EFFECT OF FTO rs9939609 POLIMORPHISM ON OBESITY IN TURKISH POPULATION

Meliha Merve HIZ ÇİÇEKLIYURT* & Sevilay OĞUZ KILIÇ** & Levent ELMAS***

* Çanakkale Onsekiz Mart University, Faculty of Medicine, Department of Medical Biology, TURKEY, e-mail: mmervemeliha@comu.edu.tr
ORCID ID: https://orcid.org/0000-0003-4303-9717

** Çanakkale Onsekiz Mart University, Faculty of Medicine, Department of Dermatology, TURKEY, e-mail: drsevilayoguz@hotmail.com
ORCID ID: https://orcid.org/0000-0003-3560-849X

*** Bakırçay University, Faculty of Medicine, Department of Medical Biology, TURKEY, e-mail: elmas.levant@gmail.com
ORCID ID: https://orcid.org/0000-0002-6865-6466

Received: 26 September 2020. Accepted: 6 October 2020

ABSTRACT

Obesity is a disease that is affected by environmental conditions as well as genetic predisposition. This is a case-control study that aimed to investigate the relationship between FTO rs9939609 polymorphism and obesity. The relation between the rs9939609 polymorphism and obesity in 80 over-weight (BMI≥30) and 131 under/normal weight (BMI<30) subjects was examined. The allele and genotype frequencies of each group were determined by the allele counting method. The relationship between single nucleotide exchange and obesity risk was calculated using the odds ratio. Statistical analyses were performed with SPSS 18.0.

Results: The frequency of T allele was 0.58 and 0.45 respectively in the healthy and overweight group when allele frequencies of both groups were compared. The change in allele frequency increased the obesity rate by 1.8 fold. The rs9939609 polymorphism increases obesity risk by 2.7 fold in the homozygotes model.

Conclusions: In this research, we found statistically significant results in allele frequency difference and allele positivity. The relationship between rs9939609 polymorphism and obesity will be highlighted by larger population studies.

Keywords: Adiposity, FTO, rs9939609, BMI; Body composition.
1. INTRODUCTION

Obesity is a major problem in the 21st century affecting the public health and is directly related to increased risk of premature death, coronary heart diseases, diabetes mellitus, cancer, hypertension, dyslipidemia and shock (Finkelstein et al. 2009). Obesity cases have been increasing in the last three decades, the reason for this rise pointed as malnutrition, unhealthy lifestyles, immobility, and overeating (Hu, 2003; Rizzi et al., 2016).

Usually, lifestyle changes enhance treatment success and decrease obesity. However, with nutritional genomics, the importance of lifestyle changes gradually decreased. The discovery of obesity-related genes inspires in an enhanced personalized treatment regime (Doaei et al., 2017). It’s predicted that genetic factors are responsible for 40-90% of patients who has body mass index (BMI) variation (Fawcett and Barroso, 2010).

One of these genes is the FTO gene, which exists only in vertebrates and marine algae. The expression of the FTO gene is associated with food intake and energy balance. (Doaei et al., 2017, Guifang et al., 2008). FTO proteins were seen to have similar sequences with E. Coli enzyme AlkB (enzyme which hydroxylating DNA methyl groups and repairing DNA methylation damages) family proteins and its eukaryotic homologs. FTO gene proteins, oxidatively demethylates dsDNA3-met in the presence of iron(II), dioxygen and 2-oxoglutarate. Therefore the nucleic acid demethylase activity on DNA and RNA was noted (Fawcett and Barroso 2010; Speakman, 2015). Its affinity is primarily on RNA and thiamine/uracil due to its crystal structure (Speakman, 2015). Its thought that reversing the methylation by FTO can be a signal for gene regulation (Fawcett and Barroso, 2010; Guifang et al., 2008) and nucleic acid demethylase activity of FTO can regulate expression of metabolism enzyme genes and this dysregulation process can result in obesity (Fawcett and Barroso, 2010). FTO levels were less in starved animals while high lipid intake animals had increased FTO levels. An increase in FTO levels negatively affect food intake while a decrease in FTO levels induce food intake (Yeo, 2011). In rodents, FTO expression was bidirectional sensible to nutritional state and physical activity. Starvation state decrease FTO mRNA levels and FTO immunopositive cell numbers in the hypothalamus. This effect was recovered by intraperitoneal glucose. Functional coupling analysis has revealed that this issue may be in relationship with Brain-derived Neurotrophic Factor (BDNF) taking the role of food intake regulation (Speakman, 2015).

FTO gene has 9 exons and is located in the 16th Chromosome (16.q12.2). The most strong signal of obesity is 1st and 2nd introns of FTO gene. This region consists of 89 variants and approximately 47000 (Clausnitzer et al, 2015). In GWAS studies, in 2007, it was declared that a common variant of FTO gene (rs9939609) plays a predisposing role for Type 2 diabetes mellitus patients in the European population and that this relationship is seen to be mediated with BMI (Fawcett and Barroso 2010; Yang 2010).

Numerous evidences showed FTO SNPs are in relationship with appetite ratings, satiety, loss of eating control, obesity, diabetes and metabolic syndrome. In this study, we examined FTO rs9939609 polymorphism and obesity in Çanakale population.

2. MATERIAL AND METHOD

2.1. Ethics Committee Approval

This research has been approved by Canakkale Onsekiz Mart University Faculty of Medicine, Canakkale, Turkey ethics committee (Approval number 2011-KAEK-27/2016-E.26510) Written and verbal consent was obtained from all the participants. This research was conducted with consideration of Declaration of Helsinki (revised-2000) principles.
2.2. Study design and implementation

Case-control study is conducted for genotyping and allelic profiles of polymorphic FTO gene in general population.

2.3. Patient profile

This study includes 131 control and 80 obese patient. Including criterias were BMI <30 kg/m² for control groups and BMI ≥30 kg/m² for case group. Excluding criterias were having any medical problem such as hypertension, diabetes, metabolic syndrome, psoriasis etc. For both groups. A questionnaire was conducted for getting personal and familial medical history. Obesity diagnosis was made according to Body Mass Index (BMI).

2.4. Genotyping

All the blood samples were collected in EDTA tubes for genotyping. Genotype analyses were conducted with peripheral blood with Real-time PCR reaction after genomic DNA analysis made with commercial isolation kit according to supplier’s instructions. FTO rs9939609 polymorphism was genotyped in case and control groups by real time PCR. Real time PCR reaction was conducted with 50 ng of genomic DNA, 7.4 μl PCR-grade fluid, 1.6 μl Mg⁺² solution, 4 μl probes/primers mix and 2 μl Master mix real-time PCR in a total volume of 20 μl reaction mixture. Thermal cycling was performed under the following conditions; 10 min at 95°C (hold step), denaturation step as 45 cycles at 92°C for 15 second followed by annealing/extension at 60°C for 1 min. FTO rs9939609 T>A polymorphism was genotyped by TaqMan allelic discrimination assay.

2.5. Statistical Analysis

Allele and genotype frequencies were determined according to allele counting method. Chi-square, pearson-chi-square, fisher exact test were used for comparing subgroups. All statistical analyses were conducted via SPSS statistical software. Lower than 0.5 of p value was expressed as statistically significant. Genotype relations and relative risk was evaluated with Odds ratio using Armitage test.

3. RESULTS

In this research, we evaluated the association between FTO rs9939609 polymorphism and obesity in Turkish population. Obese patients recruited in this study (n=80) had an average of 35.93±3.54 years, and the control group consisted of 131 normal-weight volunteers with an average of 35.08±15.17 years. The BMI distribution of obese and control groups were 35.01±2.49 and 25.40±4.49 respectively. We observed the distribution of the genotypes in obese group were as follows: out of 80 cases, 18 were determined to have the wild (TT) genotype, 36 the heterozygous genotype (AT) and 26 the mutant genotype (AA). The wild allele frequency (T allele) for FTO rs9939609 polymorphism was calculated as 0.45±0.04 among obese individuals. Genotype distribution in the control group was as follows: out of 131 cases, 45 had the wild genotype (TT), 62 had heterozygous genotype (AT) and 24 had mutant genotype (AA). The frequency of the T allele was determined as 0.45 ± 0.44(Table 1).
Table 1. Genotypes and allele frequencies of the FTO gene rs99399609 polymorphism in obese individuals and healthy controls.

| Genotype | Obese \((n: 80) (\%)\) | Controls \((n: 131) (\%)\) | Odds Ratio  \\
| --- | --- | --- | --- |
| TT | 18 (16.20) | 45 (44.09) |  \\
| AT | 36 (39.60) | 62 (63.82) |  \\
| AA | 26 (24.20) | 24 (23.09) |  \\

Allele frequency

| Allele freq. difference | T allele | C.I.=[1.136-2.511]  \\
| --- | --- | --- |
|  | 0.45 ± 0.041 | 0.58 ±0.031 | Odds_ratio=1.689 |
|  | p=0.42 (Pearson) | p=0.74 (Pearson) |  \\

We observed that mutant genotype was significantly higher in obese individuals then normal-weight ones and increased obesity risk was 2.7 times higher in in homozygotous model [OR: 2.71, C.I.=[1.24-5.90] and p-value:0.01]. In addition, allele possitivit increased the obesity risk 1.8 fold more [OR:1.80, C.I.=[0.953-3.407], chi²=3.33 and p-value: 0.067]. In overal evaluation, the FTO rs99399609 increase the obesity risk 1.64 times in Turkish population. [OR:1.64, chi²=6.33, p=0.012]. In contrast, there was no significant difference found in heterozygotous comparations. [OR: 1.45, C.I.=[0.733-2.876], chi²=1.15, p=0.28](Table 2).

Table 2. Comparison of FTO rs99399609polymorphism in different models among obese patient and control subjects.

| Genotype | Obese \((n: 80)\) | Controls \((n: 131)\) |  \\
| --- | --- | --- | --- |
| Dominant Model  \\
TT : AA + AT | 18 : 62 | 45 : 86 | OR: 1.802  \\
|  \\
95%CI [0.95-3.41]  \\
p=0.068 |  \\
| Recessive Model  \\
AA : AT + TT | 26 : 44 | 24 : 107 | OR: 2.14  \\
|  \\
95 CI [1.12-4.08]  \\
z-statistics:2.32  \\
p: 0.020 |  \\
| Over-dominant Model  \\
AA + TT : AT | 44: 36 | 69 : 62 | OR:0.911  \\
|  \\
95%CI [0.52-1.59]  \\
z-statistics:0.329  \\
p: 0.74 |  \\


4. DISCUSSION and CONCLUSION

Numerous evidences showed FTO rs9939609 in relationship with apetite ratings, satiety, loss of eating control together with higher BMI across different populations (Frayling et al. 2007, Al-Serri et al 2018, Wardle et al. 2008, Hunt et al. 2008). Wardle et al. (2008) has conducted Satiety Responsiveness and Enjoyment of Food questionnaire to children and genotyped them for FTO rs9939609. The results showed that AA homozygote children have a decreased satiety response score and increased adiposity. Tanofsky-Kraff et al (2009) investigated rs9939609 polymorphism and the eating behavior of the children. Their study showed that rs9939609 polymorphism didnot affect resting/basal metabolism rates but the loss of control during eating was declared frequently by adolescents. Children with AA/AT genotype preferred energy-dense, palatable foods more than those who have the TT genotype. Aside, 34.7% of AA/AT subjects has loss of control response while 18.2% of TT subjects shown loss of control response in 190 children (Tanofsky-Kraff et al., 2009) A meta analysis collecting data from 37 research revealed FTO rs9939609 genotype significantly effect total energy and carbohydrate intake over large scale cohort including 177,330 subject (Qi et al., 2014). The relationship between energy intake, physical activity, and rs9939609 variant is researched in 1978 Afroamerican and Euroamerican subjects and significance were not noted and its seen that rs9939609 variant doesn’t affect gender, energy intake, and physical activity in adiposity related phenotypes (Liu et al., 2010). In several studies FTO rs9939609 polymorphism is linked with different obesity related causes and rs9939609. Song et al (2008) have hown the cumulative effects rs9939609 risk-allele “A” with BMI and speculated that each copy of “A” increased 0.45 kg/m(2) in BMI and waist circumference.

FTO genetic variations are associated with obesity in several populations (Yang et al 2012, Wood et al, 2016). González-Sánchez et al have evaluated FTO rs9939609 gene and obesity and shown that the “FTO AA genotype” was more frequent and related to increased waist circumference in the obese individuals among the Spanish population. Merra et al (2020) have shown the association of rs9939609 polymorphism with incerased BMI and android fat mass-FM% in Italian population. In another study, FTO rs9939609 is associated only with increased Body mass index but not obesity-related metabolic traits in Tawain population (Hsiao and Lin, 2016).

This research reveals interactions between FTO rs9939609 polymorphismwith BMI in Turkish population and our results were similar to previous studies (Ağagündüz and Gezmen-Karadag, 2019; Solak et al., 2014). It could be speculated that genetic predisposition associated with obesity. The relationship between rs9939609 polymorphism and obesity was highlighted by larger population studies previously and we confirmed that FTO rs9939609 polymorphism increased the obesity risk in Turkish population.

Acknowledgements

This work was supported by Canakkale Onsekiz Mart University The Scientific Research Projects Coordination Unit, Project number TSA-2015-417.
REFERENCES

AĞAGÜNDÜZ, D., GEZMEN-KARADAĞ, M. 2019, Association of FTO common variant (rs9939609) with body fat in Turkish individuals, Lipids in health and disease, 18 (1), 212.

AL-SERRI, A., AL-BUSTAN, S.A., KAMKAR, M., THOMAS, D., ALSMADI, O., AL-TEMAIMI, R., MOJIMINIYI, O.A., ABDELLA, N.A., 2018, Association of FTO rs9939609 with Obesity in the Kuwaiti Population: A Public Health Concern? Med Princ Pract, 27 (2), 145-151.

CLAUSNITZER, M, DANKEL, S.N., KIM, K.H., QUON, G., MEULEMAN, W., HAUGEN, C., GLUNK, V., SOUSA, I.S., BEAUDRY, J.L., PUVIINDRAN, V., ABDENNUR, N.A., LIU, J., SVENSSON, P.A., HSU, Y.H., DRUCKER, D.J., MELLGREN, G., HUI, C.C., HAUNER, H., KELLIS, M., 2015, FTO Obesity Variant Circuitry and Adipocyte Browning in Humans, N Engl J Med, 373, 895-907.

DOAEI S., KALANTARI N., MOHAMMADI N.K., TABESH G.A., GHOLAMALIZADEH M, 2017, Macronutrients and the FTO gene expression in hypothalamus, Mar-Apr, a systematic review of experimental studies, Indian Heart J, 69 (2), 277-281.

FAWCETT, K.A., BARROSO, I, 2010, The genetics of obesity: FTO leads the way, Trends in Genetics 26, 266–274.

FINKELSTEIN E.A., TROGDON J.G., COHEN J.W, 2009, Annual medical spending attributable to obesity: payer- and service-specific estimates, Health Aff, 28(5): w822–w831.

GUIFANG, J, YANG, C.G., YANG, S., JIAN, S., JIAN, X., YI, C., ZHOU, Z., HE, C., 2008, Oxidative demethylation of 3-methylthymine and 3-methyluracil in single-stranded DNA and RNA by mouse and human FTO, FEBS Letters, 582, 3313–3319.

GONZÁLEZ-SÁNCHEZ, J. L., ZABENA, C., MARTÍNEZ-LARRAD, M. T., MARTÍNEZ-CALATRAVA, M. J., PÉREZ-BARBA, M., SERRANO-RÍOS, M., 2009, Variant rs9939609 in the FTO gene is associated with obesity in an adult population from Spain, Clinical endocrinology, 70 (3), 390–393.

FRAYLING, T. M., TIMPSON, N. J., WEEDON, M. N., ZEGGINI, E., FREATHEY, R. M., LINDGREN, C. M., PERRY, J. R., ELLIOTT, K. S., LANGO, H., RAYNER, N. W., SHIELDS, B., HARRIES, L. W., BARRETT, J. C., ELLARD, S., GROVES, C. J., KNIGHT, B., PATCH, A. M., NESS, A. R., EBRAHIM, S., LAWLOR, D. A., MCCARTHY, M.I, 2007, A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science, 316 (5826), 889–894.

HSIAO T.J., LIN E, 2016, Association of a common rs9939609 variant in the fat mass and obesity-associated (FTO) gene with obesity and metabolic phenotypes in a Taiwanese population: a replication study. J Genet, 95 (3), 595-601.

HU F.B, 2003, Sedentary lifestyle and risk of obesity and type 2 diabetes, Lipids, 38 (2), 103-8.

HUNT, S.C., STONE, S., XIN, Y., SCHERER, C.A., MAGNESS, C.L., IADONATO, S.P., HOPKINS, P.N., ADAMS, T.D., 2008, Association of the FTO gene with BMI. Obesity, 16(4), 902-904.
LIU, G., ZHU, H., LAGOU, V., GUTIN, B., STALLMANN-JORGENSEN, I. S., TREIBER, F. A., DONG, Y., SNIEDER, H., 2010, FTO variant rs9939609 is associated with body mass index and waist circumference, but not with energy intake or physical activity in European- and African-American youth, BMC Medical Genetics, 11, 57.

MERRA, G., GUALTIERI, P., CIOCCOLONI, G., FALCO, S., BIGIONI, G., TARSITANO, M. G., CAPACCI, A., PICCIONI, A., COSTACURTA, M., FRANCESCHI, F., & DI RENZO, L., 2020, FTO rs9939609 influence on adipose tissue localization in the Italian population. European review for medical and pharmacological sciences, 24 (6), 3223–3235.

QI, Q., KILPELÄINEN, T. O., DOWNER, M. K., TANAKA, T., SMITH, C. E., SLUIJS, I., SONESTEDT, E., CHU, A. Y., RENSTRÖM, F., LIN, X., ÄNGQUIST, L. H., HUANG, J., LIU, Z., LI, Y., ASIF ALI, M., XU, M., AHLUWALIA, T. S., BOER, J. M., CHEN, P., DAIMON, M., … QI, L., 2014, FTO genetic variants, dietary intake and body mass index: insights from 177,330 individuals. Human molecular genetics, 23 (25), 6961–6972.

RIZZI, M., MAZZUOLI, S., REGANO, N., INGUAGGIATO, R., BIANCO, M., LEANDRO, G., BUGIANESI, E., NOË, D., ORZES, N., PALLINI, P., PETRONI, M.L., TESTINO, G., GUGLIELMI, F.W., 2016, Undernutrition, risk of malnutrition. World J Gastrointest Oncol, 8 (7), 563–572.

SPEAKMAN, JR., 2015, The ‘Fat Mass and Obesity Related’ (FTO) gene: Mechanisms of Impact on Obesity and Energy Balance, Curr Obes Rep, 4: 73–91.

SOLAK, M., OZDEMIR ERDOGAN, M., YILDIZ, S. H., UCOK, K., YUKSEL, S., ARIKAN TERZI, E. S., & BESTEPE, A., 2014, Association of obesity with rs1421085 and rs9939609 polymorphisms of FTO gene, Mol Biol Rep, 41 (11), 7381-6.

SONG, Y., YOU, N. C., HSU, Y. H., HOWARD, B. V., LANGER, R. D., MANSON, J. E., NATHAN, L., NIU, T., F TINKER, L., LIU S., 2008, FTO polymorphisms are associated with obesity but not diabetes risk in postmenopausal women. Obesity (Silver Spring), 16 (11), 2472-80.

WARDLE, J., CARNELL, S., HAWORTH, C.M., FAROOQI, I.S., O’RAHILLY, S., PLOMIN, R., 2008, Obesity associated genetic variation in FTO is associated with diminished satiety. J Clin Endocrinol Metab. 2008, 93 (9): 3640-3643.

WOOD, A.R., TYRRELL, J., BEAUMONT, R., JONES, S. E., TUKE, M. A., RUTH, K. S., GIANT CONSORTIUM, YAGHOOTKAR, H., FREATHY, R. M., MURRAY, A., FRAYLING, T. M., & WEDON, M. N., 2016, Variants in the FTO and CDKAL1 loci have recessive effects on risk of obesity and type 2 diabetes, respectively. Diabetologia, 59: 1214-1221.

YANG, J., LOOS, R. J., POWELL, J. E., MEDLAND, S. E., SPELIOTES, E. K., CHASMAN, D. I., ROSE, L. M., THORLEIFSSON, G., STEINTHORSDOTTIR, V., MÁGI, R., WAITE, L., SMITH, A. V., YERGES-ARMSTRONG, L. M., MONDA, K. L., HADLEY, D., MAHajan, A., LI G., KAPUR, K., VITART, V., HUFFMAN, J. E., … VISSCHER, P.M., 2012, FTO genotype is associated with phenotypic variability of body mass index. Nature, 490, 267-272.
YANG, Y., LIU, B., XIA, W., YAN, J., LIU, H.Y., HU, L., LIU, S.M., 2017, FTO Genotype and Type 2 Diabetes Mellitus: Spatial Analysis and Meta-Analysis of 62 Case-Control Studies from Different Regions, Genes, 8, 70.

YEO, G.S.H., 2011, FTO and Obesity: A Problem for a Billion People, Journal of Neuroendocrinology, 24, 393–394.