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ORIGINAL ARTICLE

Common polymorphisms in six genes of the methyl group metabolism pathway and obesity in European adolescents

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Abstract

Objective. The goal of the present study was to assess the relationship between the genetic variability in six genes of methyl group (CH_3) metabolism and the risk of obesity. *Methods.* Single nucleotide polymorphisms (SNP) were selected among the methylene-tetrahydrofolate reductase (*MTHFR),* methionine synthase *(MTR),* methionine synthase reductase (*MTRR)*, cystationine betha-syntase (*CBS*), transcobalamin-II (*TCN2*) and paraoxonase-1 (*PON1*) genes. The associations between SNPs and the risk of obesity were assessed in a case-control study of obese and normal-weight adolescents (age: 14.9±1.2 years), and the relationship between SNPs and body fat markers (i.e., body mass index [BMI], percentage body fat [BF%] and waist circumference [WC]) in a cross-sectional study of 1 155 European adolescents (age: 14.8±1.4 years). Genotyping was performed on an Illumina system and plasma folate level was determined by immunoassay. *Results.* In the case-control study, there was no evidence for any association between SNPs of *MTHFR*, *MTR*, *CBS*, *TCN2* and *PON1* and obesity (all p values ≥0.08). In contrast, two SNPs of *MTRR* were associated with a higher (rs10520873, Odds Ratio: 1.68 [1.18–2.39]; p=0.004) or lower (rs1801394, 0.61 [0.42–0.87]; p=0.007) risk of obesity. In the cross-sectional sample, rs1801394 was associated with lower BMI (p=0.03) and lower waist circumference (p=0.02). However, after Bonferroni correction these associations were no longer significant. No other significant association or interaction between folate levels and SNPs were detected for anthropometric variables. *Conclusion.* Our findings do not support an association between *MTHFR, MTR, CBS*, *TCN2* and *PON1* SNPs and obesity in adolescence. Further investigations are necessary to confirm the possible association between the rs1801394 variant of *MTRR* and obesity.

Key words: *Methyl group metabolism, methylene-tetrahydrofolate reductase, methionine synthase reductase, paraoxonase-1, obesity, polymorphism*

Introduction

Several studies have reported associations between low folate levels and elevated body mass index (BMI) (1–4). The mechanisms for these observations are not known. They might reflect inappropriate dietary habits characterized by high caloric and low folate intakes. Another hypothesis might be that folate influences obesity via epigenetic control of the genes implicated in the regulation of body fat storage (5,6).

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Folate plays a key role in the provision of methyl groups necessary for the methylation of cytosine residues in DNA and of amino acid residues on histones, both of which are involved in epigenetic control of gene expression (7). Besides appropriate dietary intakes, the functional integrity of the enzymes playing a role in methyl group metabolism is also required for a correct DNA methylation pattern. A defect in the genes involved in methyl group metabolism could theoretically affect the methylation of dinucleotides of genes implicated in fat storage and food intake and, thus, cellular physiology or body weight.

Recent data support the later hypothesis. Firstly, in the agouti mouse, folate supplementation is associated with the methylation of the agouti gene and body fat changes (8). Secondly, in laboratory animals and human cellular models, the expression of a number of genes implicated in fat storage, such as peroxisome proliferators-activated receptor alpha, glucocorticoid receptor, leptin and proopiomelanocortin genes, are regulated by methylation (9–12). Thirdly, methylenetetrahydrofolate reductase (*MTHFR)* (1p36.3), methionine synthase (*MTR*) (1q43) and methionine synthase reductase (*MTRR*) (5p15.2–15.3) genes are localized close to loci related to obesity and Prader-Willi syndrome (1p36.2, 1q43 and 5p15) (13). Finally, several studies have reported associations between single nucleotide polymorphisms (SNPs) of genes involved in methyl group metabolism and obesity or related phenotypes (13–15). Taken together these data support the hypothesis that a defect in methyl group metabolism might affect body weight regulation in humans.

However, the results of the genetic association studies examining the association between the genes of the methyl group metabolism pathway and obesity were not always consistent (13–17). The reasons might be that these studies were performed in different sets of populations or were carried out in samples of too limited size. Furthermore, the possible influence of folate intake on this relationship has not been reported. Therefore, the main goal of the present study was to assess the relationship between the genetic variability of six genes involved in the methyl group metabolism, namely *MTHFR, MTR, MTRR*, *CBS*, *TCN2* and *PON1* (see abbreviation list), and obesity risk or anthropometric phenotypes in adolescents and to assess the possible influence of folate status on this relationship.

Patients and methods

The Pécs case-control study on obesity

The sample consisted of 214 adolescents referred for obesity to the outpatient clinic of the University of Pécs, Department of Paediatrics (Pécs, Hungary) and 330 healthy, normal-weight adolescents recruited via Pécs schools. Participants were aged between 13 and 17 years. Obesity was defined as a BMI over the value given by Cole et al. (18), corresponding to 30 kg/m^2 at the age of 18. None of the subjects had chronic diseases and none were taking drugs or were dieting. Blood samples for DNA extraction were collected in EDTA K3 tubes. Genomic DNA was extracted from peripheral blood leukocytes, according to a standard procedure.

The HELENA cross-sectional study (HELENA Study)

Recruitment and phenotyping of the participating adolescents in the HELENA study ("Healthy Lifestyle in Europe by Nutrition in Adolescence", www.helenastudy.com) have been described elsewhere (19). Briefly, a total of 3 865 adolescents were recruited between 2006 and 2007. Data were collected in ten centres from nine European countries (20). Adolescents were randomly selected from schools using a proportional cluster sampling methodology taking into account geographical repartition in each city, private/public school ratio, and number of classes by school. One third of the classes were randomly selected for blood collection, resulting in a total of 1 155 blood samples for the subsequent clinical biochemistry assays and genetic analyses.

Anthropometry

Skinfold thicknesses were measured at six sites (biceps, triceps, sub-scapular, supra-iliac, thigh and calf) using a Holtain Caliper (21). The reliability of skinfold thickness measurements is known to be adequate for epidemiological surveys (22). Percentage body fat (BF%) was derived from the skinfold measurements according to the equations published by Parizkova et al. (23). The BMI was calculated as weight (kg) divided by height (m^2). Waist circumference (WC) was measured in triplicate at the midpoint between the lowest rib and the iliac crest with an anthropometric tape SECA 200, and was used as a surrogate of central body fat. In each centre, the same trained investigator made all measurements, and reliability was greater than 95%. The same methods were used in both studies.

Blood analysis

Blood for DNA extraction was collected in EDTA K3 tubes, stored at the Analytical Laboratory at the University of Bonn and then sent to the Genomic Analysis Laboratory at the Institut Pasteur de Lille in Lille, France (24). DNA was extracted from white

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blood cells with the Puregene kit (QIAGEN, Courtaboeuf, France) and stored at −20°C. Whole-blood folate was measured by competitive immunoassay (Immulite 2000, DPC Biermann GmbH, Bad Nauheim, Germany). The intraassay and interassay coefficients of variation were 10.7% and 14.0%, respectively, for whole-blood folate. To avoid between-assay variation, samples from each city were preferably measured by using a single kit. Red blood cell (RBC) folate concentrations were calculated according to the following equation:

RBC folate=([wholeblood folate * 100] – [plasma folate * {100 – hematocrit}])/hematocrit.

In both the *Pécs case-control study* and the *HELENA Study,* data were collected on a detailed case report form, in accordance with standardized procedures. In each centre, trained physicians carried out complete physical examinations, including weight, height and blood pressure measurements. For both studies, the protocol was approved by the local ethics committee (25). Written, informed consent was obtained from each adolescent and both of his/her parents or legal representatives. Participation in the studies was voluntary.

Gene selection and genotyping

Gene selection was based on a candidate gene approach. Genes were selected among those genes playing significant role in the methyl group metabolism pathway (26). The HapMap database (2007 release) was used to select tag and independent SNPs. We selected SNPs with a minor allele frequency (MAF) above 0.1 and tag SNPs with r^2 above 0.8. With this method, altogether 61 SNPs were selected from the six genes. Samples were genotyped by an Illumina system, 14 SNPs using the VeraCode technology and the 47 other SNPs using GoldenGate technology. Four of the selected SNPs failed the genotyping procedure either because of unknown reasons (rs13306561 and rs2303079) or because they displayed multiple clusters due to neighbouring SNPs (rs3776467 and rs7533315). The genotyping success rate for the remaining 57 SNPs (supplemental Table I) varied between 91.6 and 100%.

Statistical methods

Statistical analyses were performed with SAS software (SAS Institute Inc., Cary, NC, USA). Departure from Hardy–Weinberg equilibrium within the study groups was tested using a χ^2 test. Allele frequencies were estimated by gene-counting. In the Pècs case-control study, multivariate logistic regression was used to calculate the odds ratio for obesity for different allele exposures, using various genetic models (recessive, dominant and additive). Adjustment variables were age and gender. In the HELENA study a general linear model (GLM) was used to compare mean values of anthropometric markers for the various genotypes. Adjustment variables were age, gender and center. Recessive, dominant and additive models were tested. Three interaction terms were used separately to test heterogeneity across centers (center-genotype), between gender (gender-genotype) and with RBC folate levels (genotype-RBC folate levels expressed as a continuous variable). The statistical significance threshold was set to $p \leq 0.0008$ (after Bonferroni correction) for both studies.

Results

Table I shows the clinical characteristics of the subjects from the case-control and the cross-sectional studies. The mean ages (standard deviation [SD]) of the obese and normal-weight adolescents in the case-control study and of adolescents in the cross-sectional study were 14.7 (1.1), 14.9 (1.2) and 14.8 (1.4), respectively. In the case-control study, there was a lower proportion of girls among the obese adolescents. As expected, obese adolescents had significantly higher BMI, waist girth and percentage body fat than the normal-weight adolescents (all $p<0.001$).

In each study, all the observed SNP frequencies respected the Hardy–Weinberg equilibrium, except for two SNPs in the *PON1* gene: rs854569 and rs854567 ($p=0.004$ and 0.03, respectively) and two SNPs in the *MTRR* gene: rs326121 and rs162036 ($p=0.02$ and 0.04, respectively). These four SNPs were consequently no longer analyzed. The genotype distribution of the 53 SNPs in obese

Table I. Characteristics of the subjects.

	Obesity case-control		HELENA sectional
	Normal	Obese	
n	330	214	115
Boys/girl	161/16	116/9	552/60
Age	14.9	14.7	14.8
Weight	55.3	97.1	58.4
Height	166.8	167.5	165.0
BMI (kg/m^2)	19.8	34.5	21.3
Waist cc	70.0	95.5	72.3
BF	24.8	41.2	26.5
RBC folate	–	–	790.9
Haematocrit (%)	–	–	41.3

Data are means (standard deviation), BMI: Body mass index, Waist percentage of body fat, RBC folate: red blood.

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and normal-weight subjects from the case-control study (supplemental Table II) and in the HELENA Study (supplemental Table III) are presented in the Supplemental Tables.

The age- and gender-adjusted odds ratios (OR) and the 95% confidence interval (95% CI) for obesity using a dominant model in the case-control study are presented in Table II. We found no evidence for any significant association between SNPs of *MTHFR*, *MTR*, *CBS*, *TCN2* and *PON1* and obesity; all p values ≥0.08. In contrast, two SNPs of *MTRR* were associated with a higher (1.68 [1.18–2.39] for rs10520873; p=0.004) or lower risk (0.61 [0.42–0.87] for rs1801394; p=0.007) of obesity. After Bonferroni correction these relationhips were; however, no longer statistically significant. Similar results were observed when the analyses were performed using recessive or additive models (data not shown). No evidence for statistically significant heterogeneity between gender was observed.

The mean values of BMI (Table III), BF% and waist circumference (Supplemental Table IV) were compared according to genotype (p values in Figure 1) in the HELENA Study. The *MTRR* rs1801394 SNP was associated with lower BMI (p=0.03) and lower waist circumference (p=0.02) but not with BF% (p=0.11). Few other SNPs in *MTRR* or in the other genes were nominally associated with BMI, BF% or waist circumference. However, these associations were no longer significant after correction for multiple testing. Similar results were observed when the analyses were performed using a recessive and additive models (data not shown). There was no evidence for statistical heterogeneity across center or gender.

To explore whether folate levels might affect the relationship between the SNPs of genes involved in methyl metabolism and body composition variables, we looked for a statistical interaction between RBC folate levels and genotype. In the HELENA Study, the median value of RBC folate was 726.7 nmol/L. The cut-off values for the first and last quartile of the distribution were 556.5 and 944.6 nmol/L, respectively. Figure 2 presents the association between the SNPs and BMI according to the lower or higher quartiles of folate distribution. There was no evidence for any statistically significant interaction between RBC folate levels and any of these SNPs regarding anthropometric parameters (BMI, BF%, and WC) (all p values >0.01).

Discussion

In the present study we examined the association between 53 SNPs of six genes (*MTHFR, MTR, MTRR, CBS, TCN2 and PON1)* involved in the

Table II. Odds ratios for obesity (dominant model) in the Case-Control study.

	OR	[95% CI]	p
MTHFR			
rs9651118	1,11	0.78–1.60	0,56
rs17037390	1,24	0.82–1.85	0,31
rs2066471	0,99	0.69–1.42	0,94
rs4846052	1,28	0.87–1.88	0,21
rs1801133	0,83	0.58–1.18	0,28
rs12121543	0,97	0.68–1.38	0,88
rs1801131	1,20	0.87–1.71	0,31
rs1476413	1,04	0.73–1.47	0,84
rs22 74976	0,88	0.44–1.76	0,71
rs1537516	1,13	0.71–1.81	0,60
MTR			
rs12759827	1,37	0.96–1.95	0,08
rs4659723	0,94	0.62–1.41	0,75
rs1805087	1,01	0.70–1.46	0,97
rs4659744	1,07	0.74–1.54	0,74
rs1050996	1,06	0.74–1.51	0,77
MTRR			
rs1801394	0,61	0.42–0.87	0,007
rs7730643	0,89	0.59–1.35	0,58
rs326122	1,38	0.95–2.00	0,09
rs326123	1,03	0.71–1.47	0,89
rs1532268	1,02	0.72–1.44	0,93
rs6555501	1,44	0.96–2.17	0,08
rs162031	0,98	0.69–1.40	0,93
rs9282787	0,97	0.68–1.38	0,85
rs8659	0,97	0.69–1.37	0,86
rs10520873	1,68	1.18–2.39	0,004
CBS			
rs234706	0,92	0.65–1.31	0,64
rs1801181	0,99	0.64–1.55	0,99
TCN2			
rs11703570	0,91	0.64–1.29	0,59
rs5997703	0,93	0.65–1.34	0,71
rs757874	0,92	0.62–1.38	0,69
rs1801198	1,30	0.88–1.91	0,18
rs9621049	1,06	0.70–1.60	0,78
PON1			
rs854573	1,07	0.75–1.53	0,69
rs854572	0,81	0.55–1.18	0,28
rs854570	0,92	0.64–1.31	0,64
rs2237583	1,01	0.71–1.44	0,94
rs2299262	1,04	0.73–1.48	0,84
rs854568	1,16	0.81–1.65	0,42
rs2299261	1,23	0.86–1.77	0,26
rs2049649	0,97	0.68–1.38	0,87
rs3917490	1,09	0.73–1.61	0,68
rs22 72365	1,06	0.72–1.54	0,78
rs2074351	0,98	0.69–1.40	0,93
rs854560	0,86	0.60–1.22	0,40
rs2299257	0,84	0.59–1.21	0,35
rs3917538	1,03	0.73–1.47	0,88
rs662	1,04	0.73–1.48	0,82
rs3917550	0,92	0.60–1.39	0,68
rs854555	0,97	0.68–1.39	0,89
rs854552	0,82	0.57–1.16	0,26
rs854551	0,84	0.58–1.21	0,35
rs854549	0,89	0.73–1.26	0,51
rs3917586	1,35	0.88–2.07	0,17

OR: Odds ratio, p values are for the dominant model - i.e., using the homozygotes for the frequent allele as reference - and are adjusted on age and gender.

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Table III. Mean BMI according to the SNP genotypes in the HELENA Study.

	BMI (kg/m²)			
	11	12	22	*p*
MTHFR				
rs9651118	21.5 (3.7)	21.0 (3.8)	20.9 (3.2)	0,10
rs17037390	21.3 (3.8)	21.4 (3.7)	21.9 (2.8)	0,53
rs2066471	21.3 (3.6)	21.5 (4.0)	20.6 (3.7)	0,53
rs4846052	21.1 (3.6)	21.5 (3.9)	21.4 (3.6)	0,20
rs1801133	21.2 (3.7)	21.4 (3.7)	21.5 (3.9)	0,73
rs12121543	21.2 (3.6)	21.4 (3.9)	21.2 (3.9)	0,47
rs1801131	21.2 (3.6)	21.4 (3.9)	21.5 (3.7)	0,37
rs1476413	21.2 (3.6)	21.5 (3.9)	21.2 (4.0)	0,32
rs22 74976	21.3 (3.8)	21.0 (3.6)	25.0 (1.7)	0,85
rs1537516	21.3 (3.8)	21.1 (3.6)	23.8 (3.8)	0,89
MTR				
rs12759827	21.3 (3.8)	21.3 (3.6)	21.4 (3.8)	0,24
rs4659723	21.3 (3.8)	21.4 (3.7)	22.0 (4.9)	0,63
rs1805087	21.3 (3.7)	21.3 (3.8)	22.1 (3.9)	0,56
rs4659744	21.6 (4.0)	20.9 (3.4)	21.8 (3.8)	0,10
rs1050996	21.5 (3.8)	21.1 (3.7)	21.5 (3.9)	0,09
MTRR				
rs1801394	21.5 (4.1)	21.2 (3.6)	21.3 (3.7)	0,03
rs7730643	21.2 (3.8)	21.5 (3.5)	21.9 (4.8)	0,23
rs326122	21.3 (3.7)	21.4 (3.8)	22.1 (4.7)	0,25
rs326123	21.0 (3.1)	21.6 (3.9)	21.3 (4.2)	0,006
rs1532268	21.2 (3.5)	21.2 (3.7)	22.0 (4.6)	0,50
rs6555501	21.6 (4.1)	21.2 (3.7)	20.9 (3.1)	0,16
rs162031	21.3 (3.7)	21.4 (3.7)	21.4 (4.2)	0,67
rs9282787	21.2 (3.4)	21.5 (4.2)	21.5 (5.2)	0,05
rs8659	21.4 (3.9)	21.2 (3.6)	21.3 (3.9)	0,27
rs10520873	21.5 (4.0)	21.1 (3.5)	21.3 (3.3)	0,35
CBS				
rs234706	21.4 (3.8)	21.3 (3.7)	21.1 (3.6)	0,36
rs1801181	21.2 (3.5)	21.4 (3.9)	21.2 (3.9)	0,46
TCN2				
rs11703570	21.2 (3.8)	21.4 (3.8)	21.3 (3.4)	0,85
rs5997703	21.3 (3.9)	21.4 (3.8)	21.1 (3.5)	0,67
rs757874	21.4 (3.8)	21.2 (3.7)	20.2 (2.9)	0,39
rs1801198	21.1 (3.4)	21.4 (3.8)	21.4 (4.1)	0,15
rs9621049	21.4 (3.9)	21.2 (3.3)	20.1 (3.3)	0,84
PON1				
rs854573	21.3 (3.8)	21.4 (3.7)	20.8 (3.7)	0,94
rs854572	21.3 (3.8)	21.4 (3.8)	21.1 (3.7)	0,70
rs854570	21.4 (4.0)	21.3 (3.6)	21.0 (3.6)	0,44
rs2237583	21.5 (4.0)	21.1 (3.5)	21.4 (3.5)	0,04
rs2299262	21.4 (3.9)	21.1 (3.6)	21.6 (3.6)	0,25
rs854568	21.3 (3.8)	21.3 (3.5)	22.1 (4.2)	0,77
rs2299261	21.3 (4.0)	21.3 (3.6)	21.5 (3.4)	0,33
rs2049649	21.5 (3.8)	21.1 (3.7)	21.3 (3.6)	0,20
rs3917490	21.6 (3.7)	21.2 (3.7)	21.3 (3.8)	0,12
rs22 72365	21.3 (3.7)	21.4 (3.8)	21.1 (3.3)	0,89
rs2074351	21.3 (3.6)	21.3 (3.9)	21.4 (3.9)	0,58
rs854560	21.4 (3.8)	21.2 (3.6)	21.6 (4.1)	0,56
rs2299257	21.4 (3.7)	21.2 (3.8)	21.5 (3.8)	0,60
rs3917538	21.3 (3.6)	21.2 (3.9)	21.8 (4.4)	0,71
rs662	21.4 (3.7)	21.2 (3.8)	21.5 (4.0)	0,42
rs3917550	21.3 (3.7)	21.1 (4.0)	22.6 (4.8)	0,65
rs854555	21.4 (3.7)	21.3 (3.7)	21.1 (3.9)	0,79
rs854552	21.4 (4.0)	21.1 (3.7)	21.8 (3.8)	0,84
rs854551	21.4 (3.7)	21.1 (3.8)	22.1 (4.3)	0,98
rs854549	21.4 (3.8)	21.2 (3.7)	21.7 (3.5)	0,49
rs3917586	21.3 (3.7)	21.4 (3.8)	20.0 (3.7)	0,82

Data are mean (standard deviation). BMI: Body mass index. 1 is for major allele, 2 is for minor allele, p values are for the dominant model - i.e., comparing homozygotes for the frequent allele (11) with heterozygotes (12)+homozygotes (22) for the minor allele. P values are for general linear model (GLM) with adjustment on age, gender and center.

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methyl group metabolism and obesity or related markers in two independent studies. The rs1801394 of *MTRR* was associated with lower risk of obesity in the case-control study and with lower BMI in the cross-sectional study. There was in contrast no other association between the 52 remaining SNPs and obesity, BMI, BF% or WC in European adolescents. Given the large number of statistical comparisons performed, our results do not support an association between these SNPs and the risk of obesity.

Previous studies have directly explored the possibility of an association between SNPs in genes involved in methyl metabolism and the risk of obesity. In a small case-control study of 82 obese and 54 controls, Terruzzi et al. (13) reported a higher risk of obesity in carriers of the minor alleles of the *MTHFR* A1298C (rs1801131), *MTR* A2756G (rs1805087) and *MTRR* A66G (rs1801394) SNPs. Lambrinoudaki et al. (14) investigating 84 postmenopausal women found significant associations between the *MTHFR* C677T (rs1801133) SNP and BMI or waist-to-hip ratio. Similarly, Liu et al. reported an association between rs4846048 of *MTHFR* gene and BMI in 1873 adult men and women (15). In contrast, Lewis et al. (17) did not find any consistent relationship between the *MTHFR* C677T (rs1801133) SNP and the risk of obesity in four independent cohorts representing approximately 24 000 subjects. Finally, in a recent genome-wide association study of obesity-related traits there were no significant associations between nine SNPs of the *MTHFR* gene and BMI, weight or hip circumference (p=0.40, 0.36 and 0.15, respectively) (27). Our results are consistent with the two later investigations and extend this observation to other genes and SNPs of methyl metabolism pathway.

The *MTRR* rs1801394 SNP was associated with lower risk of obesity in the case-control study and with lower BMI levels in the cross-sectional study. However, given the larger number of statistical comparisons and the relative weakness of the associations, the possibility of a chance finding cannot be ruled out. Together these results do not support an association between genetic variability in *MTHFR*, *MTR*, *MTRR*, *CBS*, *TCN2* and *PON1* and excess body fat in European adolescents. The reasons for the difference with published studies may be related to the relative small sample sizes of the earliest, which could lead to publication bias or spurious findings. In contrast, in the present study the analyses were carried out in two independent samples of European adolescents; an approach that reduces the likelihood of spurious findings. *A posteriori* statistical power calculation using a statistical power of 80%, a p-value of 5% and two-sided tests showed that 1 100 subjects would allow the detection of a 0.6, 0.7 and 0.8 kg/m²

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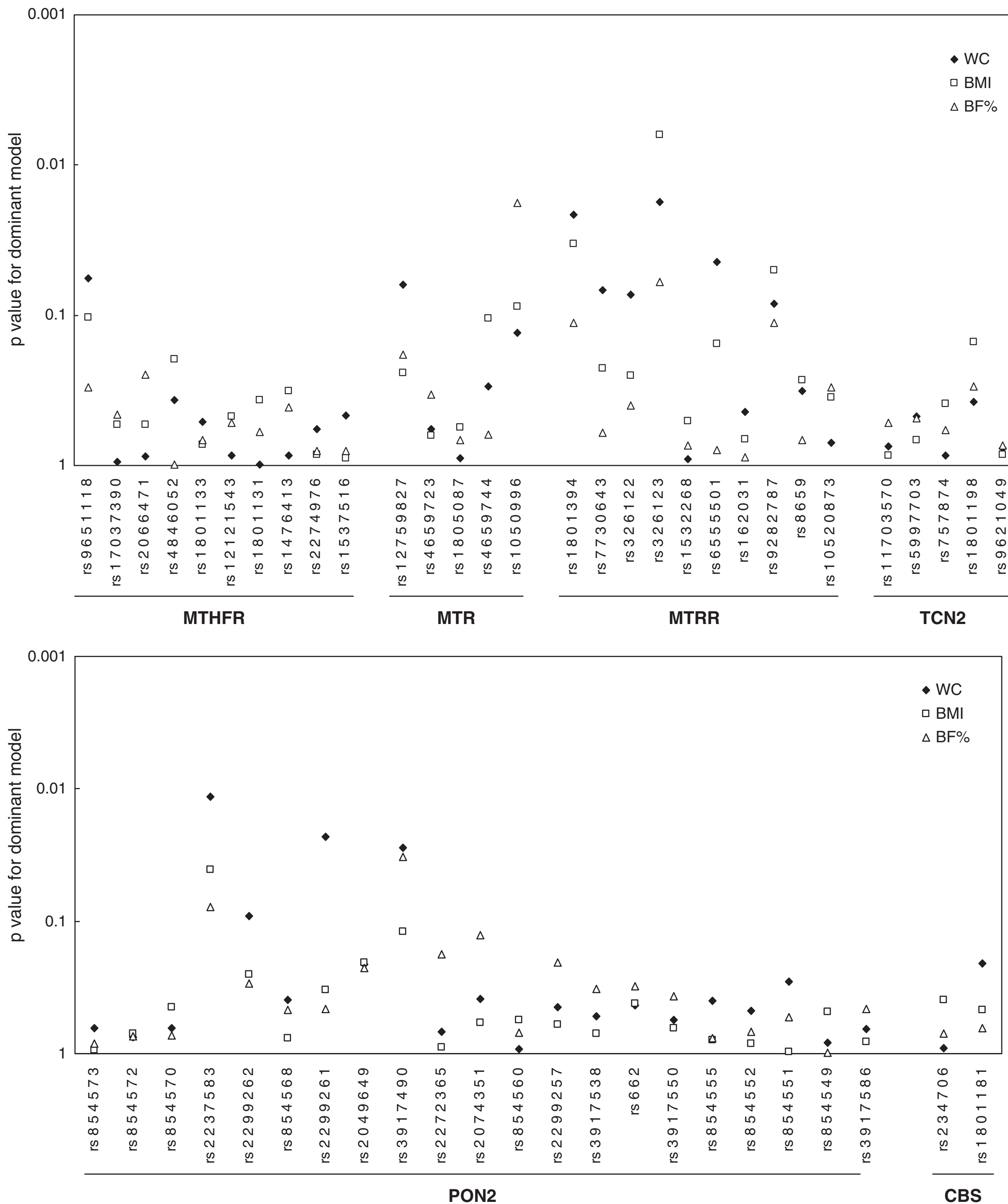

Figure 1. Probability values for the association using a dominant model between genotype and waist circumference (dark diamonds), body mass index (open squares) and percentage body fat (open circles). WC: Waist circumference, BMI: body mass index and BF%: percentage body fat. methylene-tetrahydrofolate reductase: *MTHFR,* methionine synthase*: MTR,* methionine synthase reductase: *MTRR*, transcobalamin-II: *TCN2,* paraoxonase-1: *PON1,* cystationine betha-syntase: *CBS.*

difference in BMI between carriers and not carriers of a minor allele having a frequency of 30, 20 or 10%, respectively. One concern was the selection of tag SNPs with a minor allele frequency >0.10, which does not allow the identification of rare alleles with potentially stronger influences. The results of the present study, therefore, do not exclude the possibility that small differences may exist or that other rare SNPs may be associated with body fat or obesity risk. Also, gene-gene interactions within the folate pathway might be important to consider but our studies lack statistical power to perform such analyses.

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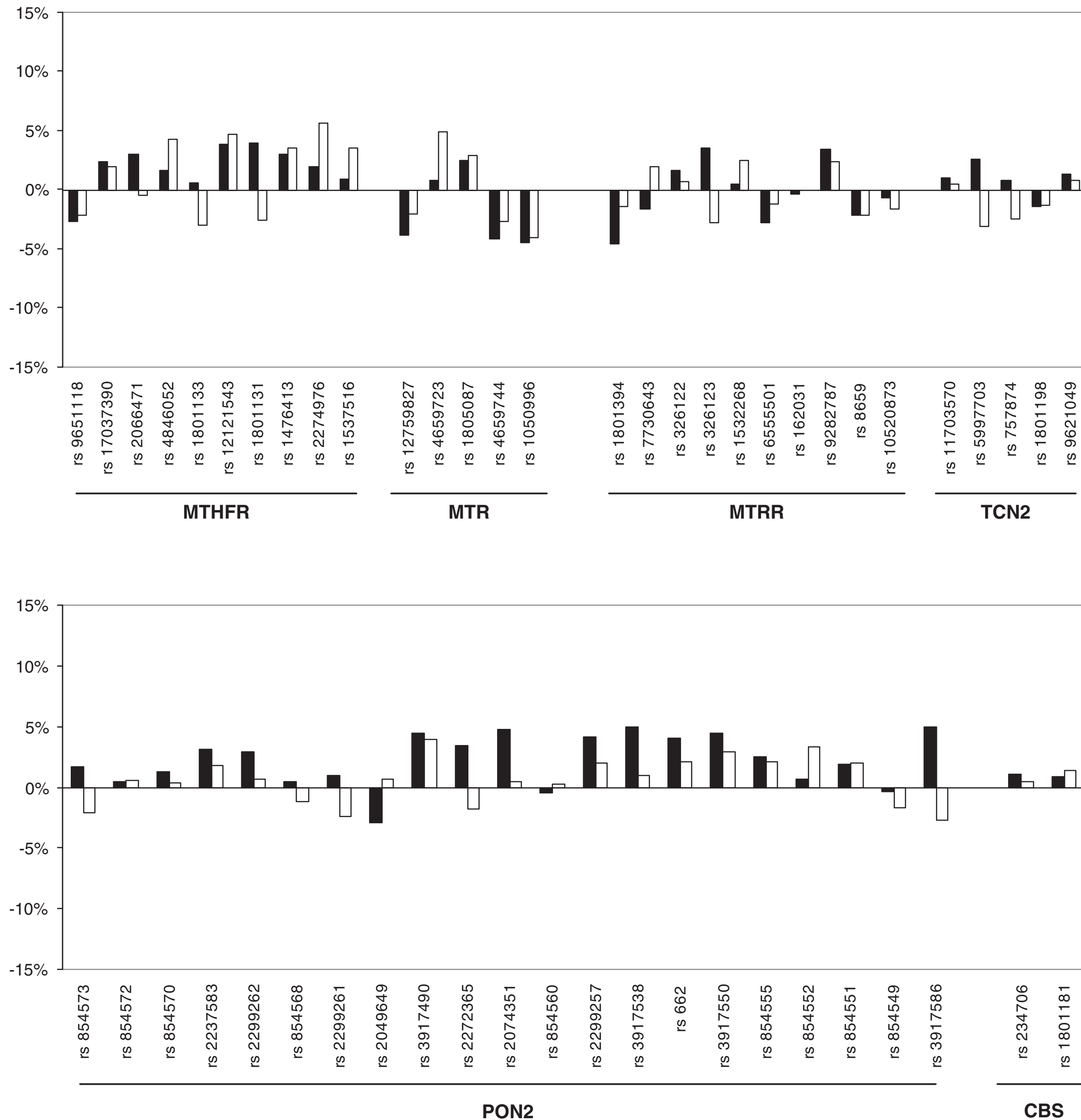

Figure 2. Graphical representation of the interaction between RBC folate levels and genotype (using a dominant model) for body mass index. Each bar represents the difference (in percent) between carriers of at least one minor allele and homozygotes for the major allele of each SNP. The white bars are for subjects in the lowest quartile of RBC folate distribution (RBC folate <556.5 nmol/L) and the dark bars are for subjects in the highest quartile of distribution (RBC folate >944.6 nmol/L). For the sake of clarity, the second and third quartiles are omitted. The interaction term was tested using RBC folate as a continuous variable. All p-values for interaction were $p>0.01$. *MTHFR,* methionine synthase: *MTR,* methionine synthase reductase: *MTRR*, transcobalamin-II: *TCN2,* paraoxonase-1: *PON1,* cystationine betha-syntase: *CBS.*

Dietary folate intake may affect the relationship between SNPs of genes involved in methyl metabolism and obesity. Friso et al. (28) showed that the *MTHFR* C677T and A1298C SNPs accounted for diminished DNA methylation only in subjects with low levels of serum/erythrocyte folate (29). Therefore, we looked for a possible interaction between RBC folate levels, a marker of folate intake, and genotypes on body composition variables (BMI, BF% and WC). Despite, important differences across extreme quartiles of RBC folate levels, we found no statistical evidence for an interaction suggesting that availability of folate as a methyl group donor is not critical in determining the association between these SNPs and anthropometric parameters. However, owing to the limited sample size to study these interactions, the results must be interpreted with caution.

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In conclusion, despite earlier indications of an association between SNPs of genes involved in methyl metabolism and risk of obesity, we found no evidence of such an association either in a case-control study of obesity or in a large representative sample of European adolescents. These data suggest that common SNPs in the genes of methyl group metabolism have no major impact on the risk of obesity in adolescents. However, these findings do not preclude the possibility that these SNPs may affect body weight at an early age or growth progression of children, or that other rare variants might have an effect on the risk of obesity. Further investigations are necessary to confirm the possible inverse association between the rs1801394 variant of *MTRR* and obesity.

Abbreviations

Methylene-tetrahydrofolate reductase: MTHFR, methionine synthase: MTR, methionine synthase reductase: MTRR, cystationine betha-syntase: CBS, transcobalamin-II: TCN2 and paraoxonase-1: PON1.

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Supplemental Material available online

Table showing collated results
HELENA Study Group

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