Original Article

Association of Obesity Susceptibility Gene Variants with Metabolic Syndrome and Related Traits in 1,443 Czech Adolescents

(single-nucleotide polymorphism / *FTO* gene / obesity, metabolic syndrome / adolescence)

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Abstract. Genome-wide association studies have revealed several gene variants associated with obesity; however, only a few studies have further investigated their association with metabolic syndrome. We performed a study of eleven variants in/near genes *TMEM18*, *SH2B1*, *KCTD15*, *PCSK1*, *BDNF*, *SEC16B*, *MC4R*, and *FTO* in Czech adolescents and analysed their association with obesity, metabolic syndrome and related traits. Genotyping was performed in 1,443 adolescents aged 13.0–17.9 years. Anthropometric parameters, biochemical parameters and blood pressure were assessed. Metabolic syndrome was defined according to the International Diabetes Federation. The *FTO* rs9939609 variant was associated with overweight/obesity (OR 1.40, 95% CI 1.21–1.63, P < 0.001). The minor allele of *TMEM18* rs7561317 was related to underweight (OR 1.78, 95% CI 1.14–2.79, P = 0.015). *BDNF* rs925946 and *MC4R* rs17782313 were associated with metabolic syndrome (OR 1.53, 95% CI 1.14–2.04, P = 0.005; 1.51, 95% CI 1.12–2.04, P = 0.009). The *PCSK1* rs6235 variant was negatively related to increased blood glucose (OR 0.69, 95% CI 0.49–0.97, P = 0.040). In conclusion, the *FTO* variant was associated with overweight/obesity in Czech adolescents. Moreover, *MC4R* and *BDNF* variants increased the risk of metabolic syndrome, probably through their effect on abdominal obesity. The *PCSK1* variant may have a protective role in the development of type 2 diabetes.

Introduction

The prevalence of obesity has been increasing worldwide in children and adolescents (Lobstein et al., 2004). This trend is alarming because obesity in childhood tends to persist to adulthood with a high incidence of cardiometabolic complications (Katzmarzyk et al., 2001). Metabolic syndrome (MS) is a cluster of risk factors for cardiometabolic diseases that includes abdominal obesity, dyslipidaemia, impaired glucose metabolism and hypertension (Zimmet et al., 2007). Due to the

current obesity epidemic, MS is often diagnosed already in children and adolescents (Bokor et al., 2008).

Genome-wide association studies (GWAS) have identified several loci associated with body mass index (BMI) and obesity. In 2007, a variant of the fat mass and obesity associated gene (*FTO*) demonstrating a strong association with BMI was discovered and until now has been considered as one of the most studied genes related to obesity (Frayling et al., 2007). The following year, single-nucleotide polymorphisms (SNPs) near the melanocortin 4 receptor gene (*MC4R*) (Loos et al., 2008) and the proprotein convertase subtilisin/kexin type 1 gene (*PCSK1*) were designated as candidate genes for obesity (Benzinou et al., 2008). Thanks to extensive GWAS, 10 other obesity susceptibility loci were identified, e.g. transmembrane protein 18 (*TMEM18*), SH2B adaptor protein 1 (*SH2B1*), potassium channel tetramerization domain containing 15 (*KCTD15*), brain-derived neurotrophic factor (*BDNF*), and SEC16 homologue B (*SEC16B*) (Thorleifsson et al., 2009; Willer et al., 2009). The last GWAS focusing on BMI associations confirmed 14 previously reported loci and revealed 18 new loci (Speliotes et al., 2010). Most of the mentioned SNPs have been replicated in children and adolescents with a similar effect on BMI (Zhao et al., 2009; den Hoed et al., 2010). Confirmation and extension of findings from GWAS in selected population represents the next step of further investigations.

The aim of the present study was to explore the association of 11 previously reported variants in/near genes *TMEM18*, *SH2B1*, *KCTD15*, *PCSK1*, *BDNF*, *SEC16B*, *MC4R* and *FTO* with different body weight statuses (underweight, normal weight, overweight, obesity), abdominal obesity, MS and related traits in Czech adolescents.

Material and Methods

Subjects

The total of 1,443 adolescents (661 boys, 782 girls) aged 13.0−17.9 years were recruited from the Czech Childhood Obesity Prevalence And Treatment (COPAT) project, focused on risk factors for obesity and MS in the adolescence. The COPAT project was conducted in different regions across the Czech Republic and included a representative cohort (N = 1,533) established by the stratified random selection and overweight/obese adolescents (N = 562) that had undergone a 4-week weight management programme in specialized clinical settings.

The present study included participants from both cohorts: 60 underweight, 713 normal weight, 194 overweight and 476 obese adolescents characterized in Table 1. Underweight, normal weight, overweight and obesity were defined by BMI percentiles according to the Czech national reference data (Kobzova et al., 2004): $\leq 10^{th}$, $10^{th}-90^{th}$, $90^{th}-97^{th}$, $\geq 97^{th}$, respectively. The standard deviation scores (SDS) of anthropometric parameters were assessed according to the national reference data (Kobzova et al., 2004). The reference percentiles of German adolescent population were used for the waist circumference (Haas et al., 2011). MS was defined according to the International Diabetes Federation (IDF) criteria for children and adolescents (Zimmet et al., 2007).

The study was approved by the Ethics Committee of the Institute of Endocrinology and was performed in accordance with the Helsinki Declaration. All participants and their parent(s) provided written informed consent.

Anthropometric and biochemical characteristics

Body height was measured by a stadiometer (precision 0.1 cm); body weight was measured by Tanita

Table 1. Characteristics of the studied cohort

Variable	Underweight	Normal weight	Overweight	Obese
Number	60	713	194	476
Boys/girls	23/37	337/376	85/109	216/260
Age (years)	16.4 (15.5–17.1)	16.0 (15.1–16.9)	15.8 (14.6–17)	15.4 (14.2–16.6)
Height-SDS	−0.3 (−0.9–0.3)	−0.1 (−0.7–0.6)	0.0 (−0.5–0.8)	0.2 (−0.6–0.8)
Weight-SDS	−1.4 (−1.7–1.0)	−0.1 (−0.6–0.5)	1.5 (1.1–1.9)	3.2 (2.4–4.4)
BMI-SDS	−1.4 (−1.6–1.2)	0.0 (−0.5–0.5)	1.5 (1.3–1.8)	3.3 (2.6–4.6)
Waist circumference-SDS	−1.3 (−1.6–1.0)	−0.4 (−0.8–0)	0.7 (0.4–1.2)	2.3 (1.5–3.1)
Abdominal circumference-SDS	−0.5 (−1–0.2)	0.4 (−0.1–1)	2.3 (1.6–2.8)	4.3 (3.3–5.4)
Hip circumference-SDS	−1.2 (−1.4–0.7)	0.0 (−0.5–0.6)	1.4 (1.1–1.9)	2.8 (2.1–3.7)
Arm circumference-SDS	−1.3 (−1.7–0.9)	0.1 (−0.3–0.7)	1.7 (1.2–2.2)	3.1 (2.4–3.9)
Total body fat (%)	15.2 (12.4–18.5)	20.1 (15.1–24.4)	29.3 (23.1–32.1)	35.4 (30.6–40)
Trunk fat (%)	11 (8.1–13)	15.2 (11.3–19.5)	23.8 (19.4–27.3)	30.4 (25.8–35.9)
Systolic blood pressure (mmHg)	114 (105–124)	121 (111–128)	120 (111–128)	122 (113–131)
Diastolic blood pressure (mmHg)	76 (69–81)	77 (72–83)	76 (72–82)	78 (73–84)
Glucose (mmol/l)	5.0 (4.7–5.4)	5.0 (4.7–5.2)	5.0 (4.7–5.3)	5.0 (4.7–5.3)
C-peptide (mmol/l)	0.7 (0.6–0.8)	0.7 (0.6–0.8)	0.8 (0.7–0.9)	0.9 (0.7–1.2)
Insulin (mmol/l)	9.2 (6.4–12.8)	9.7 (7.3–12.8)	11.8 (8.8–15.4)	14.7 (10.7–21.5)
Triglycerides (mmol/l)	0.7 (0.6–1.0)	0.8 (0.6–1.1)	0.9 (0.7–1.1)	1.1 (0.8–1.6)
HDL-cholesterol (mmol/l)	1.5 (1.2–1.7)	1.4 (1.2–1.6)	1.3 (1.1–1.5)	1.2 (1–1.4)
Total cholesterol (mmol/l)	4.3 (3.7–4.6)	4.0 (3.6–4.6)	4.1 (3.7–4.7)	4.3 (3.7–4.9)
LDL-cholesterol (mmol/l)	2.1 (1.7–2.5)	2.1 (1.7–2.6)	2.3 (1.9–2.8)	2.4 (2.0–3.0)

Data are expressed as a median (lower–upper quartile).

BC-418 MA (Tanita Corporation, Tokyo, Japan; precision 0.1 kg). Body circumferences were determined by a tape measure (precision 0.1 cm) in a horizontal level; waist circumference midway between the upper iliac crest and the lower rib, abdominal circumference at the level of umbilicus, hip circumference over the maximum buttocks diameter, arm circumference in the middle of relaxed arm. The percentage of total body and trunk fat was measured by bioelectric impedance Tanita BC-418 MA and Tanita AB-140 ViScan (Tanita Corporation, Tokyo, Japan). We previously demonstrated that trunk fat determined by bioelectric impedance in Czech adolescents highly correlated with both dual-energy X-ray absorptiometry and magnetic resonance imaging measurements (Zamrazilova et al., 2010).

Peripheral blood samples were taken from the cubital vein after an overnight fast. Glucose, insulin, C-peptide, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides were determined using the Cobas® 6000 instrument and commercial kits (Roche Diagnostics GmbH, Mannheim, Germany).

Blood pressure was measured by the arm sphygmomanometer with appropriate cuff size. Two sets of measurements were performed and an average value was calculated.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using QuickGene DNA whole blood kit (Fujifilm, Tokyo, Japan). Genotyping of SNPs: rs7561317 (*TMEM18*), rs7498665 (*SH2B1*), rs29941 (*KCTD15*), rs6232 and rs6235 (*PCSK1*), rs925946 and rs4923461 (*BDNF*), rs10913469 (*SEC16B*), rs12970134 and rs17782313 (*MC4R*), rs9939609 (*FTO*) was performed using the TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA). The assays were run in a Biomark instrument (Fluidigm, South San Francisco, CA) and rs12970134, rs17782313, and rs9939609 in a LightCycler 480 (Roche, Basel, Switzerland). To ensure consistency among runs, samples of known genotypes and negative controls were used in every run. The genotype distributions of all SNPs followed the Hardy-Weinberg equilibrium ($P > 0.2$). The SNPs located in *PCSK1* (rs6232, rs6235) and *BDNF* (rs925946, rs4923461) were incompletely linked ($r^2 = 0.16$ and 0.11, respectively). The SNPs near *MC4R* (rs12970134, rs17782313) were in high linkage disequilibrium ($r^2 = 0.79$).

Statistical analysis

The statistical software NCSS 2004 (NCSS LLC, Kaysville, Utah) and Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA) were used. P values (two-tailed) < 0.05 were considered statistically significant. The Hardy-Weinberg equilibrium was tested using the χ^2 test. Linkage disequilibrium was estimated using Haploview V3.2. (Barrett et al., 2005). Genotype and allele frequencies were compared by χ^2 test with Yates

correction. Odds ratios (ORs) and 95% confidence intervals (CI) were calculated to determine associations of each SNP and weight status (underweight, overweight, obesity) using normal weight subjects as controls; for MS and its components, non-carriers were used as controls. Association of SNPs and quantitative traits (anthropometric, biochemical characteristics, blood pressure) were analysed by general linear models after adjustments for sex and age. Anthropometric traits were additionally adjusted for BMI. Due to the non-Gaussian distribution, data are expressed as medians with lower and upper quartiles.

Results

Associations with body weight categories

The observed genotype and allele frequencies of SNPs in the overweight/obese and underweight/normal weight adolescents are shown in Table 2. Significant differences were found for the *FTO* rs9939609 variant ($P < 0.001$) and the *SEC16B* rs10913469 variant ($P = 0.033$). The *FTO* rs9939609 A-allele was associated with overweight and/or obesity (Table 3). ORs were 1.27 (95% CI 1.01–1.59, $P = 0.046$) for overweight and 1.46 (95% CI 1.24–1.73, $P < 0.001$) for obesity. No other variants showed significant associations. In the case of *SEC16B* rs10913469 there was a tendency towards an association with overweight and obesity (OR 1.21, 95% CI 0.99–1.47, $P = 0.066$).

Associations between each SNP and underweight are demonstrated in Table 3. The *TMEM18* rs7561317 variant was found to be significantly related to underweight. The minor allele increased the risk of underweight (OR 1.78, 95% CI 1.14–2.79, $P = 0.015$).

Associations with metabolic syndrome

The prevalence of MS in our cohort of 1,443 Czech adolescents was 7.7 % (65 boys, 46 girls). MS was only present in overweight and obese adolescents in whom its prevalence reached 16.6 %. Table 3 shows associations of the 11 investigated variants with MS. The *BDNF* rs925946 T-allele ($P = 0.005$), the *MC4R* rs12970134 A-allele ($P = 0.035$), and the *MC4R* rs17782313 C-allele ($P = 0.009$) increased the risk of MS. However, the gender-specific analyses revealed that the increased risk was only significant in boys with OR of 1.77 (95% CI 1.22–2.59, $P = 0.004$ vs. girls $P = 0.412$) for *BDNF* rs925946 and OR of 1.56 (95% CI 1.05–2.33, $P = 0.036$ vs. $P = 0.156$) for *MC4R* rs17782313. The same tendency for the *MC4R* rs12970134 variant ($P = 0.069$) was also demonstrated in boys only.

A detailed case-control study was performed to determine the association of gene variants with individual risk factors of MS (carriers vs. non-carriers). Abdominal obesity was significantly related to the *FTO* variant (OR 1.39, 95% CI 1.19–1.64, $p < 0.001$). Furthermore, only in boys the minor alleles of *BDNF* rs925946 (OR 1.42, 95% CI 1.09–1.84, $p = 0.010$), *MC4R* rs12970134 (OR

Table 2. Differences of genotype and allele frequencies between overweight/obese and underweight/normal weight adolescents

Variant	Locus	Group	Genotypes (N)			P^a	RAF (%)	P^a
			AA	AG	GG		G	
rs7561317	*TMEM18*	UW/NW	14	208	551	0.611	84.7	0.477
		OW/OB	13	165	492		85.7	
			AA	AG	GG		G	
rs7498665	*SH2B1*	UW/NW	249	388	136	0.461	42.7	0.235
		OW/OB	234	329	106		40.4	
			AA	AG	GG		G	
rs29941	*KCTD15*	UW/NW	63	328	369	0.853	70.1	0.876
		OW/OB	57	274	326		70.5	
			CC	CT	TT		C	
rs6232	*PCSK1*	UW/NW	3	65	702	0.143	4.6	0.098
		OW/OB	2	77	589		6.1	
			CC	CG	GG		G	
rs6235	*PCSK1*	UW/NW	407	316	49	0.243	26.8	0.514
		OW/OB	375	246	49		25.7	
			GG	GT	TT		T	
rs925946	*BDNF*	UW/NW	401	318	54	0.520	27.6	0.831
		OW/OB	359	257	53		27.1	
			AA	AG	GG		A	
rs4923461	*BDNF*	UW/NW	468	263	40	0.673	77.8	0.859
		OW/OB	415	215	39		78.1	
			CC	CT	TT		C	
rs10913469	*SEC16B*	UW/NW	18	208	546	0.080	15.8	**0.033**
		OW/OB	20	213	437		18.9	
			AA	AG	GG		A	
rs12970134	*MC4R*	UW/NW	46	281	437	0.412	24.4	0.309
		OW/OB	51	241	364		26.1	
			CC	CT	TT		C	
rs17782313	*MC4R*	UW/NW	59	297	407	0.554	27.2	0.337
		OW/OB	51	256	312		28.9	
			AA	AT	TT		A	
rs9939609	*FTO*	UW/NW	139	387	240	**<0.001**	43.4	**<0.001**
		OW/OB	176	338	152		51.8	

[a]P value was calculated by χ^2 test with Yates correction.
OW/OB – overweight/obese adolescents; UW/NW – underweight/normal weight adolescents; RAF – risk allele frequency

1.39, 95% CI 1.06–1.82, p = 0.020) and *MC4R* rs17782313 (OR 1.40, 95% CI 1.07–1.83, P = 0.018) increased the risk of abdominal obesity. Increased blood glucose was negatively associated with *PCSK1* rs6235 (OR 0.69, 95% CI 0.49–0.97, P = 0.040). The protective effect of this variant was only found in boys (OR 0.61, 95% CI 0.39–0.95, P = 0.035).

Table 3. Association of 11 SNPs with underweight, overweight and/or obesity, and metabolic syndrome

Variant	Locus	Risk allele	RAF (%)	Underweight		Overweight		Obesity		Overweight/obesity		Metabolic syndrome	
				OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
rs7561317	TMEM18	G	85	**0.56 (0.36–0.88)**	**0.015**	1.08 (0.78–1.49)	0.704	1.01 (0.80–1.27)	0.998	1.03 (0.83–1.27)	0.846	0.89 (0.61–1.29)	0.611
rs7498665	SH2B1	G	42	1.23 (0.85–1.79)	0.311	0.96 (0.76–1.20)	0.754	0.91 (0.77–1.08)	0.310	0.93 (0.80–1.08)	0.342	0.99 (0.75–1.30)	0.984
rs29941	KCTD15	G	71	0.75 (0.51–1.12)	0.190	1.10 (0.86–1.42)	0.480	0.95 (0.80–1.14)	0.630	0.99 (0.84–1.17)	0.969	0.84 (0.63–1.13)	0.283
rs6232	PCSK1	C	5	0.89 (0.35–2.26)	0.988	1.48 (0.93–2.37)	0.128	1.26 (0.87–1.82)	0.253	1.32 (0.95–1.85)	0.117	0.57 (0.26–1.22)	0.191
rs6235	PCSK1	G	27	0,70 (0.44–1.10)	0.152	0.86 (0.67–1.12)	0.292	0,94 (0.78–1.13)	0.563	0,92 (0.78–1.09)	0.349	0.87 (0.63–1.20)	0.459
rs925946	BDNF	T	28	0.71 (0.45–1.11)	0.162	0.90 (0.70–1.16)	0.469	0.98 (0.81–1.17)	0.838	0.95 (0.81–1.13)	0.618	**1.53 (1.14–2.04)**	**0.005**
rs4923461	BDNF	A	78	1.04 (0.66–1.63)	0.965	1.09 (0.83–1.43)	0.598	1.00 (0.82–1.22)	0.970	1.02 (0.85–1.22)	0.837	1.20 (0.85–1.70)	0.337
rs10913469	SEC16B	C	16	0.69 (0.39–1.22)	0.245	1.26 (0.95–1.69)	0.127	1.19 (0.96–1.47)	0.136	1.21 (0.99–1.47)	0.066	1.06 (0.74–1.51)	0.819
rs12970134	MC4R	A	24	1.06 (0.69–1.63)	0.877	0.99 (0.76–1.29)	0.975	1.15 (0.95–1.39)	0.162	1.10 (0.93–1.31)	0.295	**1.40 (1.03–1.89)**	**0.035**
rs17782313	MC4R	C	27	1.04 (0.69–1.59)	0.930	0.97 (0.75–1.25)	0.849	1.15 (0.95–1.39)	0.157	1.09 (0.92–1.30)	0.328	**1.51 (1.12–2.04)**	**0.009**
rs9939609	FTO	A	43	1.03 (0.71–1.50)	0.957	**1.27 (1.01–1.59)**	**0.046**	**1.46 (1.24–1.73)**	**<0.001**	**1.40 (1.21–1.63)**	**<0.001**	1.02 (0.77–1.34)	0.961

RAF – risk allele frequency

Table 4. Association of FTO and SEC16B genotypes with anthropometric parameters

FTO rs9939609	AT	AA	P
	β est ± SE	β est ± SE	
Weight-SDS	**0.25 ± 0.12**	**0.61 ± 0.15**	**<0.001**
BMI-SDS	**0.28 ± 0.12**	**0.58 ± 0.15**	**<0.001**
Waist circumference-SDS	**0.22 ± 0.10**	**0.46 ± 0.11**	**<0.001**
	0.02 ± 0.04	0.05 ± 0.05	0.533[a]
Abdominal circumference-SDS	**0.33 ± 0.13**	**0.69 ± 0.16**	**<0.001**
	0.04 ± 0.05	0.09 ± 0.06	0.305[a]
Hip circumference-SDS	**0.13 ± 0.11**	**0.47 ± 0.13**	**<0.001**
	−0.08 ± 0.05	0.02 ± 0.06	0.082[a]
Arm circumference-SDS	**0.23 ± 0.11**	**0.49 ± 0.13**	**<0.001**
	0.01 ± 0.05	0.02 ± 0.06	0.918[a]
Total body fat (%)	**1.10 ± 0.53**	**2.68 ± 0.64**	**<0.001**
	−0.17 ± 0.24	0.32 ± 0.29	0.155[a]
Trunk fat (%)	**1.16 ± 0.57**	**2.89 ± 0.68**	**<0.001**
	−0.23 ± 0.29	0.29 ± 0.35	0.237[a]

SEC16B rs10913469	CT	CC	P
	β est ± SE	β est ± SE	
Weight-SDS	**0.30 ± 0.11**	**0.22 ± 0.33**	**0.033**
BMI-SDS	**0.31 ± 0.12**	**0.22 ± 0.33**	**0.024**
Abdominal circumference-SDS	**0.32 ± 0.12**	**0.27 ± 0.35**	**0.032**
	0.03 ± 0.04	0.05 ± 0.12	0.794[a]
Arm circumference-SDS	**0.28 ± 0.10**	**0.28 ± 0.28**	**0.018**
	0.05 ± 0.04	0.10 ± 0.12	0.406[a]

P values were calculated by generalized linear regression using an additive model adjusted for sex and age. [a]Parameters were additionally adjusted for BMI.

β est – coefficient estimate (compared to the wild-type genotype); SE – standard error

Associations with anthropometric and biochemical parameters

Only significant associations of anthropometric and biochemical parameters with the studied gene variants are presented in Table 4. The *FTO* rs9939609 genotype was strongly related to body weight, BMI, body circumferences, total body and trunk fat ($P < 0.001$). The *SEC16B* rs10913469 genotype was associated with an increased body weight, BMI, abdominal and arm circumferences ($P < 0.05$). Gender-stratified analyses revealed further associations. Only in boys, associations of body weight with *MC4R* rs12970134 ($P = 0.004$) and *MC4R* rs17782313 ($P = 0.014$) and an association of abdominal circumference with the rs17782313 variant ($P = 0.032$) were found. However, after additional ad-

justment for BMI, none of the above-mentioned associations remained statistically significant.

Discussion

We evaluated 11 previously reported gene variants and their associations with weight statuses in 1,443 Czech adolescents. The association of *FTO* variants in Czech adults has already been confirmed (Hubacek et al., 2008), in contrast to the other obesity susceptibility locus in insulin-induced gene 2, which was not associated (Hubáček et al., 2010). This is the first study of other obesity gene variants performed in Czech adolescents. We confirmed the strong association of *FTO* rs9939609 with overweight and obesity with a similar effect as in previously published studies performed in European descendants (Dina et al., 2007; Frayling et al., 2007). The 10 remaining SNPs have not been found to be related to obesity. These associations have already been confirmed in children and adolescents; however, mostly in larger studies and meta-analyses (Zhao et al., 2009; den Hoed et al., 2010). We assumed that we were not able to replicate the small effects (mostly explaining 1.12 % of the total variation for BMI Z-score) of the SNPs (Zhao et al., 2009). The *SEC16B* rs10913469 variant showed the same tendency as the *FTO* variant. We demonstrated that this SNP was associated with increased anthropometric parameters. However, we suppose that the low number of minor homozygotes in our cohort might have been limiting. Variants near *MC4R* were related to body weight and abdominal circumference, but only in boys. A recent study of eight common obesity variants also found gender-specific differences (Wang et al., 2012).

Our studied population included all weight categories (obese, overweight, normal weight and underweight), and thus we were also able to perform the case-control study with underweight as the opposite extreme of BMI variability. In the GWAS of Willer et al. (2009), the major allele of *TMEM18* rs7561317 was described as the obesity risk allele. Surprisingly, the results of our study demonstrated that the minor allele of this variant significantly increased the risk of being underweight. TMEM18 is a DNA-binding protein that could possibly affect repression of the transcription of chromatin (Jurvansuu and Goldman, 2011). However, its involvement in the pathogenesis of obesity has not yet been elucidated. Our findings have to be confirmed by other independent studies. We are aware of the low frequency of the *TMEM18* minor allele. Nonetheless, we emphasize the importance of focusing further investigations on underweight individuals as well. Jacquemont et al. (2011) have recently published a study in which they suggested that severe obesity and underweight might share similar genetic background.

Metabolic syndrome

Abdominal obesity defined by waist circumference is usually considered as a crucial marker of MS (Bitsori et al., 2009). The prevalence of MS has been rapidly in-creasing in overweight and obese individuals (Weiss et al., 2004). In our study, only overweight and obese adolescents fulfilled the IDF criteria for MS. Although several GWAS have identified SNPs related to the various metabolic traits in recent years (Newton-Cheh et al., 2009; Fox et al., 2012; Tukiainen et al., 2012), there is still a lack of studies focused on MS as a complex disorder.

We found an association between the *FTO* variant and abdominal obesity. However, the association did not remain significant after the adjustment for BMI, similarly to other studied antropometric parameters. It seems that the *FTO* variant has an effect on BMI in general, but does not influence the body fat distribution (e.g. abdominal obesity) and related metabolic risks. Several studies investigated the association of SNPs in *FTO* with metabolic traits (Liem et al., 2010), but their findings were not consistent. In agreement with our results, Müller et al. (2008) found no association between the *FTO* variant and biochemical parameters. According to the meta-analyses performed by Wang et al. (2011) and Zhou et al. (2012), *FTO* variants were associated with MS but dependent on the used definition for the MS. A significant association in adult Europeans was only found when the Third Report of the National Cholesterol Education Program Adult Treatment Panel criteria (2002) of MS were applied.

According to our results, the *BDNF* rs925946 and the *MC4R* rs12970134 and rs17782313 variants increased the risk of MS. However, the association of *MC4R* rs12970134 seems to be mediated through *MC4R* rs17782313 because of the observed high linkage disequilibrium between these SNPs and a weaker association with MS for the rs12970134 variant. *BDNF* and *MC4R* variants found only in boys probably play a role in the development of abdominal obesity. BDNF, a member of the nerve growth factor family, is necessary for survival of striatal neurons in the brain and should be an important mediator of energy balance regulated by the *MC4R* gene (Zuccato et al., 2001; Xu et al., 2003). Evidences about the involvement of *BDNF* in the pathogenesis of MS have so far only been at the experimental level. Heterozygous knockout mice exhibited elevated levels of glucose, insulin and leptin (Duan et al., 2003). Human studies focused on the association of plasma BDNF with MS showed discrepancies (Levinger et al., 2008; Corripio et al., 2012). Sandholt et al. (2011) revealed association of *BDNF* rs925946 with elevated blood glucose, although more likely mediated through BMI. G protein-coupled MC4R is the key factor in the regulation of food intake and energy homeostasis in the hypothalamus (Marsh et al., 1999). rs17782313 is mapped at 188 kb downstream of the *MC4R* gene, but it is still unclear whether it modulates MC4R expression (Loos et al., 2008). The association of *MC4R* variants with MS and related traits has not been clearly demonstrated. However, in studies of males, rs17782313 was shown to be associated with lower HDL cholesterol (Kring et al., 2010) and elevated diastolic blood pres-

sure (Vogel et al., 2011). In agreement with our results, Zobel et al. (2009) found no associations with blood glucose, insulin, C-peptide and lipids in the study of 14,940 Danes.

PCSK1 is a neuroendocrine convertase that activates hormones and neuropeptides. Mutations in the *PCSK1* gene cause monogenic obesity accompanied by malabsorption and impaired glucose homeostasis (Jackson et al., 1997). In our cohort, the obesity risk allele of *PCSK1* rs6235 was less frequent in subjects with elevated blood glucose. A similar finding was shown in a study of middle-aged Danes, in whom the risk allele carriers had significantly lower fasting glucose (Gjesing et al., 2011). Our data support the finding of Gjesing and the hypothesis that the variants in *PCSK1* may be protective against type 2 diabetes.

In our study, most of the associations were only observed in boys, who further demonstrated a higher frequency of MS in comparison to girls. It is well known that abdominal obesity as the key component for MS is characteristic for men, in contrast to the gynoid obesity, which is more prevalent in women and is characterized by rare cardiometabolic complications (Canoy et al., 2004). Therefore, the gender-specific effect of obesity-related SNPs should be considered in the pathogenesis of abdominal obesity and MS. GWAS focused on abdominal subcutaneous and visceral fat or the waist-to-hip ratio also revealed some loci with strong sex interactions (Heid et al., 2010; Fox et al., 2012). Further studies are necessary to confirm these findings and extend knowledge about the involvement and possible interaction of *MC4R*, *BDNF* and *PCSK1* variants in the regulation of energy metabolism. Moreover, according to our results, we could hypothesize that *PCSK1* and *TMEM18* variants may contribute to the metabolically healthy obesity (Stefan et al., 2008).

In conclusion, in our cohort of 1,443 Czech adolescents, of the 11 studied gene variants, an association with obesity was only confirmed for the *FTO* variant. Interestingly, the *TMEM18* variant was associated with underweight. *MC4R* and *BDNF* variants increased the risk of MS, probably through their effect on fat distribution represented by abdominal obesity. The *PCSK1* variant was negatively associated with elevated blood glucose, and thus seems to have a protective role in the development of type 2 diabetes.

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