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The Frequencies of Haplotypes of FTO Gene Variants and Their Association with the Distribution of Body Fat in Non-Obese Poles^{*}

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Abstract

Background. The minor allele frequencies (MAFs) of the FTO gene vary substantially among different ethnic groups, and this variation may explain, to some degree, the differences between estimates of the effects of these alleles on body fat distribution indicators.

Objectives. The aim of this study was to investigate the prevalence of fat mass and obesity associated (FTO) gene variants characterizing the structure of FTO haplotypes in a large Polish population, and to examine the influence of FTO gene variants on body fat distribution among metabolically obese normal weight (MONW) individuals, i.e. those with a normal BMI and visceral obesity.

Material and Methods. A total of 854 non-obese individuals aged from 20 to 40 years, residing in three different regions in Poland, were studied. All the patients from this group were genotyped for four FTO gene variants (rs9939609, rs9930506, rs1421085 and rs1121980). Simultaneous identification of all single nucleotide polymorphisms (SNPs) was conducted using the minisequencing method with a pair of designed specific primers.

Results. Over 90% of the diplotypes contain only the two most common haplotypes, in three combinations of haplotype pairs: CTAT/CTAT comprised 26.9% in women and 30.9% in men; CTAT/TCGA comprised 45.0% in women and 44.4% in men; and TCGA/TCGA comprised 19.3% in woman and 14.8% in men. The analysis of the variance in body fat distribution parameters shows no statistically significant differences between the three common haplotype pairs in either men or women.

Conclusions. The young Polish population is characterized by two disparate haplotypes of common FTO gene variants: TCGA (a risk SNP haplotype), and CTAT (a protective haplotype). No significant differences were found between fat distribution indicators in relation to haplotypes in either women and men (*Adv Clin Exp Med* 2016, 25, 1, 33–42).

Key words: FTO, haplotypes, MONW, body fat distribution.

The distribution of body fat plays an important role in determining the risk of numerous health disorders, even in people with a normal body mass index (BMI). Excess body fat around the waist carries more risk than fat distributed

on the hips and thighs. Metabolically obese normal weight (MONW) individuals – with body fat distribution abnormalities, but at the same time a normal BMI – are at a potentially higher risk of developing metabolic complications, such as

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insulin resistance, hyperinsulinemia, impaired glucose tolerance, an atherogenic lipid profile and hypertension. These metabolic disorders, known as metabolic syndromes, may in turn lead to cardiovascular diseases and type 2 diabetes. The etiology of MONW is multifactorial and involves genetic, endocrinological and environmental factors [1].

Common/polygenic obesity depends on various genetic factors, and the effect is intensified when variants of several risk alleles are present, especially in combination with “obesogenic” environmental conditions. Many such polygenic variants have been found to be involved in body weight regulation and adipose tissue accumulation [2]. However, the causes of excessive accumulation of visceral fat in MONW individuals have not yet been studied in depth. Genome-wide association studies (GWAS) have undoubtedly contributed to successes in identifying causal loci for polygenic/common obesity. Notably, the first intron of the fat mass and obesity associated gene (FTO) has been found to be associated with obesity-related traits [3].

The FTO gene is a large gene located on chromosome 16 at position 16q12.2; it consists of 9 exons. The intronic location of common obesity-associated single nucleotide polymorphisms (SNPs) – a 47-kb LD block – indicates that the amino acid sequence of the FTO protein does not exert its effects *via* functional mutations, but rather is likely to play a transcriptional regulatory role through its impact on the expression of the FTO gene and/or neighboring genes [3].

Numerous researchers have studied the association of FTO SNPs with obesity traits, mostly expressed in terms of the BMI. FTO minor allele frequencies (MAFs) vary substantially among different ethnic groups and this variation may explain, to some degree, the differences between estimates of the effects of these alleles on BMI, distribution of body fat and other obesity traits. Different populations are characterized by various haplotype-specific patterns of tightly-linked SNPs associated with the phenotype. Among those of European ancestry, all identified FTO SNPs significantly associated with obesity (located within the first intron) are part of a highly correlated linkage disequilibrium block. [4–7].

So far, limited data are available about the distribution of FTO gene polymorphisms, their MAFs and combinations of haplotypes in the Polish population. Excessive visceral fat accumulation still requires investigation, hence the authors hypothesized that the FTO gene is involved in this process. The research focused on four polymorphisms of the FTO gene: rs9939609, rs9930506, rs1421085 and rs1121980. According to the HapMap Project,

within the CEU population (Utah residents with Northern and Western European ancestry) these polymorphisms, covering an approximately 30kbp fragment on the first intron of chromosome 16 (chr16:52358355..52388066), are in strong linkage disequilibrium [8].

The aim of this study is therefore: 1) to investigate the distribution of the MAFs of 4 FTO SNPs and to determine the structure of haplotypes in a large sample from the Polish population with a normal BMI; 2) to examine the influence of the FTO gene on the excessive accumulation of visceral fat among individuals with a normal BMI.

Material and Methods

Study Group

A total of 854 young, normal-weight individuals (614 female and 240 men, 20 to 40 years, BMI < 25) residing in three different regions of Poland – Wrocław, Szczecin and Kraków – were included in the study. The same study group had previously been selected for a research project (KBN no. 2 P05B 044 29) evaluating the prevalence of the MONW phenotype within the Polish population. All the study subjects gave written informed consent for their participation in the study. The group underwent physical examinations, densitometry, anthropometric measurements (weight, height, waist and hip circumferences) and provided a blood sample for genetic analysis (genomic DNA). The details regarding the recruitment of the study group were described in a previous publication [9]. The clinical characteristics of the study group are presented in Table 1.

The project (no. KB-193/2012) was approved by the Bioethics Committee of Wrocław Medical University (Poland).

Body Composition and Anthropometric Measurements

The research was carried out using previously collected data regarding body composition and anthropometric measurements. The waist/hip ratio (WHR) was based on the waist and hip measurements. BMI was calculated as body weight (kg)/height (m²). Body composition and fat content (total body fat, android and gynoid fat deposits) were estimated by means of the dual X-ray absorptiometry (DXA) densitometry method, using GE-Lunar DXA scanners (General Electric, Madison, WI, USA) according to the manufacturer's standard protocol. The DXAs were cross-calibrated

Table 1. Clinical characteristics of the study population

	Men (n = 240)		Women (n = 601)	
	mean	SD	mean	SD
Age [yr.]	27.88	5.47	28.84	6.14
Height [cm]	179.86	6.65	165.02	5.71
Weight [kg]	72.51	7.72	57.60	6.63
BMI [kg/m ²]	22.39	1.73	21.13	1.97
Waist circumference [cm]	82.71	6.47	71.11	6.14
Hip circumference [cm]	97.49	4.96	94.99	5.43
WHR	0.848	0.047	0.749	0.053
Total fat [g]	12385	4701	16439	4330
Total fat [%]	17.68	5.89	29.67	5.74
Androidal deposit [g]	1068	517	1067	455
Androidal deposit [%]	18.07	6.80	23.14	6.88
Gynoidal deposit [g]	3204	1234	5232	1525
Gynoidal deposit [%]	18.96	6.04	35.45	5.47
Androidal deposit/gynoidal deposit	0.335	0.118	0.215	0.142

All values are means $\pm$ standard deviations. BMI – body mass index; WHR – waist-to-hip ratio.

using standard calibration standards. The evaluation of fat distribution has already been thoroughly described [9].

Genotyping

The genomic DNA was extracted from peripheral blood leukocytes in the blood samples according to the protocol of a commercial DNA isolation kit (QIAamp DNA Mini Kit, Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) conditions were optimized and a pair of specific primers was designed (synthesized by Generi Biotech s.r.o., Hradec Králové, Czech Republic) for the simultaneous amplification and identification of the 4 FTO gene polymorphisms.

Specific fragments of the FTO gene containing polymorphic sites were amplified by PCR, using the TaKaRa Taq DNA Polymerase Amplification Kit (Takara Bio Inc., Shiga, Japan). The PCR

mix (20 μ L) contained forward and reverse primers (shown in Table 2), 1 $\times$ PCR buffer containing 1.5 mM MgCl₂, 200 μ M dNTPs, 2 units of Taq polymerase and 200 ng of genomic DNA. Amplification was performed using a TPersonal Thermocycler (Biometra GmbH, Göttingen, Germany) with the following cycle conditions: initial denaturation at 95°C for 5 min, followed by 35 cycles of: denaturation at 95°C for 30 s, annealing at 55°C for 45 s, extension at 72°C for 45 s and final extension at 72°C for 5 min. The post-PCR products were purified of excess primers and nucleotides using a mixture of SAP and ExoI enzymes (Thermo Fisher Scientific, Waltham, MA, USA). The identification of gene polymorphisms was performed using the minisequencing method according to the protocol of an ABI PRISM[®] SNaPshot[™] Multiplex Kit (Thermo Fisher Scientific). The reaction was carried out in the presence of primers extended by single fluorescence-labeled dideoxynucleotide

Table 2. PCR primer sequences

FTO SNP	Forward primer 5'-3'	Reverse PCR primer 5'-3'	PCR product size [bp]
rs1421085	CTTCCAGGCAAAAGCAGGAG	CAGTGGAGGTCAGCACAGAG	251
rs1121980	AACAAGGAGACAGCAATGGA	CTCAGTAGATGTGTTAATGA	151
rs9930506	TGGAGAATGATGAGAATGTA	GCAATTTAAGTAATGCCTAT	171
rs9939609	CACTAACATCAGTTATGCAT	CCATTCTGACTGTTACCTA	131

Table 3. SNaPshot primer sequences

FTO SNP	Primer 5'-3'	Product size [bp]
rs1421085	GTAGCAGTTCAGGTCCTAAGGCATGA	26
rs1121980	CAGGTGGATCTGAAATCTCA	20
rs9930506	ATCCAATATTAGGGACACAAAAAGGGACATACTAC	35
rs9939609	TGTCTGAATTATTATTCTAGGTTTCCTTGC GACTGCTGTGAATTT	44

(ddNTP). This reaction consisted of 25 cycles: denaturation at 96°C for 10 s, annealing at 50°C for 5 s and extension at 60°C for 30 s. The designed specific primer sequences are shown in Table 3. Fluorescently labeled products of the reaction were separated by capillary electrophoresis in ABI PRISM® 3100 Genetic Analyzer (Thermo Fisher Scientific) and analyzed by GeneMapper® Software v. 4.0 (Thermo Fisher Scientific).

Statistical Analysis

To estimate haplotype frequencies, a standard gene-counting algorithm was used [10]. An individual's pair of haplotypes was inferred to be the most probable pair. In all but two cases, the probability of a correct inference was estimated to be at least 0.997. The two exceptions were individuals with genotypes CT, CT, AG and TT at rs1121980, rs1421085, rs9930506 and rs9939609, respectively. The probability that an individual of this genotype has haplotypes CTAT and TCGT is estimated to be 0.75. The χ^2 goodness of fit test was used

to test whether i) the genotype distributions were in line with the Hardy-Weinberg equilibrium, and ii) whether linkage disequilibrium existed between pairs of loci. Fisher's exact test was used to determine whether the MAFs depended on sex. An analysis of variance was used to test whether there was any association between the variables in Table 1 and the diplotype of an individual. A p-value less than 0.05 was considered statistically significant.

Results

FTO Allele Frequencies, Linkage Disequilibrium and Haplotypes

The χ^2 goodness of fit test showed no significant deviation from the Hardy-Weinberg equilibrium for any of the studied genotypes among either men or women – see Table 4.

In the whole study group, the MAFs of rs1121980 (T), rs1421085 (C), rs9930506 (G), rs9939609 (A) of the FTO gene were 0.451, 0.444,

Table 4. Analysis of Hardy-Weinberg equilibrium

SNP	Genotype	Observed (expected)				H-W test	
		men		women		men	women
rs1121980	C/C	76	(78.1)	169	(170.9)	p = 0.5540 $\chi^2 = 0.3502$	p = 0.7536 $\chi^2 = 0.0986$
	C/T	112	(107.7)	303	(299.2)		
	T/T	35	(37.1)	129	(130.9)		
rs1421085	C/C	35	(35.9)	127	(127.2)	p = 0.7975 $\chi^2 = 0.0658$	p = 0.9727 $\chi^2 = 0.0012$
	C/T	109	(107.2)	299	(298.6)		
	T/T	79	(79.9)	175	(175.2)		
rs9930506	A/A	75	(75.8)	182	(179.0)	p = 0.8289 $\chi^2 = 0.0467$	p = 0.6225 $\chi^2 = 0.2423$
	A/G	110	(108.4)	292	(298.0)		
	G/G	38	(38.8)	127	(124.0)		
rs9939609	A/A	34	(33.6)	120	(116.0)	p = 0.8996 $\chi^2 = 0.0159$	p = 0.5042 $\chi^2 = 0.4461$
	A/T	105	(105.9)	288	(296.1)		
	T/T	84	(83.6)	193	(189.0)		

Table 5. Linkage disequilibrium between pairs of the four SNPs

	Men	Women	Men	Women	Men	Women
rs9930506	D = 0.89 r ² = 0.89	D = 0.93 r ² = 0.93				
rs1421085	D = 0.98 r ² = 0.91	D = 0.99 r ² = 0.90	D = 0.86 r ² = 0.79	D = 0.91 r ² = 0.85		
rs1121980	D = 0.90 r ² = 0.88	D = 0.89 r ² = 0.88	D = 0.92 r ² = 0.82	D = 0.95 r ² = 0.86	D = 0.97 r ² = 0.97	D = 0.97 r ² = 0.97
	rs9939609		rs9930506		rs1421085	

D – measure of linkage disequilibrium; r² – frequency.

Table 6. Inferred haplotype frequencies

Haplotype	Frequency			Haplotype	Frequency		
	total	men	women		total	men	women
CTAT	879 (53.34%)	252 (56.50%)	627 (52.16%)	TCAT	34 (2.06%)	8 (1.79%)	26 (2.16%)
CTGA	4 (0.24%)	2 (0.45%)	2 (0.17%)	TCGA	693 (42.05%)	171 (38.34%)	522 (43.43%)
CTGT	21 (1.27%)	10 (2.24%)	11 (0.92%)	TTGT	9 (0.55%)	3 (0.67%)	6 (0.5%)
CCGA	1 (0.06%)	0	1 (0.083%)	TTAA	1 (0.06%)	0	1 (0.083%)
TCAA	1 (0.06%)	0	1 (0.083%)	TTAT	1 (0.06%)	0	1 (0.083%)
TCGT	3 (0.18%)	0	3 (0.25%)	TTGA	1 (0.06%)	0	1 (0.083%)

0.444 and 0.425, respectively; among males they were 0.408, 0.401, 0.417 and 0.388, respectively; and among females 0.467, 0.460, 0.454 and 0.439, respectively. There were no significant differences between the MAFs in relation to sex ($p = 0.095$, $p = 0.102$, $p = 0.382$ and $p = 0.177$, respectively).

An analysis of linkage disequilibrium (LD) shows an extremely strong pattern between any pair of sites from the four considered (6 pairs in total). In the study group this LD is very similar in both sexes. The data for the summary statistics describing the level of LD in both sexes is presented in Table 5.

Of the 81 possible extended genotypes of the FTO gene, based on four loci, twenty different diplotypes were observed (19 in women and 9 in men). Based on the estimates of the FTO haplotype frequencies, about 95% of the haplotypes in the whole sample are either CTAT (all protective alleles) or TCGA (all risk alleles) – see Table 6. Further analysis shows that over 90% of the diplotypes (91.2% in women and 90.1% in men) contain only the two most common FTO haplotypes: CTAT/CTAT: 26.9% in women and 30.9% in men; CTAT/TCGA: 45.0% in women and 44.4% in men; and TCGA/TCGA: 19.3% in women and 14.8% in men (see Table 7). The alleles are written in the following order: rs1121980, rs1421085, rs9930506 and rs9939609.

Analysis of Variance of Body Fat Distribution with Respect to FTO Haplotypes

The analysis of variance of the parameters of body fat distribution according to the three most common haplotype pairs ($2 \times$ CTAT as “protective”, CTAT/TCGA and $2 \times$ TCGA as “risk”) show no statistically significant differences among either men or women. There is very weak evidence for increased hip circumference ($p = 0.05$) and a trend for an increased WHR ($p = 0.07$) in women who are carriers of the risk haplotype (TCGA) – see Table 8. The alleles are given in the following order: rs1121980, rs1421085, rs9930506 and rs9939609.

Discussion

The FTO gene shares features with proteins from the Fe(II)- and 2-oxoglutarate-dependent oxygenase superfamily, suggesting nucleic acid demethylation in posttranslational modification, DNA repair and fatty acid metabolism. The FTO protein (*in vivo*) is able to catalyze demethylation in single-stranded DNA and RNA. The mRNA of FTO is particularly abundant in the hypothalamus, which is responsible for controlling food intake

Table 7. Inferred diplotype frequencies

Diplotype	Frequency			Diplotype	Frequency		
	total	men	women		total	men	women
CTAT, CTAT	231 (28.0%)	69 (30.9%)	162 (26.9%)	CTGT, TCGA	9 (1.1%)	4 (1.8%)	5 (0.8%)
CTAT, CTGT	12 (1.5%)	6 (2.7%)	6 (1.0%)	TCAT, TCGA	10 (1.2%)	2 (0.9%)	8 (1.3%)
CTAT, TCAT	24 (2.9%)	6 (2.7%)	18 (3.0%)	TCGA, TCGA	149 (18.1%)	33 (14.8%)	116 (19.3%)
CTAT, CTGA	1 (0.1%)	0	1 (0.2%)	CTAT, TTAA	1 (0.1%)	0	1 (0.2%)
CTAT, TTAT	1 (0.1%)	0	1 (0.2%)	TCAA, TCGA	1 (0.1%)	0	1 (0.2%)
TCGA, TCGT	1 (0.1%)	0	1 (0.2%)	TCGA, TTGT	1 (0.1%)	0	1 (0.2%)
TTGT, TTGT	1 (0.1%)	0	1 (0.2%)	CTAT, TCGT	2 (0.2%)	0	2 (0.3%)
CTAT, TCGA	370 (44.8%)	99 (44.4%)	271 (45.0%)	CTGA, TCGA	1 (0.1%)	0	1 (0.2%)
CTAT, TTGT	6 (0.7%)	3 (1.3%)	3 (0.5%)	TCGA, TTGA	1 (0.1%)	0	1 (0.2%)
CCGA, TCGA	1 (0.1%)	0	1 (0.2%)	CTGA, CTGA	1 (0.1%)	1 (0.4%)	0

and energy expenditure, while affecting the role of the central nervous system in predisposition to obesity [11]. There is a lot of statistical evidence that the FTO gene is involved in energy homeostasis. However, the physiological role of the FTO gene is not well known yet, although a few insights into its cellular role have recently been presented. The close proximity and linkage disequilibrium between FTO and RPGRIP1L (the human ortholog of mouse Ftm, in which FTO was first identified) may suggest that the FTO gene plays a role in the co-regulatory molecular pathway through its effect on RPGRIP1L expression. However, further studies have suggested a direct effect of the FTO gene [12]. Gulati et al. inferred that FTO acts biochemically as a cellular sensor of amino acids. FTO demethylation affects the availability of amino acids and activation of the mTORC1 signaling pathway (a protein complex, sensor of energy resources, nutrient availability and oxygen abundance) leading to the appropriate mRNA expression for protein synthesis and cell growth [13].

A large study by Frayling et al. showed that the rs9939609 SNP of the FTO gene is significantly associated with BMI, hip circumference and weight in individuals with type 2 diabetes [3]. Subsequently, Scuteri et al. found a strong association between rs9930506 and three obesity traits: BMI, hip circumference and total body weight [14]. SNPs rs1421085 and rs1121980 were also found to be associated with obesity and related traits. Numerous researchers have confirmed the association of over 60 SNPs with obesity traits, mostly expressed in terms of the BMI [4].

The aim of the present study was to investigate the MAFs of the FTO gene and determine the most commonly occurring haplotype blocks in the

region surrounding four SNPs of the FTO gene (rs1121980, rs1421085, rs9930506 and rs9939609) in a homogenous group Poles aged 20–40 with BMI < 25 kg/m² but varying body fat distribution.

According to the HapMap Project, the MAFs at rs1121980 (T), rs1421085 (C), rs9930506 (G), rs9939609 (A) of the FTO gene are 0.482, 0.464, 0.486 and 0.460, respectively [8]. In the current study group the MAFs at rs9930506 (G) and rs9939609 (A) were found to be significantly lower than in the HapMap CEU population ($p = 0.016$ and $p = 0.046$, respectively). The MAFs at rs1121980 (T), and rs1421085 (C) did not differ significantly from the CEU population ($p = 0.073$ and $p = 0.254$, respectively). In females in the study group, all the MAFs considered were similar to those in the CEU population ($p = 0.453$, $p = 0.848$, $p = 0.119$ and $p = 0.309$, respectively), but among the males in the study the MAFs were significantly lower ($p = 0.027$, $p = 0.061$, $p = 0.039$ and $p = 0.031$, respectively).

There are limited data available about the MAFs of the FTO gene and their association with obesity and metabolic parameters in the Polish population. In research on Polish children, the frequency of the A-allele of rs9939609 has been found to be somewhat higher (0.49 [15], 0.46 [16] and 0.47 [17]) than in the present study group (0.425). This may result from the fact that the study group was composed of individuals with normal BMIs, while TCGA is the risk haplotype for fat deposition.

The risk alleles of FTO rs1121980 rs1421085, rs9930506 and rs9939609 are T, C, G and A, respectively; the protective alleles are C, T, A and T, respectively [8].

The current study showed that the Polish population is characterized by two disparate

Table 8. Analysis of variance of body fat distribution in relation to FTO haplotypes

	Haplotype pair	Mean $\pm$ SD		p	
		men	women	men	women
Waist	1.00	82.9 $\pm$ 6.9	71.3 $\pm$ 6.4	a) p = 0.64	p = 0.88
	2.00	82.6 $\pm$ 6.3	71.0 $\pm$ 6.1	b) p = 0.98	p = 0.62
	3.00	83.8 $\pm$ 5.7	71.0 $\pm$ 5.7	c) p = 0.32	p = 0.80
Hip	1.00	97.8 $\pm$ 5.3	94.9 $\pm$ 5.4	a) p = 0.97	p = 0.14
	2.00	97.6 $\pm$ 4.6	94.6 $\pm$ 5.5	b) p = 0.81	p = 0.90
	3.00	97.6 $\pm$ 4.6	95.8 $\pm$ 5.1	c) p = 0.98	p = 0.05*
WHR	1.00	0.85 $\pm$ 0.05	0.75 $\pm$ 0.06	a) p = 0.40	p = 0.20
	2.00	0.85 $\pm$ 0.05	0.75 $\pm$ 0.05	b) p = 0.74	p = 0.49
	3.00	0.86 $\pm$ 0.05	0.74 $\pm$ 0.05	c) p = 0.17	p = 0.07**
C.FAT	1.00	12389 $\pm$ 5046	16384 $\pm$ 4078	a) p = 0.82	p = 0.98
	2.00	12512 $\pm$ 4260	16413 $\pm$ 4414	b) p = 0.98	p = 0.89
	3.00	11939 $\pm$ 3878	16499 $\pm$ 4275	c) p = 0.50	p = 0.83
% C.FAT	1.00	17.9 $\pm$ 6.6	29.8 $\pm$ 5.3	a) p = 0.78	p = 0.88
	2.00	17.8 $\pm$ 5.2	29.6 $\pm$ 5.8	b) p = 0.78	p = 0.79
	3.00	17.0 $\pm$ 5.0	29.8 $\pm$ 5.7	c) p = 0.45	p = 0.75
A.FAT	1.00	1076 $\pm$ 550	1067 $\pm$ 438	a) p = 0.78	p = 1.00
	2.00	1076 $\pm$ 495	1063 $\pm$ 474	b) p = 0.83	p = 0.92
	3.00	1009 $\pm$ 391	1064 $\pm$ 413	c) p = 0.40	p = 0.99
% A.FAT	1.00	18.2 $\pm$ 7.4	23.3 $\pm$ 6.5	a) p = 0.72	p = 0.74
	2.00	18.2 $\pm$ 6.2	22.9 $\pm$ 6.9	b) p = 0.82	p = 0.59
	3.00	17.2 $\pm$ 6.1	23.3 $\pm$ 6.6	c) p = 0.39	p = 0.72
G.FAT	1.00	3187 $\pm$ 1258	5292 $\pm$ 1715	a) p = 0.93	p = 0.38
	2.00	3237 $\pm$ 1175	5134 $\pm$ 1361	b) p = 0.88	p = 0.55
	3.00	3154 $\pm$ 1074	5348 $\pm$ 1671	c) p = 0.77	p = 0.37
% G.FAT	1.00	19.2 $\pm$ 6.6	35.6 $\pm$ 5.3	a) p = 0.78	p = 0.65
	2.00	19.0 $\pm$ 5.5	35.2 $\pm$ 5.3	b) p = 0.75	p = 0.66
	3.00	18.3 $\pm$ 5.3	35.7 $\pm$ 5.6	c) p = 0.46	p = 0.55
A.FAT/G.FAT	1.00	0.34 $\pm$ 0.13	0.22 $\pm$ 0.16	a) p = 0.82	p = 0.52
	2.00	0.34 $\pm$ 0.13	0.21 $\pm$ 0.08	b) p = 0.96	p = 0.83
	3.00	0.32 $\pm$ 0.07	0.23 $\pm$ 0.22	c) p = 0.39	p = 0.48
% A.FAT/% G.FAT	1.00	0.95 $\pm$ 0.26	0.65 $\pm$ 0.15	a) p = 0.87	p = 0.87
	2.00	0.96 $\pm$ 0.23	0.65 $\pm$ 0.16	b) p = 0.84	p = 0.63
	3.00	0.94 $\pm$ 0.20	0.65 $\pm$ 0.15	c) p = 0.66	p = 0.95

Haplotype pair 1.00: 2 $\times$ CTAT (protective haplotype); 2.00: CTAT/TCGA; 3.00: 2 $\times$ TCGA (risk haplotype). Assumptions made for calculating p-values: a) all diplotypes treated as different; b) diplotypes with at least one TCGA haplotype treated as being the “group at risk”; c) diplotypes with two TCGA haplotypes treated as being the “group at risk”.

* p = 0.05 indicates a tendency to significance; ** p = 0.07 indicates a trend towards significance.

haplotypes: TCGA and CTAT, and strong linkage disequilibrium ($D \geq 0.86$, $r^2 \geq 0.82$). Among the 81 possible extended genotypes of the FTO gene variants, over 90% of the diplotypes (91.2% in women and 90.1% in men) contain only the two most common haplotypes: CTAT/CTAT (26.9% in women and 30.9% in men), CTAT/TCGA (45.0% in women and 44.4% in men) and TCGA/TCGA (19.3% in women and 14.8% in men). These two common haplotypes, which differ at each SNP, are called “yin-yang” haplotypes. Yin-yang haplotype blocks usually present regions of extended homozygosity (ROHs) with neighboring homozygous SNPs. In these regions, the recombination rates are rather low [18]. The results of the current study indicate that the Polish population is genetically relatively homogeneous and is characterized by a pair of disparate haplotypes of the FTO gene – the risk haplotype TCGA, and the protective CTAT haplotype.

Because FTO gene polymorphisms have been found to be associated with BMI and related traits in many populations, including in studies in Poland [15–17, 19–22], the current authors aimed to examine whether, in this Polish study group with normal BMI, excessive accumulation of visceral fat is attributable to risk alleles/haplotypes of this gene.

Most research has clearly focused on the association of the FTO gene with obesity-related traits, especially among obese individuals (using lean individuals as a control group). Generally, it is known that individuals who have a normal BMI, but are characterized by visceral obesity, have a higher risk of developing metabolic syndrome, which in turn may lead to cardiovascular disease or type 2 diabetes [1]. These issues are often overlooked in regular and preventative medical examinations of young adults with normal BMI. The metabolic complications associated with excess accumulation of visceral adipose tissue are usually detected and treated late. Some research has been carried out on the putative genetic factors contributing to metabolic syndrome among individuals with a normal BMI [23]. However, there is little insight into the association of polymorphisms of the FTO gene with metabolic factors and the influence of these genes on body fat distribution in the general population and, in particular, among normal-BMI individuals. Some research has described

the impact of the FTO gene on body fat distribution. Livshits et al. showed a significant and consistent association between FTO gene variants and body composition phenotypes in UK twins [24]. Also, Sonestadt et al. emphasized the relationship of the FTO genotype with both fat mass and lean mass [25].

The current study investigated the differences in body fat distribution between three haplotype pairs of the FTO gene: homozygous risk ($2 \times$ TCGA), homozygous protective ($2 \times$ CTAT) and heterozygous (CTAT/TCGA). No significant differences were found between the parameters of body fat distribution – the WC, WHR, androidal deposit, gynoidal deposit, and androidal/gynoidal deposit ratio – in whole study group or in the male and female groups treated separately ($p > 0.05$). Only an insignificant trend was noted for increased hip circumference and an increased WHR in women who are carriers of the risk haplotype (TCGA).

There is some evidence that the FTO gene influences the metabolism of fat tissue and is associated with body fat accumulation [26, 27]. However, studies in various populations show that ethnic differences may contribute to differences between the frequencies of alleles of genes associated with the phenotype (determining various haplotype-specific patterns of tightly-linked SNPs), which, in turn, affects the relationship with the BMI, distribution of body fat and other obesity traits [4].

The results of the present study do not support the hypothesis that the FTO gene affects the distribution of fat tissue among individuals with normal BMI. The molecular mechanism for the excessive accumulation of visceral fat in MONW individuals may therefore be induced by other factors and be more complex than previously thought. Further investigations are required.

The authors concluded that the population of young Polish adults is characterized by two disparate haplotypes of common FTO gene variants: the risk haplotype TCGA and protective haplotype CTAT (the alleles are given in the following order: rs1121980, rs1421085, rs9930506 and rs9939609). No significant differences were found between fat distribution indicators in relation to the haplotypes of the FTO gene carried by individuals with a normal BMI.

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