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The association between the presence of fat mass and obesity-associated gene (FTO) and the incidence of obesity in Iraqi persons in Holy Kerbala

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ABSTRACT

Obesity is regarded nowadays as one of the worldwide medical and psychosocial problem so that many researches had been dealt with the underlying causes of resistant obesity and increased body mass index "BMI". Recent data demonstrate the strong association between the presence of obesity with specific gene called fat mass and obesity associated gene (FTO). Many single nucleotide polymorphisms (SNPs) was detected that are discovered that closely related to the increased incidence of obesity among populations, notably rs9939609 and rs17817449 SNPs. To evaluate the presence of these SNPs as a predisposing factor in obese persons with BMI exceeds 30. A 100 obese persons were selected randomly along with 50 body-fitted people as a control group. Molecular detection of these SNPs was done by RT-PCR followed by statistical analysis. there was significant increase in expression of AA genotype of FTO rs9939609 SNP in obese persons in comparison to non-obese group (0.60 ± 0.04 versus 0.28 ± 0.06 respectively at $P > 0.002$) and significant increase in expression of GG genotype of FTO rs1781744 SNP in obese persons in comparison to non-obese group 0.59 ± 0.04 versus 0.22 ± 0.05 respectively at $P > 0.001$).

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INTRODUCTION

Obesity is a multifactorial metabolic disorder and considered a worldwide problem because of increased incidence all over the world (Kowalska *et al.*, 2012). It expected that more than 50% of the population could be obese or overweight by 2030 (Kelly *et al.*, 2008). Obesity has many

complications, and it could lead to degenerative illnesses (Fredriksson *et al.*, 2008), e.g., Diabetes type 2, cardiovascular diseases and a few sorts of cancers (Patel *et al.*, 2014). Obesity is more prevalent in the African continent (Van Der Merwe and Pepper, 2006). Increased body mass index (BMI) in obesity is expected to be inherited by 40-70% of all cases (Cheung and Mao, 2012). Genetic factors play an important role in the incidence of diseases, specially obesity, such as the FTO (fat mass and obesity-associated) gene. This gene has different variations when associated with BMI among different individuals (Frayling *et al.*, 2007). FTO RNA transcript levels associated with food intake, suggesting the role of this gene in the focal control of energy homeostasis. Variants in FTO are associated with Diabetes type II (DM II) in white-skinned Europeans (Dina *et al.*, 2007), but there is a difference in the results of studies in Asians due to race (Ng *et al.*, 2008). The FTO gene is situated at locus 16q12.2, and it might be associated with the control of energy

homeostasis, this gene has polymorphisms that are associated with obesity (Larder *et al.*, 2011). The most important variants in the FTO gene associated with the appearance of obesity are polymorphisms rs9939609 and rs17817449 (Dougkas *et al.*, 2013), which were found to be associated with increased BMI (Haupt *et al.*, 2008).

MATERIALS AND METHODS

The study included 100 college students and middle school students in Holy Kerbala who are obese (BMI ranging 30-35) with the age of 15-20. They were selected randomly, and they were compared with 50 persons who have an optimal BMI (18.5-25) as a control group that matched carefully to the testing group to exclude confounding factors like drug intake, past medical history of endocrine disease and mode of lifestyle, then the samples were collected from 1 February to 30 December, Collect 5 mL of venous blood for molecular assays.

BMI DNA was extracted from all samples of blood, Molecular detection of single nucleotide polymorphisms (SNPs) was performed at genetic sites using PCR and PCR-RFLP techniques for two regions in the FTO gene, the primers were used to amplify the variant rs9939609 as follows:

'F': "5'-AACTGGCTCTTGAATGAAATAGGATTCAGA-3", and

'R': "5'-AGAGTAACAGAGACTATCCAAGTGCAGTAC-3"

The program used to amplification this area is one cycle at 95°C for 5 min, 35 cycles at 95°C for 30 s, 53°C for 30 s, 72°C for 45 s, and one final extension cycle at 72°C for 5 min, the PCR products was digested with restriction enzyme (ScaI) (López-Bermejo *et al.*, 2008). Then loaded by electrophoresis on a 3% agarose gel, where the wild-type (TT) genotype (wild type) has the 182 bp band, while heterozygous (AT) genotype has the 182, 154 and 28 bp bands, homozygous (AA) genotype (mutant type) has the 154 and 28 bp bands. The FTO variant rs17817449 was amplified used primer 'F': "5'-AGGACCTCCTATTTGGGACA-3'" and 'R': "5'-AGCTTCCATG GCTA GCATTA-3'" which were designed by (AlwNI) (Do *et al.*, 2008), the PCR was resolved by electrophoresis showing 498 and 330 bp bands represented wild type (TT), and heterozygous (GT) genotype has 828, 498, 330 bp bands, while the 828 bp bands was homozygous (GG) genotype.

Statistical analysis

The results were statistically analyzed using the Special Packages of Social Science (SPSS) V.22 using the means(+) standard error value to measure the

Least Significant Difference (Lsd) at $P \geq 0.01$ and $P \leq 0.05$.

RESULTS AND DISCUSSION

Molecular detection with PCR and PCR- RFLP Techniques

(Figure 1) show the electrophoresis of the FTO rs9939609 amplified by PCR after being carried on a 2% agarose at 70 V for two hours, The columns (1, 2, 3, and 5) indicate the results of FTO rs9939609 (182 bp), while the columns (4,6) did not show any result the 7 columns was referring to Size marker (100-2000 bp).

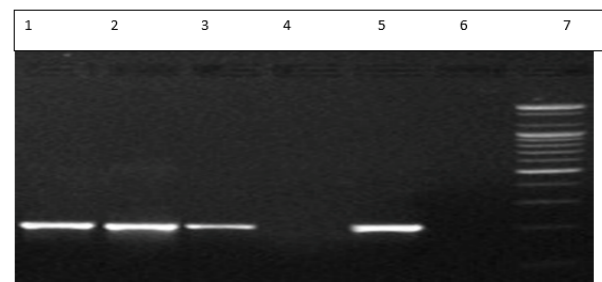


Figure 1: PCR products of FTO rs9939609

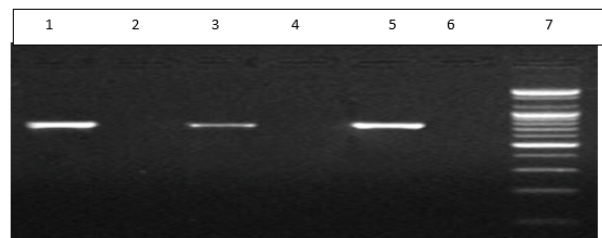


Figure 2: PCR products of FTO rs1781744

(Figure 2) referred to electrophoresis of FTO rs1781744 by PCR after being carried on a 2% agarose at 70 V for two hours, the columns (1,3,5) which represent the results of FTO rs1781744 (828 bp), while the columns (2,4,6) did not show any result, Column 7 referred to Size marker(100-2000 bp).

Figure 3 shows the results of the FTO rs9939609 polymorphism genotyping amplified by RFLP-PCR, where the product is digested by the use restriction enzyme (ScaI). The electrophoresis was done on a 3% agarose at 70 V for two hours. The column (1) indicated to (AA) genotype (154, 28 bp), while columns 2, 5, 7 refers to the genotype (AT) (182,154,28 bp). column(6) refers to the genotype (TT)(182 bp). while column (8)refers to Size marker (25-2000 bp).

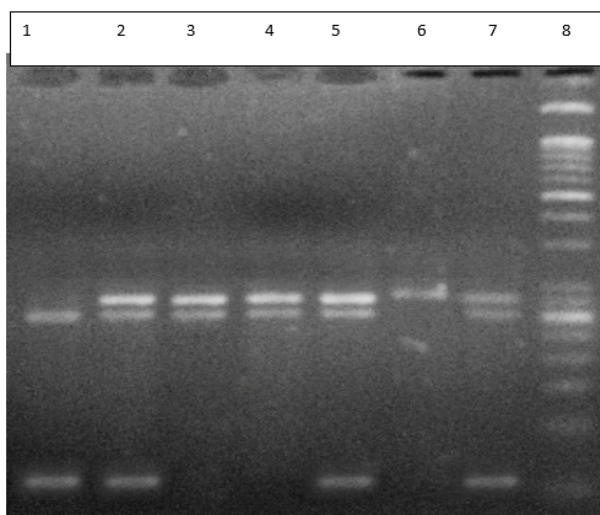
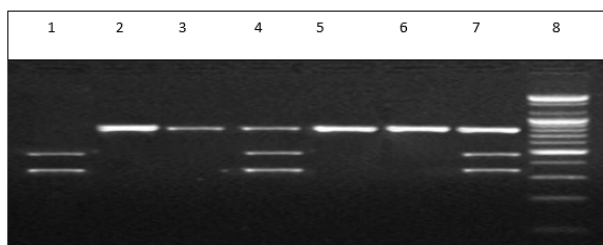
Figure 4 shows the FTO rs1781744 polymorphism genotyping, where the PCR product was digested by

Table 1: Distribution of genotypes of FTO rs9939609 in obese and control groups

TT	AT	AA	FTO rs9939609
0.19 ± 0.03	0.21 ± 0.04	0.60 ± 0.04	Obese persons
0.50 ± 0.07	0.22 ± 0.05	0.28 ± 0.06	Control group
0.1486	0.1412	0.1642	Lsd
0.0001***	0.8	0.0002***	P-value

Table 2: Distribution of FTO rs1781744 genotypes in obese and control groups

TT	GT	GG	FTO rs1781744
0.19 ± 0.03	0.22 ± 0.04	0.59 ± 0.04	Obese persons
0.62 ± 0.06	0.16 ± 0.05	0.22 ± 0.05	Control group
0.1467	0.1375	0.1611	Lsd
0.0001***	0.3	0.0001***	P-value

**Figure 3: FTO rs9939609 polymorphism genotyping after electrophoresis on 3% agarose at 70 V for two hours****Figure 4: FTO rs1781744 polymorphism genotyping after electrophoresis on 3% agarose at 70 V for two hours**

(AlwNI). The first column represents (TT) (498,330 bp). Columns (2, 3, 5, and 6) referred to the genotype (GG) (828 bp). Columns (4, 7) refer to the genotype (GT) (828,498,330 bp). Column (8) refers to the Size marker (100-2000 bp). The products elec-

trophoresed on 3% agarose gel at 70 V for two hours.

The results of the current study were shown in (Table 1), which shows the distribution of samples in obese and control group by genotypes in college students and middle school students in kerbala holy, Iraq, our results showed that FTO rs9939609, the results of our current study showed that there were significant differences between genotypes in obese and control group. There were significant differences in the two models, the (AA and TT) genotypes between the obese and the healthy, While the (AT) genotype showed no significant differences

There were significant (***) differences in the presence of targeted genotypes between obese and control groups for both (AA and TT) genotypes, While the (AT) genotype showed no significant differences. The results of our study in (Table 2) show the distribution of obese and control groups by genotypes in FTO rs1781744, there were significant differences between the obese and the control groups in the two genotypes (GG and TT), while the genotype (GT) did not show any significant difference between the obese and the control group.

There were significant differences in the (GG and TT) genotypes, while the genotype (GT) did not show any significant difference.

Up to now, obesity is regarded as a great challenge facing many peoples all over the world due to the subsequent sequel of this metabolic disorder. (Jonassaint *et al.*, 2011). Not all obese persons have a high food intake behavior suggesting that obesity may be due to genetic factors that prefer a reduced rate of energy expenditure (Church *et al.*, 2010). Many studies deal with genetic predisposition, and a lot of these studies focused on the FTO gene. In a study published by (Abdelmajed

et al., 2017) on Egyptian volunteers, it was found that (AA) genotype of FTO rs9939609 is more predominant in an obese person who are obese at the age of adolescence and younger age group. In this study, the additional result was clarified; (TT) genotype was predominant in non-obese persons suggesting the antagonizing effect of this genotype against obesity. Another point was noticed again regarding FTO rs1781744, the GG genotype of this gene was predominant in obese persons like the mentioned study, but again, the (TT) genotype was predominant in non-obese persons in Iraqi persons. In another study done on Karachi adolescents to estimate the relationship between FTO SNPs and obesity, it was found that from a total 150 samples there was 89 samples (59.3%) were "normal" (TT), whereas 49 samples (32.6%) were "heterozygous" (TA) and 11 (7.3%) were "homozygous" (AA) mutant. These results give a clue that subjects with the AA genotype had a pronounced risk of obesity and increase BMI compared with (TA) or (TT) genotype (Muhammad *et al.*, 2014).

CONCLUSION

These results ultimately counteracting our results in this study, which may be due to either racial variation or to some extent because of variable food stuff that consumed in different countries.

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