Assessment of the *FTO* gene polymorphisms in male patients with metabolic syndrome

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Conflict of interest

None declared

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Abstract

Background. Accumulating evidence indicates the potential involvement of the *FTO* gene polymorphisms in the etiology of metabolic syndrome (MetS) and related disorders.

Objectives. In this study, we aimed to investigate whether the *FTO* gene polymorphisms are associated with the risk of MetS and its simple components in a homogeneous sample of males.

Material and methods. Anthropometric and biochemical parameters were assessed in 192 males. A total of 100 males met the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) criteria for a diagnosis of MetS. The following *FTO* gene polymorphisms were genotyped: rs1421085, rs17817449, rs1558902, and rs9939609.

Results. There were significant differences between participants with distinct rs9939609 genotypes with respect to waist-to-hip ratio (WHR) and the levels of total cholesterol. Individuals with the rs1421085 CC genotype had significantly higher levels of triglycerides compared to those with other corresponding genotypes. Participants with the rs1558902 AA genotype had significantly higher body mass index (BMI), WHR, as well as the levels of total cholesterol and triglycerides. There were no significant differences in genotype distribution allelic frequencies of all tested polymorphisms between individuals with MetS and control subjects.

Conclusions. Our results indicate that the genetic variation in the *FTO* gene might be related to single metabolic disturbances. However, the *FTO* gene polymorphisms are not associated with the risk of MetS.

Key words: metabolic syndrome, obesity, polymorphism, *FTO* gene

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Introduction

Obesity is increasingly being recognized as one of the major health problems and social burdens with a potential impact on future generations.¹ In 2014, the World Health Organization (WHO) revealed that more than 1.9 billion adults are overweight worldwide. Overweight and obesity can lead to a number of metabolic and cardiovascular adversities, such as dyslipidemia, insulin resistance and hypertension, which have been clustered into the condition called metabolic syndrome (MetS). It has been reported that MetS serves as the predictor of severe cardiovascular outcomes and type 2 diabetes. Indeed, it has been estimated that 2.8 million of deaths worldwide can be attributed to the consequences of being overweight or obese.

Several lines of evidence indicate the involvement of genetic and environmental factors, including cultural patterns of dietary habits and feeding behaviors, in the development of obesity and related conditions. In recent years, a special emphasis has been placed on the role of the *FTO* gene in the development of obesity. Indeed, a number of genome-wide association studies (GWASs) have revealed that single nucleotide polymorphisms (SNPs) located in intron 1 of the *FTO* gene confer the risk of obesity in European populations. On the basis of meta-analysis, Wang et al. also revealed that certain *FTO* SNPs (rs9939609, rs8050136 and rs1421085) were associated with the risk of MetS.² The majority of more recent studies confirmed these findings.^{3–9} However, mechanisms mediating the impact of the *FTO* gene on the development of obesity and related phenotypes remain unclear. Given that these polymorphic variants are intronic, it has been postulated that the *FTO* SNPs act via interactions with genes located in their close proximity.¹⁰ Other potential mechanisms linking the *FTO* gene function with obesity include interactions with dopaminergic neurotransmission within the brain reward circuitry and ghrelin-mediated signaling, as well as the possible role of the *FTO* gene in the regulation of epigenetic processes.¹¹

Interestingly, some studies have reported gender effects in the association between the *FTO* gene SNPs and obesity-related phenotypes. For instance, in the study by Saldana-Alvarez et al., the rs1121980 polymorphism was associated with obesity in females only.¹² Another study revealed that the effects of the rs17817449 polymorphism on body weight might be limited to males and postmenopausal women.¹³ Therefore, it might be hypothesized that studies investigating the relationship between the *FTO* SNPs and MetS indices should take into account possible gender effects. Thus, the aim of this study was to examine the effects of the *FTO* gene polymorphisms (rs1421085, rs17817449, rs1558902, and rs9939609) on the risk of MetS and its single components in a homogenous sample of male individuals.

Material and methods

Subjects

We recruited 192 males aged 39.7 ± 10.2 years. A diagnosis of MetS was established in 100 males based on the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) criteria. Other participants, with body mass index (BMI) $<25 \text{ kg/m}^2$, were included in the control group. Fasting levels of total cholesterol, low-density lipoproteins (LDL) and high-density lipoproteins (HDL), triglycerides, and glucose were determined using standard procedures. The following anthropometric parameters were measured: weight, height, BMI, waist circumference, hip circumference, and waist-to-hip ratio (WHR).

Genotyping

A multiplex polymerase chain reaction (PCR) assay was designed to amplify 4 fragments of *FTO* containing investigated SNPs (rs1421085, rs17817449, rs1558902, rs9939609). Multiplex PCR was performed with the Phire Hot Start II DNA Polymerase (Thermo Scientific, Waltham, USA). Briefly, the reaction mixture contained: 2 μL of $\times 5$ reaction buffer (provides 1.5 mM MgCl_2 in the final $\times 1$ concentration) (Thermo Scientific), 0.7 μL of deoxynucleotides (dNTPs) (40 mM) (Fermentas, Burlington, Canada), 0.5 μL of each primer (10 mM) (Generi Biotech s.r.o., Hradec Králové, Czech Republic), 0.2 μL of Phire Hot Start II DNA Polymerase, 1 μL DNA, in a total volume of 10 μL .

Table 1. Genotype distributions and allelic frequencies of the *FTO* gene polymorphisms

<i>FTO</i> gene polymorphism	Patients with MetS	Controls	χ^2	p-value
rs1421085	n = 100	n = 91		
CC	27 (27.0)	33 (36.3)	1.91	0.386
CT	46 (46.0)	37 (40.6)		
TT	27 (27.0)	21 (23.1)		
C-allele	100 (50.0)	103 (56.6)	1.35	0.240
T-allele	100 (50.0)	79 (43.4)		
rs17817449	n = 100	n = 92		
GG	27 (27.0)	32 (34.8)	1.52	0.467
GT	52 (52.0)	40 (43.5)		
TT	21 (21.0)	20 (21.7)		
G-allele	106 (53.0)	104 (56.5)	0.28	0.590
T-allele	94 (47.0)	80 (43.5)		
rs1558902	n = 100	n = 90		
TT	29 (29.0)	36 (40.0)	2.69	0.261
TA	45 (45.0)	36 (40.0)		
AA	26 (26.0)	18 (20.0)		
T-allele	103 (51.5)	108 (60.0)	2.20	0.140
A-allele	97 (48.5)	72 (40.0)		
rs9939609	n = 100	n = 88		
TT	34 (34.0)	38 (43.2)	1.67	0.434
TA	45 (45.0)	34 (38.6)		
AA	21 (21.0)	16 (18.2)		
T-allele	113 (56.5)	110 (62.5)	0.89	0.350
A-allele	87 (43.5)	66 (37.5)		

Data expressed as the number of subjects (%); MetS – metabolic syndrome.

The reaction was performed in a PTC-200 thermal cycler (MJ Research Inc., St. Bruno, Canada) with the following reaction parameters: 95°C for 1 min 30 s, 35 cycles at 95°C for 20 s, 60°C for 20 s, 72°C for 15 s, and 72°C for 1 min 30 s final extension. Multiplex PCR products were checked for quality and yield by running 5 µL in 2% agarose–Tris-borate-EDTA gel, stained Sybr Green and visualized on a UV light transilluminator. Multiplex PCR products were purified using enzymatic digestion. In the study, 1 µL of multiplex PCR was treated with 1.5 µL of Clean-up enzyme mix (1 U of fast alkaline phosphatase and 10 U of Exo1) at 37°C for 15 min. SNaPshot analysis was performed using the SNaPshot Multiplex kit (Applied Biosystems, Woolston, Warrington, UK). Reactions were performed in a final volume of 5.5 µL, containing 2.5 µL of purified multiplex PCR product, 2.5 µL of SNaPshot Ready Multiplex Reaction Mix and 0.5 µL of equimolar probe mix (each probe at 2 mol/L final concentration). Multiplex single base extensions were carried out for 25 cycles with the following reaction parameters: 10 s at 96°C, 5 s at 50°C and 30 s at 60°C. SNaPshot products were then treated at 37°C for 1 h with 1 µL of fast alkaline phosphatase (1 U/µL) (Fermentas International Inc., Burlington, Canada). Fast alkaline phosphatase was inactivated for 15 min at 75°C. The labeled products were mixed with 9.5 µL of Hi-Di Formamide and 0.5 µL of Genescan-120LIZ size standard (Applied Biosystems). They were then separated using a 15-minute run on an ABI 310 DNA sequencer with POP-4 matrix (Applied Biosystems) and 10-second injection time. Fragment analyzes were performed on the Gene Marker software v. 1.85 (SoftGenetics, Centre County, State College, USA).

Statistical analysis

The Hardy-Weinberg (HWE) equilibrium was evaluated by comparing observed and expected genotype distributions using the χ^2 test. The χ^2 test was also used to compare the distribution of genotypes and allelic frequencies between patients with MetS and control subjects. Effects of distinct *FTO* genotypes on biochemical and anthropometric parameters were tested using the Kruskal-Wallis test. Adjustment for multiple testing was performed using Bonferroni correction, taking into account the number of tested polymorphisms. All tests were two-tailed with a 0.05 level of significance. Statistical analysis was performed using the STATISTICA software v. 12.5 (SPSS Inc., Chicago, USA).

Results

Genotype distributions for all polymorphisms genotyped in this study were in agreement with the HWE. There were no significant differences in genotype distributions and allelic frequencies between patients with

MetS and healthy controls (Table 1). The analysis of the *FTO* gene polymorphisms with respect to anthropometric and biochemical parameters in both groups (patients with MetS and healthy controls) is presented in Table 2. In both groups, the rs9939609 AA genotype was associated with a significantly higher WHR and total cholesterol levels compared to other corresponding genotypes. Additionally, this genotype was related to higher BMI at the trend level significance. The level of triglycerides was significantly higher in individuals with the rs1421085 CC genotype in comparison with those with other rs1421085 genotypes. Finally, the rs1558902 AA homozygotes had significantly higher BMI, WHR, total cholesterol, and triglycerides than subjects with other corresponding genotypes. However, none of these differences were significant after Bonferroni correction ($p > 0.0125$). The *FTO* rs17817449 polymorphism was not significantly associated with biochemical and anthropometric parameters.

Discussion

The *FTO* gene is located on chromosome 16 and it consists of 9 exons; however, SNPs analyzed in our study are located in intron 1. The expression of the *FTO* gene has been described in the brain and adrenal glands. It has been demonstrated that the *FTO* gene exerts biological activity via the effects on DNA methylation of genes encoding transcription factors.¹⁴ Upregulated *FTO* gene expression has been reported mainly within hypothalamic nuclei, which accounts for energy expenditure.¹⁵ In addition, the expression of the *FTO* gene might be regulated by starvation and satiety signals. The *FTO* gene might also be involved in the regulation of hypothalamic-pituitary-adrenal axis functioning and lipolysis.¹⁵ It also has been found that it might impact the sympathetic nervous system regulating the cardiovascular system and blood pressure.¹⁶ Interestingly, the *FTO* gene expression might be regulated by estrogens, pointing to the widely-reported sex differences in obesity phenotypes.¹⁷

In this study, we found that neither of the tested *FTO* gene polymorphisms was associated with the risk of MetS development. However, we demonstrated that the *FTO* gene polymorphisms, except for the rs17817449 polymorphic variant, might impact certain metabolic parameters, including BMI and WHR, as well as the levels of total cholesterol and triglycerides. Although recent meta-analyses revealed that the *FTO* gene polymorphisms (rs9939609, rs8050136 and rs1421085) might be associated with the risk of MetS, authors indicated that ethnic differences and the use of various MetS criteria might impact effect sizes.^{2,9} Therefore, the association of the *FTO* gene polymorphisms might hold true only in certain populations, depending also on MetS conceptualization.

Our findings are in agreement with previous GWASs, showing that the *FTO* gene polymorphisms might confer the risk of MetS. Importantly, in the majority of previous

Table 2. Effects of the *FTO* gene polymorphisms on metabolic parameters in the whole group of participants

Parameter	rs99396099 mean ±SD			p-value	rs 1421085 mean ±SD			p-value	rs178174499 mean ±SD			p-value	rs1558902 mean ±SD			p-value
	TT	AT	AA		TT	TC	CC		TT	TG	GG		TT	TA	AA	
Height [cm]	179 ±6.18	177 ±6.18	177 ±6.22	0.212	179 ±6.53	177 ±6.15	177 ±5.68	0.520	179 ±5.96	178 ±6.29	177 ±6.10	0.222	179 ±5.79	177 ±6.51	177 ±5.87	0.061
Weight [kg]	82.5 ±11.2	85.7 ±12.6	88.0 ±18.4	0.238	82.1 ±10.6	85.8 ±14.6	86.4 ±14.5	0.291	82.7 ±10.7	85.9 ±14.0	84.9 ±15.6	0.408	82.4 ±12.2	85.7 ±13.5	96.7 ±15.0	0.184
BMI [kg/m ²]	25.9 ±3.99	27.4 ±4.21	28.1 ±5.66	0.077	25.9 ±0.86	27.4 ±4.73	27.5 ±4.72	0.139	26 ±3.47	27.3 ±4.76	27.2 ±5.13	0.210	25.8 ±4.14	27.4 ±4.48	27.7 ±4.87	0.046*
Waist circumference [cm]	92.4 ±8.7	94.7 ±9.8	97.1 ±13.0	0.148	92.1 ±8.2	95.0 ±10.8	95.6 ±11.1	0.223	92.1 ±8.1	95.2 ±10.4	94.8 ±12.0	0.127	91.9 ±8.8	95.0 ±10.4	96.2 ±11.4	0.109
Hip circumference [cm]	103 ±5.11	103 ±5.61	104 ±8.0	0.918	103 ±4.62	104 ±6.48	104 ±6.42	0.716	103 ±5.04	104 ±5.90	103 ±7.18	0.486	103 ±5.27	104 ±6.05	104 ±6.75	0.862
WHR	0.90 ±0.06	0.91 ±0.07	0.93 ±0.06	0.032*	0.9 ±0.08	0.91 ±0.07	0.92 ±0.06	0.08	0.90 ±0.05	0.92 ±0.07	0.92 ±0.06	0.067	0.89 ±0.05	0.91 ±0.07	0.93 ±0.06	0.013*
LDL [mmol/L]	3.05 ±0.77	3.31 ±0.91	3.38 ±0.94	0.122	3.11 ±0.79	3.25 ±0.94	3.29 ±0.88	0.607	3.13 ±0.8	3.29 ±0.92	3.17 ±0.89	0.475	3.04 ±0.71	3.27 ±0.97	3.36 ±0.87	0.173
HDL [mmol/L]	1.4 ±0.33	1.47 ±0.44	1.39 ±0.28	0.393	1.39 ±0.33	1.43 ±0.41	1.51 ±0.4	0.275	1.4 ±0.33	1.43 ±0.4	1.50 ±0.42	0.549	1.44 ±0.39	1.44 ±0.43	1.44 ±0.30	0.887
Total cholesterol [mmol/L]	5.12 ±0.94	5.5 ±0.97	5.47 ±0.92	0.035*	5.09 ±0.87	5.41 ±1.05	5.55 ±0.90	0.065	5.17 ±0.99	5.40 ±1.0	5.44 ±0.88	0.259	5.1 ±0.82	5.41 ±1.04	5.59 ±0.93	0.026*
Triglycerides [mmol/L]	1.48 ±0.91	1.67 ±1.09	2.1 ±1.48	0.067	1.40 ±0.93	1.76 ±1.19	1.85 ±1.21	0.034*	1.44 ±0.94	1.70 ±1.13	1.61 ±1.32	0.096	1.36 ±0.76	1.77 ±1.22	1.96 ±1.31	0.019*
Glucose [mmol/L]	5.74 ±0.69	5.86 ±1.03	5.84 ±0.61	0.632	5.83 ±0.76	5.88 ±1.01	5.71 ±0.63	0.870	5.76 ±0.75	5.85 ±0.98	5.79 ±0.6	0.760	5.87 ±0.71	5.85 ±1.02	5.78 ±0.65	0.953

SD – standard deviation; BMI – body mass index; WHR – waist-to-hip ratio; LDL – low-density lipoproteins; HDL – high-density lipoproteins; * significant differences ($p < 0.05$).

studies, the *FTO* gene polymorphisms were related to single metabolic parameters, including anthropometric measures and the levels of glucose, HDL or triglycerides, highlighting the potential involvement of this gene in the etiology of obesity.^{18–20} In agreement with our results, previous studies have repeatedly reported the association between the *FTO* rs9939609 polymorphism and BMI.^{19–21} More specifically, the A allele within this polymorphic site has been related to higher BMI. Similar results were also obtained by Al-Attar et al., who found the association between the *FTO* rs9939609 AA genotype and higher BMI (at the trend level significance) and WHR (significant association).²² In addition, there are studies showing the impact of this polymorphism on the risk of cardiovascular diseases and type 2 diabetes.^{18,23,24} Finally, in a study by Freathy et al., this polymorphism was associated with higher levels of glucose and triglycerides as well as lower levels of HDL that appeared due to the effect on BMI.²⁵

Similar results have been reported with respect to other SNPs analyzed in this study. Regarding rs1558902, no significant effects on MetS risk have been demonstrated. However, this SNP has been related to higher BMI and fasting levels of glucose.^{6,26} Other SNPs tested in our study (rs1421085 and rs17817449) have been found to impact BMI and WHR. Interestingly, in the study by Harbron et al., risk alleles of the rs1421085 and rs17817449 SNPs predicted poor eating behaviors (higher hunger, internal locus of hunger and emotional disinhibition) and a higher intake of high fat foods and refined starches.²⁷

Our study has some limitations that should be taken into account. Firstly, our sample size was relatively low and thus it cannot be excluded that negative results with respect to the impact of the *FTO* gene SNPs on MetS risk or other metabolic parameters are simply due to a lack of statistical power. Another point is that a number of variables connected to lifestyle factors and eating behaviors have not been included in this study. Therefore, more precise conclusions on causality cannot be established. Another point is that our analysis was confined to the assessment of a limited number of metabolic parameters, restricting the conclusions regarding the biological mechanisms that might mediate the effects of the *FTO* gene SNPs on obesity and related outcomes. Finally, caution should be taken in interpreting the association between the *FTO* SNPs and anthropometric or biochemical parameters in the whole group of participants, since significant results of the statistical analysis that was unadjusted for multiple testing were not significant after Bonferroni correction.

In conclusion, the results of our study indicate that the *FTO* gene might impact single metabolic parameters, rather than a clustering of cardio-metabolic disturbances conceptualized as MetS. Future studies should investigate the effects of the *FTO* SNPs on obesity and related conditions in bigger samples with comprehensive assessment of cardio-metabolic parameters, environmental factors and eating behaviors.

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