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Assessing the Fat Mass and Obesity Associated Gene Polymorphisms (rs17817449 and rs1588413) in Obesity and Type 2 Diabetes Mellitus

Abdulhussein A. Algenabi[1],Majid K. Hussein[1], Najah R. Hadi[2], Fadhil A. Nasser[1], Ghizal Fatima[3], Hayder A. Al-Aubaidy[4*]
[1]Department of Clinical Biochemistry, College of Medicine, Kufa University, Najaf, Iraq
[2]Department of Pharmacology, College of Medicine, Kufa University, Najaf, Iraq
[3]Department of Biochemistry, Era's Medical College & Hospital, Lucknow, India
[4]School of Life Sciences, College of Science, Health and Engineering, La Trobe University, Bundoora, VIC, Australia 3086
Correspondence Author: Hayder Al-Aubaidy
E-mail: H.Alaubaidy@latrobe.edu.au

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ABSTRACT
This study aims to evaluate the effects of FTO gene polymorphism (rs17817449andrs1588413) as predictor/ risk factors of type 2 diabetes mellitus in obese Iraqi population. Four hundred obese participants clinically diagnosed with type 2 diabetes were enrolled in this study. A similar matching group of obese non-diabetic control group was also included in this study. Multi-nominal logistic regression analysis applied to study genotype and allele frequencies for FTO gene polymorphism (rs17817449, rs1588413) in patients and control groups. The homozygous genotype significantly (OR= 4.61, CI 95% 2.61-8.12, P<0.001) increases the risk of T2DM by approximately two folds with regarding to those of the wild type after adjustment for other factors (age, sex and BMI).As for the FTO gene polymorphism (rs1588413): The homozygous genotype significantly (OR= 10.68, CI 95% 5.04-22.6, P<0.001) increased the risk of T2DM. The FTO gene polymorphisms rs17817449 and rs1588413are strong predictors for the development of type 2 diabetes mellitus in obese patients.

Keywords: FTO gene polymorphisms; rs17817449; rs1588413; Type 2 diabetes; obesity

Correspondence:
Hayder A. Al – Aubaidy
School of Life Sciences, College of Science, Health and Engineering, La Trobe University, Bundoora, VIC, Australia
E-mail: H.Alaubaidy@latrobe.edu.au

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INTRODUCTION
Several studies showed there is strong association between fat mass and obesity associated (FTO) gene polymorphism with obesity and with type 2 diabetes mellitus (T2DM) [1 - 8]. Both genetic factors and environmental factors are risk predictors for the development of T2DM. The genetic factors play a vital role in pathogenesis of T2DM [9].There was a strong correlation between the sequence variation of the FTO gene located on chromosome 16 on the one hand and BMI, obesity and type 2 diabetes in European Caucasians [10].The protein consists of 505amino acids and is referred to the cell nucleus. In Vitro studies exposed that FTO to be a member of the ferrous and 2-oxoglutarate (2OG) dependent oxygen as super family [11]. The function of it includes oxygen sensing, DNA repair, metabolism of fatty acid and post-translation modifications [12-13]. In addition, its function in energy regulation [14-15], demethylation of the nucleic acid, regulating fat masses of body via lipolys is [16]. FTO expressed in hypothalamus is related to the regulation of food intake and physical activity [17]. Some studies showed no association between *FTO* gene polymorphism and the level of glucose [18], insulin levels [19], or Physical activity [20]. Other studies expressed an association between *FTO* gene polymorphism and energy expenditure [21-23]. This study aims to evaluate the effects of FTO gene polymorphism (rs17817449 and rs1588413) as predictor/ risk factors of type 2 diabetes mellitus in obese Iraqi population.

MATERIALS &METHODS
Study
This study was approved by the Human Research Ethics Committee, University of Kufa. The study group included 800 participants (four hundred obese type 2 diabetics and a matching control group).

Phenotypic data
According to WHO classification of obesity [20], body mass index (BMI) was calculated. The biochemical analyses were done including fasting plasma glucose, lipid profile and serum insulin level.

Gene analyses
Plasma samples were withdrawn and were divided into two parts, one portion (3 ml) was kept in a plain tube (without anticoagulant) and the second portion (2ml) was kept in an EDTA tube. Sera were stored at (-20 C) after centrifugation at 2000x*g* for 10 minutes, while whole blood samples were used for DNA extraction. DNA extraction was performed using Promega kit as described in our previous study [29]. Fragment length polymorphism (PCR– RFLP) for *FTO* gene using thermo cycler (Biometra, Germany). The primer sequences of rs17817449was identified fromForward5′-AGC TTC CAT GGC TAG CAT TA-3′and reverse5′-AGG ACC TCC TAT TTG GGA CA-3′. While the primer sequence of rs1588413: forward 5′-GCT CCC GTC TGC TCT GCC CT-3′ and reveres primer was5′-GCT GTG GGG AAG GGA GGT GGT-3′ [30].

Statistical analyses
Data were analyzed using Statistical package for social sciences (SPSS) version 23 (SPSS Inc., Chicago, IL). Data were presented as mean ± standard deviation. One side Student t-test was used to look for the differences between the two groups (P at ≤ 0.05).

RESULTS
Anthropometric data were shown in table (1), agarose gel electrophoresis were shown in figure (1-A) for the FTO gene

variant (rs17817449) while figure (1-B) showed the FTO gene variant (rs1588413). The FTO gene variants were showed in tables (2-3). Bothrs17817449 and rs1588413genotypes were consistent (P=0.41) and (P=0.8) respectively, the biochemical characteristics of studied individuals according to FTO gene variants rs17817449 and rs1588413.

Table 1: Clinical and biochemical characteristics of study subjects

Parameters	Obese Diabetic Subjects	Obese Control Subjects	P value
Male/Female ratio	400 (190/210)	400 (195/205)	1
Age (y)	54.8 ± 8.8	53.9 ± 9	0.7
BMI (kg/m$^{2)}$)	32.1 ± 1.8	31.9± 2	0.1
FBS (mg/dl)	239.3 ± 7	87.2 ± 7.6	< 0.001
HBA1c (%)	7.7± 0.7	4.6 ±0.5	< 0.001
Cholesterol (mg/dl)	231.5 ± 5.6	230.9 ± 6.1	0.1
Triglycerides (mg/dl)	250 ± 6.4	249.5 ± 7.3	0.3
VLDL (mg/dl)	50 ± 1.3	49.9 ± 1.5	0.3
LDL (mg/dl)	141.3 ± 6.8	141.1 ± 6.9	0.7
HDL (mg/dl)	40.3 ± 3.5	39.9 ± 3.6	0.1
Fasting plasma insulin (µU/ml)	33.2 ± 2.3	29.6 ± 5.7	< 0.001

BMI: Body mass index; FBS: Fasting blood sugar; HBA1c: Glycated hemoglobin; VLDL-C: Very low density lipoproteins-Cholesterol; LDL-C: Low density lipoproteins-Cholesterol; HDL-C: High density lipoproteins-Cholesterol

Table 2: FTO gene polymorphism (rs17817449) & Allele frequency among the study groups

rs17817449(G/T)	Obese not T2DM n=400	T2DM obese n=400	Not adjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Co dominant						
GG(Reference)	125	80				
TG	190	200	1.64 (1.16-2.31)	0.005	2.44 (1.62-3.6)	< 0.01
TT	85	120	2.2 (1.48-3.27)	< 0.01	4.61 (2.61-8.12)	< 0.01
Dominant						
TT+TG	275	320	1.58 (1.15-2.19)	0.005	1.88 (1.27-2.78)	< 0.01
Recessive						
GG+TG (Reference)	315	280				
TT	85	120	1.59 (1.15-2.12)	< 0.01	1.89 (1.27-2.28)	< 0.01
Additive						
2(TT)+GT	360	440	1.9 (1.39-2.61)	< 0.01		
Frequency of T allele	180 (45%)	220 (55%)	1.9 (1.35-2.69)	< 0.01		

Table 3: FTO gene polymorphism (rs1588413) & Allele frequency among the study groups

rs1588413 (C/T)	Obese not T2DM n=400	T2DM obese n=400	Not adjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Co dominant						
CC (Reference)	180	135				
TC	175	185	1.41 (1.04-1.91)	< 0.01	4.88 (2.64-9.01)	< 0.01
TT	45	80	2.37 (1.54-3.63)	< 0.01	10.68 (5.04-22.6)	< 0.01
Dominant						
TT+TC	220	265	1.97 (1.32-2.92)	< 0.01	2.18 (1.42-3.37)	< 0.01
Recessive						
CC+TC(Reference)	355	320				
TT	45	80	1.98 (1.32 -2.92)	< 0.01	2.19 (1.42-3.37)	< 0.01
Additive						
2(TT)+CT	265	345	1.62 (1.25-2.11)	< 0.01		

Frequency of T allele	132 (33%)	172 (43%)	1.73 (1.26-2.38)	< 0.01	

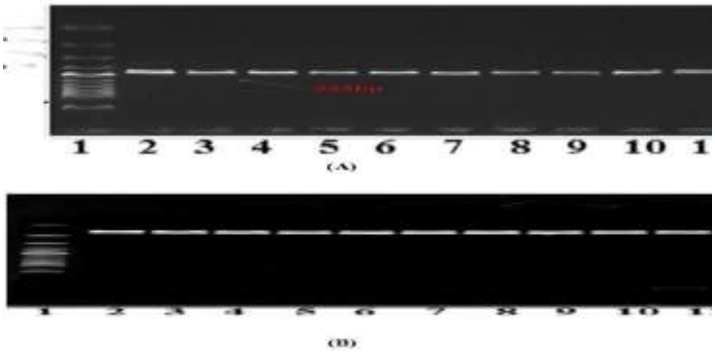

Figure 1: AGE showing the product analysis of A. rsl17817449 & B.rsl588413 SNPs specific primers template DNA with matrix mix

In addition, the FTO gene (rs17817449) possesses significant effects on glycemic markers and serum lipid profile, table (4). However, FTO gene (rs1588413) genotype possessed more significant effects on LDL-C levels.

Table 4: Characteristics of study group 1 according to *FTO* (rs17817449) polymorphism

Parameters	GG (n=80)	TG (n=200)	TT (n=120)	P value
Age (years)	54.27 ± 9.02	54.01± 8.76	54.21± 8.93	0.96
BMI (kg/m^2)	30.15 ± 1.82	32.09 ± 1.84	34.2 ± 1.79	0.000
Cholesterol (mg/dl)	230.77 ± 5.84	232.08 ± 5.49	231.28± 5.47	0.16
Triglycerides (mg/dl)	250.08 ± 6.49	250.06 ± 6.4	249.99 ± 6.48	0.99
VLDL (mg/dl)	50.01 ± 1.29	50.01 ± 1.28	49.99 ± 1.29	0.98
LDL(mg/dl)	138.45 ± 7.6	141.79± 6.29	143.07 ± 7.06	0.000
HDL(mg/dl)	42.3 ± 3.48	40.27± 3.49	38.2 ± 3.49	0.000
Fasting plasma insulin (µU/ml)	31.9 ± 2.27	32.9 ± 2.26	34.76 ± 2.44	0.000
HBA1c (%)	7.67 ± 0.72	7.73± 0.72	7.71± 0.73	0.82
FBS mg/dl	241± 9.65	240.92 ± 5.21	235.45 ± 6.04	0.000

BMI: Body mass index; FBS: Fasting blood sugar; VLDL-C: Very low density lipoproteins-Cholesterol; LDL-C: Low density lipoproteins-Cholesterol; HDL-C: High density lipoproteins-Cholesterol; HBA1c: Glycated hemoglobin.

DISCUSSION

Type 2 diabetes mellitus is a chronic medical condition affecting millions of individuals globally [24]. T2DM is more common on people with obesity [24]. Both conditions have common risk factors such as genetic predisposition [25], and environmental factors [26]. Previous studies looked for the association between weight gain and T2DM [27] and illustrated the role of the FTO protein in regulation of the body weight [28]. Studies published earlier by our team showed the significant association between the FTO (rs9939609 and rs918031) gene polymorphisms in patients with diabetes [29-30]. Statistically, the SNPs represent a significant risk factor for diabetes, however, this is largely dependent on other parameters (like the individual characteristics of the participants in this study) [31]. In the current study, FTOrs17817449 SNP was significantly correlated with obesity and T2DM. The T Allele FTOrs17817449gene may be a good predictor for obesity and diabetes in Iraqi population when compared to the TG and GGAlleles [32-34], table (2). Similarly, the SNP rs1588413 (The Allele T) showed to be another strong predictor for the development of T2DM among the obese Iraqi population, table 3. The FTO rs17817449 and rs1588413 SNPs significantly affects the markers of glycemic control and dyslipidemia. This indicates that the obese Iraqi diabetics who have (TT/TGrs17817449 and TT/TC rs1588413) genotypes are more prone for diabetic complications (both micro & microvascualr), via several pathways, most commonly via the adipocytokines activation causing fatty acid deposition in the liver and skeletal muscles and produce insulin resistance [35].

CONCLUSION

The FTO gene SNPsrs17817449 and rs1588413 can be used as a predictor for the development of diabetes among the obese Iraqi population via its effects on serum lipids, insulin and glucose levels.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest: The authors declare that they have no potential conflict of interest.

AUTHOR'S ROLE

Dr Abdulhussein Algenabi, Dr Majid Hussein and Dr Najah Hadi contributed to the process of ethics approval, recruitment of participants, collection of data, blood sample

analysis and writing the manuscript. Dr Fadhil A. Nasser, Dr Ghizal Fatima, and Dr Hayder A. Al-Aubaidy contributed to data analysis and critical writing and finalizing the manuscript for publication.

REFERENCES

1. Rees SD, Islam M, Hydrie MZ, Chaudhary B, Bellary S, Hashmi S, et al. An FTO variant is associated with Type 2 diabetes in South Asian populations after accounting for body mass index and waist circumference. Diabet Med.2011;28(6): 673-680.
2. Dina C, Meyre D, Gallina S, Durand E, Korner A, Jacobson P, et al. Variation in FTO contributes to childhood obesity and severe adult obesity. Nat Genet.2007;39: 724±726. doi: 10.1038/ng2048 PMID: 17496892
3. Heard-Costa NL, Zillikens MC, Monda KL, Johansson A, Harris TB, Fu M, et al. NRXN3 is anovel locus for waist circumference: a genome-wide association study from the CHARGE Consortium. PLOS Genet.2009;5: e1000539. doi: 10.1371/journal.pgen.1000539 PMID: 19557197
4. Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, et al. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat Genet.2010;42: 937±948. doi: 10.1038/ng.686 PMID: 20935630
5. Hinney A, Nguyen TT, Scherag A, Friedel S, Bronner G, Muller TD, et al. Genome wide association (GWA) study for early onset extreme obesity supports the role of fat mass and obesity associated gene (FTO) variants. PLOS One.2007;2: e1361. doi: 10.1371/journal.pone.0001361 PMID: 18159244
6. Yang J, Loos RJ, Powell JE, Medland SE, Speliotes EK, Chasman DI, et al. FTO genotype is associated with phenotypic variability of body mass index. Nature.2012;490: 267±272. doi: 10.1038/ nature11401 PMID: 22982992
7. Zhang X, Qi Q, Zhang C, Smith SR, Hu FB, Sacks FM, et al. FTO genotype and 2-year change in body composition and fat distribution in response to weight-loss diets: the POUNDS LOST Trial. Diabetes.2012;61: 3005±3011. doi: 10.2337/db11-1799 PMID: 22891219
8. Abdullah A, Peeters A, de Courten M, Stoelwinder J (2010) The magnitude of association between overweight and obesity and the risk of diabetes: a meta-analysis of prospective cohort studies. Diabetes Res Clin Pract.2010;89: 309±319. doi: 10.1016/j.diabres.2010.04.012 PMID: 20493574
9. 9.Yang W, Ji Q, Zhu D, Weng J, Jia W, Ji L, Xiao J, Shan Z, Liu J, Tian H, Ge J, Lin L, Chen L, Guo X, Zhao Z, Li Q, Zhou Z, Shan G, He J; China National Diabetes and Metabolic Disorders Study Group. Prevalence of diabetes among men and women in China. N Engl J Med.2010;362: 1090-1101.
10. Kudabayeva K, Kosmuratova R, Sakhanova S, Bazargaliyev Y. DNA damage and their connection with excessive body mass and obesity. Georgian Med News. 2019;(292-293):49-53.
11. 11.Speakman JR. The 'Fat Mass and Obesity Related' (FTO) gene: Mechanisms of Impact on Obesity and Energy Balance. Curr Obes Rep. 2015;4(1):73-91.
12. Clifton IJ, McDonough MA, Ehrismann D, Kershaw NJ, Granatino N. Structural studies on 2-oxoglutarate oxygenases and related doublestranded betahelix fold proteins. J Inorg Biochem. 2007;100:644–669.
13. Clifton IJ, McDonough MA, Ehrismann D, Kershaw NJ, Granatino N, Schofield CJ. Structural studies on 2-oxoglutarate oxygenases and related doublestranded beta-helix fold proteins. J Inorg Biochem. 2006;100:644–69.
14. Fredriksson, R, Hagglund M, Olszewski PK, Stephansson O, Jacobsson JA, Olszewska AM, Levine AS, Lindblom J, Schioth HB et al. The obesity gene, FTO, is of ancient origin, up-regulated during food deprivation and expressed in neurons of feeding-related nuclei of the brain. Endocrinology. 2008;149(5):2062-71.
15. Naaz K, Kumar A, Choudhury I. Assessment of FTO Gene Polymorphism and its Association with Type 2 Diabetes Mellitus in North Indian Populations. Am J Physiol Regul Integr Comp Physiol. Indian J Clin Biochem. 2019;34(4):479-484.
16. Wahlen K, Sjolin E, Hoffstedt J. The common rs9939609 gene variant of the fat mass- and obesity-associated gene FTO is related to fat cell lipolysis. J Lipid Res. 2008;49(3):607–11.
17. Dina C, Meyre D, Gallina S, Durand E, Körner A, Jacobson P, Carlsson LM, Kiess W, Vatin V, Lecoeur C, Delplanque J, Vaillant E, Pattou F, Ruiz J, Weill J, Levy-Marchal C, Horber F, Potoczna N, Hercberg S, Le Stunff C, Bougnères P, Kovacs P, Marre M, Balkau B, Cauchi S, Chèvre JC, Froguel P. Variation in FTO contributes to childhood obesity and severe adult obesity. Nat Genet. 2007;39:706–707.
18. Andreasen, C.H,. Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body fat accumulation. Diabetes. 2008;57(1):95-101.
19. Wing, M.R. Analysis of FTO gene variants with measures of obesity and glucose homeostasis in the IRAS Family Study. Hum Genet. 2009;125(5-6):615-26.
20. Kurisu S, Ikenaga H, Watanabe N, Higaki T, Shimonaga T, Ishibashi K, Dohi Y, Fukuda Y, Kihara Y: Implications of World Health Organization classification for body mass index on the correlations between common electrocardiographic indexes for left ventricular hypertrophy and left ventricular mass. Clin Exp Hypertens. 2016;38(8):715-720.
21. Yang Jiao, Jingying Zhang 2, Lunjie Lu 2, Jiaying Xu and Liqiang Qin. The FTO Gene Regulates the Proliferation and Differentiation of Pre-Adipocytes in Vitro. Nutrients. 2016;1/13.
22. Haupt, A. Variation in the FTO gene influences food intake but not energy expenditure. Exp Clin Endocrinol Diabetes. 2009;117(4):194-7.
23. Hakanen, M, O. T. Raitakari. FTO genotype is associated with body mass index after the age of seven years but not with energy intake or leisure-time

physical activity. J Clin Endocrinol Metab. 2009;94(4):1281-7.
24. Kaftan AN, Hussain MK. Association of adiponectin gene polymorphism rs266729 with type two diabetes mellitus in Iraqi population. A pilot study. Gene. 2015;570(1):95-9.
25. Ng MCY, Graff M, Lu Y, Justice AE, Mudgal P, Liu CT, et al. Discovery and fine-mapping of adiposity loci using high density imputation of genome-wide association studies in individuals of African ancestry: African Ancestry Anthropometry Genetics Consortium. PLoS genetics. 2017;13(4):e1006719.
26. Go MJ, Lee Y, Park S, Kwak SH, Kim BJ, Lee J. Genetic-risk assessment of GWAS-derived susceptibility loci for type 2 diabetes in a 10 year follow-up of a population-based cohort study. J Hum Genet. 2016;61(12):1009-1012.
27. O'Neil PM, Miller-Kovach K, Tuerk PW, Becker LE, Wadden TA, Fujioka K, et al. Randomized controlled trial of a nationally available weight control program tailored for adults with type 2 diabetes. Obesity (Silver Spring, Md). 2016;24(11):2269-77.
28. Loos RJ, Yeo GS. The bigger picture of FTO: the first GWAS-identified obesity gene. Nature reviews Endocrinology. 2014;10(1):51-61.
29. Nasser FA, Algenabi AA, Hadi NR, Hussein MK, Fatima G, Al-Aubaidy HA. The association of the common fat mass and obesity associated gene polymorphisms with type 2 diabetes in obese Iraqi population. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2019;13(4):2451-5.
30. Younus LA, Algenabi AHA, Abdul-Zhara MS, Hussein MK. FTO gene polymorphisms (rs9939609 and rs17817449) as predictors of Type 2 Diabetes Mellitus in obese Iraqi population. Gene. 2017;627:79-84.
31. Namkung J, Won S. Single Marker Family-Based Association Analysis Not Conditional on Parental Information. Methods Mol Biol. 2017;1666:409-439.
32. Antonio J, Knafo S, Kenyon M, Ali A, Carson C, Ellerbroek A, et al. Assessment of the FTO gene polymorphisms (rs1421085, rs17817449 and rs9939609) in exercise-trained men and women: the effects of a 4-week hypocaloric diet. J Int Soc Sports Nutr. 2019;16(1):36.
33. Vimaleswaran KS, Bodhini D, Lakshmipriya N, Ramya K, Anjana RM, Sudha V, et al. Interaction between FTO gene variants and lifestyle factors on metabolic traits in an Asian Indian population. Nutrition & metabolism. 2016;13:39.
34. Li T, Wu K, You L, Xing X, Wang P, Cui L, Liu H, Cui Y, Bian Y, Ning Y, Zhao H, Tang R, Chen ZJ.Common variant rs9939609 in gene FTO confers risk to polycystic ovary syndrome. BMC medical genetics. PLoS One. 2013;8(7):e66250.
35. Yeo GS. The role of the FTO (Fat Mass and Obesity Related) locus in regulating body size and composition. Mol Cell Endocrinol. 2014;397(1-2):34-41.