Influence of common variants near INSIG2, in FTO, and near MC4R genes on overweight and the metabolic profile in adolescence

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Influence of common variants near *INSIG2*, in *FTO*, and near *MC4R* genes on overweight and the metabolic profile in adolescence: the TRAILS (TRacking Adolescents' Individual Lives Survey) Study[1–3]

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ABSTRACT

Background: Overweight is a complex trait in which both environmental and genetic factors play a role.

Objective: We aimed to evaluate the influence of common genetic variants identified by genome-wide association studies on overweight and the metabolic profile in adolescence.

Design: In a population-based cohort of 663 girls and 612 boys aged 16 y, weight, height, skinfold thicknesses, percentage body fat, waist circumference, blood pressure, glucose, insulin, lipid profile, and DNA were obtained. We defined overweight according to international criteria. We performed multiple linear and logistic regression analyses to assess the influence of candidate single nucleotide polymorphisms near the *INSIG2,* in the *FTO*, and near the *MC4R* genes and repeated-measures analyses of available body mass index (BMI) and skinfold thickness data across 3 visits at ages 11, 13.5, and 16 y.

Results: A total of 15.1% of participants were overweight or obese at age 16 y. No associations with *INSIG2* were found. Common variation in the *FTO* gene was associated with sex-specific *z* scores of BMI (B: 0.11; 95% CI: 0.03, 0.19), sum of skinfold thicknesses (B: 0.12; 95% CI: 0.04, 0.20), percentage body fat (B: 0.11; 95% CI: 0.03, 0.19), waist circumference (B: 0.11; 95% CI: 0.03, 0.19), fasting glucose (B: 0.10; 95% CI: 0.00, 0.20), and overweight (odds ratio: 1.34; 95% CI: 1.06, 1.69) at age 16 y. Repeated-measures analyses confirmed the associations for BMI and sum of skinfold thicknesses, and physical activity did not modify these associations. Common variation near the *MC4R* gene was associated with BMI in cross-sectional (B: 0.11; 95% CI: 0.02, 0.20) and repeated-measures (B: 0.12; 95% CI: 0.03, 0.20) analyses.

Conclusions: Common variation in the *FTO* gene is associated with overall and abdominal adiposity. Variation near the *MC4R* gene is associated with BMI. These findings in adolescents strengthen and extend the results from previous research. *Am J Clin Nutr* 2010;91:321–8.

INTRODUCTION

Overweight is associated with an increased risk of diabetes, hypertension, dyslipidemia, and cardiovascular disease. It is known that childhood overweight tends to track into adolescence and adulthood (1, 2). Moreover, epidemiologic studies have shown that, already in childhood, total and abdominal fat appear to be significantly associated with an unfavorable metabolic profile, including insulin resistance, elevated LDL cholesterol, and decreased HDL cholesterol (3, 4). Thus, childhood overweight poses a major public health concern.

Lifestyle factors such as increased energy intake and decreased physical activity are probably the main determinants of the increased prevalence of childhood overweight. Genetic background predicts an individual's susceptibility to weight change resulting from a certain lifestyle (5, 6). Multiple genes are involved, probably interacting with each other and with environmental factors, implying a multifactorial trait. Recently, genome-wide association studies in large cohorts have identified common variants associated with overweight. The variant near *INSIG2* was the first to be associated with BMI in a genome-wide association study (7). Although it was shown that variation near *INSIG2* predicted the amount of weight loss during treatment of obese children and adolescents (8), the association between the variant near *INSIG2* and overweight was not confirmed by other

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[2] This study was performed within the Groningen Expert Center for Kids with Obesity, supported by Hutchison Whampoa Ltd and by the University Medical Center Groningen. This research is part of the TRacking Adolescents' Individual Lives Survey (TRAILS). Participating centers of TRAILS include various departments of the University Medical Center and University of Groningen, the Erasmus University Medical Center Rotterdam, the University of Utrecht, the Radboud Medical Center Nijmegen, and the Trimbos Institute, all in the Netherlands. Principal investigators: J Ormel (University Medical Center Groningen) and FC Verhulst (Erasmus University Medical Center). TRAILS has been financially supported by various grants from the Netherlands Organization for Scientific Research NWO (Medical Research Council program grant GB-MW 940-38-011; ZonMW Brainpower grant 100-001-004; ZonMw Risk Behavior, and Dependence grants 60-60600-98-018 and 60-60600-97-118; ZonMw Culture and Health grant 261-98-710; Social Sciences Council medium-sized investment grants GB-MaGW 480-01-006 and GB-MaGW 480-07-001; Social Sciences Council project grants GB-MaGW 457-03-018, GB-MaGW 452-04-314, and GB-MaGW 452-06-004; and NWO large-sized investment grant 175.010.2003.005); the Sophia Foundation for Medical Research (projects 301 and 393), the Dutch Ministry of Justice (WODC), and the participating universities.

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studies (9–13). An association between common variation in the *FTO* gene and BMI was identified by 3 groups (14–16), 2 of which used genome-wide association studies (15, 16). This was confirmed in multiple follow-up studies (17–25). Recently, common variants near the *MC4R* gene have been found to influence BMI in whites (26) and waist circumference and insulin resistance in Indian Asians (27).

In most of these studies, an association with childhood overweight was also found, not only for *FTO* (14, 15, 19, 24, 25) but also for variants near the *INSIG2* (7) and *MC4R* (26) genes. In 2 pediatric studies (15, 24), overweight was assessed on the basis of BMI and a dual-energy X-ray absorptiometry scan, more specifically measuring overall adipose tissue. However, few studies have evaluated abdominal fat (28) or overweight-related metabolic profiles (29) in children. These are important because they confer an increased risk of later metabolic complications such as diabetes and cardiovascular disease. Because results obtained by Wåhlén et al (30) suggest that *FTO* could be involved in body weight regulation through lipolysis, it would also be interesting, from a pathophysiologic point of view, to evaluate whether these genetic variants are associated with metabolic traits, such as lipids, and, if so, whether these associations are driven by BMI, as was found in studies of adults (31).

The aim of this study was to assess the influence of common genetic variants found through genome-wide association studies, more specifically variants near the *INSIG2*, in the *FTO*, and near the *MC4R* genes, on overweight and its related metabolic traits at age 16 y. We assessed overweight by BMI, total body fat by sum of skinfold thicknesses and total body impedance analysis, abdominal adiposity by waist circumference, and metabolic profile by blood pressure, glucose, and lipids. In addition, BMI (at ages 11 and 13.5 y) and sum of skinfold thicknesses (at age 11 y) were available from earlier visits. Finally, we aimed to evaluate possible modification of genetic effects by physical activity.

SUBJECTS AND METHODS

Study population

Our study was performed in the TRAILS (TRacking Adolescents' Individual Lives Survey) population, an ongoing Dutch prospective cohort study assessing both psychosocial and physical health from preadolescence into adulthood. Sample selection was described elsewhere (32). In brief, children were recruited through community registers and through their schools to obtain a representative sample. The present study included mainly data from the third assessment visit, during which most overweight-related data were collected. This visit took place in 2005–2007 at a mean (±SD) age of 16.2 ± 0.67 y. In addition, during 2 previous visits (in 2001–2002 at age 11.1 ± 0.55 y and in 2003–2004 at age 13.5 ± 0.52 y), weight and height (first and second visits) and skinfold thicknesses (first visit only) were measured, which we included in our repeated-measures analyses. For this study we included participants for whom DNA was available (n = 1460). We excluded all participants who were not of northern European ancestry (n = 161) and the second of all siblings within the cohort (n = 25), which resulted in a population of 1275 adolescents (52.0% girls). All procedures were approved by the Dutch Central Committee on Research Involving Human Subjects. Written informed consent, including specific consent to undertake genetic analyses, was obtained from participants and their parents or custodians.

Measures

We measured weight and height using regularly calibrated equipment (models 770 and 214, respectively; Seca, Hamburg, Germany). Body mass index (BMI; in kg/m^2) was also calculated. We defined overweight and obesity according to international age- and sex-adjusted BMI criteria (equivalent to the Dutch 94th and 99.7th percentiles in 1980 for overweight and obesity, respectively) (33). We obtained triceps, biceps, subscapular, and suprailiac skinfold thicknesses with a Harpenden skinfold caliper (CMS Instruments, London, United Kingdom); and the sum of 4 thicknesses was calculated. We measured waist circumference at the midpoint between the lower costal margin and the iliac crest. We performed all measurements in duplicate, and, if the difference between these measurements exceeded a predefined value, a third measurement was performed. All available measurements were used to calculate means. We performed a hand-to-foot bioelectrical impedance analysis (type BIA 101; Akern, Pontassieve, Italy), from which percentage body fat (%BF) was calculated by using the Deurenberg equation (34). Systolic (SBP) and diastolic (DBP) blood pressure were measured in duplicate with a Dinamap Critikon 1846SX (Critikon Inc, Tampa, FL), from which we calculated means.

We obtained a blood sample after ≥8 h of fasting for the measurement of glucose (Roche Diagnostics, Basel, Switzerland), insulin (Diagnostic Systems Laboratories Inc, Webster, TX), triglycerides, total cholesterol, and HDL cholesterol (Roche Diagnostics). LDL cholesterol was calculated according to Friedewald's equation (35). The presence of the metabolic syndrome was determined according to the International Diabetes Federation (IDF) criteria (36, 37), based on age-specific cutoffs for waist circumference, triglycerides, HDL cholesterol, blood pressure, and glucose.

Questionnaires were filled out to assess pubertal stage (Physical Development Scale questionnaire; 38) and physical activity. We asked how many days per week the adolescents participated in ≥60 min of moderate or vigorous physical activity, from which "sufficient physical activity" was determined as ≥5 d/wk, in accordance with international recommendations (39).

Genotyping

We extracted DNA from buffy coats (n = 1216) or buccal swabs (Cytobrush) (n = 59) using a manual salting out procedure similar to the protocol described by Miller et al (40). Genotyping was performed on the Illumina BeadStation 500 platform (Illumina Inc, San Diego, CA) by laboratory personnel blinded to the true identity of the individual samples. Scan data were analyzed and genotyped in BeadStudio 3.0 (Illumina Inc, San Diego, CA). For this study, we used genotype data from rs7566605 (*INSIG2*), rs9939609 (*FTO*), rs17782313 (*MC4R*), and rs17700633 (*MC4R*). Call rates were 100% for all but rs17782313, which could be genotyped in 99.9% of the participants. Genotyping accuracy for our single nucleotide

TABLE 1
Genotype and minor allele frequencies[1]

Gene	*n*	rs	Genotype frequency 11	12	22	MAF	HWE *P* value
INSIG2	1275	7566605	0.48	0.43	0.10	0.31	0.968
FTO	1275	9939609	0.39	0.47	0.14	0.38	0.967
MC4R	1274	17782313	0.56	0.37	0.06	0.25	0.837
MC4R	1275	17700633	0.49	0.43	0.09	0.30	0.562

[1] 1 = major allele, 2 = minor allele. MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium (determined by Pearson's chi-square test).

polymorphisms (SNPs), as determined by concordance between duplicates, was 100%. Genotype frequencies of all SNPs were in Hardy-Weinberg equilibrium (**Table 1**).

Data analysis

Weight, BMI, thicknesses, waist circumference, SBP, and triglycerides were log transformed to obtain a better approximation of the normal distribution, before calculating age- and sex-specific *z* scores with the use of means and SDs.

We performed multiple linear regression analyses for weight, height, BMI, skinfold thicknesses, and %BF *z* scores and all components of the metabolic syndrome, ie, waist circumference, SBP, DBP, glucose, HDL cholesterol, and triglycerides *z* scores. On the basis of previous reports (7, 15, 26), *INSIG2* genotypes were analyzed under a recessive model; *FTO* and *MC4R* genotypes under an additive model. We adjusted all models for age and pubertal stage. To evaluate the influence on overweight (including obesity) and the metabolic syndrome, we performed multiple logistic regression analyses. In all regression analyses, we evaluated the interaction of genotypes with sex and physical activity by adding a multiplicative term to the models. We also evaluated the interaction between genotypes.

Because weight and height were also measured at age 11 y and age 13.5 y and skinfold thicknesses at age 11 y, we additionally performed repeated-measures analyses (ie, linear mixed-effect models) for weight, height, BMI, and skinfold thicknesses to assess the association between the genotypes and changes in weight, height, and BMI (3 time points) and thicknesses (2 time points) from age 11 y to age 16 y. Simultaneous adjustment for age and pubertal stage in these repeated-measures analyses was not feasible because of multicollinearity. Because pubertal stage had the highest number of missing values, the models were adjusted only for age. In subanalyses, we evaluated the interaction between genotypes and the interaction between genotypes and physical activity and sex by adding multiplicative terms.

We used Quanto to calculate the power available to detect main effects and interaction effects in our cross-sectional analyses (*see* supplementary Tables 1 and 2 under "Supplemental data" in the online issue) (41). All other statistical analyses were performed by using SPSS version 14.0 (SPSS, Chicago, IL). The level of statistical significance was set at a probability of <0.05.

RESULTS

Our population consisted of 663 girls and 612 boys, with a mean (±SD) age of 16.2 ± 0.67 y. At this age, 12.4% were overweight and 2.7% were obese (**Table 2**). Compared with girls, boys showed less advanced pubertal stage, were more physically active, were heavier and taller, and had a lower BMI, sum of skinfold thicknesses, and %BF (Table 2). The prevalence of the metabolic syndrome was 4.5%. Significant sex differences were found for all metabolic characteristics (**Table 3**). To evaluate selection bias, we compared the 1275 participants included in this study with the original sample of 1868 children who participated in the BMI measurements at age 11 y. Compared with the 593 who were either excluded ($n = 25$) or lost to

TABLE 2
Sociodemographic and anthropometric characteristics according to sex

	All		Girls		Boys		
	Value	*n*	Value	*n*	Value	*n*	*P* for sex effect[1]
Age (y)	16.2 ± 0.7[2]	1275	16.3 ± 0.7	663	16.2 ± 0.6	612	0.17
Pubertal stage in 3 categories (%)[3]	15.9/28.7/55.4	1199	0.8/0.3/98.9	631	32.7/60.2/7.0	568	<0.001
Physical activity (% sufficient)[4]	29.8	1230	26.4	644	33.6	586	0.01
Socioeconomic status in 3 categories (%)[5]	19.4/50.3/30.3	1263	18.5/52.4/29.0	658	20.3/47.9/31.7	605	0.28
Weight (kg)	63.1 (57.7–70.3)[6]	1244	60.6 (55.5–66.0)	645	66.0 (60.0–74.6)	599	<0.001
Height (cm)	174.5 ± 8.9	1248	169.2 ± 6.3	649	180.3 ± 7.6	599	<0.001
BMI (kg/m^2)	20.75 (19.20–22.55)	1244	21.21 (19.56–22.93)	645	20.27 (18.81–22.19)	599	<0.001
Overweight/obese (%)	12.4/2.7	1244	14.0/2.2	645	10.7/3.2	599	0.13
Sum of skinfold thicknesses (mm)	47 (32–65)	1236	59 (47–73)	642	32 (26–47)	594	<0.001
Body fat (%)	28.2 ± 5.6	1222	31.3 ± 4.3	631	25.0 ± 4.8	591	<0.001
Waist circumference (cm)	73.8 (70.0–78.9)	1243	73.8 (69.7–79.4)	647	73.8 (70.3–78.3)	596	0.51

[1] *P* values were obtained by using a chi-square test for pubertal stage, physical activity, socioeconomic status, and overweight or obese; a *t* test for age, height, and percentage body fat; and a Mann-Whitney *U* test for weight, BMI, sum of skinfold thicknesses, and waist circumference.
[2] Mean ± SD (all such values).
[3] Measured by the Physical Development Scale questionnaire and classified as pre/early pubertal, midpubertal, and late/postpubertal.
[4] Sufficient physical activity defined as ≥60 min of moderate or vigorous physical activity ≥5 d/wk, in accordance with international recommendations.
[5] On the basis of questionnaires and classified as low, medium, and high.
[6] Median; interquartile range in parentheses (all such values).

TABLE 3
Metabolic characteristics according to sex

	All		Girls		Boys		
	Value	*n*	Value	*n*	Value	*n*	*P* for sex effect[1]
Systolic blood pressure (mm Hg)	117 (109–127)[2]	1252	113 (107–122)	652	122 (113–132)	600	<0.001
Diastolic blood pressure (mm Hg)	61 ± 7[3]	1252	62 ± 7	652	61 ± 7	600	<0.001
Glucose (mmol/L)	4.5 ± 0.4	955	4.5 ± 0.4	504	4.6 ± 0.4	451	<0.001
Insulin (mU/L)	12.0 (9.1–15.3)	948	12.1 (9.5–16.0)	503	11.0 (8.5–15.0)	445	0.003
Total cholesterol (mmol/L)	3.8 ± 0.7	956	4.0 ± 0.7	505	3.6 ± 0.7	451	<0.001
HDL cholesterol (mmol/L)	1.5 ± 0.3	956	1.5 ± 0.3	505	1.4 ± 0.3	451	<0.001
LDL cholesterol (mmol/L)	2.2 ± 0.6	956	2.4 ± 0.6	505	2.2 ± 0.6	451	<0.001
Triglycerides (mmol/L)	0.69 (0.52–0.92)	956	0.72 (0.56–0.96)	505	0.63 (0.49–0.88)	451	<0.001
Metabolic syndrome (%)[4]	4.5	—	4.5	—	4.5	—	0.96

[1] *P* values were obtained by using a chi-square test for the metabolic syndrome; a *t* test for diastolic blood pressure, glucose, total cholesterol, and HDL and LDL cholesterol; and a Mann-Whitney *U* test for systolic blood pressure, insulin, and triglycerides.
[2] Median; interquartile range in parentheses (all such values).
[3] Mean ± SD (all such values).
[4] Defined according to the International Diabetes Federation criteria on the basis of age-specific cutoffs for waist circumference, triglycerides, HDL cholesterol, blood pressure, and glucose.

follow-up ($n = 568$) between the first and third assessment visits, there were no statistically significant differences in BMI *z* score ($P = 0.19$) and sum of thicknesses *z* score ($P = 0.07$).

Because the analyses of sum of skinfold thicknesses provided results similar to those from the analyses of all 4 thicknesses separately, only analyses regarding the former were reported. For none of the models, the interaction with sex was significant. Therefore, we did not report these results.

INSIG2

We found no associations between the SNP near *INSIG2* and measures of overweight or metabolic traits, neither in the cross-sectional analyses (**Table 4**) nor in the repeated-measures analyses (**Table 5**).

FTO

Linear regression analyses under an additive model, adjusted for sex and pubertal stage, showed that rs9939609 was significantly associated with weight (B: 0.11; $P = 0.01$), BMI (B: 0.11; $P = 0.01$), sum of skinfold thicknesses (B: 0.12; $P = 0.004$), % BF (B: 0.11; $P = 0.01$), waist circumference (B: 0.11; $P = 0.01$), and fasting glucose (B: 0.10; $P = 0.04$) (Table 4). *FTO* explained 0.5–0.7% of the variance in these outcome measures. Adjustment for BMI or %BF in the model for waist circumference resulted in nonsignificant results for *FTO* genotype. Adjustment for BMI, %BF, or waist circumference in the association between *FTO* and glucose did not change the results (all B values = 0.10; $P = 0.048$, 0.044, and 0.042, respectively). No significant modification by physical activity was found in the associations

TABLE 4
Associations between genotypes and overweight-related measures

	n	*INSIG2* rs7566605: *CC* vs *GG/GC*	*FTO* rs9939609: per *A* allele	*MC4R* rs17782313: per *C* allele	*MC4R* rs17700633: per *A* allele
Overweight[1]	1160	0.88 (0.50, 1.56)	1.34 (1.06, 1.69)[2]	1.20 (0.93, 1.54)	1.18 (0.92, 1.51)
Metabolic syndrome[3]	886	2.28 (0.96, 5.41)	2.05 (1.27, 3.31)[2]	1.43 (0.86, 2.38)	0.99 (0.58, 1.67)
Weight *z* score[4,5]	1173	−0.06 (−0.25, 0.12)	0.11 (0.03, 0.19)[2]	0.05 (−0.04, 0.14)	0.07 (−0.02, 0.16)
Height *z* score[4]	1176	−0.13 (−0.32, 0.06)	0.02 (−0.06, 0.11)	−0.08 (−0.17, 0.01)	−0.02 (−0.11, 0.07)
BMI *z* score[4,5]	1173	0.01 (−0.19, 0.19)	0.11 (0.03, 0.19)[2]	0.11 (0.02, 0.20)[2]	0.09 (0.00, 0.18)
Skinfold thickness *z* score[4,5]	1166	−0.05 (−0.24, 0.14)	0.12 (0.04, 0.20)[2]	0.05 (−0.04, 0.14)	0.06 (−0.03, 0.15)
% Body fat *z* score[4]	1154	0.04 (−0.15, 0.23)	0.11 (0.03, 0.19)[2]	0.04 (−0.05, 0.14)	0.06 (−0.02, 0.15)
Waist circumference *z* score[4,5]	1172	−0.05 (−0.24, 0.14)	0.11 (0.03, 0.19)[2]	0.07 (−0.02, 0.16)	0.06 (−0.03, 0.15)
Systolic blood pressure *z* score[4,5]	1178	0.02 (−0.17, 0.21)	0.07 (−0.02, 0.15)	0.08 (−0.01, 0.18)	0.03 (−0.07, 0.11)
Diastolic blood pressure *z* score[4]	1178	0.09 (−0.10, 0.28)	0.02 (−0.07, 0.10)	0.04 (−0.06, 0.13)	−0.05 (−0.14, 0.04)
Glucose *z* score[4]	906	0.09 (−0.13, 0.31)	0.10 (0.00, 0.20)[2]	0.04 (−0.07, 0.15)	−0.04 (−0.15, 0.06)
HDL-cholesterol *z* score[4]	906	−0.03 (−0.25, 0.19)	0.02 (−0.08, 0.11)	−0.04 (−0.14, 0.07)	−0.11 (−0.21, −0.00)[2]
Triglycerides *z* score[4,5]	906	−0.01 (−0.23, 0.20)	0.01 (−0.08, 0.11)	−0.04 (−0.14, 0.07)	0.03 (−0.07, 0.13)

[1] Odds ratios (95% CIs) are reported from multiple logistic regression analyses adjusted for pubertal stage.
[2] $P < 0.05$.
[3] Odds ratios (95% CIs) are reported from multiple logistic regression analyses adjusted for age, sex, and pubertal stage.
[4] B values (95% CIs) are reported from multiple linear regression analyses adjusted for age and pubertal stage.
[5] Log-transformed before calculation of *z* scores to obtain a better approximation of the normal distribution.

TABLE 5
Associations between genotypes and BMI or sum of skinfold thicknesses in repeated-measures analyses[1]

	n	*INSIG2* rs7566605: *CC* vs *GG/GC*	*FTO* rs9939609: per *A* allele	*MC4R* rs17782313: per *C* allele	*MC4R* rs17700633: per *A* allele
Weight *z* score	1273	0.02 (−0.14, 0.19)	0.06 (−0.01, 0.13)	0.05 (−0.03, 0.13)	0.06 (−0.02, 0.13)
Height *z* score	1274	−0.10 (−0.28, 0.07)	0.01 (−0.07, 0.08)	−0.08 (−0.16, 0.00)	−0.01 (−0.09, 0.07)
BMI *z* score[2]	1273	0.07 (−0.11, 0.24)	0.09 (0.01, 0.16)[3]	0.12 (0.03, 0.20)[3]	0.08 (0.00, 0.16)[3]
Skinfold thickness *z* score[2]	1258	0.03 (−0.15, 0.20)	0.10 (0.02, 0.18)[3]	0.06 (−0.03, 0.15)	0.04 (−0.04, 0.12)

[1] All values are B values (95% CIs) that were reported from linear mixed models including age.
[2] Outcome variables were log-transformed before calculating *z* scores to obtain a better approximation of the normal distribution.
[3] $P < 0.05$.

between *FTO* genotype and overweight measures or glucose (*see* supplementary Table 3, A–E, under "Supplemental data" in the online issue).

Logistic regression analyses showed that *FTO* was significantly associated with overweight and the metabolic syndrome after adjustment for sex and pubertal stage (Table 4). Per *A* allele, the OR of being overweight at age 16 y was 1.34 (P = 0.01), and the OR of developing the metabolic syndrome at age 16 y was 2.05 (P = 0.003). Adjustment for BMI in the metabolic syndrome model resulted in a nonsignificant OR of 1.66 (P = 0.09).

Repeated-measures analyses for BMI and sum of skinfold thicknesses, also under an additive model, showed the same pattern of results (Table 5). There were no significant interactions between *FTO* and age, which indicated that the associations of *FTO* with BMI and sum of skinfold thicknesses remained similar from 11 to 16 y of age (**Figure 1**A). As is clear from the figure, a recessive model for *FTO* gives the best description of our data (B: 0.21; P = 0.01). However, in line with the original articles and to limit the number of tests, we only presented results from the additive models. We found no significant interaction effect with physical activity, neither in the model for BMI (B = −0.06; 95% CI: −0.24, 0.11; P = 0.47) nor in the model for sum of skinfold thicknesses (B: 0.01; 95% CI: −0.16, 0.19; P = 0.88) (*see* supplementary Table 4, A and B, under "Supplemental data" in the online issue).

MC4R

Cross-sectional regression analyses adjusted for sex and pubertal stage showed that under an additive model, rs17782313 was significantly associated with BMI per minor allele increase in *z* score (OR: 0.11; P = 0.02), but not with overweight (OR: 1.20; P = 0.17). The variance explained by rs17782313 was 0.5%. In addition, rs17700633 was associated with HDL cholesterol (B: −0.11, P = 0.04), and there was a trend for BMI (B: 0.09, P = 0.05). Adjustment for BMI in the model for HDL cholesterol resulted in nonsignificant results. No association was found between the SNPs near *MC4R* and height. We evaluated possible modification by physical activity in the associations with BMI and HDL cholesterol, but we found no significant interactions (P values ranging from 0.09 to 0.55). (*See* supplementary Table 3, A and F, under "Supplemental data" in the online issue.)

Repeated-measures analyses for BMI, also under an additive model, showed similar effect sizes compared with the cross-sectional analyses for rs17782313 (B: 0.12, P = 0.01) and rs17700633 (B: 0.08, P = 0.047) (Table 5). In the model containing both SNPs, only rs17782313 remained significantly associated with BMI *z* score (rs17782313, B: 0.10, P = 0.03; and rs17700633, B: 0.04, P = 0.33). There were no interactions between *MC4R* genotypes and time, which suggested a stable association between age 11 y and age 16 y (Figure 1B). We found no interactions with physical activity and no significant associations between variation near *MC4R* and sum of skinfold thicknesses, similar to the cross-sectional analyses.

When we included the *FTO* SNP (rs9939609) and rs17782313 in the same linear regression model for BMI at age 16 y, we

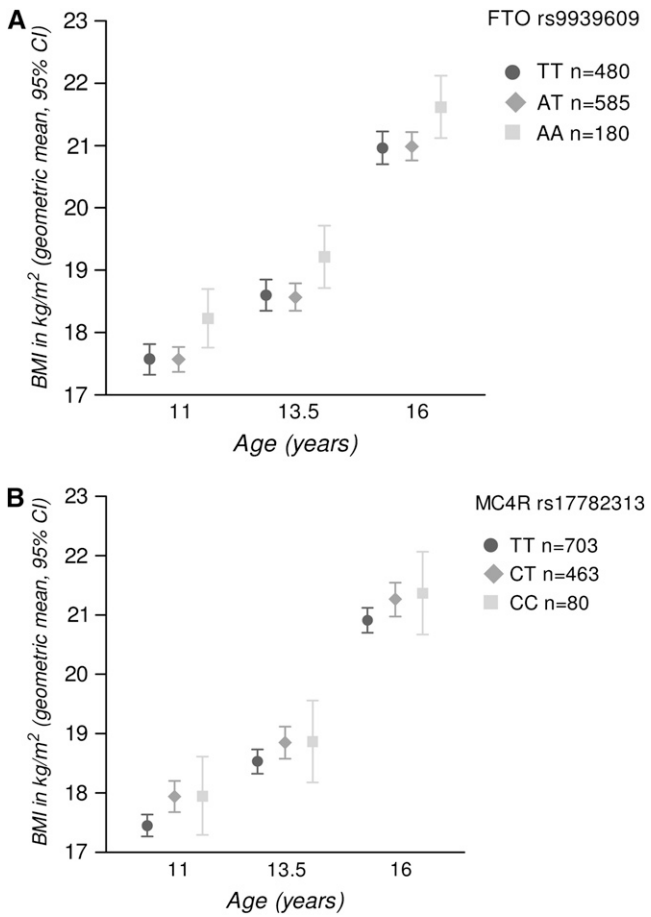

FIGURE 1. Association between *FTO* single nucleotide polymorphism rs9939609 and BMI (A) and between *MC4R* single nucleotide polymorphism rs17782313 and BMI (B) from ages 11 to 16 y.

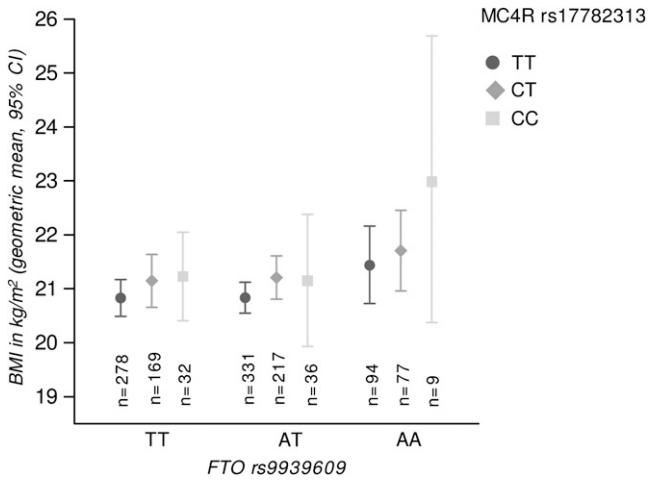

FIGURE 2. Association between rs9939609 (*FTO*) and rs17782313 (*MC4R*) genotypes at age 16 y. Both single nucleotide polymorphisms showed a significant additive effect: rs9939609 ($P = 0.01$) and rs17782313 ($P = 0.02$).

found no evidence for interaction ($P = 0.88$). In the model containing both SNPs, rs9939609 (B: 0.11; $P = 0.01$) and rs17782313 (B: 0.11; $P = 0.02$) were independently associated with BMI (**Figure 2**). Similar findings were obtained from the repeated-measures analyses (B values of 0.08 and 0.12 and P values of 0.03 and 0.01, respectively).

DISCUSSION

We studied the association of common variation in 3 genes discovered through genome-wide association studies with overweight and its associated metabolic traits in adolescence. In line with other large studies (9–11, 13, 42), we found no associations with the SNP near *INSIG2*, which supports the hypothesis that an important role for *INSIG2* in the etiology of childhood overweight is unlikely. In contrast, we were able to replicate associations for *FTO* and the variants near *MC4R*.

FTO

The *A* allele of SNP rs9939609 was associated with higher BMI, sum of skinfold thicknesses, %BF, waist circumference, and fasting glucose. For BMI and sum of skinfold thicknesses we were able to establish that these associations were already present at age 11 y and persisted throughout adolescence to age 16 y. In addition, for each *A* allele in the *FTO* SNP, the risk of adolescent overweight increased by 1.34, and the risk of the metabolic syndrome increased by 2.05. Adjustment for BMI in the metabolic syndrome model rendered the association nonsignificant, which suggests that the effect of *FTO* variation on the metabolic syndrome was mediated by BMI. Our findings are in line with previous research in adolescents in which each *A* allele was found to be associated with an increase in BMI *z* score of 0.05–0.12 and with an OR of 1.27 of being overweight (15). Furthermore, similar variances in BMI have been reported (14, 15, 21). In addition, we found no associations with lipid measurements, which agreed with the findings of a previous study in morbidly obese adults (17). However, Freathy et al (31) found statistically significant associations of *FTO* with glucose, insulin, triglycerides, and HDL. These associations were all driven by BMI. Furthermore, the minor allele frequency (MAF) in our study (0.38) was slightly lower than that reported in HapMap (0.45 in Europeans), but lower frequencies were found in other studies (19, 21). Finally, in line with one of the original articles on *FTO* (15), we found no interaction with sex in the association with BMI.

Our results from both the cross-sectional and repeated-measures analyses suggest that physical activity does not modify the association between *FTO* variation and overweight. This finding is in contrast with that of other studies in a middle-aged Danish population ($n = 5554$) (12), in Amish adults (43), and more recently in a large UK population ($n = 20{,}374$) (44). This discrepancy may partly be explained by our smaller population, which resulted in a lower power to detect significant effects (*see* supplementary Table 2 under "Supplemental data" in the online issue). In addition, we measured physical activity differently. Because no gold standard exists for measuring physical activity by questionnaire, we used a measure based on international recommendations to divide the participants into clear subgroups of those with insufficient (70.2%) and those with sufficient (29.8%) exercise. However, the subgroup that does not sufficiently exercise is rather large, which could have influenced our findings. Cauchi et al (45) found an interaction between *FTO* and physical activity in their adolescent Finnish population ($n = 4780$), but not in their middle-aged French population ($n = 3167$). In line with our study, Jonsson et al (46) found no interaction between rs9939609 and physical activity on BMI in a large study among 15,925 Swedish and 2511 Finnish adults. This is also supported by a study in twins, in which the *FTO* × environment interaction was studied in general (47).

The function of the *FTO* gene remains unknown. Whereas some studies suggest that it plays a role in central regulation of body weight (14, 48), Wåhlén et al (30) studied *FTO* with regard to fat cell function and adipose tissue gene expression. Their results suggest that *FTO* could be involved in body weight regulation through lipolysis. However, our results and those of others (17), in which no association was found with triglycerides or cholesterol, do not support this hypothesis (31).

MC4R

Variation near *MC4R* was associated with BMI *z* score. The per minor allele increase of 0.11 in BMI *z* score we found for rs17782313 was similar to the value of 0.10–0.13 described by Loos et al (26) in children aged 7–11 y. Also, similar to their findings, the effects were stronger for rs17782313 than for rs17700633, ie, the effect was driven by rs17782313. Additionally, the associations between the SNPs near *MC4R* and BMI were stable between ages 11 and 16 y. The MAFs we found for the SNPs near *MC4R* were in line with the frequencies previously reported (26). Although no direct evidence exists for a functional role of these variants (or the variants they tag) in *MC4R* expression, it has been described that the phenotypic pattern (positive association with height, which we were not able to replicate) is similar to the phenotype caused by rare *MC4R* mutations (26). In addition, the larger effect sizes found in children than in adults (26) suggest an association with early-onset obesity, similar to the effect of rare *MC4R* mutations.

The rs17782313 in *MC4R* was significantly associated with only BMI, and — although the association is in the same direction

— was not significantly associated with other measures of body fat, unlike *FTO*. This finding was probably due to the (near-significant) negative association between rs17782313 and height, which is larger than its positive effect on weight. Thus, the minor allele of rs17782313 is associated with a higher BMI through its combined effect on lower height and higher weight, which suggests that *MC4R* influences BMI in a different manner than *FTO*.

Including the *FTO* SNP and rs17782313 in the same model showed that they were independently associated with BMI, which suggests that their effects are additive. This has also been shown by Loos et al (26).

The main strength of our study was that we genotyped a homogeneous population of reasonable size in which multiple phenotypic measurements of overall and abdominal adiposity as well as associated metabolic traits were obtained. Power calculations using previously reported effect sizes for BMI *z* scores showed that, with an α of 0.05, our sample size (n = 1275) had a power of 28% to detect a cross-sectional association for rs7566605, 84% for rs9939609, 81% for rs17782313, and 15% for rs17700633 (*see* supplementary Table 1 under "Supplemental data" in the online issue). To our knowledge, this was the first population-based study to evaluate the influence of both *FTO* and variation near *MC4R*, not only on overall adiposity but also on abdominal adiposity and its related metabolic traits. In addition, we were able to evaluate associations with BMI and sum of skinfold thicknesses at both age 11 y and 16 y in repeated-measures analyses, which strengthens our findings for all outcome measures at age 16 y.

A potential limitation of our study was the dropout rate in TRAILS (31.7%), which was mainly due to a refusal to participate. Evaluation of selection bias did not show statistically significant differences between the participants and the group lost to follow-up, but a difference in sum of skinfold thicknesses cannot be excluded entirely. The fact that we found lower MAFs than reported in HapMap could suggest that a leaner population participated in the follow-up visit. Nevertheless, it seems unlikely that this would affect the associations between genetic variants and the outcome variables of interest. In addition, the fact that our associations of BMI and sum of skinfold thicknesses were consistent across assessment visits renders it unlikely that a selection bias affected our findings. Another point of discussion is the use of the IDF criteria for the metabolic syndrome. Although dichotomizing an outcome measure does have its disadvantages, such as loss of power (49), we used the IDF criteria to enhance the comparability with other studies.

In conclusion, in our population of 16-y-old adolescents, we found that variation near the *MC4R* gene was positively associated with BMI. In addition, variation in the *FTO* gene was not only positively associated with measures of overall fat but also with measures of abdominal fat. The associations were stable between ages 11 and 16 y. Our findings strengthen and extend the results from previous genome-wide association studies.

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