

Effect of FTO *rs1121980* to body mass index (BMI)

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The aim of this study was to investigate the effect of selected polymorphism FTO *rs1121980* on body mass index in humans. In the study participated 79 people from Slovakia with some genetic relatedness. The column kit has been used to isolate the genomic DNA from a buccal swab. Genotyping of single nucleotide polymorphism *rs1121980* of FTO gene was performed by amplification-refractory mutation system (ARMS). The most common genotype was found in heterozygous form (CT = 0.4051). The least frequent genotype in FTO was CC (0.2911) and the minor C allele reached frequency 0.49365. The FTO gene had increased frequency of T allele (0.50635). According to the results it can be assumed that the genotype CC (FTO *rs1121980*) has a protective effect on the prevalence of obesity compared to the other genotypes. With adding of the anthropometric measurements, blood test and extension of the group, the statistical relevance could increase in the future in relation to obesity.

Keywords: body mass index (BMI), FTO gene, human obesity, SNP polymorphism, *rs1121980*

1 Introduction

The nowadays incidence of obesity is increasing as in children population, same in the adult one. Is a worldwide chronic disease and there should be take into account many aspects they are influencing the prevalence also with consideration of non-nutritive origin.

Promising approaches such as whole exome and eventually whole genome sequencing, in addition to studies exploring short and long-range interactions between genes leading to obesity have the potential to guide to an exhaustive map of obesity predisposing genes in the near future. Gene identification efforts using the genetic studies have provided a more broad aspect to understand the biological mechanisms involved in the development of obesity and this information can be important not only for scientists and clinicians but for a general population too (Srivastava A., Srivastava N., Mittal, 2015).

Zhang et al. (2010) confirmed the association of common variants of FTO gene with “body fatness” measures in an isolated island population. They also observed evidence of pleiotropic effects of FTO gene on fat-free mass, such as frame size and muscle mass assessed by bicondilar upper arm width and upper arm circumference respectively and these pleiotropic effects might be influenced by variants that are different from the ones associated with “body fatness”. Hinney et al. (2007) found that variation in FTO strongly contributes to early onset obesity.

Some from the single nucleotide polymorphisms (SNP) in the FTO gene are showing a much stronger association with all-cause mortality than expected from its association with body mass index (BMI), body fat mass index (FMI) and waist circumference (WC). This finding implies that the SNP has strong pleiotropic effects on adiposity and adiposity-independent pathological pathways that leads to increased mortality, but Zimmermann et al. (2014) did not found that FTO SNP is associated with all-cause mortality independently of the adiposity phenotypes in comparing with previous investigated studies.

Zimmermann et al. (2014) found that the minor allele of the FTO SNP was associated with higher BMI ($n = 169,551$; 0.32 kg m^{-2} ; 95 % CI 0.28–0.32, $P < 1.0 \times 10^{-32}$), WC ($n = 152,631$; 0.76 cm; 0.68–0.84, $P < 1.0 \times 10^{-32}$) and FMI ($n = 48,192$; 0.17 kg m^{-2} ; 0.13–0.22, $P = 1.0 \times 10^{-13}$). Subsequently, Cox proportional hazard regression analyses for mortality showed that the hazards ratio (HR) for the minor

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allele of the FTO SNPs was 1.02 (1.00–1.04, $P = 0.097$), but the apparent excess risk was eliminated after adjustment for BMI and WC (HR: 1.00; 0.98–1.03, $P = 0.662$) and for FMI (HR: 1.00; 0.96–1.04, $P = 0.932$). Zimmermann did find that FTO SNP is not associated with all-cause mortality independently of the adiposity phenotypes in contrary to previous studies.

The aim of this study was to investigate the effect of selected polymorphism FTO *rs1121980* on body mass index in humans.

2 Material and methods

The target group consisted of people with different age structures and with certain genetic similarity, which created relatedness between individuals. Because of the formation of a general examination of overweight and obesity in humans, there were 79 people evaluated, belonging to 14 families.

Genomic DNA was isolated from the buccal swabs using a commercial available Qiagen DNA Mini Kit (Qiagen). FTO selected single nucleotide polymorphism *rs1121980* was performed by amplification-refractory mutation system (ARMS) analysis of point mutation, following the methodology Shabana and Hasnain (2015).

The PCR amplification of allele specific fragments which represent specific alleles C and T of SNP C4685T were analysed using ARMS-PCR method. The sequence of using PCR primers were designed by Shabana and Hasnain (2015). The primers sequences were: forward outer primer 5'-AAAAGCCAGATAAGGAGACTACTG-3', reverse outer primer 5'-GTGCCACCATATCTACCTCTTC-3', forward inner primer 5'-GCAGGTGGATCTGAAATCTAAT-3', and reverse inner primer 5'-TAGTCACGTGTCTTGGTACTCTG-3'. The reaction mixture in the total volume 25 μ l contained 100 ng DNA, 1 U Taq DNA polymerase (Fermentas), 1 x PCR buffer $(\text{NH}_4)_2\text{SO}_4$, 2 mM MgCl_2 , 800 μ M dNTP, 5 pM of each outer primers and 15 pM of each inner primers. The PCR reaction was optimized in the gradient thermocycler C1000TM Thermal Cycler (Biorad). The following amplification parameters were applied: 94 °C for 5 minutes followed by 25 cycles: 94 °C for 30 seconds, 56 °C for 30 seconds, 72 °C for 30 seconds. The reaction was completed by the final synthesis: 72 °C for 10 minutes. Allelic specific fragments were loaded on 2% agarose gel (Invitrogen) containing GelRed dye (Biotium) at 150 V in 1x sodium borate buffer for 15 minutes and the gel was analyzed in the UV rays.

Age, height, weight were assessed in the target group and then the body mass index (BMI) was calculated according to the following formula:

$$\text{BMI} = \text{weight (kg)} / \text{body height}^2 \text{ (m)}$$

Basic statistical analysis of gene polymorphism was performed by the following relationships:

- A. Alleles frequencies for double allelic system according to the Hardy-Weinberg law

$$p_A = (2.AA + AB) : 2N \quad q_B = (2.BB + AB) : 2N$$

p_A , q_B - the frequency of each allele, N - total number of individuals

- B. Genotypes frequencies according to the Hardy-Weinberg law

$$(p_A + q_B)^2 = p_A^2 + 2 p_A q_B + q_B^2 = 1$$

- C. Genotypic balance was verified by χ^2 – test

$$\chi_{(n-1)}^2 = \sum \frac{(e - t)^2}{t}$$

n - total number of phenotypic classes, e - observed number of genotypes, t - theoretical number of genotypes

- D. The polymorphic information content (PIC) of Botstein et al., (1980)

$$P = 1 - \sum (p^2 + q^2) - \left(\sum_{i=1}^{n-1} \sum_{j=i+1}^n 2 p_i^2 p_j^2 \right)$$

- E. Expected heterozygosity ($H_{e_{exp}}$) by Nei, (1978)

$$H_{e_{exp}} = 1 - \sum(p^2 + q^2)$$

The relationship of body mass index (BMI) and the polymorphism studied genes was analyzed on the basis of a linear model:

$$BMI_{ijk} = \mu + SEX_i + LEP_j + b(age)_{ijk} + e_{ijk}$$

BMI – body mass index, μ – mean value, SEX_i – fixed effect of sex, LEP_j – fixed effect of genotype (gene LEP), $b(age)_{ijk}$ – effect of age in the form of linear regression, e_{ijk} – residual effect

For the numerical expression of the alleles and genotypes, we used the following substitution: gene FTO (allele C = 0, T = 1, genotype CC = 0, genotype CT = 1, TT = 2). Numerical expression of the genes was used because of the expression of genetic predisposition obesity to individuals and families by individual analysed genes studied separately and also together, based on expected positive or negative effect from the previous studies.

Laboratory processing of samples and the DNA analyses were carried out at the Department of Genetics and Animal Breeding Biology, Slovak University of Agriculture in Nitra. Statistical analysis was performed in the program SAS Enterprise Guide 5.1 and the program SAS 9.2.

3 Results and discussion

3.1 Analysis of frequency and basic statistical characteristics

The group consisted of 79 people of people of different age structures, usually with a known family relatedness, including 48 women and 31 men.

Table 1 The basic statistical characteristics and the basic statistical characteristic with minimum age-20*

Gender	Indicator	Mean	Mean *	s _x	s _x *	min.	min.*	max.	max.*	n	n*
Men	Weight (kg)	80.85	87.80	23.21	15.49	14.00	64.00	128.00	128.00	31	26
	Height (m)	1.71	1.77	0.20	0.07	0.88	1.55	1.91	1.91	31	26
	BMI (kg m ⁻²)	27.9	28.10	5.35	4.87	18.8	21.14	39.51	39.51	31	26
	Age (year)	42.29	48.65	20.82	16.00	2.00	20.00	79.00	79.00	31	26
Women	Weight (kg)	69.23	73.00	18.29	12.96	12.00	51.00	113.00	113.00	48	44
	Height (m)	1.64	1.67	0.14	0.06	0.98	1.54	1.80	1.80	48	44
	BMI (kg m ⁻²)	25.37	26.30	5.99	5.25	11.15	17.86	43.06	43.06	48	44
	Age (year)	38.65	41.52	18.42	16.37	2.00	20.00	74.00	74.00	48	44

The average age of people was 40.08 years and the mean of BMI was 26.04 kg m⁻² (79 reviews people). For persons who have reached the minimum age of 20 years the average age of 44.17 years was found and the mean of BMI was 26.97 kg m⁻² (70 reviews people).

In a study conducted on a population of people in the US, an average BMI of 27.8 kg m⁻² was reported (Morland and Evenson, 2008). Dukát (2007) states that in Slovakia, the mean BMI is 26.94 kg m⁻². In comparison with these values the first set of users has only a slightly lower mean BMI. Similar results were recorded in the first group of people by gender. When considering the minimum age of 20 years, we find the mean of BMI 26.97 kg m⁻² is virtually identical to the average for Slovakia.

The age structure of the group was very diverse. In the case of men it was from 2 to 79 years and in women from 2 to 74 years. The diverse age structure is justified from the standpoint of families and needed (existence of a joint analysis of one or two generations of ancestors).

According to our results, the age is not a limiting factor in the analysis of the relationship of BMI of the individual genes and polymorphisms. When comparing the body mass index of men (27.09) and women (25.37) of all subjects and average values of subjects who had at least 20 years (male 28.10 and female 26.30) there was a difference of about only 1 point. In any case, since individual human

body measurements are significantly affected by age, also the effect of age was taken into account in the analysis.

The classification of individuals analyzed according to the international classification of underweight, normal weight, overweight and obesity is presented in Table 2.

Table 2 Classification of overweight and obesity

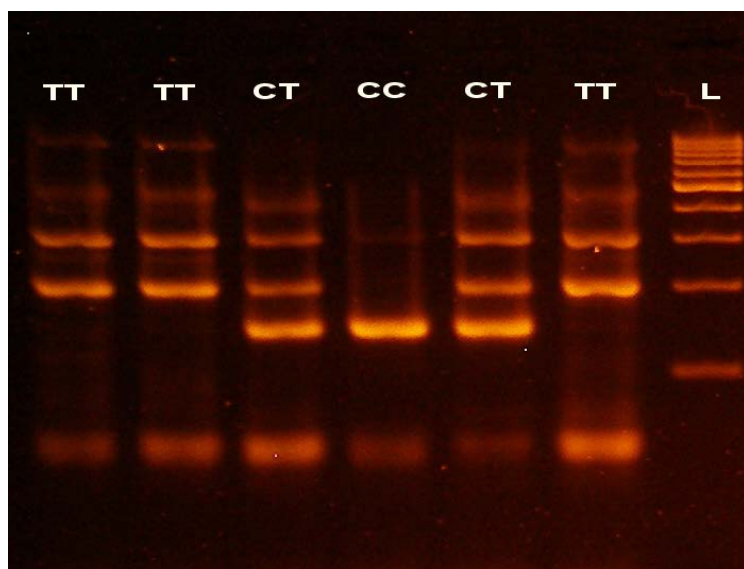
BMI (kg m ⁻²)	Men	%	Women	%	Count	%
< 25	12	38.7	23	47.9	35	44.3
25–30 (overweight)	10	32.3	15	31.3	25	31.6
30≥ (obesity)	9	29.0	10	20.8	19	24.1

Based on the classification of overweight and obesity we can conclude that, overall, 55.7 % of people (61.3 % men and 52.1 % women) we analyzed, suffer from overweight and obesity.

3.2 Molecular-genetic analysis of gene *FTO* rs1121980

The principle of ARMS-PCR method is using two outer primers which produce outer control fragment of PCR reaction and two inner primers. The primers are designed such that the two primer pairs overlap at a SNP location but each match perfectly to only one of the possible allele. The allele specific primers differentiated alleles C and T. Genotype CC was detected by specific 148 bp fragment for allele C and one control fragment with size 311 bp. The genotype TT was detected on the base of presence specific 208 bp fragment for allele T and one 311 bp control fragment.

The heterozygous genotype CT was detected by specific 148 bp and 208 bp fragments and one control fragment with size 311 bp (Figure 1). We analysed 79 samples of human for SNP polymorphism *rs1121980* of *FTO* gene by ARMS-PCR method in relation to human obesity based on BMI values with respect to gender and age.



CC – homozygous genotype (148 bp , 311 bp), CT – heterozygous genotype (148 bp, 208 bp, 311 bp), TT – homozygous genotype (208 bp, 311 bp), L – 100 bp DNA ladder (Fermentas),

Figure 1 Representative results of analysis ARMS-PCR for *rs1121980* of human *FTO* gene on 2% agarose gel

Basic statistical analysis of analysed rs 1121980 FTO is presented in Table 3 and shown in Figure 2.

Table 3 Frequency of genotypes and alleles of the FTO gene

Gene	Genotype	Count	Frequencies			PIC	He _(obs)	He _(exp)	χ ² – test
			Genotype	Allele					
FTO <i>rs1121980</i>	CC	23	0.2911	C	T	0.375	0.4051	0.4999	2.57
	CT	32	0.4051	0.49365	0.50635				
	TT	24	0.3038	±0.0447	±0.0447				

The most frequent genotype for FTO was heterozygous genotype (40.51 %). Testing frequencies of genotypes according to the Hardy-Weinberg law χ² test confirmed the maintenance of equilibrium in the evaluated group of people.

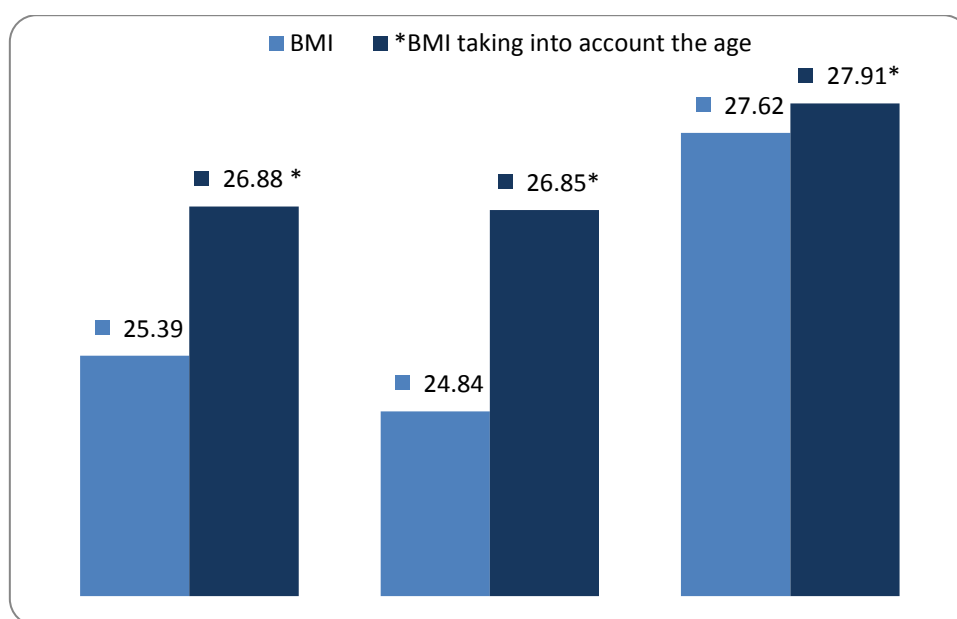


Figure 2

Vimalleswaran et al. (2009) have confirmed the effect of polymorphism FTO *rs1121980* to BMI and also to the waist circumference. They found a highly significant association of BMI with allele T and increasing the BMI level in average 0.40–0.66 points per risk allele. They considered bigger effect of the FTO gene variant in less physical active people.

Using the numerical substitution for the risk allele in the FTO *rs1121980* (T allele) we can see increased BMI level in the group, also with age consideration (Figure 3). In separate consideration of genders and also of the age* (Figure 3, 4 and 5) we found the highest increase of BMI in men (1.25 kg m⁻²).

The correlation between selected polymorphism and influence to BMI level has shown considerable effect. With gender consideration comes to decreasing of statistical relevance cause of the decreasing number in each group (M = 31, W = 68). Our results confirm the findings of Li et al. (2010) that in the FTO locus was shown the effect to BMI. Common analysed polymorphism variants have small effects on obesity measures and they have cumulative effects but their predictive value for obesity risk is kindly limited.

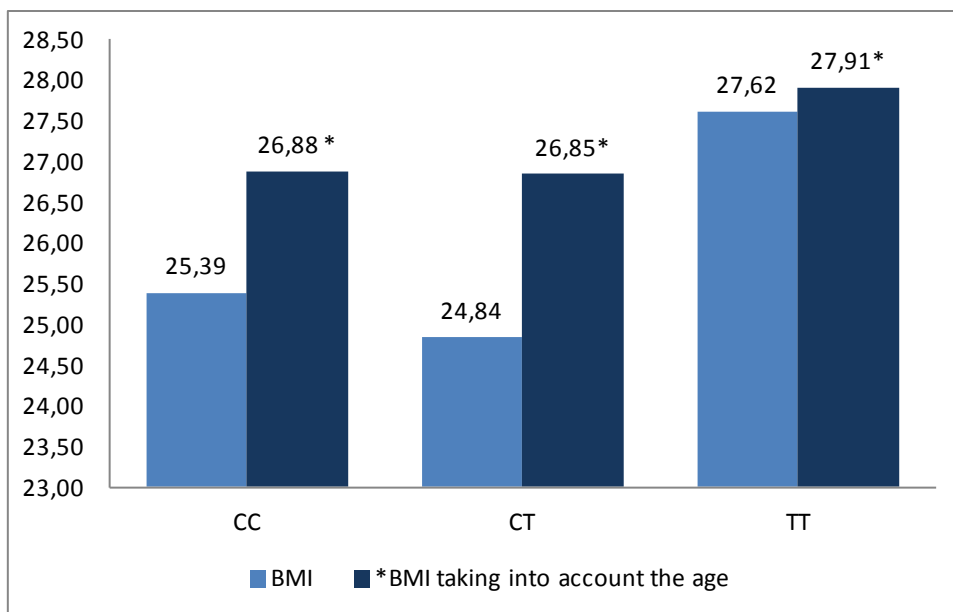


Figure 3 The average of BMI level for FTO rs1121980 without and with consideration of age

In previous research (Trakovická et al., 2015; Candráková, Trakovická, 2016), we also found connection between polymorphisms in the LEP (G2548A), LEPR (Gln223Arg) and GHR (171T/C) genes and BMI level. But mainly due to the limited sample and polygenic character of evaluated traits further research is needed.

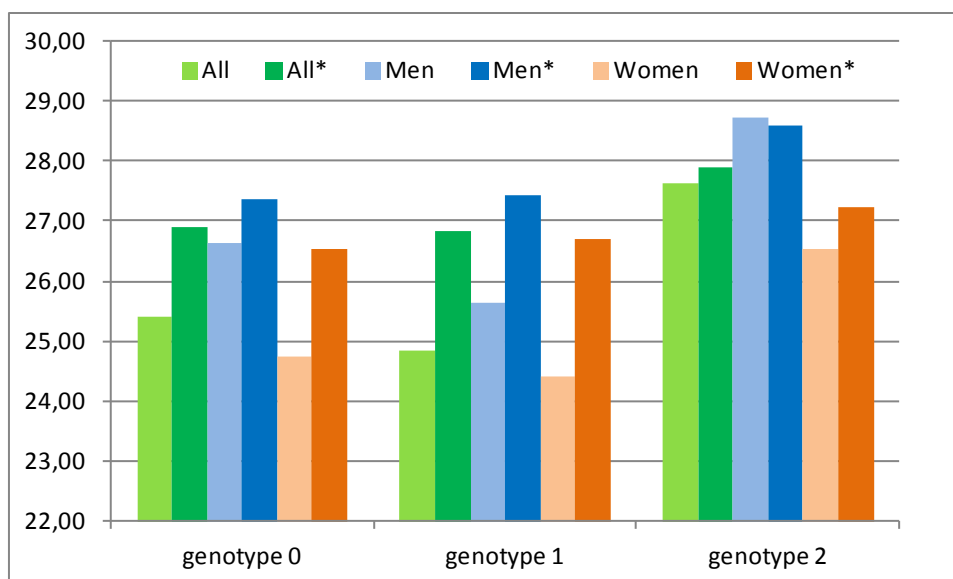


Figure 4 The impact of considered negative allele and genotypes on BMI (kg m^{-2})

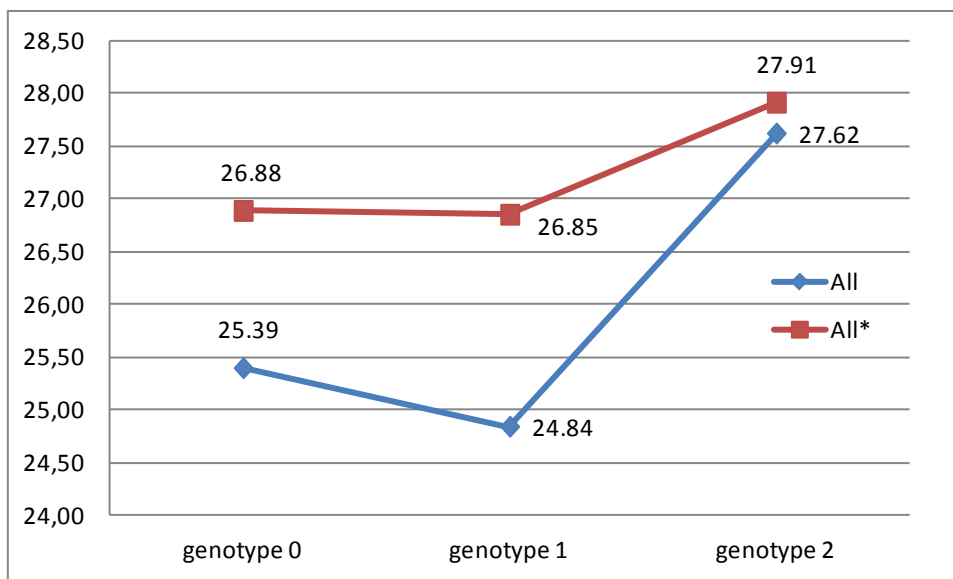


Figure 5 The impact of considered negative allele and genotypes on BMI (kg/m²) in all group

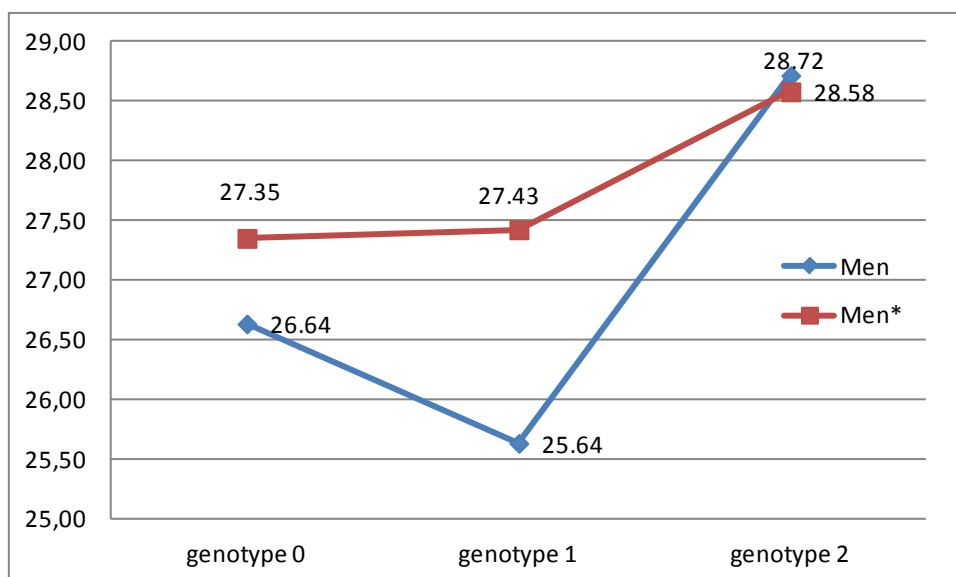


Figure 6 The impact of considered negative allele and genotypes on BMI (kg/m²) in men

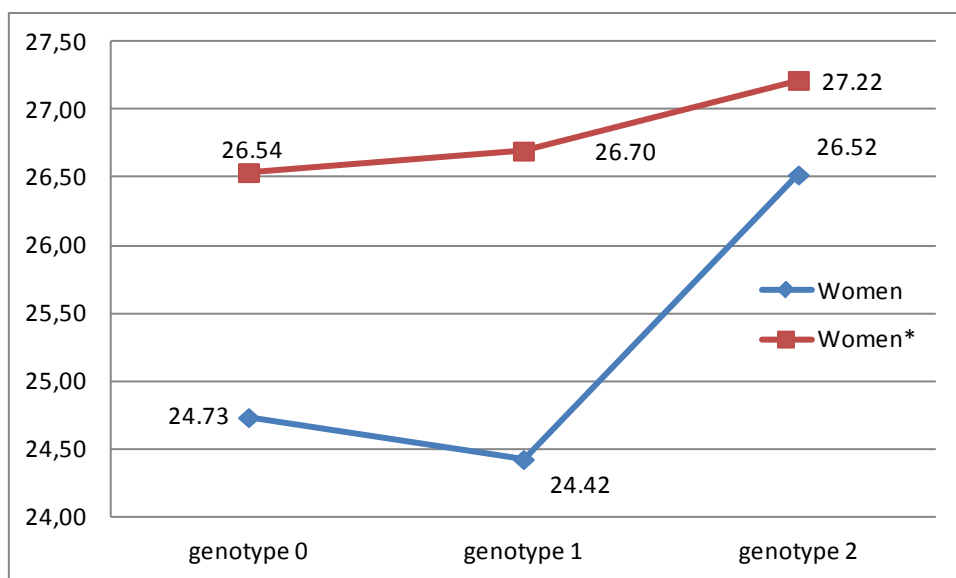


Figure 7 The impact of considered negative allele and genotypes on BMI (kg/m²) in women

4 Conclusions

The results achieved in analysis of the relation for body mass index and polymorphisms studied gene allow us to confirm the hypothesis that the FTO polymorphism is related to obesity expressed by human body mass index (BMI). For a more comprehensive evaluation of polymorphisms studied genes and their impact on obesity, we suggest future conduct additional anthropometric measurements, blood tests in connection with the nutritional history and in particular the extension samples of observed people.

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