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ORIGINAL ARTICLE

Genetic susceptibility for insulin resistance among Egyptian women

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Abstract The fat mass and obesity-associated (*FTO*) gene is recognized as the strongest predictor of obesity related traits such as insulin sensitivity and plasma glucose. The aim of this study was to investigate the association of the *FTO rs17817449* genetic variant (G > T) polymorphism with risk of insulin resistance (IR) among Egyptian women. The variants in FTO rs17817449 were genotyped in 301 Egyptian women comprising two study groups, 150 women with IR and 151 healthy controls. The polymorphism of FTO rs17817449 was tested for association with IR. The frequencies of the FTO genotypes differed significantly between IR patients and healthy controls. Results revealed a significant association of TT genotype (OR, 2.33; 95% CI, 1.38–3.92; p = .001) and T-allele (OR, 1.55; 95% CI, 1.11–1.72; p .007) with IR. BMI, waist circumference, waist to hip and, body fat % were the highest in homozygotes TT genotype and the lowest in GG homozygotes in IR women but not observed in control subjects. Moreover, other abnormal metabolic risk parameters were significantly higher in TT carriers compared to GT and GG carriers in IR group. Association between *FTO* SNP (*rs17817449*) and IR was observed under recessive model.

Conclusion: The present study suggests that *FTO* rs17817449 may have an important role in development of IR in Egyptian women.

1. Introduction

Large energy intake and low physical action are major causes of obesity on a population level, but studies on twins have obviously established that obesity also has an important genetic component of 70% [15]. Several genome wide association studies identified variants in the FTO (fat mass and obesity associated) gene to be associated with body mass index (BMI) [3,2]. Fat mass and obesity-associated *FTO* gene stands out as the most vigorous and significant genetic contributor to polygenic obesity [22,10].

For several decades, obesity and type 2 diabetes mellitus (T2D) have been worldwide issues because of their alarming increased prevalence and their associated morbidity (mainly

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essential hypertension, dyslipidemia, cardiovascular diseases) and mortality. Over the last three decades, the prevalence of overweight and obesity have increased rapidly and the World Health Organization (WHO) estimates that 1.6 billion adults were overweight and 400 million were obese which are predicted to rise.

The association of *FTO* single nucleotide polymorphisms (SNPs) was confirmed with obesity in French Caucasians [20] and with BMI, hip circumference, and weight in Sardinians, European Americans, and Hispanic Americans but not African Americans [20]. The role of *FTO* gene in type 2 diabetes is less clear, as other genome-wide association studies have shown that SNPs in the gene were not associated with type 2 diabetes in French Caucasian subjects [21], Finnish individuals [19], or in a combination of Finnish and Swedish samples [18]. Waist circumference was also strongly associated with *FTO* genotype, as described in other studies [21,19].

The aim of the present was to assess the association of the *FTO* SNP (rs17817449) with measures of obesity and insulin resistance (IR) risk in the Egyptian women.

2. Materials and methods

The variants in FTO SNPs (rs17817449) were genotyped in 301 females comprising two study groups: (1) a case group consisting of 150 women with IR enrolled at the outpatient clinic of Medical services unit of National Research Centre, Egypt. (2) The control group comprising 151 of Egyptian females. Subjects with overweight/obesity or chronic diseases were excluded from the control group. All subjects provided written informed consent. This study protocol was approved by the ethics committee board of the National Research Centre of Egypt (No. 10/223).

2.1. Genotyping

Genomic DNA was extracted from peripheral blood leucocytes from the samples collected for the purpose of this study using salting-out modified method [1]. The SNP variant rs17817449 was genotyped by a PCR–RFLP method. The primers used were as follows (Forward) 5-′AGGACCTCC TATTTGGGACA-3′ and (reverse) 5′AGCTTCCATGGCTA GCATTA-3′ [4].

Each PCR reaction contained 25ul final volumes consisting of the following 250 ng genomic DNA, 200 uMdNTPS, 0.5 unit of DNA polymerase (DyNAZyme II, FINZYMES) and 20 pmol of each primer. The thermocycling conditions consisted of initial denaturation at 94 °C for 5 min followed by 40 cycles of 30 s at 94 °C, 30 s at 57 °C, and for 30 s at 72 °C, followed by final extension step consisted of 15 min at 72 °C. PCR products were digested with AlwNI restriction endonuclease (Fermentas, Germany) overnight at 37 °C, giving 240 bp-bp product whereas the GG was present and two fragments of 216 and 24 bp when T allele was present and 220, 216 bp and 24 bp when the GT was present. The products of the digest were then visualized on a 2.5% agarose gel stained with ethidium bromide. Polymorphisms were confirmed by sequencing analysis and analytical validity was analyzed.

To ensure the accuracy of genotyping, one plate (containing 100 DNA samples) was analyzed twice within one week with 100% conformity.

2.2. Anthropometry and laboratory methods

Anthropometric and body composition measurements were performed with the subject wearing light clothing and without shoes. For all subjects, body weight and height were measured using a scale and a wall-mounted stadiometer to the nearest 0.5 kg and 0.5 cm respectively. Body mass index (BMI) was computed as weight (in kilograms) divided by height (in squared meters). Waist circumference (cm) was measured in the middle between the 12th rib and the iliac crest, and hip circumference (cm) was measured around the buttocks, at the level of the maximum extension. The waist-to-hip ratio was then calculated. Skin fold thickness was measured using Holtain skin fold Caliper, calibrated to 0.2 mm, but the measurement can be conveniently estimated to the last completed 0.1 mm by the same investigator at the following sites: triceps – half-way between the acromion and the olecranon; and subscapular – 1 cm below the inferior angle of the scapula dial of the caliper were illustrated and the sum of skinfold thickness was calculated.

Body fat % was assessed by Tanita Body Composition Analyzer (SC-330).

Blood pressure was measured three times and was averaged for analysis. Fasting plasma glucose and insulin were measured by enzymatic colorimetric methods using a Hitachi auto analyzer 704 (Roche Diagnostics Switzerland).

Insulin resistance was then determined by the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) calculated as the product of the fasting plasma insulin level (μU/mL) and the fasting plasma glucose level (mmol/L), divided by 22.5.

HOMA-IR more than 3.4 was used for diagnosis of insulin resistance in the present study.

2.3. Statistical analyses

All clinical data were expressed as mean ± SD. The distributions of the variables were examined using the Kolmogorov–Smirnov test. Some discreet and continuous variables showed skewed distribution, thus we applied nonparametric tests to compare the differences between groups: Mann–Whitney *U* test. Comparison of means between FTO genotypes was tested. We used least squares means to evaluate potential associations between FTO polymorphisms and obesity measures as continuous traits. Differences in anthropometric and metabolic measures were analyzed with ANOVA.

Genotype and allele distribution was compared between IR and control subjects using $\times 2$ test, the odds ratios (ORs) and 95% confidence intervals (CIs) were also estimated.

Pearson's chi square-test (2 d.f) was applied to test for deviation from Hardy–Weinberg equilibrium in both the case and control groups. Analysis of variance (ANOVA) followed by Fisher's post hoc PSLD test was used in order to examine obesity related phenotypes for differences between genotype groups assuming a recessive model and adjusting for significant covariates.

All P-values reported are from two-sided tests and significance was set at $p < 0.05$.

All statistical analyses were performed applying SPSS 16.0 package for Windows (SPSS Inc., Chicago, IL).

3. Results

The frequencies of the FTO genotypes differed significantly between IR patients and healthy controls. The frequency of the GG, GT, and TT genotypes were 31.13, 49.01, 19.86, in control group and 26, 37.33, and 36.66 in IR group (Table 1). We therefore studied the association of FTO variants with metabolic parameters in IR patients and in controls. The *FTO* genotypic distribution did not differ from the expected values of the Hardy–Weinberg equilibrium. The *FTO rs17817449* showed an association with IR, 36.66% of cases were TT-homozygote as compared to 19.86% of the control subject ($p < .001$). Results revealed a significant association of TT genotype (OR, 2.33; 95% CI, 1.38–3.92; $p = .001$) and T-allele (OR, 1.55; 95% CI, 1.12–2.14; p .007) with IR.

Association of BMI, waist circumference and WHR and skin fold thickness with the FTO rs17817449 genotypes in IR patients and controls are shown in Table 2. The mean BMI for IR cases was 30.3 ± 2.11 (kg/m^2), and ranged 29–33 (kg/m^2), their mean age was 28.8 ± 1.30 years. Control group had normal weight and their BMI was 22.3 ± 3.12. The TT carriers had significantly higher values of BMI, waist circumference, waist to hip ratio and skin folds values compared to GT and GG carriers among IR cases; however this association was not significant in the control group.

Table 3 shows Association of BMI, waist circumference, WHR and body fat% with the FTO rs17817449 genotypes in IR patients and controls. Analysis showed that *TT* carriers had significant higher levels of SBP, DBP, fasting glucose, insulin, HOMA-IR levels and body fat% than *GT* and *GG* genotypes in IR group. However, no significant difference was found in the control group.

4. Discussion

In the present study, we confirmed the association of *FTO rs17817449* with IR and metabolic risk factors among Egyptian women. The FTO gene variants were primarily described to be associated with BMI levels in Caucasian studies, with potentially sex specific effects. It is possible that the part of the FTO risk associated with IR development is caused by elevated BMI, in the current study the mean BMI values of IR patients significantly increased compared to controls. The association between IR and elevated BMI has been observed in this study which is in agreement with several previous studies [3,2,1]. The present study suggests that both obesity and genetic risk factors contribute to IR risk and both are of high importance.

Obesity is a major health problem in recent years, and obesity prevalence increased dramatically over the last decades. Generally, obesity results from the interactions between unhealthy life style (abundant energy intake and low physical activity) and genetic predispositions. Among the newly detected genes, particular interest is focused on the *FTO* gene, the first locus unequivocally associated with BMI values.

The *FTO* gene is one of the few genes to show consistent association with obesity as in Asian [13,16] and African populations [8]. The genome-wide association studies analyzed the variants *rs9939609*, *rs1421085* and *rs17817449*, and alleles A (*rs9939609*), C (*rs1421085*), and *G* (*rs17817449*) found strong association with obesity phenotypes. It has been shown that all three variants are in almost complete linkage disequilibrium. The *FTO* gene codes for a 2-oxoglutarate-dependent-nucleicacid demethylase and is widely expressed in human tissues [7]. Moreover, FTO variants could enhance the risk of

Table 1 Genotype and allele frequency of FTO SNPs (rs17817449) gene polymorphism in controls and IR subjects.

Genotype	Controls ($n = 151$) *n* (%)	IR ($n = 150$) *n* (%)	*p*-Value	OR (95% CI)
GG	47 (31.13)	39 (26%)	Reference	Reference
GT	74 (49.01)	56 (37.33%)	0.22	1.22 (0.76–1.90)
TT	30 (19.86%)	55 (36.66%)	.001	2.33 (1.38–3.92)
Allele				
G	168 (55.62)	134 (44.66%)	Reference	Reference
T	134 (44.37%)	166 (55.33%)	.007	1.55 (1.12–2.14)

Table 2 Mean of BMI, waist circumference, WHR and sum of skin folds by the FTO rs17817449 genotype in IR and controls.

	N	%	Controls ($n = 151$)			
			BMI (kg/m^2)	Waist (cm)	Waist to hip ratio	Sum of skin folds (mm)
GG	47	31.13	20.3 ± 3.11	76.91 ± 5.01	.76 ± .09	27.05 ± 5.07
GT	74	49.01	22.34 ± 4.15	78.3 ± 5.61	.76 ± .15	28.11 ± 3.11
TT	30	19.86	24.34 ± 2.13	78.9 ± 8.13	.77 ± .13	29.51 ± 5.14
			$p = 0.0\ 6$	$p = 0.07$	$p = 0.06$	$p = 0.07$
			IR ($n = 150$)			
GG	39	26	29 ± 3.51	85.91 ± 4.88	.91 ± .08	31.55 ± 4.70
GT	56	37.33	30.3 ± 3.11	86.87 ± 5.01	.94 ± .09	38.55 ± 5.17
TT	55	36.66	33.34 ± 2.10	122.3 ± 4.11	.99 ± .13	43.55 ± 6.44
			$p = 0.0\ 2$	$p = 0.04$	$p = 0.05$	$p = 0.05$

$p \leqslant 0.05$ for ANOVA between genotypes in controls and IR cases.

Table 3 Association of BMI, waist circumference, WHR and body fat% with the FTO rs17817449 genotypes in IR patients and controls.

Variables	IR				Controls			
	GG $n = 39$	GT $n = 56$	TT $n = 55$	Recessive p-value	GG $n = 47$	GT $n = 74$	TT $n = 30$	Recessive p-value
SBP (mmHg)	114.2 ± 5.2	134.4 ± 6.4	154.1 ± 7.7	.05	110.2 ± 5.2	111.4 ± 6.4	112.2 ± 6.7	.06
DBP (mmHg)	80.6 ± 11.7	85.5 ± 9.1	90.4 ± 7.2	.02	79.6 ± 8.7	81.5 ± 9.8	82.4 ± 7.2	.07
Glucose (mg/dl)	82.9 ± 9.8	87.6 ± 9.3	118.6 ± 9.3	.03	80.9 ± 9.8	81.61 ± 9.5	84.6 ± 8.3	.08
Insulin (μU/mL)	10.6 ± 6.9	12.4 ± 5.91	16.5 ± 5.1	.05	9.6 ± 3.9	10.41 ± 5.9	11.5 ± 5.8	.06
HOMA-IR	1.8 ± .1.1	2.4 ± .89	4.4 ± .82	.02	1.4 ± .1.2	2.3 ± .8	2.4 ± .9	.09
Body fat %	25.5 ± 4.7	33.5 ± 6.5	42.5 ± 5.5	03	20.5 ± 6.5	22.3 ± 6.5	23.5 ± 5.4	06

Recessive mode indicates TT vs. GT and GG for rs17817449.
SBP: systolic blood pressure; DBP: diastolic blood pressure; HOMA-IR: homeostasis model assessment insulin resistance; IR: insulin resistance.

IR through another mechanism, namely through its possible effect on DNA methylation (i.e. the epigenetic status of the organism). Animal experiments show clearly that different nutritional and lifestyle factors affect the methylation (epigenetic) status of many genes, thus most likely also genes influencing IR development and methylation therefore play a significant regulatory role in the wide spectrum of human diseases.

Our results confirm previous findings of the association of *FTO* SNP (rs17817449) with several measures of adiposity and metabolic risk parameters. Several publications have confirmed the influence of *FTO* polymorphisms on fat mass in different population samples [19,1]. Variants in the first intron of the *FTO* gene were associated with the BMI in the Western European and North American populations [9], but the Oceanic populations [16], Chinese [11], or African Americans [10] displayed no significant association between the FTO polymorphisms and BMI. SNPs in the FTO gene have recently been associated with obesity-related phenotypes [21,5] and type 2 diabetes [19]. The present study focused on *FTO*SNP (rs17817449) to investigate its association with IR among Egyptian women which was shown to be mediated by its effect on obesity. We analyzed metabolic traits among the IR women and found association between FTO (rs17817449) and obesity parameters as well as metabolic risk parameters, such as systolic and diastolic blood pressure and HOMA-IR.

Previous genetic association-based studies have shown that SNPs in the *FTO* gene are associated with increased body mass index (BMI) [2,20,23] and/or other metabolic-related traits, such as higher fasting insulin [6], glucose, triglycerides, lower HDL cholesterol, waist circumference [4] and weight.

Meta-analysis investigated the associations between five *FTO* polymorphisms (*rs9939609*, *rs1421085*, *rs8050136*, *rs17817449*, and *rs1121980*) and obesity risk in 41,734 cases and 69,837 controls from 59 studies, counting the cases and control subjects from each study only once [17]. They found significant evidence for a modest increase in the risk of obesity associated with the five polymorphisms in various ethnic populations.

Our results confirm previous studies that reported associations between *FTO* polymorphisms and obesity-related measures. The strength of the *FTO* genotype effect on BMI in this study was similar to results of previous reports. Earlier studies in whites found that each risk allele was associated with a BMI increase between 0.4 and 0.7 kg/m^2, and homozygotes for the risk allele were approximately 0.9–1.2 kg/m^2 heavier than those without a risk allele [12].

Egypt and the countries of the Middle East in general, are typical of many middle income developing countries that have experienced a rapid rise in the prevalence of obesity. Obesity in the Eastern Mediterranean Region has reached 'alarming levels' according to the World Health Organization [20]. Martorell et al. [14] reported that women in Egypt have the highest proportion of overweight (31.7%), as well as the highest proportion of obesity (20.1%). *FTO*SNP (rs17817449) the present study detected strong associations between *FTO* variants and measures of obesity in IR patients which is similar to results of previous reports [3]. This is the first study indicating major differences with regard to the effect of this SNP on the prevalence of IR as well as for the associated phenotypes in Egyptian women.

In conclusion, the present study suggests that FTO variants play an important role in the adiposity measures in Egyptian women and have an indicative value for predicting IR development.

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