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Association between rs9930506 polymorphism of the fat mass & obesity-associated (*FTO*) gene & onset of obesity in Polish adults

Małgorzata Wrzosek, Anna Zakrzewska, Lech Ruczko*, Beata Jabłonowska-Lietz* & Grażyna Nowicka

*Department of Pharmacogenomics, Division of Biochemistry & Clinical Chemistry, Medical University of Warsaw, Warsaw & *National Food & Nutrition Institute, Warsaw, Poland*

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***Background & objectives*: The fat mass and obesity-associated (*FTO*) gene is known to be associated with obesity. However, no data are available on the relation between *FTO* rs9930506 polymorphism and obesity in Polish population. The aim of this study was to evaluate an association between rs9930506 variants of the *FTO* gene and obesity in Polish adults.**

***Methods*: The study group consisted of 442 adults, aged 33.9 ±12.7 yr, with mean BMI 27.2 ± 5.4 kg/m^2. The following variables were determined for each subject: fasting blood glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides. Real-time PCR was used to detect the A/G alleles of the rs9939506 polymorphism in the *FTO* gene. An association between the rs9930506 polymorphism and obesity was determined using codominant, dominant, and recessive models. The odds ratio (OR) was calculated to determine the risk of obesity associated with this polymorphism.**

***Results*: It was observed that the presence of *FTO* rs9939506 G allele was associated with increased risk for obesity and this association was found significant in both recessive (OR = 1.72, *P* = 0.014) and co-dominant (OR = 1.36, *P* = 0.031) models of inheritance. The *FTO* rs9939506 GG homozygotes had a significantly higher BMI than those with other genotypes.**

***Interpretation & conclusions*: This study shows that *FTO* rs9939506 GG genotype is related to higher BMI and is associated with obesity in Polish adults.**

Key words Adults - BMI - *FTO* gene - obese - obesity - polymorphism

Obesity has become a serious public health issue and its prevalence is increasing in both developed and developing countries[1]. The World Health Organization (WHO) has defined obesity as a condition with excessive fat accumulation in the body, corresponding to a body mass index (BMI) ≥of 30 kg/m^2 in Caucasians[2]. The main adverse consequences of obesity are cardiovascular disease, type 2 diabetes, and several cancers[3]. Environmental factors including excessive energy intake and lack of physical activity are known to play a key role in obesity development. However, person-to-person variations seen in response to an obesogenic environment suggest the existence of a genetic predisposition to the excessive accumulation of adipose tissue[4]. The twin and family studies suggest that genetic factors may have a strong effect on the variations seen in body mass index (BMI) and body fat percentage[5-7]. Genetic susceptibility to

obesity was also revealed by genome-wide association studies (GWAS)[8,9]. The fat mass and obesity-associated (*FTO*) gene is recognized as associated with enhanced adiposity and seems to influence the risk of obesity in various populations[9,10].

The *FTO* gene is located in chromosome region 16q12.2[11] and encodes the nucleic acid demethylase. Recombinant human nucleic acid demethylase can demethylate 3-methylthymine in single-stranded DNA and 3-methyluracil in RNA[12,13]. However, the exact mechanism by which *FTO* variants influence metabolism and lead to obesity is still unknown. The catalytic activity of FTO may regulate the transcription of genes involved in metabolism by nucleic acid demethylation. It was found that *FTO*-dependent demethylation of specific mRNAs *in vivo* relates to the control of the dopaminergic signaling pathway[14]. This is important because reward behaviour and the motivation behind feeding behaviour seem to be mediated by dopamine neurons located in the midbrain[15]. Previous studies have reported an association between variations in the *FTO* gene and obesity phenotypes, and have highlighted the role of *FTO* rs9939609 single nucleotide polymorphism (SNPs)[16,17]. A meta-analysis of 17.037 white European individuals revealed associations between *FTO* variants not only with BMI, but also with fasting insulin, glucose, triglycerides, and HDL-cholesterol concentrations[18]. Some studies, however, did not confirm the importance of the *FTO* gene as a genetic candidate for higher BMI[19,20]. The influence of ethnic variation is often cited as the cause of these differences[21-23]. Other polymorphisms like rs9930506 have also been observed to lead to the increased risk of obesity[9,24]. There are no data regarding the frequency of genetic variations in rs9930506 polymorphism of the *FTO* gene, nor its relation to BMI and the occurrence of obesity in the Polish population. Therefore, the aim of our study was to assess the frequency of genotypes and alleles of the rs9930506 polymorphism of the *FTO* gene and to investigate the association between this polymorphism and the onset of obesity.

Material & Methods

Obese and non-obese unrelated individuals were consecutively recruited on the basis of clinical investigation, between September 2012 and December 2013 from patients who had been directed to the Outpatient Clinic at the National Food and Nutrition Institute, Warsaw, Poland, due to obesity or for a routine general health screening. The individuals included in the study had no signs or symptoms of thyroid or other endocrine diseases, renal and hepatic disorders, as well as diabetes or history of hypoglycaemic treatment, were free from any psychotropic medication, did not receive medications known to influence plasma lipid levels and body mass, and women did not use hormonal therapy. They were asked not to take part in weight loss programmes and not to successfully lower their body mass. Demographic and clinical variables were recorded: age, weight, height, BMI= weight/ hight^2 (kg/m^2), blood pressure. Obesity was defined as BMI $\geq$30 kg/m^2 according to WHO classification[2]. Obese subjects were consecutively selected from patients attending the Outpatients clinic. Non-obese subjects were age- and sex matched subjects who came for annual medical checkup. Eleven obese and 19 non-obese individuals did not give consent, therefore, they were not qualified for the study.

All participants underwent a comprehensive medical evaluation including clinical history, physical examination, anthropometric parameters and blood pressure (BP) measurements. They completed a questionnaire concerning smoking habits, physical activity, medications and dietary supplements.

All individuals provided written informed consent prior to inclusion in the study. The study protocol was approved by the local research ethics committee (KB/127/2012, Medical University of Warsaw, N151923 Grant National Food and Nutrition Institute in Warsaw).

Peripheral fasting blood samples (5 ml) were collected in commercially available vacuum tubes. The plasma was separated by low speed centrifugation and used for glucose and lipid analyses. Fasting plasma glucose (FPG) was determined by the glucose oxidase method[25]. Enzymatic methods were used to determine concentrations of total cholesterol (Chol) and triglycerides (TG)[26]. HDL-cholesterol was measured after precipitation of apolipoprotein B containing lipoproteins, and LDL-cholesterol level was calculated using Fridewald formula[27].

Genomic DNA was extracted from peripheral whole blood (1ml) using the Blood Mini genomic DNA purification kit (A&A Biotechnology, Poland) according to the manufacturer's instructions. DNA concentration and purity were determined with UV spectrophotometry, measuring absorbance ratios of 260/280 nm. High quality DNA was considered to have an A260/A280 ratio of 1.85 - 2.10. All genomic DNA was diluted to a final concentration of

20 ng/µl. Genotyping of polymorphism rs9930506 of *FTO* gene was performed by TaqMan allelic discrimination real-time PCR[28]. Validated TaqMan SNP genotyping assays were obtained from Life Technologies (Thermo Fisher Scientific, USA). The initial step of the allelic discrimination genotyping assay protocol included: 95°C for 10 min, 40 cycles of 15 sec each at 95°C and 60°C for 1 min. More than 50 per cent of the 442 genotypes were determined twice, and genotyping was 100 per cent concordant.

Statistical analysis: The data were analyzed using Statistica Software, version 10.0 (StatSoft Inc, Tulsa, Oklahoma, USA). Allelic frequencies were calculated by gene counting. Quantitative variables were expressed as mean ± standard error (SE). Baseline characteristics and the differences between obese and non-obese groups were assessed using Student t test. A link between the polymorphism rs9930506 in the *FTO* gene and obesity was determined by using codominant (genotype test), recessive (increased risk in GG *vs.* AG + AA) and dominant (increased risk in GG or AG *vs.* AA) models of inheritance[29]. Differences in minor allele frequencies and genotype distributions among obese and non-obese patients with corresponding odds ratios (OR) and the 95 % confidence interval (CI) were analyzed by likelihood ratio tests with calculation of the *P* value by Chi-square (χ^2) approximation to its distribution using Web-Assotest program (*http://www.ekstroem.com*). *P* values for a model fit (P_{fit}) were calculated and $P_{fit} < 0.05$ indicated that given model of inheritance should be rejected.

Analysis of variance (ANOVA), Tukey post-hoc analysis and the t test were applied to test the differences in BMI and studied parameters (FPG, Chol, LDL, HDL, TG) across the genotypes and alleles of rs9930506 polymorphism.

Results

Altogether, 163 obese subjects and 279 non-obese subjects matched for age and sex participated in the study. The obese participants with class I/II obesity had mean BMI = 33.2 ± 2.6 kg/m^2. The group of non-obese had mean BMI 23.7 ± 3.2 kg/m^2. No difference in body height between obese and non-obese participants was observed. Obese compared to non-obese participants had significantly ($P<0.001$) higher systolic and diastolic blood pressure, fasting blood glucose concentrations, plasma triglycerides ($P<0.05$) and significantly ($P<0.001$) lower HDL-cholesterol concentrations (Table I). All these differences are commonly related to differences in BMI and body fatness.

Distribution of the *FTO* rs9930506 genotypes, presented in Table II, did not deviate from Hardy-Weinberg equilibrium in both obese ($p = 0.60$, $\chi^2 = 0.27$, df = 1) and non-obese ($p = 0.21$, $\chi^2 = 1.61$, df = 1) groups. The frequency of rs9930506 G allele among obese (56%) was higher than among non-obese (49%, OR = 1.34, $P = 0.033$). The statistical analysis revealed a significant association between polymorphism rs9930506 and obesity in a recessive (OR = 1.72, $P = 0.014$) and a co-dominant models of inheritance (OR = 1.36, $P = 0.031$). The dominant model was sufficiently different from the general model ($P_{fit} = 0.025$) as it did not produce a good fit and could be rejected (Table II). Our study could detect with power of 78.9% ($\alpha = 0.05$) the genotypic association conferring OR= 1.72.

Mean BMI levels were found to be significantly higher ($P = 0.012$) in subjects with the GG genotype than in carriers of other genotypes (AA and AG). Homozygotes for the G allele of the *FTO* rs9930506 polymorphism differed from carriers of the A allele by, on average, about 1.5 BMI units (kg/m^2) (Table III).

There were no significant differences between the three genotypes of *FTO* rs9930506 polymorphism among the entire group of 442 subjects in concentrations of fasting plasma glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, and plasma triglycerides. There was also no association between the *FTO* rs9930506 G allele and the biochemical parameters tested (FPG, Chol, LDL, HDL, TG).

Discussion

The *FTO* as the susceptibility gene recognized by genome-wide association studies has attracted much attention in obesity research. The mechanism underlying the increased risk of obesity in presence of a specific allele remains unclear. It is suggested that the risk variant influences ghrelin mRNA expression and the levels of circulating ghrelin, and the failure to suppress hunger is related to loss of control over eating and to the selection of energy dense food[30,31]. Among the various SNPs in the *FTO* gene reported to be associated with obesity, polymorphism rs9939609 has been of particular interest[16,17]. However, under specific conditions, both environmental and ethnic, particular genetic variants may have different degrees of influence on body fatness and BMI measurements. In the Polish

Table I. Characteristics of obese and non-obese study participants

	Obese n = 163	Non-obese n = 279
Age (yr)	32.8 ± 12.9	34.7 ± 12.7
Males/Females (n)	62/101	118/161
Body weight (kg)	94.9 ± 13.4***	66.8 ± 11.6
Height (cm)	168.6 ± 10.3	167.5± 9.7
BMI (kg/m^2)	33.2 ± 2.6***	23.7 ± 3.2
SBP (mmHg)	129.9 ± 12.4***	119.1 ± 14.3
DBP (mmHg)	82.9 ± 7.8***	75.3 ± 8.9
Fasting plasma glucose (mg/dl)	86.3 ± 7.9***	81.5 ± 11.5
Total cholesterol (Chol) (mg/dl)	176.5 ±37.5	182.9 ± 40.1
LDL-cholesterol (mg/dl)	102.9 ± 31.3	105.7 ± 35.4
HDL-cholesterol (mg/dl)	49.7 ± 12.0***	58.0 ± 14.4
Triglycerides (TG) (mg/dl)	120.8 ± 62.8*	98.9 ± 122.2

Data presented as mean ± standard deviation. P *<0.05; ***<0.001 compared to non-obese group.
BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure

Table II. Genotype distribution and allele frequency of the rs9930506 *FTO* polymorphism in obese and non-obese participants

FTO rs9930506 (A/G)			
Groups	Genotypes n (%)		
	AA	AG	GG
Obese	33 (20)	77 (47)	53 (33)
Non-obese	68 (24)	150 (54)	61 (22)
Co-dominant model OR (CI) P, P_{fit}	1.36, (1.03–1.80), 0.031, 0.238		
Groups	Genotype n (%)		
	GG	AG + AA	
Obese	53 (33)	110 (67)	
Non-obese	61 (22)	218 (78)	
Recessive model OR (CI) P, P_{fit}	1.72, (1.12–2.66), 0.014, 0.825		
Groups	Genotype n (%)		
	GG + AG	AA	
Obese	130 (80)	33 (20)	
Non-obese	211 (76)	68 (24)	
Dominant model OR (CI) P, P_{fit}	1.27, (0.79–2.03), 0.316, 0.025		
Groups	Alleles n (frequency)		
	A	G	
Obese	143 (0.44)	183 (0.56)	
Non-obese	286 (0.51)	272 (0.49)	
Additive model OR (CI) P	1.34 (1.02–1.77), 0.033		

A, wild; G, polymorphic; OR, odds ratio; CI, 95% confidence interval for the OR

Table III. Distribution of BMI (body mass index) in studied adults (n=442) stratified by *FTO* rs9930506 genotype

Gene/SNP	Genotype	BMI (kg/m^2) Mean±SD	95% CI	*P* value
FTO/rs9930506	GG	28.3±5.4	27.3 – 29.3	
	GA	26.8±5.6	26.1 – 27.6	0.043
	AA	26.8±5.0	25.8 – 27.8	
FTO/rs9930506	GG	28.3±5.4	27.3 – 29.3	0.012
	AA +AG	26.8 ± 5.5	26.2 – 27.4	

t-test, ANOVA were performed where appropriate

population, the presence of AA genotype of rs9939609 polymorphism on the *FTO* gene was reported to be associated with higher BMI in both children and adults[32,33], however, no data on polymorphism rs9930506 have been presented previously in the Polish population. Studies on Sardinian and Italian samples[9,24] have revealed significant associations of rs9930506 polymorphism of the *FTO* gene with BMI and obesity. Our study showed an association between this rs9930506 A/G polymorphism, and BMI and obesity among Polish adults. In the present study, the G allele of rs9930506 was found to be associated with higher BMI, and a 1.5 kg/m^2 increase in BMI per this allele copy was recognized. A similar association between rs9930506 *FTO* polymorphism and BMI was reported in Italian subjects, where the mean difference in BMI level between the AA genotype and other genotypes was 1.4 kg/m^2,[24] and in Sardinians subjects, where the two homozygotes (AA vs. GG) differed, on average, by 1.5 BMI units[9].

The frequency of the G allele in the obese participants was significantly higher compared to non-obese group. The allele frequency observed in the Polish adults was similar to that observed in Italian and Sardinian population[9,24]. In contrast, in the Asian population, a lower frequency of the G allele of the rs9930506 polymorphism of the *FTO* gene was found: a G allele frequency of 0.20 in a Chinese Han Population[23] and 0.23 in a Beijing population[34], and no association between *FTO* genetic variants and BMI and obesity was revealed[23].

In our study, carriers of the GG genotype had an increased risk of obesity compared to other genotypes. A similar association was reported by Sentinelli *et al*[24]. in the Italian individuals, where the G allele of *FTO* rs9930506 was significantly associated with class I/II obesity. No significant differences were observed in concentrations of fasting plasma glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides between carriers of the A allele and GG homozygotes in our study, while higher BMI was observed in GG homozygotes. Similar results were reported for the Italian subjects by Sentinelli *et al*[24]. The possible explanation for these observations may be the relatively young age of the participants in both studies, and, as suggested by Sentinelli *et al*[24], these subjects may develop metabolic abnormalities in the future. It indicates that differences in biochemical parameters between obese and non-obese subjects are caused by enhanced adipose tissue accumulation, rather than the occurrence of specific variants in *FTO* gene, which interact with obesity-promoting environmental factors and influence the risk for obesity.

In conclusion, our study reported significant association between *FTO* rs9939506 GG genotype and BMI and obesity in the Polish population. In favour of the role of *FTO* gene in obesity, Smemo *et al*[35] showed that FTO was functionally connected with regulation of *IRX3* gene expression. *IRX3* encodes a transcription factor highly expressed in brain and is an important determinant of body mass and metabolism. Our results indicate that parts of the Polish population are carriers of a genetic variant which, in an obesogenic environment, may significantly enhance the risk of developing obesity. This is an additional argument indicating the need to make continuous and intensive effort to promote changes in lifestyle and dietary habits to stop the epidemic of obesity.

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Conflicts of Interest: None.

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Reprint requests: Dr Małgorzata Wrzosek, Department of Pharmacogenomics, Division of Biochemistry & Clinical Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Banacha St. 1, 02-097 Warsaw, Poland
e-mail: malgorzata.wrzosek@wum.edu.pl