Development of overweight in adolescence

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Chapter 2.1

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

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ABSTRACT

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

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

Design: In a population-based cohort of 663 girls and 612 boys aged 16 years, weight, height, skinfold thicknesses, % 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 (SNPs) near the *INSIG2 (insulin induced gene 2),* in the *FTO*, and near the *MC4R* genes and repeated measures analyses of available BMI and skinfold thickness data across three visits at ages 11, 13.5, and 16 years.

Results: A total of 15.1% of participants were overweight or obese at the of age 16 years. No associations with *INSIG2* were found. Common variation in the *FTO* gene was associated with sex-specific standard deviation scores (SDSs) of BMI (B=0.11; 95% CI, 0.03 – 0.19), sum of skinfold thicknesses (B=0.12; 95% CI, 0.04 – 0.20), %BF (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 with overweight (OR=1.34; 95% CI, 1.06 – 1.69) at age 16 years. Repeated measures analyses confirmed the associations for BMI and sum of skinfold thicknesses, and corroborated that 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.

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, epidemiological studies have demonstrated that, already in childhood, total and abdominal fat appear to be significantly associated with an unfavorable metabolic profile, including insulin resistance, elevated low density lipoprotein cholesterol (LDLC), and decreased high density lipoprotein cholesterol (HDLC).[3,4] Thus, childhood overweight poses a major public health concern.

Life style 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 (GWAS).[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 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* genes[26]. In 2 pediatric studies,[15,24] overweight was assessed by BMI as well as DEXA scan, which more specifically measures overall adipose tissue. However, few studies have evaluated abdominal fat[28] or overweight related metabolic profile[29] in children. These are important because they confer increased risk of later metabolic complications such as diabetes and cardiovascular disease. Since results obtained by Wåhlén and colleagues suggest that *FTO* could be involved in body weight regulation through lipolysis,[30] it would also be interesting, from a pathophysiologic point of view, to evaluate if 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 among adults.[31]

The aim of this study was to assess the influence of common genetic variants found through GWASs, specifically variants near the *INSIG2*, in the *FTO*, and near the *MC4R* genes, on overweight and its related metabolic traits at age 16 years. 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) and sum of skinfold thicknesses (at age 11) 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 population, an ongoing Dutch prospective cohort study, assessing both psychosocial and physical health from preadolescence into adulthood. Sample selection has been described elsewhere.[32] In short, 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 years. In addition, during two previous visits (in 2001-2002, at age 11.1 ± 0.55; and in 2003-2004, at age 13.5 ± 0.52 years), weight and height (first and second visit) and skinfold thicknesses (first visit only) were measured, which we included in our repeated measures analyses. For this study we included participants of whom DNA and BMI were available (n=1413). We excluded all participants who were not from Northern European ancestry (n=158) and also excluded the second of all siblings within the cohort (n=11), resulting in a population of n=1244 adolescents (51.8% girls). All procedures were approved by the Dutch Central Committee on Research Involving Human Subjects (CCMO). 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 with the use of regularly calibrated equipment (models 770 and 214, respectively; Seca, Hamburg, Germany). Body mass index (BMI; in kg/m^2) was calculated. We defined overweight and obesity according to international age- and sex-adjusted BMI criteria (equivalent to the Dutch 94^{th} and 99.7^{th} percentile in 1980 for overweight and obesity, respectively).[33] We obtained triceps, biceps, subscapular, and supra-iliac skinfold thicknesses with a Harpenden skinfold caliper (CMS instruments, London, UK); and the sum of 4 thicknesses was calculated. We measured waist circumference at the mid-point 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 (BIA), type BIA 101 (Akern®, Italy), from which %BF was calculated with the use of the Deurenberg equation.[34] Systolic (SBP) and diastolic (DBP) blood pressure were measured

in duplicate with the use of a Dinamap Critikon 1846SX (Critikon Inc., Tampa, FL, USA), from which we calculated means.

We obtained a blood sample after at least 8 hours of fasting, for measurement of glucose (Roche Diagnostics, Basel, Switzerland), insulin (Diagnostic Systems Laboratories Inc., Texas, USA), triglycerides, total cholesterol, and HDLC (Roche Diagnostics). LDLC was calculated according to Friedewald's equation.[35] The presence of the metabolic syndrome was determined according to the IDF criteria,[36,37] based on age-specific cut-off points for waist circumference, triglycerides, HDLC, 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 at least 60 minutes of moderate or vigorous physical activity, from which 'sufficient physical activity' was determined as ≥ 5 days per week, in accordance with international recommendations.[39]

Genotyping

We extracted DNA from buffy coats (n=1042) or buccal swabs (Cytobrush®) (n=202) using a manual salting out procedure similar to the protocol described by Miller and colleagues.[40] Genotyping was performed on the Illumina BeadStation 500 platform (Illumina Inc., San Diego, CA, USA) 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, USA). 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 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, skinfold thicknesses, waist circumference, SBP, and triglycerides were ln-transformed, to obtain a better approximation of the normal distribution, before calculating age- and sex-specific SDSs, with the use of means and standard deviations.

Table 1. Genotype and minor allele frequencies.

Gene	n	rs	Genotype frequency			MAF	HWE P-value
			11	12	22		
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 = major allele, 2=minor allele

MAF = minor allele frequency

HWE = Hardy Weinberg Equilibrium (determined by Pearson's chi-squared test)

We performed multiple linear regression analyses for weight, height, BMI, skinfold thicknesses, and %BF SDSs and all components of the metabolic syndrome, i.e., waist circumference, SBP, DBP, glucose, HDLC, and triglycerides SDSs. 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 interaction of genotypes with sex and physical activity by adding a multiplicative term to the models. We also evaluated interaction between genotypes.

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

We used Quanto to calculate power available to detect main effects and interaction effects in our cross-sectional analyses (Supplementary tables 1 & 2).[41] All other statistical analyses were performed using SPSS version 14.0 (SPSS, Chicago IL, USA). 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 years. At this age, 12.4% was overweight and 2.7% was obese (Table 2). Compared with girls, boys showed less advanced pubertal stage, were more physically active, were heavier, 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 1244 participants included in this study with the original sample of 1868 children who participated in the BMI measurements at age 11 years. Compared with the 624 who were either excluded (n=25) or lost to follow-up (n=599) between the first and third assessment visits, there were no statistically significant differences in BMI SDS (p=0.19) and sum of thicknesses SDS (p=0.07).

Since analyses of sum of skinfold thicknesses provided similar results compared with analyses of all 4 thicknesses separately, only analyses regarding the former were reported.

Table 2. Sociodemographic and anthropometric characteristics according to sex.

	All		Girls		Boys		Sex effect
		n		n		n	P-value
Age (yrs)	16.2 ± 0.7	1244	16.3 ± 0.7	645	16.2 ± 0.6	599	0.17
Pubertal stage (% in 3 categories) *	15.9/28.9/55.2	1173	0.8/0.3/98.9	615	32.6 /60.4/7.0	558	<0.001
Physical activity (% sufficient)**	29.8	1203	26.2	627	33.9	576	0.01
Weight (kg)	63.1 (57.7 – 70.3)	1244	60.6 (55.5 – 66.0)	645	66.0 (60.0 – 74.6)	599	<0.001
Height (cm)	174.5 ± 8.9	1244	169.2 ± 6.3	645	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

All data are means ± SD or median (interquartile range) unless otherwise indicated.

* Measured by the Physical Development Scale questionnaire, divided into pre/early pubertal, midpubertal and late/post pubertal.

** 'Sufficient physical activity' was defined as at least 60 minutes of moderate or vigorous physical activity ≥5 days/week, in accord with international recommendations.

P-value from chi-square test for pubertal stage, physical activity, and overweight or obese;
t test for age, height, and % body fat;
Mann-Whitney U test for weight, BMI, sum of skinfold thicknesses, and waist circumference.

For none of the models, interaction with sex was significant. Therefore, we do not report these results.

INSIG2

We did not find any 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

Table 3. Metabolic characteristics according to sex.

	All		Girls		Boys		Sex effect
		n		n		n	P-value
Systolic blood pressure (mmHg)	117 (109 – 127)	1252	113 (107 – 122)	652	122 (113 – 132)	600	<0.001
Diastolic blood pressure (mmHg)	61 ± 7	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
HDLC (mmol/l)	1.5 ± 0.3	956	1.5 ± 0.3	505	1.4 ± 0.3	451	<0.001
LDLC (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* (%)	4.5	955	4.5	504	4.5	451	0.96

HDLC SDS = high density lipoprotein cholesterol SD score; LDLC SDS = low density lipoprotein cholesterol SD score.
All data are means ± SD or median (interquartile range) unless otherwise indicated.
* Defined according to the International Diabetes Federation criteria, based on age-specific cut-off points for waist circumference, triglycerides, HDLC, blood pressure and glucose.
P-value from chi-square test for metabolic syndrome;
t test for diastolic blood pressure, glucose, total cholesterol, HDLC and LDLC;
Mann-Whitney U test for systolic blood pressure, insulin, and triglycerides.

respectively). No significant modification by physical activity was found in the associations between *FTO* genotype and overweight measures or glucose (Supplementary tables 3a-e).

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 was 1.34 (p=0.01); and the OR of suffering from the metabolic syndrome at age 16 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, indicating that the associations of *FTO* with BMI and sum of skinfold thicknesses remained similar from 11 to 16 years of age (Figure 1a). 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 papers and to limit the number of tests we only present results from 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) (Supplementary tables 4a & 4b).

Table 4. Associations between genotypes and overweight related measures.

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

Significant associations are in bold.

[1] Odds ratio (95% CI) are reported from multiple logistic regression analyses adjusted for pubertal stage.

[2] Odds ratio (95% CI) are reported from multiple logistic regression analyses adjusted for age, sex, and pubertal stage.

[3] B's (95% CI) are reported from multiple linear regression analyses adjusted for age and pubertal stage.

[4] Ln-transformed before calculation of SDSs to obtain a better approximation of the normal distribution.

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 SDS 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 HDLC (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 HDLC 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 HDLC, but we did not find any significant interactions (p-values ranging from 0.09 to 0.55). (Supplementary tables 3a & 3f)

Repeated measures analyses on 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 SDS (rs17782313: B=0.10, p=0.03; and rs17700633: B=0.04, p=0.33). There were no interactions between *MC4R* genotypes and time, suggesting a stable association between age 11 and age 16 years (Figure 1b). We

Table 5. Associations between genotypes and BMI / sum of skinfold thicknesses in repeated measures analyses.

		INSIG2 rs7566605	***FTO*** rs9939609	***MC4R*** rs17782313	***MC4R*** rs17700633
	n	CC vs. GG/GC	per A allele	per C allele	per A allele
Weight SDS	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 SDS	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 SDS[1]	1273	0.07 (-0.11 – 0.24)	**0.09 (0.01 – 0.16)**	**0.12 (0.03 – 0.20)**	**0.08 (0.00 – 0.16)**
Skinfold thicknesses SDS[1]	1258	0.03 (-0.15 – 0.20)	**0.10 (0.02 – 0.18)**	0.06 (-0.03 – 0.15)	0.04 (-0.04 – 0.12)

Significant associations are in bold.

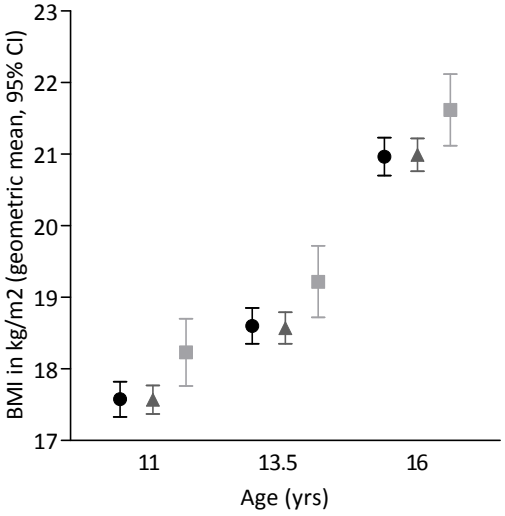
B's (95% CI) are reported from linear mixed models including age.

[1] Outcome variables were ln-transformed before calculating SDSs to obtain a better approximation of the normal distribution.

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 years, we 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

Figure 1a. Association between *FTO* SNP rs9939609 and body mass index (BMI) from age 11 to age 16 years.



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 did not find 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, and waist circumference, and with a higher fasting glucose. For BMI and sum of skinfold thicknesses we were able to establish that these associations were already present at age 11 and persisted throughout adolescence to age 16 years. Additionally, for each A-allele in the *FTO* SNP, the risk of adolescent overweight increased with 1.34, and the risk of the metabolic syndrome increased with 2.05. Adjustment for BMI in the

Figure 1b. Association between *MC4R* SNP rs17782313 and body mass index (BMI) from age 11 to age 16 years.

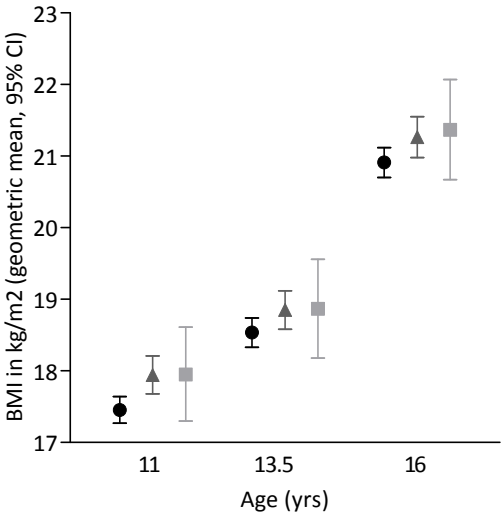
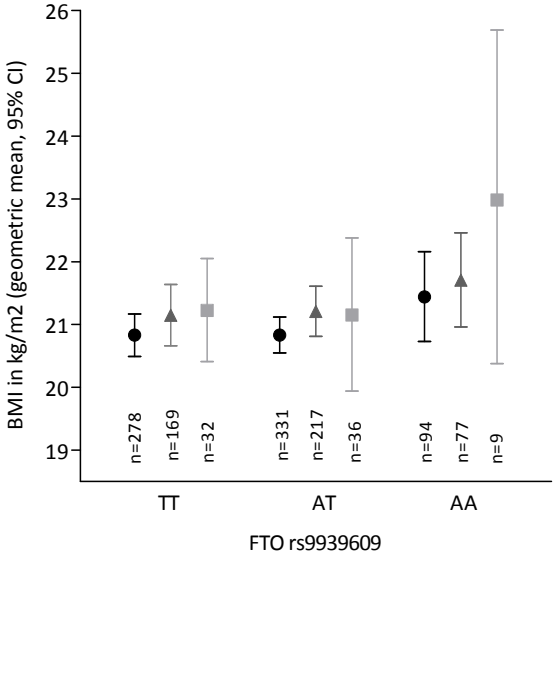


Figure 2. Effects of rs9939609 (*FTO*) and rs17782313 (*MC4R*) genotypes on BMI at age 16 years. The 2 SNPs show a significant additive effect: rs9939609, p=0.01; rs17782313, p=0.02.



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 SDS of 0.05 to 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 did not find any associations with lipid measurements, which was in accord with a previous study in morbidly obese adults.[17] However, Freathy and colleagues found statistically significant associations of *FTO* with glucose, insulin, triglycerides, and HDLC.[31] These associations were all driven by BMI. Furthermore, the minor allele frequency (MAF) in our study (0.38) was slightly lower than reported in HapMap (0.45 in Europeans), but lower frequencies have been found in other studies.[19,21] Finally, in line with one of the original papers on *FTO*,[15] we did not find an 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 is in contrast to other studies in a middle-aged Danish population (n=5,554),[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, resulting in a lower power to detect significant effects (Supplementary table 2). Additionally, 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 insufficient (70.2%) versus sufficient (29.8%) exercise. However, the subgroup which does not sufficiently exercise is rather large, which could have influenced our findings. Cauchi and colleagues found an interaction between *FTO* and physical activity in their adolescent Finnish population (n=4,780), but not in their middle-aged French population (n=3,167).[45] In line with our study, Jonsson and colleagues did not find an interaction between rs9939609 and physical activity on BMI in a large study among 15,925 Swedish and 2,511 Finnish adults.[46] This is also supported by a study in twins, evaluating *FTO* x environment interaction 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 and colleagues studied *FTO* with regard to fat cell function and adipose tissue gene expression.[30] 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 SDS. The per minor allele increase of 0.11 in BMI SDS we found for rs17782313 was similar to the value of 0.10 to 0.13 Loos and colleagues described in children aged 7 to 11 years. Also, similar to their findings, the effects were stronger for rs17782313 than for rs17700633, i.e., the effect was driven by rs17782313. Additionally, the associations between the SNPs near *MC4R* and BMI were stable between age 11 and 16 years. 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 compared with adults[26] suggest an association with early-onset obesity, similar to the effect of rare *MC4R* mutations.

The rs17782313 in *MC4R* is only significantly associated with BMI, and - although the association is in the same direction – was not significantly associated with measures of body fat, unlike *FTO*. This finding is 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, suggesting 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 and colleagues.[26]

The main strength of our study is 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 SDSs showed that with an alpha 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 (Supplementary table 1). To our knowledge, this was the first population-based study evaluating the influence of both *FTO* and variation near *MC4R*, not only on overall 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 and 16 years in repeated measures analyses, which strengthens our findings for all outcome measures at age 16 years.

A potential limitation of our study is the drop-out rate in TRAILS (31.7%), mainly due to 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 can not 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-year-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 but also with measures of abdominal fat. The associations were stable between ages 11 and 16 years. Our findings strengthen and extend the results from previous genome wide association studies.

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SUPPLEMENTARY TABLES

Supplementary table 1. Power calculations for SNP effects on BMI SDS.

Gene	SNP	MAF	Model	Effect size*	Power (%)
		as reported in our study		as reported by previous studies	
INSIG2	rs7566605	0.31	recessive	0.13	28
FTO	rs9939609	0.38	additive	0.12	84
MC4R	rs17782313	0.25	additive	0.13	81
MC4R	rs17700633	0.30	additive	0.04	15

Power calculations are based on: $\alpha = 0.05$, n = 1275.

* Effect sizes from models for BMI SDS.[7,15,26]

Supplementary table 2. Power calculations for SNP x environment interactions on BMI SDS.

Gene	SNP	R_{GxE}	Power (%)
INSIG2	rs7566605	1.50	6
		1.75	7
		2.00	9
FTO	rs9939609	1.50	10
		1.75	17
		2.00	27
MC4R	rs17782313	1.50	9
		1.75	15
		2.00	23
MC4R	rs17700633	1.50	10
		1.75	16
		2.00	25

Power calculations are based on the following assumptions:

- $\alpha = 0.05$, n = 1275;
- main genetic effects on BMI SDS of B = 0.13, 0.12, 0.13, and 0.04 for rs7566605, rs9939609, rs17782313, and rs17700633 respectively.[7,15,26] Based on a recessive model for rs7566605; and on an additive model for rs9939609, rs17782313, and rs17700633;
- main physical activity effect of -0.12, as was found in our study (data not shown).

Supplementary table 3a. SNP x environment interactions for body mass index (BMI) in cross-sectional analyses.

Gene	SNP	Cross-sectional associations for BMI		
		Interaction effect	SNP effect	
		B_{GxE}, (95% CI), p	B_G, (95% CI), p physically passive	B_G, (95% CI), p physically active
INSIG2	rs7566605	-0.27 (-0.67 – 0.14), 0.20	0.10 (-0.13 – 0.33), 0.41	-0.17 (-0.50 – 0.17), 0.32
FTO	rs9939609	-0.08 (-0.26 – 0.11), 0.41	0.13 (0.03 – 0.23), 0.01	0.05 (-0.11 – 0.21), 0.50
MC4R	rs17782313	-0.08 (-0.29 – 0.12), 0.43	0.13 (0.03 – 0.24), 0.01	0.05 (-0.13 – 0.23), 0.57
MC4R	rs17700633	-0.06 (-0.26 – 0.14), 0.55	0.11 (0.004 – 0.21), 0.04	0.05 (-0.12 – 0.22), 0.58

Supplementary table 3b. SNP x environment interactions for sum of skinfold thicknesses in cross-sectional analyses.

Gene	SNP	Cross-sectional associations for sum of skinfold thicknesses		
		Interaction effect	SNP effect	
		B_{GxE}, (95% CI), p	B_G, (95% CI), p physically passive	B_G, (95% CI), p physically active
INSIG2	rs7566605	-0.28 (-0.69 – 0.13), 0.18	0.05 (-0.18 – 0.28), 0.69	-0.23 (-0.57 – 0.11), 0.18
FTO	rs9939609	-0.08 (-0.27 – 0.10), 0.39	0.14 (0.05 – 0.24), 0.004	0.06 (-0.09 – 0.22), 0.43
MC4R	rs17782313	-0.10 (-0.31 -0.11), 0.35	0.09 (-0.02 – 0.19), 0.12	-0.01 (-0.19 – 0.17), 0.88
MC4R	rs17700633	-0.04 (-0.24 – 0.17), 0.74	0.07 (-0.03 – 0.18), 0.18	0.04 (-0.13 – 0.21), 0.66

Supplementary table 3c. SNP x environment interactions for % body fat in cross-sectional analyses.

Gene	SNP	Cross-sectional associations for % body fat		
		Interaction effect	SNP effect	
		B_{GxE}, (95% CI), p	B_G, (95% CI), p physically passive	B_G, (95% CI), p physically active
INSIG2	rs7566605	-0.05 (-0.46 – 0.35), 0.79	0.06 (-0.17 – 0.29), 0.60	0.01 (-0.33 – 0.33), 0.97
FTO	rs9939609	-0.14 (-0.32 – 0.04), 0.12	0.15 (0.06 – 0.25), 0.002	0.01 (-0.14 – 0.17), 0.88
MC4R	rs17782313	-0.15 (-0.36 – 0.06), 0.15	0.09 (-0.02 – 0.20), 0.11	-0.06 (-0.24 – 0.11), 0.48
MC4R	rs17700633	-0.15 (-0.35 – 0.05), 0.14	0.12 (0.02 – 0.22), 0.03	-0.03 (-0.20 – 0.14), 0.74

Supplementary table 3d. SNP x environment interactions for waist circumference in cross-sectional analyses.

Gene	SNP	Cross-sectional associations for waist circumference		
		Interaction effect	SNP effect	
		B_{GxE}, (95% CI), p	B_G, (95% CI), p physically passive	B_G, (95% CI), p physically active
INSIG2	rs7566605	-0.15 (-0.56 – 0.25), 0.46	0.01 (-0.22 – 0.24), 0.91	-0.14 (-0.47 – 0.19), 0.41
FTO	rs9939609	-0.08 (-0.26 – 0.11), 0.41	0.12 (0.03 – 0.22), 0.01	0.05 (-0.11 – 0.20), 0.55
MC4R	rs17782313	-0.09 (-0.29 – 0.12), 0.42	0.09 (-0.01 – 0.20), 0.08	0.01 (-0.17 – 0.19), 0.92
MC4R	rs17700633	-0.08 (-0.28 – 0.12), 0.44	0.09 (-0.01 – 0.19), 0.09	0.01 (-0.16 – 0.18), 0.90

Supplementary table 3e. SNP x environment interactions for blood glucose in cross-sectional analyses.

Gene	SNP	Cross-sectional associations for blood glucose		
		Interaction effect	SNP effect	
		B_{GxE},(95% CI), p	B_G,(95% CI), p physically passive	B_G,(95% CI), p physically active
INSIG2	rs7566605	0.08 (-0.39 – 0.55), 0.72	0.07 (-0.20 – 0.34), 0.60	0.16 (-0.23 – 0.54), 0.43
FTO	rs9939609	-0.07 (-0.29 – 0.14), 0.50	0.11 (-0.01 – 0.23), 0.06	0.04 (-0.14 – 0.22), 0.68
MC4R	rs17782313	-0.10 (-0.34 – 0.14), 0.42	0.06 (-0.07 – 0.19), 0.36	-0.04 (-0.25 – 0.17), 0.70
MC4R	rs17700633	0.002 (-0.23 – 0.23), 0.99	-0.05 (-0.17 – 0.08), 0.49	-0.04 (-0.24 – 0.15), 0.66

Supplementary table 3f. SNP x environment interactions for blood high density lipoprotein cholesterol (HDLC) in cross-sectional analyses.

Gene	SNP	Cross-sectional associations for blood HDLC		
		Interaction effect	SNP effect	
		B_{GxE},(95% CI), p	B_G,(95% CI), p physically passive	B_G,(95% CI), p physically active
INSIG2	rs7566605	0.18 (-0.28 – 0.65), 0.44	-0.09 (-0.36 – 0.17), 0.49	0.09 (-0.30 – 0.47), 0.65
FTO	rs9939609	-0.09 (-0.30 – 0.12), 0.41	0.05 (-0.07 – 0.16), 0.41	-0.04 (-0.22 – 0.14), 0.66
MC4R	rs17782313	0.16 (-0.08 – 0.40), 0.20	-0.09 (-0.21 – 0.04), 0.17	0.07 (-0.13 – 0.27), 0.50
MC4R	rs17700633	-0.20 (-0.43 – 0.03), 0.09	-0.06 (-0.18 – 0.07), 0.38	-0.25 (-0.44 – -0.06), 0.01

Supplementary table 4a. SNP x environment interactions for body mass index (BMI) in mixed models.

Gene	SNP	Mixed effects model for BMI		
		Interaction effect	SNP effect	
		B_{GxE},(95% CI), p	B_G,(95% CI), p physically passive	B_G,(95% CI), p physically active
INSIG2	rs7566605	-0.28 (-0.66 – 0.10), 0.15	0.19 (-0.03 – 0.40), 0.09	-0.09 (-0.40 – 0.22), 0.57
FTO	rs9939609	-0.06 (-0.24 – 0.11), 0.47	0.11 (0.02 – 0.20), 0.02	0.05 (-0.10 – 0.19), 0.53
MC4R	rs17782313	-0.05 (-0.25 – 0.14), 0.59	0.13 (0.03 – 0.23), 0.01	0.08 (-0.09 – 0.24), 0.34
MC4R	rs17700633	-0.01 (-0.20 – 0.17), 0.88	0.10 (0.01 – 0.20), 0.04	0.09 (-0.07 – 0.25), 0.27

Supplementary table 4b. SNP x environment interactions for sum of skinfold thicknesses in mixed models.

Gene	SNP	Mixed effects model for sum of skinfold thicknesses		
		Interaction effect	SNP effect	
		B_{GxE},(95% CI), p	B_G,(95% CI), p physically passive	B_G,(95% CI), p physically active
INSIG2	rs7566605	-0.32 (-0.70 – 0.07), 0.11	0.14 (-0.07 – 0.36), 0.20	-0.18 (-0.50 – 0.14), 0.28
FTO	rs9939609	0.01 (-0.16 – 0.19), 0.88	0.10 (0.01 – 0.19), 0.03	0.11 (-0.03 – 0.26), 0.13
MC4R	rs17782313	-0.09 (-0.29 – 0.11), 0.37	0.08 (-0.02 – 0.19), 0.10	-0.005 (-0.17 – 0.16), 0.95
MC4R	rs17700633	-0.04 (-0.23 – 0.14), 0.64	0.08 (-0.02 – 0.17), 0.13	0.03 (-0.13 – 0.19), 0.69

Chapter 2.2

Influence of *DRD2* and *SLC6A4* genes on impulsivity and adiposity in adolescence

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ABSTRACT

Objective: The aim of this study was to assess whether common variation near the *dopamine receptor D2 (DRD2)* gene and in the *serotonin transporter (SLC6A4)* gene influences adiposity via impulsivity in a population-based cohort of adolescents.

Methods: In a population-based cohort of 645 girls and 599 boys aged 16.2 years, DNA, weight, height, skinfold thicknesses, % body fat, waist circumference, and an impulsivity questionnaire were obtained. We calculated BMI in kg/m^2 and defined overweight according to international criteria. We performed multiple linear and logistic regression analyses to assess the association between impulsivity and adiposity measures; and the influence of polymorphisms near the *DRD2* gene and in the *SLC6A4* gene on impulsivity and adiposity measures. We corrected for sex, pubertal stage, and socioeconomic status.

Results: 15.1% of participants were overweight or obese at age 16.2 years. Impulsivity was associated with significantly increased BMI (B=0.06; 95% confidence interval (CI), 0.01 – 0.10), % body fat (B=0.08; 95% CI, 0.01 – 0.15), and waist circumference (B=0.16; 95% CI, 0.03 – 0.28); and with a trend for being overweight or obese (OR 1.04; 95% CI, 0.99 – 1.08). There were no significant associations of genetic variation near the *DRD2* gene and in the *SLC6A4* gene with impulsivity or adiposity measures.

Conclusion: we found that impulsivity was associated with measures of general and abdominal adiposity in adolescence, in which genetic variation near the *DRD2* gene and in the *SLC6A4* gene did not have a role.

INTRODUCTION

Childhood overweight is a major public health concern because it is associated with adult overweight and with cardiovascular morbidity and mortality. The rise in prevalence of childhood overweight in the past decades across the Western world[1,2] is considered a reflection of the changes in lifestyle that have accompanied the growing economies, specifically decreased amounts of physical exercise and increasingly unhealthy diets. Genetic background determines an individual's vulnerability to develop overweight.[3,4]

Genetic variation might exert part of its influence through variation in increased energy intake.[4,5] Apart from appetite and satiety, impulsivity might have a role in the development of overweight.[6] Studies regarding the association between impulsive traits and overweight in population-based cohorts are scarce.[7] Overweight children seeking treatment exhibit lower impulse control than normal weight children;[7] and the most impulsive children lose less weight during treatment programs.[6,8] Lack of self-regulation has also been associated with faster childhood weight gain.[9] Although impulsivity is a multifaceted construct,[10] it is considered a very stable personality trait.[11] Results from a twin and sibling sample suggested that genetic influence explains the stability of impulsivity across time.[11] Heritability estimates of 0.30 to 0.45 have been reported.[12,13]

Research regarding genetic variation underlying impulsivity has focused mainly on the dopaminergic and serotonergic systems. The *Taq1A* polymorphism (rs1800497) near the *DRD2* gene was shown to be associated with impulsivity, although findings are conflicting.[14,15] The *Taq1A* polymorphism near the *DRD2* gene has also been implicated in overweight.[16,17] Thus, it is possible that genetic variation in dopaminergic activity influences overweight risk via differences in impulsivity. The *DRD2* gene encodes the D2 subtype of the dopamine receptor. Low availability of this receptor in the striatum has been associated with high impulsivity in rats[18] and with obesity in humans.[19] The *Taq1A* polymorphism is located 9.4kb downstream from the coding region of the *DRD2* gene.[14] Although no evidence exists for linkage disequilibrium with another polymorphism directly associated with *DRD2* gene expression, it was established that the A1 allele is associated with decreased *DRD2* availability in the striatum.[20]

Decreased serotonergic activity has also been related to impulsivity. Common variation in the *SLC6A4* gene, including the insertion-deletion polymorphism in promoter region (*5-HTTLPR*) has been related to impulsive behavior.[21,22] The short (S) allele (as compared with the long (L) allele) of the *5-HTTLPR* polymorphism, leading to decreased serotonin activity, was also associated with measures of general and abdominal adiposity in adolescence.[23] Recently, a single nucleotide substitution (rs25531, A>G) was found near the insertion-deletion polymorphism which creates an allele (L(G)) that is functionally equivalent to the S-allele due to reduced transcription.[24,25]

The aim of our study was to assess the influence of common variation near the *DRD2* gene and in *5-HTTLPR* on the association between impulsivity and adiposity in a population-based cohort of adolescents. Specifically, we aimed to evaluate whether this genetic variation exerts its influence on adolescent adiposity via impulsive personality characteristics. We included pubertal stage and socioeconomic status (SES) as possible confounders.[9]

METHODS

Study population

Our study was performed within the TRAILS survey, which studies psychosocial and physical health from preadolescence into adulthood. Sample selection has been described elsewhere.[26] In short, children were recruited through community registers and through their schools to obtain a representative sample. The present study included data from the third assessment visit, which was performed from October 2005 to December 2007, at a mean age of 16.2 (SD=0.7) years. For this study we included participants of whom DNA and BMI were available (n=1413). We excluded all participants who were not from Northern European ancestry (n=158) and the second of all siblings within the cohort (n=11), resulting in a population of n=1244 adolescents (51.8% girls).

All procedures were approved by the Dutch Central Committee on Research Involving Human Subjects (CCMO). Written informed consent was obtained from participants and their parents or custodians.

Measures

Weight and height were measured with the use of regularly calibrated scales (Model 770, Seca, Hamburg, Germany) and stadiometers (Model 214, Seca, Hamburg, Germany), while wearing light clothing and no shoes. Body mass index (BMI) was calculated in kg/m^2. We defined overweight and obesity according to international age- and sex-adjusted BMI criteria (equivalent to the Dutch 94th and 99.7th percentile in 1980 for overweight and obesity, respectively).[27] Triceps, biceps, subscapular, and supra-iliac skinfold thicknesses were measured with the use of a Harpenden skinfold caliper (CMS instruments, London, UK); and the sum of four skinfolds was calculated. Waist circumference was measured on bare skin 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. A hand-to-foot BIA, type BIA 101 (Akern®, Italy) was performed from which %BF was calculated with the use of the Deurenberg equation.[28]

Impulsivity was measured by a subscale of the Revised Neuroticism-Extraversion-Openness Personality Inventory (NEO-PI-R), which is a 240-item questionnaire assessing

the five facets of personality. The impulsivity subscale of 8 items includes statements such as 'Sometimes I'll eat until I'm nauseous' and 'I seldom give in to impulses', to which the participants can answer on a five point scale ranging from 'I strongly disagree' to 'I strongly agree'. It results in a score ranging from 5 to 40.

Questionnaires were filled out to assess SES and pubertal stage. Pubertal stage was measured by the Physical Development Scale questionnaire and divided into 3 categories: pre/early pubertal, midpubertal, and late/post pubertal.[29] SES was calculated as the mean of SD scores on family income and mother's and father's levels of education and levels of occupation based on the International Standard Classification of Occupations.[30] The lowest 25%, intermediate 50%, and 25% highest were considered as to represent low, medium, and high SES, respectively.

Candidate polymorphism selection

We performed a literature search to find reports of genetic association studies on overweight and impulsivity. Based on previous research, the S/L-allele[21-23,25] and rs25531[24,25] in the *SLC6A4* gene; and the *Taq1A* polymorphism near the *DRD2* gene were selected.[20,31]

Genotyping

We extracted DNA from buffy coats (n=1042) or buccal swabs (Cytobrush®) (n=202) with the use of a manual salting out procedure similar to the protocol described by Miller and colleagues.[32] Genotyping of rs1800497 (*DRD2*) was performed on the Illumina BeadStation 500 platform (Illumina Inc., San Diego, CA, USA) by laboratory personnel blinded to the true identity of the individual samples. Scan data were analyzed in BeadStudio 3.0 (Illumina Inc., San Diego, CA, USA).

Genotyping of the *5-HTTLPR* polymorphism in the promoter region of the *SLC6A4* gene was performed by simple sequence length analysis on an automated capillary sequencer (ABI3730, Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) using standard conditions. The single nucleotide substitution (A>G) located immediately upstream of the insertion-deletion polymorphism (rs25531) was genotyped with the use of a custom-made Taqman assay (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). We genotyped the SNP on a 7500 Fast Real-Time PCR (polymerase chain reaction). Genotypes were scored with the use of the algorithm and software supplied by the manufacturer (Applied Biosystems). The assay was validated by digesting the *SLC6A4* PCR product with MspI (New England Biolabs, Ipswich, USA) and separating the restriction fragments on a 2% agarose gel.

Call rates were 99.5% for rs1800497 and 96.5% for rs25531. Genotyping accuracy, as determined by concordance between duplicates, was 100% for all polymorphisms. Genotype frequencies of all polymorphisms were in Hardy-Weinberg equilibrium (Table 1).

Table 1. Genotype and allele frequencies.

Gene	Polymorphism	Genotype frequency			Minor allele frequency	HWE P-value
		11	12	22		
DRD2	rs1800497	0.62	0.34	0.04	0.21	0.416
SLC6A4	L/S	0.34	0.49	0.17	0.41	0.883
	rs25531	0.26	0.50	0.24	0.49	0.844
	composite*	0.26	0.50	0.24	0.49	0.922

* Homozygote L(A)/L(A), heterozygote L(A)/S or L(A)/L(G), homozygote S/S or L(G)/L(G) for 5-HTTLPR and rs25531.

Data analysis

We evaluated the role of impulsivity on BMI and skinfolds, %BF, and waist circumference in multivariate linear regression analyses. Weight, BMI, skinfolds, and waist circumference were ln-transformed in view of not normally distributed residuals. Mean effect sizes for ln-transformed outcomes were back-transformed to report them on their original scale. They were calculated as mean increase in the average value of the outcome measure per unit increase in the predictor: *mean effect size = median* $_{outcome\ measure}$ $* (e^{\beta} -1)$.[33]

We also evaluated genotypes as predictors of both impulsivity and adiposity measures. Genotypes were tested under a general (codominant, 2 degrees of freedom) model and if significant results were found, additive, dominant, and recessive (1 degree of freedom) models were evaluated. Finally, we evaluated impulsivity as a possible mediator by entering it as a covariate in the association between genotypes and anthropometric measurements. In all analyses, we corrected for age and sex. We evaluated sex as a potential effect modifier by adding a multiplicative term to the models. Since SES and pubertal stage showed missing values (n=12 and n=71, respectively), we corrected for these variables in a second step. Similarly, we performed multivariate logistic regression analyses, with overweight and obese participants defined together as cases.

We used Quanto to calculate power available to detect main genetic effects.[34] All other statistical analyses were performed using SPSS version 16.0 (SPSS, Chicago IL, USA). The level of statistical significance was set at a probability of < 0.05.

RESULTS

Our population consisted of 645 girls and 599 boys, with a mean (± SD) age of 16.2 ± 0.7 years. Mean BMI was 20.75 (interquartile range (IQR), 19.20 – 22.55); and 12.4% was overweight and 2.7% was obese (Table 2). There was no difference in prevalence between boys and girls (p=0.14). Compared with girls, boys showed less advanced pubertal stage, were heavier, taller, and had a lower impulsivity score and a lower BMI, sum of skinfolds and %BF (Table 2).

Table 2. Sociodemographic and anthropometric characteristics, and impulsivity according to sex.

	All		Girls		Boys		Sex effect
		n		n		n	P-value
Age (yrs)	16.2 ± 0.7	1244	16.2 ± 0.7	645	16.2 ± 0.6	599	0.18
Pubertal stage (% in 3 categories)*	15.9 / 28.9 / 55.2	1173	0.8 / 0.3 / 98.9	615	32.6 / 60.4 / 7.0	558	<0.001
SES (% in 3 categories)**	19.1 / 50.0 / 30.9	1232	18.8 / 51.6 / 29.7	640	19.4 / 48.3 / 32.3	592	0.50
Impulsivity score***	23 ± 4	1212	24 ± 4	631	23 ± 4	581	0.001
Weight (kg)	63.1 (57.7 – 70.3)	1244	60.6 (55.5 – 66.0)	645	66.0 (60.0 – 74.6)	599	<0.001
Height (cm)	174.5 ± 8.9	1244	169.2 ± 6.3	645	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	0.14
Sum of skinfolds (mm)	47 (32 – 65)	1233	59 (47 – 73)	639	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)	1240	73.8 (69.7 – 79.4)	644	73.8 (70.3 – 78.3)	596	0.46

All data are means ± SD or median (interquartile range) unless otherwise indicated.

* Measured by the Physical Development Scale questionnaire, divided into pre/early pubertal, midpubertal and late/post pubertal.

** Socioeconomic status was based on questionnaires and classified as low, medium, and high SES.

*** Measured by the impulsiveness subscale (8 items) of the NEO-PI-R questionnaire, resulting in a score of 5 to 40.

**** Overweight or obese as defined by international age- and sex-adjusted BMI criteria (equivalent to the Dutch 94th and 99.7th percentile in 1980 for overweight and obesity, respectively).

P-value from chi-square test for pubertal stage, effect on overweight / obesity, and socioeconomic status;
t test for age, height, % body fat, and impulsivity score;
Mann-Whitney U test for weight, BMI, sum of skinfolds, and waist circumference.

Impulsivity and adiposity

Overall impulsivity was associated with increased measures of general as well as abdominal adiposity, also after correction for possible confounders, i.e. SES and pubertal stage (Table 3). For example, an increase in the impulsivity score of 4 points, corresponding to 1 SD, was associated with a mean increase of 0.24 kg/m^2 in BMI, of 0.32 % in %BF, and of 0.64 cm in waist circumference. Impulsivity was associated with a trend for a higher risk

Table 3. Linear regression of impulsivity on measures of general and abdominal adiposity.

	Overall impulsivity	
	B (95% CI), p	
	adjusted for sex	adjusted for sex, SES and pubertal stage
BMI* (kg/m^2)	0.06 (0.01 – 0.10), 0.02	0.07 (0.02 – 0.11), 0.004
Sum of skinfolds* (mm)	0.19 (-0.09 – 0.48), 0.19	0.25 (-0.03 – 0.54), 0.08
Body fat (%)	0.08 (0.01 – 0.15), 0.03	0.09 (0.02 – 0.16), 0.02
Waist circumference* (cm)	0.16 (0.03 – 0.28), 0.01	0.19 (0.06 – 0.31), 0.003

* Ln-transformed in view of not normally distributed residuals; back-transformed using: *mean effect size = median* $_{outcome\ measure}$ ** (e^{β} -1).*[33]

of being overweight (OR 1.04; 95% CI, 0.99 – 1.08). Since age was not significant in any of these models, we present models without adjustment for age. We did not find significant interaction terms between impulsivity and sex.

Influence of genetic variation on impulsivity and adiposity measures

Rs1800497 near the *DRD2* gene was not associated with the impulsivity score; neither was the composite polymorphism of *5-HTTLPR* and rs25531 in the promoter of the *SLC6A4* gene (Table 4).

We did not find significant associations between rs1800497 and BMI, sum of skinfolds, %BF, and waist circumference (Table 4). There was also no significant influence of the composite polymorphism of *5-HTTLPR* and rs25531 near the *SLC6A4* gene on these anthropometric measurements (Table 4). We therefore did not conduct any mediation analyses.

Table 4. Results from logistic and linear regression of genotypes on impulsivity, overweight and measures of adiposity.

Gene	Polymorphism	Model	Impulsivity	Overweight/ obese	BMI (kg/m²)*	Sum of skinfolds (mm)*	Body fat (%)	Waist circumference (cm)*
			statistic,** p	statistic,** p	statistic,** p	statistic,** p	statistic,** p	statistic,** p
DRD2	rs1137101	codominant	0.10, 0.90	1.87, 0.39	0.52, 0.60	1.58, 0.21	0.16, 0.85	0.29, 0.75
SLC6A4	composite***	codominant	0.83, 0.44	0.70, 0.71	0.10, 0.91	1.10, 0.33	0.39, 0.68	0.95, 0.39

In all models, adjustments were made for sex, pubertal stage and socioeconomic status.

* Ln-transformed in view of not normally distributed residuals; back-transformed using: *mean effect size = median* $_{outcome\ measure}$ $* (e^{\beta} - 1)$.[33]

** Influence of genotypes in codominant models (2 degrees of freedom) was assessed by means of the Wald statistic for logistic regression analyses and the F change statistic for linear regression analyses.

*** Homozygote L(A)/L(A), heterozygote L(A)/S or L(A)/L(G), homozygote S/S or L(G)/L(G) for *5-HTTLPR* and rs25531.

DISCUSSION

In our population-based cohort of adolescents we found significant associations between impulsivity and measures of overall and abdominal adiposity. We did not find any evidence for associations between genetic variation near the *DRD2* gene and in the *SLC6A4* gene and impulsivity; nor for associations between variation near the *DRD2* gene and in the *SLC6A4* gene and adiposity measures.

To our knowledge, this is the first report of an association between impulsivity and adiposity in a population-based cohort of healthy adolescents. Case-control studies also provided support for such an association. Results from a case-control study comparing 56 normal weight 13-year-olds and 56 overweight children seeking treatment, showed that overweight children had higher impulsivity levels compared with normal weight children.[7] Another case-control study of 32 overweight children participating in a treatment program for overweight and a control group of 31 children, also showed that impulsivity was as-

sociated with overweight at age 13 years.[6] Specifically, overweight children were more sensitive to reward and showed less inhibitory control than normal weight children. In the same population, less inhibitory control was also associated with a less successful response to overweight treatment. In a quasi-experimental study, exposure to a wide variety of obesogenic foods led to overeating only in more impulsive, reward sensitive children.[35]

We did not find significant interaction terms between impulsivity and sex, which is in line with one of the case-control studies.[7] In contrast, Francis and colleagues did report an association only in girls between lack of self-regulation and weight gain, when conducting separate models according to sex.[9]. We found a similar trend of consistently higher effect sizes in girls compared with boys (data not shown). These differences decreased after correction for SES and pubertal stage.

We hypothesized that genetic variation could influence adiposity via an effect on impulsivity. Genetic variation near the *DRD2* gene and in the *SLC6A4* gene did not show an association with impulsivity. In addition, we were not able to replicate influences of common polymorphisms near the *DRD2* gene and in the *SLC6A4* gene on overweight.

The *Taq1A* polymorphism near the *DRD2* gene has been associated with overweight in various studies.[16,17,31,36] However, others have refuted an association, [37,38] in line with our findings. Also with regard to its influence on impulsivity, contrasting findings have been reported. In adolescents, admitted to a psychiatric clinic for suicidal thoughts or behaviors, the minor allele A1 was associated with increased impulsivity as measured by a delay discounting task,[14] in which participants are asked to choose between an immediate smaller reward and a delayed larger reward. In contrast, in a study among alcohol dependent patients, higher impulsivity scores as measured by a questionnaire (the Barrett Impulsiveness Scale, BIS) were found among the major allele A2 carriers.[15]

Evidence for an association between the *serotonin transporter* gene and overweight was found by Sookoian and colleagues.[23] They reported that the S-allele in the *SLC6A4* promoter was associated with overweight in a population-based study of Argentinean children and adolescents from European ancestry (n=172). The association was confirmed in a US population-based study.[37] In line with our own results, no association was reported in a Turkish case-control study (262 cases, 138 controls).[39] Evidence for the influence of the *SLC6A4* gene on impulsivity was found in alcohol dependent patients, in whom an association was established between a SNP in the promoter region and impulsive behavior as measured by a questionnaire (BIS).[21] In a population-based sample of Estonian adolescents (n=429) a significant association was found between the S-allele of *5-HTTLPR* polymorphism and impulsivity as measured by a cognitive visual comparison test (in which speed-accuracy was determined); but not when impulsive traits were assessed by the BIS questionnaire.[22] In contrast, another study reported an association between L-allele and impulsiveness measured by the BIS questionnaire.[40] The only study integrating variation in *5-HTTLPR*, impulsivity, and overweight used a family based method in a small sample size

of 50 obese individuals and their parents.[41] Results suggested that in obese females the L-allele is associated with impulsivity. This could have been a false positive finding, because of the small sample size. Although these conflicting findings could have been caused by differences in sample selection and the multifaceted aspect of impulsiveness, they could also be because of a misclassification bias because recent studies found that rs25531 near the *5-HTTLPR* polymorphism also influences *SLC6A4* transcription.[24,25] We evaluated the composite polymorphism based on the *5-HTTLPR* polymorphism and rs25531 and did not find a significant influence on adiposity measures.

The main strength of our study is that we assessed common genetic variation, impulsivity and various, accurately determined measures of adiposity in a large population of healthy adolescents. Power calculations showed that with a power of 80% and an alpha of 0.05, our sample size (n=1244) was able to detect explained variances of as low as 0.63%. For example, we had a power of 99.7 to 100% to detect the magnitude of the association between *5-HTTLPR* and BMI as reported by Sookoian and colleagues.[23] Thus, in the etiology of adolescent overweight, important influences of the *Taq1A* polymorphism near the *DRD2* gene and of the polymorphic promoter region of the *SLC6A4* gene are unlikely.

A limitation is that our study was restricted to the most commonly reported polymorphisms related to dopaminergic and serotonergic activity. It is possible that other genetic variants determine dopaminergic and serotonergic dysfunction; and thereby influence the association between impulsivity and adiposity. The *Taq1A* polymorphism is actually located in the *Ankyrin Repeat and Kinase Domain Containing 1* (*ANKK1*) gene in which it causes an amino-acid substitution.[14] The effects of this substitution are unknown, but it could influence interactions of *ANKK1* proteins with other proteins, including *DRD2*. It is possible that there are better markers of *DRD2* gene expression. A second limitation is the fact that our study is cross-sectional. Impulsivity and adiposity measures were both obtained at age 16. We are therefore unable to distinguish if impulsivity precedes adiposity, although we conclude that a relationship does exist between impulsivity and adiposity. Further research on this topic should focus on the longitudinal association. Preventive strategies and treatment programs might be more successful when individualized and adapted to specific personality characteristics, such as impulsivity.

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