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Genes and lifestyle factors in obesity: results from 12 462 subjects from MONICA/KORA

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Abstract

Background—Data from meta-analyses of genome-wide association studies provided evidence for an association of polymorphisms with body mass index (BMI), and gene expression results indicated a role of these variants in the hypothalamus. It was consecutively hypothesized that these associations might be evoked by a modulation of nutritional intake or energy expenditure.

Objective—It was our aim to investigate the association of these genetic factors with BMI in a large homogenous population-based sample to explore the association of these polymorphisms with lifestyle factors related to nutritional intake or energy expenditure, and whether such lifestyle factors could be mediators of the detected single-nucleotide polymorphism (SNP)-association with BMI. It was a further aim to compare the proportion of BMI explained by genetic factors with the one explained by lifestyle factors.

Design—The association of seven polymorphisms in or near the genes *NEGR1*, *TMEM18*, *MTCH2*, *FTO*, *MC4R*, *SH2B1*and *KCTD15* was analyzed in 12 462 subjects from the population-based MONICA/KORA Augsburg study. Information on lifestyle factors was based on standardized questionnaires. For statistical analysis, regression-based models were used.

Results—The minor allele of polymorphism rs6548238 C>T (TMEM18) was associated with lower BMI (−0.418 kg/m^2, p=1.22×10−8), and of polymorphisms rs9935401 G>A (FTO) and rs7498665 A>G (SH2B1) with increased BMI (0.290 kg/m^2, p=2.85×10−7 and 0.145 kg/m^2, p=9.83×10−3). The other polymorphisms were not significantly associated. Lifestyle factors were correlated with BMI and explained 0.037 % of the BMI variance as compared to 0.006 % of explained variance by the associated genetic factors. The genetic variants associated with BMI

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were not significantly associated with lifestyle factors and there was no evidence of lifestyle factors mediating the SNP-BMI association.

Conclusions—Our data first confirm the findings for TMEM18 with BMI in a single study on adults and also confirm the findings for FTO and SH2B1. There was no evidence for a direct SNP-lifestyle association.

Keywords

TMEM18; *FTO*; *SH2B1*; lifestyle; obesity

Introduction

Obesity is caused by a prolonged maintenance of a positive energy balance in which energy intake is higher than energy expenditure. Lifestyle factors are the main modulators of body weight control and obesity risk. From twin, adoption, and family studies, genetic components are also known to have an important role and reported to be responsible for up to 90 % of body weight variation (1), whereas the detection of polygenes with small effects on body weight is ongoing (2).

Willer et al (3) recently reported six novel obesity loci and a gene expression mainly in the brain. Most of these loci were also reported by Thorleifsson et al (4). The authors hypothesized that these loci might convey an effect on obesity through the central nervous system (CNS). As appetite and satiety are regulated in the hypothalamus, it was further deduced that these loci could have a role in modulating energy homeostasis. The hypothesis of a role of these loci in the central nervous system would be in line with previous functional evidence on these loci: (a) The neuronal growth regulator 1 (NEGR1) protein has a role in the development of the central nervous system (5). (b) The transmembrane 18 (*TMEM18*) gene modulates cell migration (6). (c) Tumour phenotypes are influenced by the mitochondrial carrier homolog 2 (*MTCH2*) gene (7) and the MTCH2 protein might have a role in mitochondrial apoptosis (8). (d) Variants within the Src-homology-2 (SH2) domain containing the putative adaptor protein 1 (*SH2B1*) gene were associated with serum leptin and obesity-related phenotypes (9). In mice, SH2B is a key regulator of leptin sensitivity, energy balance, and body weight (10), and knockout mice develop a disordered glucose metabolism (11). (e) Knowledge about potassium channel tetramerisation domain containing 15 (*KCTD15*) gene and about (f) glucosamine-6-phosphate deaminase 2 (*GNPDA2*) gene and their proteins is limited.

The two strongest genetic risk factors previously described (12,13), the fat mass and obesity associated (*FTO*) gene and the melanocortin-4-receptor (*MC4R*) gene, are reported to control energy expenditure (14,15) and to modulate dietary habits (16-22). The *FTO* gene has been reported to code for an oxygenase involved in DNA methylation (23) and MC4R is a G-protein-coupled receptor, which has, as part of the melanocortinergic pathway, a crucial role in energy homeostasis (24).

We hypothesize that the association of these genetic loci with obesity might be exerted through a direct association of the loci on energy intake or energy expenditure. In epidemiological studies, energy intake and expenditure can be assessed by questionnaires on food intake frequency and physical activity scores. Data on the direct association of these genetic variants with such lifestyle variables in a large population-based study was lacking up to now. A recent Dutch study in females (n=1 700) showed a borderline significant association of two obesity-related genetic loci with fat and carbohydrate intake, but the results were rather inconclusive (25). Given the expected moderate associations and the

difficulty of meta-analysis due to different lifestyle assessment tools, a large homogeneous population-based study sample is best suited to investigate this hypothesis.

Therefore we investigated the six novel obesity loci reported by Willer et al complemented by *FTO* and *MC4R* in our large homogenous population-based sample of 12 462 subjects with regard to body mass index (BMI), and lifestyle factors including carbohydrate intake score, fat intake score, smoking, alcohol consumption, and physical activity. Our main research questions were whether we could replicate the BMI association and whether there was a direct association of these polymorphisms with lifestyle factors. We also investigated whether the polymorphisms have an effect on obesity by modulating lifestyle factors.

Subjects and Methods

Study population

As part of the World Health Organization Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) project and the Cooperative Health Research in the Region of Augsburg (KORA) project, four independent cross-sectional population-based surveys (S1-S4) were conducted in the city of Augsburg and two adjacent counties. This study is based on 12 462 genotyped participants (6 271 men and 6 191 women) with German passports aged 25-74 years from the surveys S2 (1989/90), S3 (1994/95), and S4 (1999-2001). All of them gave written informed consent to genetic analysis. The potential of population stratification was reported to be small in KORA (26). Given genome-wide data on a subset of the KORA subjects, the lambda factor for the single nucleotide polymorphism (SNP)-BMI association was 1.02, indicating no major population stratification in this study. Details of the study population have been previously described (27,28).

Assessment of demographic, lifestyle, and clinical characteristics

Standardized interviews to obtain demographic and lifestyle variables and medical examination were conducted by trained medical staff. BMI (kg/m^2) was calculated as body weight in kg measured in light clothing to the nearest 0.1 kg divided by squared body height in m^2 measured to the nearest 0.5 cm.

A four-category seasonal physical activity score was assessed from questions on leisure time sports in summer and winter: 1 = regularly >2 h, 2 = regularly about 1 h, 3 = irregularly about 1 h, 4 = no sports on a weekly basis during leisure time (29). From self-reported alcohol intake for the previous workday and the previous weekend, alcohol consumption was calculated in gram per day (g per day) (30,31). Scores of the frequency of consuming fat or carbohydrate containing foods were constructed based on a validated qualitative food frequency questionnaire with 24 items. The subjects were asked for the frequency (almost daily, several times per week, about once a week, several times per month, once a month or less and never) of the usual intake of food groups (32).

Genotyping

The top signals reported by Willer et al (rs6548238 near *TMEM18*, rs10938397 near *GNPDA2*, rs10838738 within *MTCH2*, rs7498665 within *SH2B1* and rs11084753 near *KCTD15)* or polymorphisms highly correlated with them (rs10789336 (r^2=0.93 with rs2815752) near *NEGR1*, rs9935401 (r^2=1.0 with rs9939609) within *FTO*, and rs17700144 (r^2=0.84 with rs17782313) near *MC4R*) were selected for genotyping (3).

Samples were genotyped with the MassARRAY system using the iPLEX Gold chemistry (Sequenom, San Diego, CA, USA). The allele-dependent primer extension products were loaded onto one 384-element chip using a nanoliter pipetting system (SpectroCHIP,

SpectroPOINT Spotter, Sequenom). The samples were analyzed in a matrix-assisted laser desorption ionisation time of flight mass spectrometer (MALDI TOF MS, Bruker Daltonik, Leipzig, Germany). The discordance of the 12.5 % double-genotyped samples was lower than 0.5 %. Fisher's exact test was used to test for deviation from Hardy Weinberg equilibrium (HWE). Three SNPs (rs10789336 (*NEGR1*), rs7498665 (*SH2B1*), rs11084753 (*KCTD15*)) violated HWE ($p<0.05$). One SNP (rs10938397 (*GNPDA2*)) was not genotyped successfully. For all analyzed SNPs, genotyping success rate was 94 %.

Statistical analysis

Linear regression models were used to analyze association of the polymorphisms with BMI, and logistic regression models for the association with dichotomized lifestyle variables. Lifestyle factors were evaluated for their potential as mediators in the association of the polymorphism with BMI according to the guidelines for surrogacy analyses (33) and as applied previously for genetic data (19). Briefly, this involves the following criteria: the genotype is associated with the outcome BMI (model 1); the mediator (lifestyle variable) is associated with the outcome (model 2); the genotype is associated with the mediator (model 3); including the mediator as an additional covariate into model 1, the genotype-outcome association is abolished (model 4). With regard to model 2, we applied two approaches: modelling each lifestyle factor separately ("single lifestyle factor model") as well as modelling all lifestyle factors together ("multiple lifestyle factor model"). Gene-environment or gene-gene interactions were calculated including an interaction term (i) of each genotype and each lifestyle factor (SNP*lifestyle factor) or (ii) of the *TMEM18* genotype and each other genotype (*TMEM18**SNP) or of the *FTO* genotype and each other genotype (*FTO**SNP) or (iii) of *TMEM18* genotype and *FTO* genotype and each lifestyle factor (*TMEM18***FTO**lifestyle factor) in the model. *TMEM18* and *FTO* SNP were selected because of their strong association with BMI. All analyses were adjusted for sex, age, and survey, also conducted by gender, and an additive genetic effect was assumed. The significance level was set to 0.7 % to account for the seven polymorphisms tested. Given the BMI effect sizes, as well as minor allele frequencies (MAFs) reported by Willer et al (3) (*NEGR1*, rs2815752, 0.10 kg/m^2, MAF=0.38 / *TMEM18*, rs6548238, 0.26 kg/m^2, MAF=0.16 / *MTCH2*, rs10838738, 0.07 kg/m^2, MAF=0.34 / *FTO*, rs9939609, 0.33 kg/m^2, MAF=0.41 / *MC4R*, rs17782313, 0.20 kg/m^2, MAF=0.21 / *SH2B1*, rs7498665, 0.15 kg/m^2, MAF=0.41 / *KCTD15*, rs11084753, 0.06 kg/m^2, MAF=0.33), our power to detect these associations was 99 % for *FTO*, 92 % for *TMEM18*, 82 % for *MC4R*, 74 % for *SH2B1*, 40 % for *NEGR1*, 21 % for *MTCH2*, and 17% for *KCTD15*. Power analysis was carried out using the program QUANTO (version 1.2.4., University of Southern California, Los Angeles, CA, USA; http://hydra.usc.edu/gxe). Statistical analyses were carried out using SAS Version 9.1 (SAS Institute, Cary, NC, USA).

Results

Association of polymorphisms with BMI

The baseline characteristics of the study population are given in Table 1. Table 2 summarizes the associations between polymorphisms and BMI (model 1). Significant results were detected for rs6548238 near the *TMEM18* gene (-0.418 kg/m^2, $p=1.22\times10^{-8}$) and rs9935401 within the *FTO* gene (0.290 kg/m^2, $p=2.85\times10^{-7}$). Results were similar for men and women. The polymorphism rs7498665 (*SH2B1*) showed a borderline significant association using a two-sided test. Applying a one-sided test for the direction reported by Willer et al (3), the association reached significance (0.145 kg/m^2, $p=4.92\times10^{-3}$), but was not pronounced in men. None of the other polymorphisms showed a significant association with BMI. Gene-gene interaction tests (*TMEM18* SNP with each other SNP or *FTO* SNP

with each other SNP) showed no statistically significant associations with BMI (P-values between 0.03 and 0.93).

The proportion of the variance of BMI explained by rs6548238 (*TMEM18*) and rs9935401 (*FTO*) and rs7498665 (*SH2B1*) together was 0.006 %.

Association of lifestyle factors with BMI

There were significant associations between lifestyle factors and BMI (model 2) both in the "single lifestyle factor model" (p-values ranging from 8.10×10^{-4} to 6.77×10^{-31}; data not shown) and in the "multiple lifestyle factor model" (P-values ranging from 1.82×10^{-3} to 5.08×10^{-28}; Table 3). High carbohydrate score, high fat score, high alcohol consumption, smoking, and high physical activity were significantly associated with decreased BMI. Interestingly, high fat score was associated with decreased BMI ("single lifestyle factor model: −0.432 kg/m^2, P=1.00×10^{-7}), but less strongly associated when adjusting for carbohydrate score ("multiple lifestyle factor model: −0.265 kg/m^2, P=1.82×10^{-3}). The association of all investigated lifestyle factors with BMI was stronger among women compared with men. There were significant differences between men and women for the association of fat score (P-value for gender difference=3.76×10^{-4}), alcohol consumption (P=1.78×10^{-17}), smoking (P=2.52×10^{-10}) and physical activity (P=4.08×10^{-6}) with BMI, but not for carbohydrate score (P=0.06). Lifestyle factors together explained 0.037 % of the variance of BMI. Age and sex explained together 0.10 %. All lifestyle factors together with age, sex, and survey explained 0.121 % of BMI variance.

Association of polymorphisms with lifestyle factors

There was no evidence for the association between genetic variants and lifestyle factors (model 3), although polymorphisms rs6548238 (*TMEM18*) and rs11084753 (*KCTD15*) showed a trend toward an association with fat score (overall: OR=1.081, P=0.03 and OR=1.066, P=0.03, respectively; Table 4). Polymorphism rs9935401 (*FTO*) showed a trend toward an association with smoking (overall: OR=0.936, P=0.02) and rs10789336 (*NEGR1*) was weakly associated with alcohol consumption (overall: OR=0.903, P=1.50×10^{-3}; Table 4). Gene-environment interaction tests showed no significant association with BMI (data not shown). A trend was seen for the interaction rs9935401 and alcohol consumption (−0.411 kg/m^2, P=2.64×10^{-3}. The more complex interaction terms including *TMEM18* SNP, *FTO* SNP and one lifestyle factor showed no significant association (P>0.05).

Mediator analyses: Lifestyle factors as covariates in the genotype-outcome association

Results with lifestyle factors as covariates in the genotype-outcome (BMI) association model (model 4) are shown in Supplementary Table 1. Adjustment for carbohydrate score, fat score, alcohol consumption or physical activity did not change the associations between genotype and BMI. Including the covariate smoking slightly lowered the P-value for the association of rs6548238 (*TMEM18*) on BMI. Gender-specific analysis provided similar results (data not shown).

Discussion

Our major findings are the confirmed associations of the *TMEM18*, *FTO* and *SH2B1* polymorphisms with BMI in a large homogenous study on adults. Our data underscore the strong role of age, sex, and lifestyle factors as correlates with BMI, compared with which the genetic factors have a minor contribution in the general population. There is weak evidence for an association of the *TMEM18* SNP with fat intake and of the *FTO* SNP with smoking. There is no evidence that lifestyle factors act as a mediator within the association between genotype and BMI.

Genetic risk factors

This is the first study positively replicating *TMEM18* (rs6548238) as a locus for obesity in adults in a homogenous study sample apart from the two initial reports from meta-analyses of genome-wide association studies (3,4). However, it should be noted that there was an overlap of our sample with the gene discovery analysis (3) of 13 % (n=1 600). In our study, BMI was reduced by −0.4 kg/m^2 per minor allele T of rs6548238, which corresponds to 2.4 kg for a person with height of 1.70 m. The odds for a person to be obese (BMI ≥ 30 kg/m^2) were decreased by 14 % per minor allele. An association between *TMEM18* variants and BMI was recently reported in children (n=6 078) (34). In Dutch females (n=1 700) and Swedish adults (n=3 885), the effect of *TMEM18* gene on obesity risk could not be confirmed, which might be due to low power (25,35).

With regard to *FTO*, multiple replication studies have already substantiated a strong association between the *FTO* gene and BMI (36,37). Per minor allele of the polymorphism rs9935401, which was highly correlated with the leading variant in the gene discovery study (rs9939609), we found a BMI increase by 0.3 kg/m^2 and an increased OR for obesity of 17 %.

The association between *SH2B1* (rs7498665) could already be replicated in Swedish adults (35), but failed replication in other reports (25,34), also most likely due to limited power. An association of the other obesity-related loci reported with BMI by Willer et al. (*NEGR1, MTCH2, MC4R,* and *KCTD15)* could not be confirmed in this MONICA/KORA sample, which could be due to a limited power for the small associations of these variants despite our substantial sample size. It could also be due to violation of HWE for our *NEGR* and *KCTD15* SNP genotypes, which might have derived from these SNPs being within or near copy number variations as already described for *NEGR1* (3).

Lifestyle risk factors

Our data are in line with a predominant association of lifestyle factors on BMI, which was more pronounced in women. The strongest association was found for high versus low physical activity with a decrease of −0.9 kg/m^2 in BMI, which is similar to previous reports (29,38). The picture for dietary variables is more complex: high carbohydrate intake was associated with decreased BMI. This might point toward a beneficial or antiobesogenic effect of a high carbohydrate diet. However, this view is disrupted by the lack of association of low fat intake with decreased BMI (Table 3). A reason for the more difficult pattern of dietary variables could be a high measurement error in these variables: first, quantitative assessment of food intake is difficult and – independently of the method used – associated with a high error rate of up to 75 % (39). Second, the intake of healthy foods might often be overestimated and that of fat-containing foods underestimated due to ignoring hidden fats (for example in salad dressings). Measurement error could even be differential between obese and nonobese subjects because of a different intentional or unintentional attempt of more obese persons to underreport the amount of food or fat intake (40,41).

Most notably, high fat intake score was associated with a lower BMI, which was to some part confounded by the association between higher carbohydrate intake score and lower BMI. This points towards a close relationship between lifestyle factors and the need to view these as a system rather than studying them separately.

Our data are in line with previous studies showing a significantly lower BMI in smokers compared with never smokers (42,43) and an inverse relation between alcohol consumption and BMI. Interestingly, the inverse alcohol-BMI relation is only seen in women, which might be due to the different selection of alcoholic beverages between men and women (44,45).

Comparison of the BMI variance explained by genetic factors versus lifestyle factors

We find the percentage of BMI variance explained by lifestyle factors to be substantially larger than the BMI variance explained by the genetic factors explored here, which can be assumed to be the strongest common genetic factors for obesity in the general population. The clear discrepancy between the heritability estimated as 70 percent (46) from twins raised apart as compared with the percentage of BMI variance explained by currently known genetic factors is a subject of high debate. One explanation is that unknown rare variants might have a role in obesity, which are hard to detect by current methodology. In any case, our data underscore the great importance of lifestyle factors compared with these common genetic factors studied here.

Genetic associations on BMI mediated through lifestyle factors?

There was neither evidence in our sample that lifestyle factors were mediators of the association between genotypes and BMI, nor was there a clear direct association between genotype and lifestyle factors. This could be due to low power – considering the small effects and the potentially high assessment error in lifestyle factors – or due to a real lack of an association.

Beside the deliberate hypothesis of a potential role in the central nervous system, the physiological role of the genetic variants within obesity-related loci is not clear. For *FTO*, mouse models were carried out to explain their role in physiological systems such as energy homeostasis (14,47). For the *TMEM18* gene, no functional studies are available and the SH2B protein has a role in leptin signalling (10). For *FTO* and *MC4R*, there is diverging evidence from association studies that they have an effect on energy intake and expenditure. A Danish study revealed that low physical exercise might accentuate the effect of the *FTO* gene on body weight (48). In contrast, in Swedish and Finnish adults, there was no significant interaction between physical activity and the *FTO* variant rs9939609 on BMI (49). There are findings that genetic variants (*FTO*, *MC4R*) influence dietary intake (17,18,20,50) and satiety (16,51), but there are also studies in which no association between genetic variants near the *MC4R* and energy or dietary intake could be detected (25,52).

It is a great opportunity to investigate human data in a large homogeneous study in the attempt to learn about associations between the genetic polymorpisms on energy intake or expenditure. Until now, there is only one study addressing the same focus in a substantially smaller sample (n=1 700) (25). Our results underscore that attempts to seek replication for the reported obesity-loci requires a substantial sample size that not even our study with > 10,000 individuals fulfills completely. Our results also indicate that the genetic associations on BMI cannot easily be pinpointed to lifestyle factors by epidemiological studies.

Strengths and limitations of this study

The strengh of our study is that we analyzed a large homogenous population-based and well-phenotyped cohort. Furthermore, our mediator analysis is a systematic approach to examine the potential mediator role of lifestyle factors in the relationship between genetic variants and obesity. The limitations of our data are the missing information of total energy intake and the lack of information on absolute carbohydrate and fat intake in gram, which may have given a better insight into real dietary habits. Total energy intake adjustment may lead to a more precise association between nutritional factors and BMI. Despite the large sample of more than 12 000 subjects, the limited power and the violation of HWE in three of the investigated polymorphisms also need to be considered as limitations.

Conclusion

In conclusion, our data provide evidence for genetic (*TMEM18*, *FTO*, *SH2B1*) and environmental (dietary habits, alcohol consumption, smoking, physical activity) factors being associated with BMI in a large homogenous population-based study. We find great value in attempting to support the pathways with epidemiological data, but there were no clear associations of the polymorphisms with lifestyle factors directly, nor were lifestyle factors clear mediators of the genetic association with BMI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of the study population

	Overall		Men		Women	
	n	**Mean ± s.d. or %**	**n**	**Mean ± s.d. or %**	**n**	**Mean ± s.d. or %**
General factors						
Age (years)	12462	49.38 ± 13.97	6271	49.82 ± 14.10	6191	48.94 ± 13.82
Education (< 12 years) *1)*	12462	68.73 %	6271	60.87 %	6191	76.69 %
Anthropometric factors						
BMI (kg/m^2)	12357	26.97 ± 4.49	6231	27.32 ± 3.81	6126	26.61 ± 5.07
Height (cm)	12421	167.92 ± 9.32	6249	174.25 ± 7.06	6172	161.51 ± 6.52
Lifestyle factors						
High carbohydrate score (≥median) *2)*	12426	54.30 %	6250	55.55 %	6176	52.80 %
High fat score (≥median) *2)*	12423	58.75 %	6248	59.57 %	6175	57.93 %
Smoking (ever smokers) *3)*	12458	55.47 %	6268	69.10 %	6190	41.68 %
High alcohol ≥40g/d (men) / ≥20g/d (women) *4)*	12438	22.09 %	6271	26.76 %	6191	17.28 %
High physical activity (scores 1 and 2) *5)*	12441	43.47 %	6257	45.02 %	6184	41.90 %

Abbreviation: BMI, body mass index.

1) Educational level was categorized according to <12or ≥12 years of schooling including job training

2) Dichotomized at medians (high carbohydrate score: ≥44 for overall, ≥43 for men, ≥45 for women; high fat score: ≥20 for overall, men, and women)

3) Subjects currently smoking at least one cigarette per day were defined as smokers

4) Alcohol intake was dichotomized as ≥40 g/day for men and ≥20 g/day for women

5) Physical activity was dichotomized as 2 = high activity (scores = 1 and 2) and 1 = low activity (scores = 3 and 4)

Mean ± s.d. (standard deviation) or percentage (%) is shown.

Table 2

Association of SNPs (*genotype*) with BMI (*outcome*) – model 1

					Overall			Men			women		
SNP	**Gene**	**Chr.**	**Minor allele**	**MAF [%]**	**n**	**Estimate [kg/m^2]**	**P-value**	**n**	**Estimate [kg/m^2]**	**P-value**	**n**	**estimate [kg/m^2]**	**p-value**
rs10789336	*NEGR1*	1	G	39	11290	−0.035	0.54	5650	−0.053	0.44	5640	−0.022	0.80
rs6548238	*TMEM18*	2	T	17	11687	−0.418	1.22×10^{-8}	5856	−0.350	1.03×10^{-4}	5831	−0.475	3.30×10^{-5}
rs10838738	*MTCH2*	11	G	33	11771	−0.064	0.27	5916	−0.111	0.12	5855	−0.015	0.87
rs9935401	*FTO*	16	A	41	11701	0.290	2.85×10^{-7}	5875	0.206	2.82×10^{-3}	5826	0.364	4.08×10^{-5}
rs17700144	*MC4R*	18	A	23	11693	0.101	0.13	5863	0.157	0.06	5830	0.067	0.52
rs7498665	*SH2B1*	18	G	39	11683	0.145	9.83×10^{-3}	5851	0.043	0.53	5832	0.236	7.89×10^{-3}
rs11084753	*KCTD15*	19	A	33	11814	0.012	0.83	5922	−0.045	0.52	5892	0.076	0.41

Abbreviations: BMI, body mass index; Chr., chromosome; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

Beta-estimates per minor allele and P-value from linear regression of SNP on outcome BMI, adjusted forage, sex, and survey are given for overall and gender specific analyses;

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Table 3

Association of lifestyle factors (*mediator*) with BMI (*outcome*) – model 2

	Overall(n=12297)		Men (n=6200)		Women (n=6103)	
Lifestyle factor	**Estimate [kg/m^2]**	**P-value**	**Estimate [kg/m^2]**	**P-value**	**Estimate [kg/m^2]**	**P-value**
High carbohydrate score	−0.422	3.19×10^{-7}	−0.282	5.18×10^{-3}	−0.465	2.91×10^{-4}
High fat score	−0.265	1.82×10^{-3}	−0.179	0.08	−0.284	0.04
High alcohol consumption	−0.477	3.19×10^{-7}	0.099	0.35	−1.228	1.15×10^{-14}
Ever smoking	−0.273	7.26×10^{-4}	0.230	0.02	−0.495	9.57×10^{-5}
High physical activity	−0.861	5.08×10^{-28}	−0.657	6.85×10^{-12}	−1.052	1.84×10^{-17}

Abbreviation: BMI, body mass index. Association estimates and P-values from linear regression of lifestyle factors on the outcome BMI, adjusted for age, sex, survey and all lifestyle factors are shown for the effects of high (≥median) versus low (reference) carbohydrate or fatscore, high (men: ≥40 g per day; women: ≥ 20 g per day) versus low (reference) alcohol consumption, ever versus never smokers (reference), high (scores 1 and 2) versus low (reference) physical activity.

Table 4

Association of SNPs (*genotype*) with lifestyle factors (*mediator*) – model 3

		Carbohydrate score		Fat score		Alcohol consumption		Smoking behaviour		Physical activity	
SNP	**Gene**	**OR**	**P-value**	**OR**	**P-value**	**OR**	**p-value**	**OR**	**P-value**	**OR**	**P-value**
Overall											
rs10789336	NEGR1	1.033	0.22	0.999	0.96	0.903	1.50×10^{-3}	0.979	0.45	0.973	0.31
rs6548238	TMEM18	1.035	0.32	1.081	0.03	1.009	0.83	0.951	0.17	1.027	0.45
rs10838738	MTCH2	1.020	0.47	1.018	0.54	0.979	0.53	0.969	0.28	1.036	0.21
rs9935401	FTO	0.989	0.69	1.003	0.91	0.954	0.14	0.936	0.02	0.975	0.34
rs17700144	MC4R	1.042	0.19	0.998	0.95	0.985	0.69	1.035	0.29	1.005	0.89
rs7498665	SH2B1	1.020	0.46	0.974	0.35	0.996	0.89	1.031	0.27	0.962	0.16
rs11084753	KCTD15	1.039	0.16	1.066	0.03	1.006	0.86	1.016	0.59	0.988	0.67
Men											
rs10789336	NEGR1	1.058	0.13	0.952	0.21	0.933	0.10	1.037	0.37	0.995	0.89
rs6548238	TMEM18	1.065	0.20	1.027	0.61	0.967	0.55	0.945	0.28	0.995	0.93
rs10838738	MTCH2	1.030	0.45	1.067	0.11	0.911	0.03	0.937	0.12	1.050	0.22
rs9935401	FTO	1.016	0.68	1.020	0.62	0.961	0.35	0.934	0.09	0.953	0.21
rs17700144	MC4R	1.031	0.50	0.969	0.51	0.938	0.21	1.036	0.47	1.006	0.90
rs7498665	SH2B1	1.046	0.24	0.965	0.36	1.008	0.85	1.047	0.25	0.980	0.60
rs11084753	KCTD15	1.047	0.23	1.089	0.03	0.994	0.89	1.001	0.97	1.005	0.89
Women											
rs10789336	NEGR1	1.038	0.32	1.049	0.23	0.867	3.85×10^{-3}	0.932	0.07	0.953	0.21
rs6548238	TMEM18	1.041	0.41	1.137	0.01	1.062	0.34	0.941	0.24	1.059	0.25
rs10838738	MTCH2	1.020	0.61	0.971	0.48	1.067	0.20	0.992	0.83	1.018	0.65
rs9935401	FTO	0.966	0.36	0.988	0.77	0.943	0.24	0.941	0.12	0.995	0.90
rs17700144	MC4R	1.023	0.61	1.025	0.59	1.043	0.46	1.019	0.69	1.003	0.94
rs7498665	SH2B1	1.034	0.38	0.987	0.75	0.982	0.71	1.028	0.48	0.944	0.13
rs11084753	KCTD15	0.996	0.93	1.041	0.34	1.026	0.61	1.031	0.46	0.971	0.46

Abbreviations: OR, odds ratio; SNP, single-nucleotide polymorphism.

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ORs and P-values show the effect per minor allele; lifestyle factors were dichotomized with higher versus lower (reference) scores for carbohydrate and fat intake, alcohol consumption, and physical activity,with smoking versus never smoking