Bosch, J. & Pogue, J. Rationale, design and recruitment characteristics of a large, simple international trial of diabetes prevention: the DREAM trial. *Diabetologia* **47**, 1519-1527, doi:10.1007/s00125-004-1485-5 (2004).

214 Anand, S. S. *et al.* Glucose levels are associated with cardiovascular disease and death in an international cohort of normal glycaemic and dysglycaemic men and women: the EpiDREAM cohort study. *Eur J Prev Cardiol* **19**, 755-764, doi:10.1177/1741826711409327 (2012).

215 Keating, B. J. *et al.* Concept, design and implementation of a cardiovascular gene-centric 50 k SNP array for large-scale genomic association studies. *PLoS One* **3**, e3583 (2008).

216 Wu, Y. *et al.* Genome-wide association study for adiponectin levels in Filipino women identifies CDH13 and a novel uncommon haplotype at KNG1-ADIPOQ. *Hum Mol Genet* **19**, 4955-4964, doi:10.1093/hmg/ddq423 (2010).

217 Genuth, S. *et al.* Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* **26**, 3160-3167 (2003).

218 Janssens, A. C. *et al.* The impact of genotype frequencies on the clinical validity of genomic profiling for predicting common chronic diseases. *Genet Med* **9**, 528-535, doi:10.1097GIM.0b013e31812eece0 (2007).

219 Robiou-du-Pont, S. *et al.* Contribution of 24 obesity-associated genetic variants to insulin resistance, pancreatic beta-cell function and type 2 diabetes risk in the French population. *Int J Obes (Lond)* **37**, 980-985, doi:10.1038/ijo.2012.175 (2013).

220 McCaffery, J. M., Papandonatos, G. D., Bond, D. S., Lyons, M. J. & Wing, R. R. Gene X environment interaction of vigorous exercise and body mass index among male Vietnam-era twins. *Am J Clin Nutr* **89**, 1011-1018, doi:10.3945/ajcn.2008.27170 (2009).

221 Corella, D. *et al.* Statistical and biological gene-lifestyle interactions of MC4R and FTO with diet and physical activity on obesity: new effects on alcohol consumption. *PLoS One* **7**, e52344, doi:10.1371/journal.pone.0052344 (2012).

222 Yang, J. *et al.* FTO genotype is associated with phenotypic variability of body mass index. *Nature* **490**, 267-272, doi:10.1038/nature11401 (2012).

223 Ronn, T. *et al.* A six months exercise intervention influences the genome-wide DNA methylation pattern in human adipose tissue. *PLoS Genet* **9**, e1003572, doi:10.1371/journal.pgen.1003572 (2013).

224 Nitert, M. D. *et al.* Impact of an exercise intervention on DNA methylation in skeletal muscle from first-degree relatives of patients with type 2 diabetes. *Diabetes* **61**, 3322-3332, doi:10.2337/db11-1653 (2012).

225 Ioannidis, J. P. Why most published research findings are false. *PLoS Med* **2**, e124, doi:10.1371/journal.pmed.0020124 (2005).

226 Lee, H. J. *et al.* Effects of common FTO gene variants associated with BMI on dietary intake and physical activity in Koreans. *Clin Chim Acta* **411**, 1716-1722, doi:10.1016/j.cca.2010.07.010 (2010).

227 Karasawa, S. *et al.* Association of the common fat mass and obesity associated (FTO) gene polymorphism with obesity in a Japanese population. *Endocr J* **57**, 293-301 (2010).

228 Moore, S. C. *et al.* Common genetic variants and central adiposity among Asian-Indians. *Obesity (Silver Spring)* **20**, 1902-1908, doi:10.1038/oby.2011.238 (2012).

229 Luke, A. & Cooper, R. S. Physical activity does not influence obesity risk: time to clarify the public health message. *Int J Epidemiol* **42**, 1831-1836, doi:10.1093/ije/dyt159 (2013).

230 Wareham, N. J. & Brage, S. Commentary: Physical activity and obesity; scientific uncertainty and the art of public health messaging. *Int J Epidemiol* **42**, 1843-1845, doi:10.1093/ije/dyt164 (2013).

231 Speakman, J. R. & O'Rahilly, S. Fat: an evolving issue. *Dis Model Mech* **5**, 569-573, doi:10.1242/dmm.010553 (2012).

232 Janssen, I. *et al.* Comparison of overweight and obesity prevalence in school-aged youth from 34 countries and their relationships with physical activity and dietary patterns. *Obes Rev* **6**, 123-132, doi:10.1111/j.1467-789X.2005.00176.x (2005).

233 Ladabaum, U., Mannalithara, A., Myer, P. A. & Singh, G. Obesity, Abdominal Obesity, Physical Activity, and Caloric Intake in U.S. Adults: 1988-2010. *Am J Med*, doi:10.1016/j.amjmed.2014.02.026 (2014).

234 Li, S. *et al.* Physical activity attenuates the genetic predisposition to obesity in 20,000 men and women from EPIC-Norfolk prospective population study. *PLoS Med* **7** (2010).

235 Luan, J. A., Wong, M. Y., Day, N. E. & Wareham, N. J. Sample size determination for studies of gene-environment interaction. *Int J Epidemiol* **30**, 1035-1040 (2001).

236 Peters, T. *et al.* Validity of a short questionnaire to assess physical activity in 10 European countries. *Eur J Epidemiol* **27**, 15-25, doi:Doi 10.1007/S10654-011-9625-Y (2012).

237 Suchanek, P. *et al.* Which index best correlates with body fat mass: BAI, BMI, waist or WHR? *Neuroendocrinol Lett* **33**, 78-82 (2012).

238 Rankinen, T., Rice, T., Teran-Garcia, M., Rao, D. C. & Bouchard, C. FTO genotype is associated with exercise training-induced changes in body composition. *Obesity (Silver Spring)* **18**, 322-326, doi:10.1038/oby.2009.205 (2010).

239 Rampersaud, E. *et al.* Physical activity and the association of common FTO gene variants with body mass index and obesity. *Arch Intern Med* **168**, 1791-1797, doi:10.1001/archinte.168.16.1791 (2008).

240 Rothman KJ, G. S. *Modern epidemiology*. 2nd edn edn, ( Lippincott Williams & Wilkins,, 1998).

241 Mitchell, J. A. *et al.* FTO genotype and the weight loss benefits of moderate intensity exercise. *Obesity (Silver Spring)* **18**, 641-643, doi:10.1038/oby.2009.311 (2010).

242 Grimes, D. A. & Schulz, K. F. Cohort studies: marching towards outcomes. *Lancet* **359**, 341-345, doi:Doi 10.1016/S0140-6736(02)07500-1 (2002).

243 Steinthorsdottir, V. *et al.* A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. *Nat Genet* **39**, 770-775, doi:10.1038/ng2043 (2007).

244 Li, Y. Y. *et al.* CDKAL1 gene rs7756992 A/G polymorphism and type 2 diabetes mellitus: a meta-analysis of 62,567 subjects. *Sci Rep* **3**, 3131, doi:10.1038/srep03131 (2013).

245 Ortega-Alonso, A., Sipila, S., Kujala, U. M., Kaprio, J. & Rantanen, T. Genetic influences on change in BMI from middle to old age: a 29-year follow-up study of twin sisters. *Behav Genet* **39**, 154-164 (2009).

246 Gray, J. *et al.* Hyperphagia, severe obesity, impaired cognitive function, and hyperactivity associated with functional loss of one copy of the brain-derived neurotrophic factor (BDNF) gene. *Diabetes* **55**, 3366-3371, doi:10.2337/db06-0550 (2006).

247 Kernie, S. G., Liebl, D. J. & Parada, L. F. BDNF regulates eating behavior and locomotor activity in mice. *Embo J* **19**, 1290-1300, doi:10.1093/emboj/19.6.1290 (2000).

248 Jonsson A, F. P. Obesity, FTO gene variant, and energy intake in children. *N Engl J Med* **360**, 1571–1572; author reply 1572 (2009).

249 Neel, J. V. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? *Am J Hum Genet* **14**, 353-362 (1962).

250 Southam, L. *et al.* Is the thrifty genotype hypothesis supported by evidence based on confirmed type 2 diabetes- and obesity-susceptibility variants? *Diabetologia* **52**, 1846-1851, doi:10.1007/s00125-009-1419-3 (2009).

251 Jonsson, A. & Franks, P. W. Obesity, FTO gene variant, and energy intake in children. *N Engl J Med* **360**, 1571-1572; author reply 1572, doi:10.1056/NEJMc090017 (2009).

252 Belcher, B. R. *et al.* Physical activity in US youth: effect of race/ethnicity, age, gender, and weight status. *Med Sci Sports Exerc* **42**, 2211-2221, doi:10.1249/MSS.0b013e3181e1fba9 (2010).

253 Craig, C. L. *et al.* International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* **35**, 1381-1395, doi:10.1249/01.MSS.0000078924.61453.FB (2003).

254 Thorburn, A. W. Prevalence of obesity in Australia. *Obes Rev* **6**, 187-189, doi:10.1111/j.1467-789X.2005.00187.x (2005).

255 Mamun, A. A. *et al.* Associations of excess weight gain during pregnancy with long-term maternal overweight and obesity: evidence from 21 y postpartum follow-up. *Am J Clin Nutr* **91**, 1336-1341, doi:10.3945/ajcn.2009.28950 (2010).

256 Gore, S. A., Brown, D. M. & West, D. S. The role of postpartum weight retention in obesity among women: a review of the evidence. *Ann Behav Med* **26**, 149-159 (2003).

257 Williamson, D. F., Kahn, H. S., Remington, P. L. & Anda, R. F. The 10-year incidence of overweight and major weight gain in US adults. *Arch Intern Med* **150**, 665-672 (1990).

258 Loria, C. M., Signore, C. & Arteaga, S. S. The need for targeted weight-control approaches in young women and men. *Am J Prev Med* **38**, 233-235, doi:10.1016/j.amepre.2009.11.001 (2010).

259 Thangaratinam, S. *et al.* Interventions to reduce or prevent obesity in pregnant women: a systematic review. *Health technology assessment* **16**, iii-iv, 1-191, doi:10.3310/hta16310 (2012).

260 Ogden, C. L. *et al.* Prevalence of overweight and obesity in the United States, 1999-2004. *Jama* **295**, 1549-1555, doi:10.1001/jama.295.13.1549 (2006).

261 Institute of Medicine. Nutrition during pregnancy. Part I, weight gain. Washington, DC: National Academy Press 1990.

262 Viswanathan, M. *et al.* Outcomes of maternal weight gain. *Evid Rep Technol Assess (Full Rep)*, 1-223 (2008).

263 Linne, Y. & Neovius, M. Identification of women at risk of adverse weight development following pregnancy. *Int J Obes (Lond)* **30**, 1234-1239, doi:10.1038/sj.ijo.0803258 (2006).

264 Walker, L. O. Predictors of weight gain at 6 and 18 months after childbirth: a pilot study. *Journal of obstetric, gynecologic, and neonatal nursing : JOGNN / NAACOG* **25**, 39-48 (1996).

265 Nohr, E. A. *et al.* Combined associations of prepregnancy body mass index and gestational weight gain with the outcome of pregnancy. *Am J Clin Nutr* **87**, 1750-1759 (2008).

266 Streuling, I., Beyerlein, A. & von Kries, R. Can gestational weight gain be modified by increasing physical activity and diet counseling? A meta-analysis of interventional trials. *Am J Clin Nutr* **92**, 678-687, doi:10.3945/ajcn.2010.29363 (2010).

267 Abrams, B., Altman, S. L. & Pickett, K. E. Pregnancy weight gain: still controversial. *Am J Clin Nutr* **71**, 1233S-1241S (2000).

268 Taveras, E. M. *et al.* First steps for mommy and me: a pilot intervention to improve nutrition and physical activity behaviors of postpartum mothers and their infants. *Matern Child Health J* **15**, 1217-1227, doi:10.1007/s10995-010-0696-2 (2011).

269 van der Pligt, P. *et al.* Systematic review of lifestyle interventions to limit postpartum weight retention: implications for future opportunities to prevent maternal overweight and obesity following childbirth. *Obes Rev* **14**, 792-805, doi:10.1111/obr.12053 (2013).

270 Lan-Pidhainy, X., Nohr, E. A. & Rasmussen, K. M. Comparison of gestational weight gain-related pregnancy outcomes in American primiparous and multiparous women. *Am J Clin Nutr* **97**, 1100-1106, doi:10.3945/ajcn.112.052258 (2013).

271 Davis, E. M., Zyzanski, S. J., Olson, C. M., Stange, K. C. & Horwitz, R. I. Racial, ethnic, and socioeconomic differences in the incidence of obesity related to childbirth. *Am J Public Health* **99**, 294-299, doi:10.2105/AJPH.2007.132373 (2009).

272 Harris, H. E., Ellison, G. T. & Clement, S. Do the psychosocial and behavioral changes that accompany motherhood influence the impact of pregnancy on long-term weight gain? *Journal of psychosomatic obstetrics and gynaecology* **20**, 65-79 (1999).

273 Harris, H. E., Ellison, G. T. & Holliday, M. Is there an independent association between parity and maternal weight gain? *Ann Hum Biol* **24**, 507-519 (1997).

274 Rooney, B. L. & Schauberger, C. W. Excess pregnancy weight gain and long-term obesity: one decade later. *Obstet Gynecol* **100**, 245-252 (2002).

275 Linne, Y., Dye, L., Barkeling, B. & Rossner, S. Weight development over time in parous women--the SPAWN study--15 years follow-up. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* **27**, 1516-1522, doi:10.1038/sj.ijo.0802441 (2003).

276 Lederman, S. A. *et al.* Body fat and water changes during pregnancy in women with different body weight and weight gain. *Obstet Gynecol* **90**, 483-488 (1997).

277 Gunderson, E. P. & Abrams, B. Epidemiology of gestational weight gain and body weight changes after pregnancy. *Epidemiol Rev* **22**, 261-274 (2000).

278 Keppel, K. G. & Taffel, S. M. Pregnancy-related weight gain and retention: implications of the 1990 Institute of Medicine guidelines. *Am J Public Health* **83**, 1100-1103 (1993).

279 Auwerx, J. & Staels, B. Leptin. *Lancet* **351**, 737-742, doi:10.1016/S0140-6736(97)06348-4 (1998).

280 Stein, T. P., Scholl, T. O., Schluter, M. D. & Schroeder, C. M. Plasma leptin influences gestational weight gain and postpartum weight retention. *Am J Clin Nutr* **68**, 1236-1240 (1998).

281 Gaillard, R. *et al.* Risk factors and outcomes of maternal obesity and excessive weight gain during pregnancy. *Obesity (Silver Spring)* **21**, 1046-1055, doi:10.1002/oby.20088 (2013).

282 Stuebe, A. M. *et al.* Obesity and diabetes genetic variants associated with gestational weight gain. *Am J Obstet Gynecol* **203**, 283 e281-217, doi:10.1016/j.ajog.2010.06.069 (2010).

283 Benedict, C. *et al.* Brief communication: The fat mass and obesity-associated gene (FTO) is linked to higher plasma levels of the hunger hormone ghrelin and lower serum levels of the satiety hormone leptin in older adults. *Diabetes*, doi:10.2337/db14-0470 (2014).

284 Cederlof, R. & Kauer, L. The effect of childbearing on body-weight. A twin control study. *Acta Psychiatr Scand* **219,**, 47-49 (1970).

285 Gunderson, E. P. & Abrams, B. Epidemiology of gestational weight gain and body weight changes after pregnancy. *Epidemiol Rev* **21**, 261-275 (1999).

286 Olson, C. M., Strawderman, M. S., Hinton, P. S. & Pearson, T. A. Gestational weight gain and postpartum behaviors associated with weight change from early pregnancy to 1 y postpartum. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* **27**, 117-127, doi:10.1038/sj.ijo.0802156 (2003).

287 Gunderson, E. P., Abrams, B. & Selvin, S. The relative importance of gestational gain and maternal characteristics associated with the risk of becoming overweight after pregnancy. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity* **24**, 1660-1668 (2000).

288 Butte, N. F., Ellis, K. J., Wong, W. W., Hopkinson, J. M. & Smith, E. O. Composition of gestational weight gain impacts maternal fat retention and infant birth weight. *Am J Obstet Gynecol* **189**, 1423-1432 (2003).

289 Jokela, M., Elovainio, M. & Kivimaki, M. Lower fertility associated with obesity and underweight: the US National Longitudinal Survey of Youth. *Am J Clin Nutr* **88**, 886-893 (2008).

290 Cramer, D. W., Barbieri, R. L., Xu, H. & Reichardt, J. K. Determinants of basal follicle-stimulating hormone levels in premenopausal women. *The Journal of clinical endocrinology and metabolism* **79**, 1105-1109, doi:10.1210/jcem.79.4.7962282 (1994).

291 Frisch, R. E. Body fat, menarche, fitness and fertility. *Hum Reprod* **2**, 521-533 (1987).

292 Grodstein, F., Goldman, M. B. & Cramer, D. W. Body mass index and ovulatory infertility. *Epidemiology* **5**, 247-250 (1994).

293 Rich-Edwards, J. W. *et al.* Physical activity, body mass index, and ovulatory disorder infertility. *Epidemiology* **13**, 184-190 (2002).

294 Rich-Edwards, J. W. *et al.* Adolescent body mass index and infertility caused by ovulatory disorder. *Am J Obstet Gynecol* **171**, 171-177 (1994).

295 Robker RL, A. L., Bennett BD, Thrupp PN, Chura LR, Russell DL, Lane M, Norman RJ. Obese Women Exhibit Differences in Ovarian Metabolites, Hormones, and Gene Expression Compared to Moderate Weight Women. *J Clin Endocrinol Metab* **94**, 533–540 (2009).

296 Ziomkiewicz, A., Ellison, P. T., Lipson, S. F., Thune, I. & Jasienska, G. Body fat, energy balance and estradiol levels: a study based on hormonal profiles from complete menstrual cycles. *Hum Reprod* **23**, 2555-2563, doi:10.1093/humrep/den213 (2008).

297 Chakravarthy, M. V. & Booth, F. W. Eating, exercise, and "thrifty" genotypes: connecting the dots toward an evolutionary understanding of modern chronic diseases. *J Appl Physiol* **96**, 3-10, doi:10.1152/japplphysiol.00757.2003 (2004).

298 Bravard, A. *et al.* FTO contributes to hepatic metabolism regulation through regulation of leptin action and STAT3 signalling in liver. *Cell Commun Signal* **12**, 4, doi:10.1186/1478-811X-12-4 (2014).

299 Khoury, M. J. & Wacholder, S. Invited commentary: from genome-wide association studies to gene-environment-wide interaction studies--challenges and opportunities. *Am J Epidemiol* **169**, 227-230; discussion 234-225, doi:10.1093/aje/kwn351 (2009).

300 Liu, C. Y., Maity, A., Lin, X., Wright, R. O. & Christiani, D. C. Design and analysis issues in gene and environment studies. *Environ Health* **11**, 93, doi:10.1186/1476-069X-11-93 (2012).

301 Joseph, P. G., Pare, G. & Anand, S. S. Exploring gene-environment relationships in cardiovascular disease. *The Canadian journal of cardiology* **29**, 37-45, doi:10.1016/j.cjca.2012.10.009 (2013).

302 Rothman, N., Garcia-Closas, M., Stewart, W. T. & Lubin, J. The impact of misclassification in case-control studies of gene-environment interactions. *IARC Sci Publ*, 89-96 (1999).

303 Morimoto, L. M., White, E. & Newcomb, P. A. Selection bias in the assessment of gene-environment interaction in case-control studies. *Am J Epidemiol* **158**, 259-263 (2003).

304 Yokoyama, A. *et al.* Reliability of a flushing questionnaire and the ethanol patch test in screening for inactive aldehyde dehydrogenase-2 and alcohol-related cancer risk. *Cancer Epidemiol Biomarkers Prev* **6**, 1105-1107 (1997).

305 Reich, D. E. & Goldstein, D. B. Detecting association in a case-control study while correcting for population stratification. *Genet Epidemiol* **20**, 4-16, doi:10.1002/1098-2272(200101)20:1<4::AID-GEPI2>3.0.CO;2-T (2001).

306 Thomas, D. C. & Witte, J. S. Point: population stratification: a problem for case-control studies of candidate-gene associations? *Cancer Epidemiol Biomarkers Prev* **11**, 505-512 (2002).

307 Laird, N. M., Horvath, S. & Xu, X. Implementing a unified approach to family-based tests of association. *Genet Epidemiol* **19 Suppl 1**, S36-42, doi:10.1002/1098-2272(2000)19:1+<::AID-GEPI6>3.0.CO;2-M (2000).

308 Weinberg, C. R. & Umbach, D. M. Choosing a retrospective design to assess joint genetic and environmental contributions to risk. *Am J Epidemiol* **152**, 197-203 (2000).

309 Franks, P. W. & Nettleton, J. A. Invited commentary: Gene X lifestyle interactions and complex disease traits--inferring cause and effect from observational data, sine qua non. *Am J Epidemiol* **172**, 992-997; discussion 998-999, doi:10.1093/aje/kwq280 (2010).

310 Wang, K. *et al.* A genome-wide association study on obesity and obesity-related traits. *PLoS One* **6**, e18939, doi:10.1371/journal.pone.0018939 (2011).

311 Wen, W. *et al.* Meta-analysis identifies common variants associated with body mass index in east Asians. *Nat Genet* **44**, 307-311, doi:10.1038/ng.1087 (2012).

312 Franks, P. W., Mesa, J. L., Harding, A. H. & Wareham, N. J. Gene-lifestyle interaction on risk of type 2 diabetes. *Nutr Metab Cardiovasc Dis* **17**, 104-124, doi:10.1016/j.numecd.2006.04.001 (2007).

313 Heitmann, B. L. *et al.* Obesity: lessons from evolution and the environment. *Obes Rev* **13**, 910-922, doi:10.1111/j.1467-789X.2012.01007.x (2012).

314 Crossa, J. From genotype x environment interaction to gene x environment interaction. *Curr Genomics* **13**, 225-244, doi:10.2174/138920212800543066 (2012).

315 Williamson, E., Ponsonby, A. L., Carlin, J. & Dwyer, T. Effect of including environmental data in investigations of gene-disease associations in the presence of qualitative interactions. *Genet Epidemiol* **34**, 552-560 (2010).

316 Khoury, M. J., Adams, M. J., Jr. & Flanders, W. D. An epidemiologic approach to ecogenetics. *Am J Hum Genet* **42**, 89-95 (1988).

317 Kraft, P., Yen, Y. C., Stram, D. O., Morrison, J. & Gauderman, W. J. Exploiting gene-environment interaction to detect genetic associations. *Hum Hered* **63**, 111-119 (2007).

318 Murcray, C. E., Lewinger, J. P. & Gauderman, W. J. Gene-environment interaction in genome-wide association studies. *Am J Epidemiol* **169**, 219-226 (2009).

319 Aschard, H., Hancock, D. B., London, S. J. & Kraft, P. Genome-wide meta-analysis of joint tests for genetic and gene-environment interaction effects. *Hum Hered* **70**, 292-300 (2011).

320 Franks, P. W. Gene x environment interactions in type 2 diabetes. *Curr Diab Rep* **11**, 552-561, doi:10.1007/s11892-011-0224-9 (2011).

321 Brito, E. C. *et al.* Previously associated type 2 diabetes variants may interact with physical activity to modify the risk of impaired glucose regulation and type 2 diabetes: a study of 16,003 Swedish adults. *Diabetes* **58**, 1411-1418, doi:10.2337/db08-1623 (2009).

322 Pare, G., Cook, N. R., Ridker, P. M. & Chasman, D. I. On the use of variance per genotype as a tool to identify quantitative trait interaction effects: a report from the Women's Genome Health Study. *PLoS Genet* **6**, e1000981, doi:10.1371/journal.pgen.1000981 (2010).

323 Arcos-Burgos, M. & Muenke, M. Genetics of population isolates. *Clin Genet* **61**, 233-247 (2002).