

# FTO First Intron rs1558902 Variant and Platelets Count in White Middle-aged Women: Prague Pre- and Post-Menopausal Females (3PMFs) Study

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**Abstract:** The polymorphisms within the *FTO* gene play an important role in the genetic determination of body weight and body mass index and have been associated with cardiovascular disease, but the causal mechanism is still a matter of debate. The possible effect on the platelet count as a marker of hemocoagulation status as a possible cardiovascular risk factor was suggested in Japanese population. We have analyzed both rs1558902 *FTO* polymorphism (T > A) and platelet counts in the Prague Pre and Post Menopausal Females (3PMFs) study, including those of 669 women (mean age,  $55.7 \pm 2.7$  years). The frequencies of the *FTO* genotypes were similar to other populations (TT, 30.4%; TA, 48.1%; and AA, 21.5%). We have not detected a significant association between the *FTO* rs1558902 variant and platelet counts in white women (TT,  $242 \pm 55 \times 10^9$ ; TA,  $246 \pm 67 \times 10^9$ ; and AA,  $247 \pm 55 \times 10^9$ ;  $F[2,642] = 0.30$ ,  $P = 0.75$ ). At least in white persons, platelet count seems not to be a link between the *FTO* variation and risk of cardiovascular disease.

**Key Words:** *FTO* gene, polymorphism, platelets, white persons

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Cardiovascular diseases (CVDs) are the most common cause of mortality and morbidity in developed countries. Genetics plays a substantial role in development of CVD, but particular knowledge is far from the entire estimate of genetic predisposition of CVD, which should be approximately 50%. Near the “classical” candidate genes, for example, genes for apolipoprotein E<sup>1,2</sup> or apolipoprotein A5<sup>3</sup> (where the association is based on the known physiological effects on plasma lipids), high throughput methods (especially chips used in genomewide association studies) lead to the detection of the new genes of interest, mainly, however, without clear biochemical or pathological links between the gene and disease.

Among these genes belongs also the gene for *FTO* (fat mass and obesity-associated protein, OMIM ID, 610966; gene ID, 79068), primarily recognized as body mass index (BMI)-associated gene (for review, see Fawcett and Barroso<sup>4</sup>). A cluster of single-nucleotide polymorphisms (SNPs) located within the first intron of the *FTO* gene was recognized as

sufficient for the detection of the obesity-associated *FTO* variant. This variant could be detected by the analysis of 1 of the 3 tagging SNPs (rs9939609, rs1421085, and rs17817449; these SNPs are in almost complete linkage disequilibrium).

Later, the importance of the first intron variants within the *FTO* gene in determination of noncommunicable diseases, especially, but not only, of CVD, was detected.<sup>5–8</sup> Interestingly, the association between *FTO* polymorphism and CVD is at least partially independent on common risk factors such as obesity, diabetes mellitus, or smoking.<sup>5,6</sup> Despite the intensive research and the fact that some regulatory functions of the *FTO* are supposed,<sup>9,10</sup> the mechanism linking the *FTO* gene to the CVD development remains unclear.

Recently, an association between the rs1558902 (also located within the first *FTO* intron) and platelet counts was detected in Japanese individuals.<sup>11</sup> Obesity-associated A allele was associated also with the approximately 10% increase of platelet count in contrast to the values observed in the “normal” homozygotes.

Especially because the relative low number of included individuals (209), an important limitation of the original finding<sup>1</sup> is the lack of the confirmatory study. It was described that even large studies are prone to be false positive (type I error), and their results need to be replicated.<sup>12</sup>

To confirm the original study, we have analyzed the potential association between the rs1558902 *FTO* polymorphism and (not only) platelet counts in a group of middle-aged white women.

## MATERIALS AND METHODS

Individuals included within the Prague Pre and Post Menopausal Females study (3PMFs) were analyzed.<sup>13</sup> Briefly, 5% representative random sample (individuals with severe kidney or liver disease were not included in the study) of the population consisting of 29,440 women aged 45 to 54 years living in Prague was selected from the registers of health insurance companies. From a random sample of 1472 women, 908 gave their informed consent to participate in the study and were primarily examined. In 2010/2011, 669 women (73.7%) participated in the second survey of the study.

Anthropometrical parameters and blood pressure were measured according the standardized protocols. Plasma lipids were analyzed in fasting plasma by the WHO Regional Lipid Reference Centre, IKEM, Prague, on a Roche COBAS-MIRA autoanalyzer (Hoffmann-La Roche, Basel, Switzerland), using reagents from Boehringer Mannheim Diagnostics (Mannheim, Germany), and Hoffmann-La Roche. For the hematological analysis, vials containing tripotassium ethylenediaminetetraacetic acid (EDTA) were used. Parameters were automatically determined by an impedance method using COULTER LH-750 analyzer (Beckman Coulter, Brea, CA).

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Genomic DNA was extracted from EDTA blood by a standard method.<sup>14</sup> Rs1558902 variant within the first intron of the *FTO* gene (T > A) was analyzed using the polymerase chain reaction restriction fragment length polymorphism method. Briefly, oligonucleotides 5'-TAG CAA CTG CGA TAC AAG TGT TAG ATA TC and 5'-TAG GGT ACG TTG CAG CAA TAA CCT ACC CGA were used for amplification of 181-base pair (bp) DNA fragment at annealing temperature of 64°C. Treatment with restriction enzyme *Mbo*I distinguished between the common T allele (uncut polymerase chain reaction product of 181 bp) and minor A allele (restriction fragments of 150 and 31 bp). To ensure the accuracy of the methods for genotyping, one plate, obtaining 94 samples, was genotyped twice within 1 week with 100% conformity.

Data are presented as percentages for categorical variables and means  $\pm$  SD for continuous variables. Between-group comparison of continuous variables was performed using analysis of variance, and analysis of covariance was performed to exclude potential confounding by age. Discrete variables were tested by  $\chi^2$  statistics.

The protocol of the study was approved by institutional ethic committee. Clinical characteristics of the analyzed women are presented in Table 1.

## RESULTS

Frequencies of the rs1558902 genotypes in our study (TT, 30.4%; AT, 48.1%; and AA, 21.5%) are within the expected range and are in agreement with the Hardy-Weinberg equilibrium ( $P = 0.43$ ).

Of the 669 individuals included in the second survey of the 3PMFs study, both the rs1558902 *FTO* genotype and valid platelet counts were available in 646 individuals (96.4%).

The *FTO* variant rs1558902 (Table 1) was not significantly associated with BMI, waist circumference, subcutaneous fat, or plasma lipids.

Furthermore, we did not detect any association between the *FTO* rs1558902 polymorphism and platelet counts (TT,  $242 \pm 55 \times 10^9$ ; TA,  $246 \pm 67 \times 10^9$ ; and AA,  $247 \pm 55 \times 10^9$ ;  $F[2.642] = 0.30$ ,  $P = 0.74$ ). Of the other parameters analyzed, AA homozygotes have slightly lower plasma levels of total cholesterol ( $F[2.643] = 3.19$ ,  $P = 0.043$ ) and hemoglobin ( $F[2.643] = 3.12$ ,  $P = 0.045$ ) than others, but the significance disappeared after adjustment for multiple testing. Other anthropometric, lipid and hematological parameters were independent of the *FTO* rs1558902 genotypes (for more details, see Table 1).

## DISCUSSION

In our study, we did not confirm the original finding of Kotani et al.<sup>11</sup> who have detected an increased platelet counts in carriers of the obesity-associated AA (rs1558902) *FTO* genotype.

There are a couple of plausible explanations for the observed difference. At first, the original study has included only slightly more than 200 individuals, and such studies are prone to false-positive results, especially if any correction for multiple testing was performed. Furthermore, age and sex differences between the studies could cause the observed differences, as we have included only women of narrow age range.

In contrast, it is not very likely that the ethnical differences (whites vs Japanese) could be the reason for the observed differences, as the *FTO* variants have the similar effects on BMI values across the different ethnics. In the case of the rs1558902 variant, no association between the SNP and BMI was observed both in our study and in the Japanese study.

Based on our results, it seems not likely that an effect of the *FTO* variant on platelet count could at least partially explain the mechanism, how *FTO* variants affect the risk of development of a couple of noncommunicable diseases.

**TABLE 1.** Clinical Characteristics of the Analyzed Women in 3PMFs

Clinical Characteristic	TT	TA	AA	P
<i>FTO</i> rs1558902				
n (%)	196 (30.3)	311 (48.1)	139 (21.5)	
Age, yrs*	55.5 $\pm$ 2.7	55.7 $\pm$ 2.7	55.9 $\pm$ 2.8	0.402
BMI, kg/m <sup>2</sup> *	26.8 $\pm$ 4.8	26.5 $\pm$ 4.7	27.07 $\pm$ 5.3	0.548
WHR*	0.856 $\pm$ 0.079	0.841 $\pm$ 0.069	0.845 $\pm$ 0.075	0.077
Diabetes, n (%)	8 (4.1)	7 (2.3)	7 (5.0)	0.265
Hypertension, n (%)	99 (51.0)	143 (46.4)	67 (48.9)	0.597
Smoking prevalence, n (%)	39 (19.9)	87 (28.0)	34 (24.5)	0.121
Total cholesterol, mmol/L*	5.71 $\pm$ 1.02	5.59 $\pm$ 0.93	5.36 $\pm$ 0.93	0.043†
Triglycerides, mmol/L*	1.46 $\pm$ 0.81	1.32 $\pm$ 0.64	1.34 $\pm$ 0.68	0.112
HDL cholesterol, mmol/L*	1.66 $\pm$ 0.39	1.67 $\pm$ 0.37	1.63 $\pm$ 0.42	0.444
Glycemia*	5.47 $\pm$ 0.93	5.37 $\pm$ 0.60	5.49 $\pm$ 0.91	0.201
Insulin*	7.17 $\pm$ 3.95	6.95 $\pm$ 4.29	7.66 $\pm$ 5.71	0.314
Hemoglobin, g/L*	139.3 $\pm$ 8.3	138.9 $\pm$ 8.6	136.9 $\pm$ 10.4	0.045†
Platelets, $\times 10^9/L$ *	242.2 $\pm$ 55.3	246.0 $\pm$ 64.7	246.6 $\pm$ 54.9	0.737
Leukocytes, $\times 10^9/L$ *	6.17 $\pm$ 1.64	6.16 $\pm$ 1.84	6.31 $\pm$ 1.68	0.680
Neutrophils, $\times 10^9/L$ *	3.47 $\pm$ 1.20	3.47 $\pm$ 1.46	3.67 $\pm$ 1.26	0.309
Lymphocytes, $\times 10^9/L$ *	1.97 $\pm$ 0.60	1.97 $\pm$ 0.55	1.93 $\pm$ 0.59	0.741
Erythrocytes, $\times 10^{12}/L$ *	4.41 $\pm$ 0.30	4.41 $\pm$ 0.31	4.40 $\pm$ 0.31	0.915

\*Results are presented as mean  $\pm$  SD.

†Not significant after Bonferroni correction for multiple testing.

HDL indicates high-density lipoprotein; WHR, waist-hip ratio.

Variants within the first intron of the *FTO* gene (especially the rs1421085, rs17817449, and rs9939609 variants) are between the exciting pleiotropic genetic variants. They were primarily recognized as a risk factor of obesity development.<sup>15–17</sup> A short time after, *FTO* variants were described to be (at least partially), independently of BMI, associated also with CVD,<sup>5,6</sup> diabetes mellitus type 2,<sup>7,18</sup> end-stage renal disease,<sup>19</sup> some but not all types of cancer,<sup>8,20,21</sup> and total mortality.<sup>22</sup> Despite an intensive research during the past years, the underlying mechanism remains unclear. The first experiments were focused on the possible effect on dietary habits, physical activity (reviewed by Dlouha et al.<sup>23</sup>), or basal metabolic rate<sup>24</sup> and led to inconsistent results. Recent publications suggest that the *FTO* is more likely a regulatory protein, exhibiting low DNA demethylase activity<sup>9</sup> functioning like a possible transcriptional cofactor<sup>10</sup> and potentially influencing also the telomere length<sup>25</sup> and, thus, the biological aging.

In summary, we have not confirmed a potential association between the first intron SNP rs1558902 and platelet counts in white middle-aged women. The underlying mechanism connecting the *FTO* first intron tagging polymorphisms and enhanced risk of noncommunicable diseases needs to be examined in future studies.

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