A Decade of the Common FTO Rs9939609 Polymorphism: A Systematic Review

Indira Baručija-Özçoban¹, Adna Ašić¹, Larisa Bešić¹, Sabina Halilović¹, Damir Marjanović¹,², Serkan Dogan¹

¹Department of Genetics and Bioengineering, International Burch University, Sarajevo, Bosnia and Herzegovina
²Institute for Anthropological Research, Zagreb

ABSTRACT

Several association studies focusing on FTO gene polymorphisms have been published in the past years; however, the association between FTO-related conditions and FTO gene variants remains unexplained. Population genetics and association studies of different populations provide a valuable tool for further research. Thus, the aim of this systematic review is to summarize current knowledge on the FTO SNP rs9939609, and its association with presumably related conditions. The study included original research articles collected from PubMed and ResearchGate databases that were published in the period between 2007 and November 2017, and that provide information on rs9939609 mutant allele frequency and its probable association with any condition suspected of being related to the mutant allele. Genotype data was extracted and analyzed, and missing data was obtained from secondary sources. Short summaries of relevant studies from primary sources are organized in an overview table. The results of the systematic review suggest that mutant allele A is the most prevalent in European populations and least frequent on the Far East. In addition, it has been concluded that allele A is a good tool for the prediction of an increased risk of higher-than-normal BMI in a person carrying it, as well as that allele A should be further analyzed as a possible risk marker for type 2 diabetes mellitus and polycystic ovary syndrome development.

Keywords: FTO, rs9939609, body mass index (BMI), type 2 diabetes mellitus (T2DM), polycystic ovary syndrome (PCOS), population genetics

Introduction

Since obesity and many other weight-related disorders have reached epidemic levels, there is a large interest in the causes of excess body weight as well as various disorders that share this specific symptom. The FTO (Fat mass and obesity-associated protein) gene, which was identified as one of the possible genetic triggers, is a large gene containing nine exons that span over 400 kb on the long arm of chromosome 16 at position 12.2, and encodes for 2-oxoglutarate-dependent dioxygenase which functions in the repair of alkylated DNA and RNA by oxidative demethylation. It was first identified in the mouse genome on chromosome 8, and is strongly conserved across various vertebrate species⁵. FTO was shown to act as a demethylase with a strong preference for 3meU (methyluracil) and 3meT (mononucleotide 3-methylthymidine) in single-stranded RNA and DNA, respectively⁶. In addition, the gene plays an important role in global metabolism and maintaining homeostasis, and is known to be unequivocally associated with adiposity and body fat accumulation control, as well as the control of adipocyte differentiation into white or brown fat cells⁷. FTO variants can interact with IRX3 (Iroquois homeobox 3) and even cause an increase in IRX3 and IRX5 (Iroquois homeobox 5) expression during adipocyte differentiation⁸.

The FTO gene has 68 identified single nucleotide polymorphisms (SNPs)⁹. The FTO rs9939609 SNP is one of the ten SNPs in the first intron of the FTO gene, which was found to be associated with increased weight, raised body mass index (BMI) and related traits⁹. The FTO rs9939609 SNP has two alleles, T (wild-type) and A (mutant), which were shown to be associated with the development of obesity⁹.
The link between FTO and eating disorders and type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) susceptibility genes and multiple SNPs located in the first intron of FTO were identified to be associated with T2DM in 2007, which led to a number of studies examining the effect of FTO SNPs on this and related conditions.

The rs9939609 SNP was found to be connected to an increased risk of T2DM, although this influence could also be explained by its effect on BMI in affected and unaffected individuals, since FTO was also found to influence appetite and food intake. Furthermore, Gerken et al. suggested that, since present particularly in hypothalamic nuclei governing energy balance, FTO mRNA levels in the arcuate nucleus might be regulated by feeding and fasting. It has also been reported that FTO SNPs are associated with insulin resistance and that association can be observed even after the adjustment of BMI.

The function of the FTO protein product has not been completely understood yet. Most studies are association studies, and mechanisms of relations between gene variants and traits remain obscure. Numerous studies confirmed that the FTO genotype has an impact on BMI, which in turn has an effect on metabolic traits. However, there is still a lack of linkage between the genotype and the resulting metabolic trait.

A recent study depicted that the FTO gene has a rapid turnover in the pancreatic β cells involved in the regulation of insulin secretion under glucose stimulation. Additionally, according to the same study, FTO plays an important role in the function regulation of pancreatic β cells. However, the functional role of FTO in pancreatic β cells and related molecular mechanism are still unclear.

As the strongest common genetic determinants of adiposity, FTO variants partially act by influencing dopaminergic signaling in the brain and promote increased food intake. FTO A allele is linked to central insulin resistance, and peripheral insulin sensitivity regulation is controlled by central insulin. Dopamine neurons also express FTO proteins and inactivation of the FTO gene impairs dopamine receptor type 2 (D2R) and type 3 (D3R; collectively, ‘D2-like receptor’)-dependent control of neuronal activity and behavioral responses.

There appears to be an interaction between FTO and ANKK1 polymorphisms that are responsible for midbrain activity during reward learning as both FTO and ANKK1 confer risk for obesity by their influence on D2 receptors. ANKK1 indicates reduced D2 receptor availability, which was shown to lead to an FTO variation-related increase in body fat and waist circumference, as well as reduced peripheral insulin sensitivity. This event confers an increase in the risk of obesity in the presence of any of the two risk alleles.

Eating disorders

FTO was recently identified as a potential candidate for the molecular association with eating disorders (ED). A study published in 2017 suggests that individuals with the A allele are more likely to develop ED or to express emotional eating. The two FTO SNPs assumed to have the largest effect on BMI variations between individuals are rs9939609 and rs9930506. The recent genome-wide association study (GWAS) for BMI, which included data from up to 247,796 European individuals, found rs1558902 to be the most significantly associated SNP (PGWAS < 10−60).

FTO and polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is frequently accompanied by insulin resistance and glucose intolerance. In addition, 38% to 88% of women with PCOS are overweight or obese. Consequently, T2DM and PCOS share common symptoms, which led to the investigation of the connection between FTO gene variants and individuals’ risks of PCOS. Recently published studies have shown that the FTO gene variants might have large effects on obesity, insulin resistance, and glucose intolerance among patients with PCOS phenotypes. The association between FTO and PCOS, however, still remains unclear and decrease in severity of phenotypic manifestations of PCOS is often linked to BMI improvement with weight loss. In a study completed in 2014, Louwers et al. demonstrated a lack of connection between 12 BMI risk alleles and PCOS.

The effect of FTO rs9939609 combined with MC4R rs17782313 has been discussed in relation to PCOS after

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**Figure 1. A flowchart for systematic review**
studies on their effect on obesity\textsuperscript{29}. A ten-kg weight difference was reported between AA and TT homozygotic PCOS carriers\textsuperscript{30}. In addition, the FTO rs9939609 polymorphism was found to be significantly associated with the risk of PCOS in Asians and Caucasians\textsuperscript{31}. Li et al. (2013) also found that the mutant allele A of rs9939609 leads to a higher risk of PCOS (Pcombined= 1.26E-11, ORs = 1.44; Pmeta= 6.89E-12), even after the adjustment for differences in BMI (P = 1.82E-6)\textsuperscript{32}.

We have therefore aimed to collect previously published data regarding the mutant allele A in different regions around the world and to summarize them in the form of a systematic review in order to investigate whether T2DM and PCOS onset and increase in BMI might be related to rs9939609 genotype.

Materials and Methods

This review is based on research papers collected from relevant databases. Terms “FTO”, “SNP”, “diabetes”, “insulin”, “resistance”, “PCOS”, “gene”, “variant”, “BMI”, and “hypothalamus”, were searched in PubMed and Research-
Gate with a focus on papers published from the period of 2007 up to the end of November 2017.

In order to analyze the abundance of the mutant FTO allele, the authors searched for genotyping data of particular populations in the articles. From 46 papers, 14 studies concentrated on non-target SNPs or contained data which was not represented adequately for further analysis, and were eliminated as non-eligible studies. Therefore, a total of 32 studies were accepted for this review. Data extraction was performed in order to investigate the frequency of mutant alleles in specific regions. All selected articles provided data on the distribution of FTO alleles in the studied population. Exclusion and inclusion criteria are briefly explained in the Figure 1.

In order to investigate the possibility of FTO rs9939609 being an indicator for mutant allele-related disorders, A allele distribution was compared among populations in the world and data was linked to associated disorders for particular populations. Participants in the included studies were patients with conditions compared to control healthy subjects, or overweight/obese individuals compared to underweight/normal weight individuals. Different types of genotyping methods were used, such as Taqman allelic discrimination, restriction enzyme length polymorphism (RFLP), TaqMan assays, Qiagen, tetra-primer amplification refractory mutation system polymerase chain reaction (ARMS-PCR), or agarose gel electrophoresis. For result generation, we used sample sizes, genotype frequencies, allele frequencies, region and year of study publication.

In the first data extraction, a total of 32 data sets were obtained. Reorganization of obtained data provided distinguished data sets according to investigated populations, regions, and mutant allele-associated disorders. Results are presented in graphical form.

Results and Discussion

Most association studies reporting the effects of FTO variants were concerned with the gene variant effects on obesity and PCOS. A summary of all performed studies is given in Table 1.

In order to investigate the possibility of FTO rs9939609 being an indicator of mutant allele-related disorders, A allele distribution was compared among worldwide populations, and data was associated to the disorders of interest in those same populations. The data extraction provided 32 cohorts with genotype and allele frequencies from a total of 24 different populations. The highest mutant allele A frequency (49.5%) was observed in a combined French and Romanian population study. The lowest A allele frequency was reported in South Korean populations in a study conducted by Kim et al., followed by the
<table>
<thead>
<tr>
<th>Study population</th>
<th>Scope</th>
<th>Findings of interest</th>
<th>Reference</th>
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<tr>
<td>UK Obese, UK Control</td>
<td>ED</td>
<td>UK children participated in research regarding food intake. They were genotyped for rs9939609 and their eating behavior was analyzed. There was no significant relation between FTO genotype and any physical activity characteristics. However, it was shown that A allele is likely affecting appetite.</td>
<td>[34]</td>
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<tr>
<td>Czech Republic General Population PCOS</td>
<td></td>
<td>Authors proposed that the FTO gene is associated with higher OGTT-stimulated glycaemia and leptin levels besides obesity-related quantitative traits. In this study, samples of participants from the Czech Republic were included. The most present genotype in population was TA, and mutant allele frequency was 43.2%.</td>
<td>[58] Retrieved from [30]</td>
</tr>
<tr>
<td>Mexico Nonobese, Mexico Class I/II BMI Obese, Mexico Class III Obese, Mexico Obese Healthy, Mexico Nonobese Healthy, Mexico T2DM, Mexico Healthy</td>
<td></td>
<td>Subcutaneous and visceral adipose tissue biopsies from class III obesity patients were investigated for FTO and ABCA1 mRNA expression. This was the first report giving evidence of FTO and ABCA1 variant interactions affecting BMI. FTO and ABCA1 risk alleles revealed a lack of significant association with BMI or WC.</td>
<td>[38]</td>
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<td>USA Caucasians European Ancestry T2DM Male, USA Caucasians European Ancestry Healthy Male, USA Caucasians European Ancestry T2DM Female, USA Caucasians European Ancestry Healthy Female</td>
<td></td>
<td>Study subjects were genotyped for rs9939609 in a larger research cohort. Measurements also included plasma adiponectin and leptin for both diabetic men and women. The authors observed a decrease in the level of association between rs9939609 and BMI in individuals older than 65.</td>
<td>[36]</td>
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<tr>
<td>Denmark Treatment Naïve, Denmark Caucasians, Denmark NGT, Denmark T2DM</td>
<td>T2DM</td>
<td>Danish participants from five different study groups were genotyped for FTO rs9939609. It was confirmed that this variation is associated with T2DM even without BMI adjustment.</td>
<td>[33]</td>
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<tr>
<td>France and Romania General Population</td>
<td>PCOS</td>
<td>From a study completed by Wojciechowski (2012), combined genotypes of French and Romanian populations previously published in Attaoua (2008) were analyzed for a total of 189 genotypes. Attaoua (2008) reported very similar incidence in both wild and mutant alleles. The most present genotype was TA. Attaoua (2008) reported association between MetS and PCOS.</td>
<td>[59] Retrieved from [30]</td>
</tr>
<tr>
<td>China PCOS, China Control</td>
<td>PCOS</td>
<td>PCOS patients and healthy controls participated in this case-control association study of linkage between FTO rs9939609 and PCOS development. The difference in allele frequencies between the groups was statistically significant with a higher frequency of mutant allele in the PCOS group. The mutant allele was found to influence PCOS development by causing an increase in the carrier’s BMI.</td>
<td>[50] Retrieved from [31]</td>
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<tr>
<td>Poland General Population</td>
<td>PCOS</td>
<td>PCOS patients were the subject of anthropometric measurement, euglycaemic–hyperinsulinaemic clamp, oral glucose tolerance tests and sex hormone assessments. The study group was also genotyped for the FTO rs9939609 polymorphism. The FTO gene risk variant seems to have more impact on obesity and related traits in patients with PCOS than in others.</td>
<td>[52]</td>
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<tr>
<td>Austria General Population</td>
<td>PCOS</td>
<td>The impact of FTO variants on metabolic and endocrine parameters in PCOS women were investigated in PCOS patients. Alongside genotyping, metabolic and hormonal measurements, oral glucose tolerance test, hirsutism score, and lipometry were performed. The A allele was associated with a significant increase in free testosterone, BMI, 2-hour glucose, 1-hour insulin, and AUCins, as well as body fat percentage. The authors demonstrated that rs9939609 influences hyperandrogenemia and anthropometric parameters in women with PCOS.</td>
<td>[54]</td>
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<td>UK combined OBB/UKBS, UK Control OBB, UK control UKBS, UK PCOS</td>
<td>PCOS</td>
<td>This was a genetic association study of the FTO rs9939609 variant with case–control analyses conducted on UK PCOS patients. Authors provided the first report about predisposing variants to common obesity, which also may lead to PCOS, mediated through adiposity. Authors reported a significant association between FTO genotype and PCOS status.</td>
<td>[60]</td>
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Subjects from families having a history of PCOS had samples taken for genotyping of 15 gene polymorphisms. Transmission/disequilibrium test (TDT) was used to assess linkage between PCOS and gene variants. In the independent case-study, PCOS women and healthy women were also analyzed. SNPs in FTO seemed to be associated with BMI in patients with PCOS, but to the reproductive phenotypes of PCOS.

The aim of this study was to analyze the association of rs9939609 and obesity among 9- to 12-year-old children in Malaysia. No association between FTO rs9939609 polymorphism and BMI categories (p=0.9014), WC (p=0.6828), body fat percentage (p=0.9011), or gender (p=0.3345) was confirmed.

The objective of this study was to investigate the association between FTO polymorphism and PCOS in non-obese women. It was concluded that this gene variant affects the development of PCOS by acting to increase BMI, but that rs9939609 is not the main determinant for developing PCOS throughout the lifetime.

T2DM patients and healthy controls participated in this study to examine the correlation between FTO, T2DM and BMI. The authors concluded that A allele of rs8050136 and rs9939609 (both from FTO gene) may be associated with the T2DM and obesity prevalence.

The study included PCOS patients and women with regular ovulation cycles. The correlation of FTO SNPs rs9939609 and rs8050136 with PCOS incidence was not found, while the presence of the risk allele in both SNPs was associated with higher fasting glucose levels.

In the cross-sectional study, samples from children at age between 7 and 17 were genotyped for the study FTO SNP, along with recording information on the family history of obesity. The results implied that children with AA genotype were overweight/obese in 57.4% of all cases. On the other hand, TA and TT had lower percentage of overweight/obese children (33.1% and 28.9%, respectively).
Lithuanian individuals between 25 and 64 years were genotyped for rs9939609 in order to investigate the association between genotypes and obesity, and metabolic syndromes. The study confirmed a relationship between AA gene variants and high BMI.

Obesity was genotyped on Taiwanese adults to show that the TT genotype is the most abundant (73.3%), while AA was present in 1.5% study participants. Results proved that the FTO rs9939609 SNP may be linked with the risk of obesity.

Two FTO SNPs, rs7206790 and rs9939609, were analyzed in the study of osteoporosis risk. The osteoporosis patients and healthy controls were genotyped. It was shown that rs9939609 SNP does not relate to the risk of osteoporosis development during the lifetime.

In order to detect if there is any association between FTO variants and anthropometric and metabolic characteristics, Romanian children (obese and non-obese), were tested for allele variants. The results confirmed a significant association between obesity and rs9939609 genotype.

In order to analyze A allele distribution in more depth, allele frequencies from different study cohorts were added together and joint results are presented in Figure 3. The allele distribution patterns imply that geographically close populations exhibit similar allele frequencies for rs9939609 SNP. Considering the fact that European populations are rich in FTO rs9939609 SNP, those populations were linked with the risk of obesity.

Chinese population and the Taiwanese population.

In the investigation of allele frequencies among the study populations (Figure 2), it was observed that the highest frequency of A allele is present in European population on other continents or in populations that are likely to be of European descent.

In order to analyze A allele distribution in more depth, allele frequencies from different study cohorts were added together and joint results are presented in Figure 3. The
from China, this population truly differs from its neighboring populations, as confirmed in the present study (Figures 5 and 6a) using the FTO SNP rs9939609. Dogan et al. (2014) analyzed Uygurs in a study of 46 worldwide populations on the basis of autosomal short tandem repeats (STRs) to confirm its uniqueness in the region\(^n\). On the other hand, it is interesting to note that Egyptian and Indian populations have similar frequencies of A and T alleles, while Pakistani population tends to differ from these two (Figure 6b). However, it was not feasible to assess the allele frequency ratios in Egypt and its closest neighbors, as Egypt is the only population of Arabs analyzed in this study (Figure 6b).

Brazilians of European origin exhibit results similar to those from European and US populations\(^36,55\). In addition, it has been observed that Brazilian Caucasian subpopulation differs by 8.2% from other Brazilian populations in terms of mutant allele A frequency. The lowest incidence of FTO allele A in the American continent was observed in Mexico (Figure 7).
The focus of extensive research has been the relation between rs9939609 and T2DM. Numerous studies have been conducted on FTO resulting in confirmed effects of gene variants on weight and BMI. A limitation of the present study is that data on FTO variants and their association with T2DM were obtained from only four populations, namely Denmark, US Caucasian, Uygur, and North Indian. However, it was observed that allele A frequencies were similar in the T2DM groups and in control groups in all populations (Figure 8). In Denmark, the T2DM group had a 6.3% higher frequency of allele A when compared to the control. The mutant allele was more prevalent than wild-type allele T in the other three populations with a frequency difference of 4.4%, 3% and 0.5% in Uygur, Indian and US Caucasian populations, respectively (Figure 8).

Despite the fact that the overall frequency of allele A is rather low in Chinese population, PCOS patients from the same country tend to have a significant increase in the frequency of this allele. On the other hand, Brazilian populations have a relatively high allele A frequency, however...
the difference between PCOS patients and controls is far less pronounced than in Chinese population (Figure 9).

Villalobos-Comparán et al. (2008) concluded that rs9939609 has been associated only with class III obesity and not with overall obesity or BMI in admixed Mexican adults38. The data available for the present analysis allowed the comparison of obese individuals with controls in only three populations (UK, Mexican, and Indian)34,36,37, On the basis of this limited dataset, it has been concluded that the obese and non-obese groups within the study populations positively correlate with A allele frequency in those populations. The most prominent difference in allele A frequency between obese and non-obese individuals was observed in the UK with allele A being 14.69% more prevalent in obesity group.

Conclusions and Future Perspectives

The main objective of the present study was to collect and discuss previously published data on FTO SNP rs9939609, which has been associated with weight-related health conditions. The results of original studies show a higher incidence of mutant A allele in European populations, as well as in populations of European origin inhabiting other continents when compared to the other regions of the world. In addition, it has been found that the frequency of the mutant allele of this polymorphism positively correlates with increased BMI in study participants. Other conditions that were suspected of being associated with rs9939609 are PCOS and T2DM. However, as obesity is usually one of the most prominent symptoms of both PCOS and T2DM, there is a necessity of normalizing patients’ data according to BMI. In addition, there is a need for selecting patients and healthy controls with similar demographic and other parameters in larger study cohorts, which would enable researchers to follow the association between FTO polymorphism and a single condition. This is especially true for Arabic populations, as studies on these populations are scarce in current literature.

REFERENCES

Nekoliko asocijacijskih studija koje se fokusiraju na polimorfizme FTO gena su objavljene u proteklih godina; međutim, povezanost između stanja vezanih s FTO-om i varijante gena FTO-a ostaje neobjašnjena. Populacijska genetika i asocijacijska studija različitih populacija pružaju vrijedan alat za daljnja istraživanja. Stoga je cilj ovog sustavnog pregleda povezanost između stanja vezanih s FTO-om i varijante gena FTO-a ostaje neobjašnjena.