



FULL LENGTH ARTICLE

Association analysis of FTO gene polymorphisms and obesity risk among Egyptian children and adolescents



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Abstract Obesity is a common disorder that has a significant impact on human health as it may lead to many serious diseases and sometimes morbidity. Previous genome-wide association studies (GWAS) confirmed that there is a relationship between some variants in the first intron of the fat mass and obesity associated (FTO) gene and obesity in adults and children in different ethnic groups. In our study, the association of the FTO rs9939609 and rs17817449 variants with obesity was investigated in Egyptian children and adolescents. We examined rs9939609 and rs17817449 polymorphisms in 100 control and 100 obese cases, we used the restriction fragment length polymorphism (RFLP) technique to genotype the samples. The current study showed that there were no significant differences ($P > 0.05$) between the cases and controls in both variants of rs17817449 and rs9939609 polymorphisms.

However, there were significant correlations between rs17817449 and cholesterol and between rs9939609 and LDL. In Current Study although the two variants (rs9939609 and rs17817449) didn't show an association with obesity, but there was a correlation between the lipid profile and these two variants.

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Introduction

Obesity is a multifactorial disease and considered a global concern due to its increase prevalence globally between adults and children, as it is expected that more than 50% of the worldwide population could be obese or overweight by 2030,¹ Obesity also has a major effect on health, as it could lead to degenerative diseases such as Diabetes type 2 (DT2) fatty liver, cardiovascular diseases and some sorts of cancers.²

Obesity is highly prevalent in Egypt,^{3,4} and as per the WHO statistics the estimated prevalence of overweight and obesity (BMI ≥ 25 kg/m²) is 61–70% of the whole population aged 20 and above, with percentage 65% of males and 76% of females aged 15 and above.⁵

Ellabany have labelled obesity as one of the top contributors to the national mortality of Egyptians along with other non-communicable diseases (NCDs) such as hypertension and diabetes.⁴ Chronic NCDs cause 41% of all mortality in Egypt.⁶

The growing prevalence of obesity and its co-morbidities worldwide in recent decades highlights the need to clarify the factors involved in its development,^{1,7} high energy intake and low physical activity are considered the main reasons for high BMI.

However, Genetics play an important role in the inception of obesity, as many studies have revealed that polymorphisms of some genes are correlated with a predisposition to obesity, yet, these observations have not been replicated in all populations, proposing that different ethnic backgrounds could show the variability observed in association studies.⁷

The FTO (fat mass and obesity associated) gene was first exposed in a genome wide association study (GWAS) for its contribution in early onset of obesity⁸ and also GWAS showed that Different FTO (Fat mass and obesity associated gene) variants have been correlated with Body Mass Index (BMI) in different populations.^{9,10}

Single nucleotide polymorphisms (SNPs) found in the first intron of FTO indicated the strongest association with obesity stated up till now and they have been investigated more than any other common variants related to human obesity.¹¹

The FTO gene is located at locus 16q12.2 and it encodes a demethylation enzyme which cleaves the methyl groups from DNA and RNA nucleotides, and it may be involved in physiological procedures such as the control of energy homeostasis, DNA methylation, and abiogenesis.¹¹

However, its role in the pathophysiology of obesity still under exploration,^{12,13} In murine models, FTO RNA transcript levels correlated with food intake, suggesting the role that this gene might play in the central control of energy homeostasis.

FTO is expressed in the hypothalamus of the brain, which regulates the food intake and energy expenditure,¹⁴ studies of FTO variants with obesity are supported by animal studies, studies in mouse models proposed that the FTO is the gene for obesity, loss of function and/or expression of FTO was correlated with a lean phenotype, whereas over-expression lead to obesity.¹⁵

FTO polymorphisms rs9939609 and rs17817449 are of the most important variants that have been examined by many

genome wide association studies, where alleles A (rs9939609) and G (rs17817449) were strongly associated with obesity phenotype.¹³ Data from studies showed that Single nucleotide polymorphisms (SNPs) of FTO showed a relationship with increased BMI values in different populations such as Europeans.¹⁶

Although these associations have not been confirmed yet in other ethnic groups such as African Americans.¹⁷

Because of these controversies, we investigated the association of the FTO rs9939609 and rs17817449 variants gene polymorphism with obesity and obesity-related parameters in Egyptian children and adolescents.

Materials and methods

Subjects

The study included 200 Egyptian participants (100 obese and 100 control), originally born in Egypt with age range from 2 years to 17 years old (Mean \pm SD = 9.93 \pm 3.06). Cases have been collected from governmental schools and hospitals and a written informed consent was provided by the parents of the participants. Inclusion criteria for patients were obese children and adolescents aged 2–17 years. Obese cases had body mass index greater than 95th percentile for age and gender according to WHO references data. Controls were randomly collected from the general population from the same region of residence and free of any medical complications. An informed written consent was obtained from all participants in accordance with the World Medical Association's Declaration of Helsinki. Medical assessments were performed for participants, including a history of symptoms covering various systems, and a physical examination was performed looking for characteristic abnormalities.

Anthropometric measurements and metabolic parameters

Clinical and biochemical parameters were measured by standard laboratory procedures. Anthropometric variables including height, weight, waist and hip were measured. Body weight was measured with the patients in light clothing and without shoes. Patients' height was measured with them standing leaning their backs against the stadiometer of the scale. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m²) and Z-score values were calculated based on the WHO recommendations. Hip circumference (HC) and waist circumference (WC) were measured in centimeter using a plastic, non-stretchable tailor's tape. Waist circumference (WC) was measured with light clothing at a level midway between the lower rib margin and the iliac crest standing and breathing normally while hip circumference (HC) was measured at the widest circumference over the buttocks. Subsequently, the waist hip ratio (WHR) was calculated by dividing WC by HC.

Anthropometric measurements were obtained according to standardized equipment and following the recommendations of the International Biological Program.¹⁸ Z-score

was calculated according to the WHO references with sex–age-specific cut-points.

Venous blood samples were obtained for serum biochemical analyses total cholesterol [TC], triglycerides [TG], LDL and HDL were collected after overnight fasting. Plasma concentrations of TC and TG were measured by standard enzymatic methods LDL-C was calculated using the Friedewald formula and Fasting blood glucose was measured by the standard glucose oxidase method.

Genotyping

Genomic DNA was extracted from blood using the standard salting out method, Genotyping of both polymorphisms in the FTO gene was carried out using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis which was performed as following, these primers were used to amplify the variant rs9939609 Forward primer: 5'-AACTGGCTCTTGAATGAAATAGGATTCAGA-3' and Reverse primer: 5'-AGAGTAACAGAGACTATCCAAGTGCAGTAC-3', successfully amplified PCR products was digested with restriction enzyme *ScaI*¹⁹ then resolved by electrophoresis on a 2.5% agarose gel, where the T allele produced 182 bp band and the A allele produced 154 bp and 28 bp bands.

It means that the wild-type TT genotype has the 182 bp band, heterozygous TA genotype has the 182, 154 and 28 bp bands, and homozygous mutated AA genotype has the 154 and 28 bp bands. While the FTO variant rs17817449 was amplified with forwarding primer 5'-AGGACCTCTATTTGG-GACA-3' and Reverse primer 5'-AGCTTCCATGGCTAGCATT-3' which were designed by Primer 3 software, and digested with restriction enzyme *AlwNI*,²⁰ and it was resolved by electrophoresis on a 2.5% agarose gel, where the T allele produced 498 bp and 330 bp bands and the G allele produced 828 bp band. Which means that the wild type TT genotype has 498 and 330 bp bands, heterozygous GT genotype has got the 828,498,330 bp bands and the homozygous mutated GG genotype has the 828 bp bands.

Statistical analysis

The statistical analysis of sample data was obtained by using Statistical Package for Social Sciences (SPSS version 16.). Quantitative variables were expressed as mean \pm S.D., and qualitative variables were expressed as percentages. Allele frequencies in patients and controls of FTO rs9939609 and rs17817449 with respect to BMI status were assessed for association by Pearson's Chi-square test. Anthropometric measurements between genotypes and alleles were compared using one way analysis of variance (ANOVA) and student's t test, respectively. P value (<0.05) was considered as statistically significant.

Results

In the current study, we successfully replicated the association of the FTO variants rs9939609 and rs17817449 SNP with the risk of obesity among Egyptian children and adolescents, our results showed that FTO rs9939609 and rs17817449 weren't associated with obesity risk in the Egyptian children subjects in terms of BMI values. However,

we also observed that FTO rs9939609 and rs17817449 had a significant association with some of the obesity-related metabolic traits (see Table 1).

As shown in (Table 2) that the variant rs9939609 showed significant association ($p < 0.05$) with p-value (0.039) with the LDL which is mostly a main consequence of obesity and unhealthy diet, regarding the other variant rs17817449 it showed a correlation with high cholesterol levels in patients as its p-value was 0.030 ($p < 0.05$). In addition, its value with the LDL levels was 0.068 although it's not significant but we can consider that this variant (rs17817449) has an effect on its levels.

Although there was no significant correlation between the two polymorphisms rs9939609 and rs17817449 and BMI but there was and a trend of association between biochemical and anthropometric values and the different genotypes, as shown in (Table 3) in case of rs17817449, total cholesterol (Mean \pm SD) in cases with TT was 27.73 ± 9.45 and was 28.76 ± 5.27 in GT while for the mutant homozygous GG it was 31.33 ± 8.76 we can see that there was an increase in the values. A significant association between was observed with polymorphic allele carriers with higher total cholesterol levels in the G allele carriers as compared to non carriers. Same as rs9939609 regarding LDL-C (Mean \pm SD), in TT cases it was 28.52 ± 5.73 , in AT was 30.02 ± 2.72 and AA was 31.09 ± 9.75 . The increasing levels of LDL-C values showed significant association between higher total LDL-C levels and the A allele. That there is some relation between the variants and obesity related lipid parameters as the wild type has the lowest values of total cholesterol and LDL-C levels as compared to polymorphic allele carriers in both variants, particularly homozygous carriers had the highest values.

Table 1 Frequency distribution of genotypes and alleles between the obese and control subjects.

rs9939609 (T/A)		Groups	
		Patients N = 100 n (%)	Controls N = 100 n (%)
Genotypes	TT	18 (18)	33 (33)
	AT	11 (11)	27 (27)
	AA	71 (71)	40 (40)
Alleles	A	153 (76.5)	93 (46.5)
	T	47 (23.5)	107 (53.5)
rs17817449 (T/G)		Groups	
		Patients N = 100 n (%)	Controls N = 100 n (%)
Genotypes	GG	44 (44)	33 (33)
	GT	35 (35)	50 (50)
	TT	21 (21)	17 (17)
Alleles	G	123 (61.5)	116 (58)
	T	77 (38.5)	84 (42)

In rs9939609 (T/A) AA denotes homozygous carriers of the A allele, AT heterozygous carriers, and TT noncarriers, while in rs17817449 (T/G) GG denotes homozygous carriers of the G allele, GT heterozygous carriers, and TT noncarriers.

Table 2 p-values of biochemical and anthropometric parameters regarding rs9939609 and rs17817449 polymorphisms.

Parameters	p-value	
	rs17817449	rs9939609
BMI	0.632	0.726
Z-Score	0.367	0.766
TG	0.669	0.336
LDL-C	0.068	0.039*
HDL-C	0.55	0.489
Total cholesterol	0.030 [§]	0.172
FBS	0.855	0.751
WC	0.165	0.738
HC	0.134	0.963
WHC	0.107	0.270

*Associations are considered significant when $P < 0.05$, A allele carriers vs. non A allele carriers.

[§]Associations are considered significant when $P < 0.05$, G allele carriers vs. non G allele carriers.

Discussion

Traditional methodologies for managing obesity have not been so efficient for the last decades. Therefore, prevention is the most promising strategy to face the obesity epidemic. Thus, from this perspective, having genetic knowledge for individuals to predict who have a high risk of obesity and obesity associated diseases is the hope to prevent obesity. Since the FTO gene has been the most strongly related gene with obesity so far. Lately, the basic pathophysiology behind how this gene may perform to elevate the risk of being obese became the research scope to clarify it,²¹ as gene identification studies have delivered more broad aspect to understand the biological mechanisms involved in the development of obesity. For instance, the researches in genetics have found that people vary in their perceptions to hunger and satiety based on their genetics and that

predispose some people in different populations to be more susceptible to obesity than others.²²

In Egypt, we have high susceptibility to obesity due to our culture, as per Asfaw,²³ and other studies concerning the Middle East.^{3,4} The estimated prevalence of overweight and obesity ($BMI \geq 25 \text{ kg/m}^2$) is 61–70% of the whole population aged 20 and above.

Many studies have been applied for inspecting different FTO gene variants including rs9939609 and rs17817449 and their correlation with obesity and obesity related parameters in different Ethnic groups some supported our results and showed no correlation and other showed a strong association with obesity. In Egypt, there are no studies that confirmed the association between genetic variants and common obesity in children, which allow us to compare these data with data from other populations.

A prior meta-analysis²⁴ indicated that there is a strong association between the FTO gene and adiposity in childhood and adolescence, this was consistent with the results of a longitudinal study that confirmed a stronger linkage among BMI and the FTO rs9939609 A allele in children aged 11 years.²⁵

From the studies that have validated the association of rs9939609 or rs17817449 are studied on Portuguese children,²⁶ Turkish,²⁷ Dutch,²⁸ French Canadians,²⁰ Germans,¹⁶ Sardinian,²⁹ Salvician Eastern Europe³⁰ and U.K population.⁹

Supporting our results, it was confirmed that there was no significant association between the rs9939609 and rs17817449 and obesity in individuals of different ethnicity, including a study that proved that Mexican children who have variants rs17817449 & rs9939609 showed no association between these SNPs and obesity.³¹

However, studies on Asian populations were contradictory as some proved association,^{32,33} while others not, as six Oceanic populations displayed no significant association between the FTO polymorphisms and BMI, as these variants were in strong linkage disequilibrium.³⁴

Opposing these studies, a study on Indian population found an association between FTO rs9939609 variant with

Table 3 Demographic and metabolic characteristics of study patients.

Parameters	rs17817449			rs9939609		
	TT	GT	GG	TT	TA	AA
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
BMI	27.73 \pm 9.45	28.76 \pm 5.27	31.33 \pm 8.76	28.52 \pm 5.73	30.02 \pm 2.72	31.09 \pm 9.75
Z-Score	2.52 \pm 0.40	2.31 \pm 0.46	2.75 \pm 1.03	2.47 \pm 0.34	2.58 \pm 0.49	2.52 \pm 0.30
TG	82.80 \pm 28.82	83.45 \pm 27.81	93.86 \pm 26.23	82.00 \pm 26.76	101.67 \pm 36.25	82.24 \pm 29.47
LDL-C	85.29 \pm 22.91	83.0 \pm 30.16	109 \pm 10.1	77.33 \pm 24.94	103.50 \pm 34.33	103.50 \pm 34.33
HDL-C	40.36 \pm 7.42	42.09 \pm 12.92	37.00 \pm 7.09	44.44 \pm 11.98	42.83 \pm 10.99	40.70 \pm 7.10
Total cholesterol	135.57 \pm 22.18	140 \pm 28.23	165 \pm 13.46	140.44 \pm 27.59	168.00 \pm 35.99	155.70 \pm 26.97
FBS	89.0 \pm 12.37	88.45 \pm 10.37	86.29 \pm 5.56	87.76 \pm 7.01	87.76 \pm 7.01	87.61 \pm 10.488
WC	81.00 \pm 15.35	84.33 \pm 15.95	90.50 \pm 6.608	86.00 \pm 12.033	92.50 \pm 13.43	97.50 \pm 38.89
HC	85.00 \pm 15.78	95.67 \pm 15.373	110.75 \pm 14.50	97.83 \pm 20.79	103.50 \pm 14.85	99.50 \pm 45.96
WHC	0.824 \pm 0.08	0.87 \pm .0280	0.95 \pm 0.068	0.89 \pm .082	0.89 \pm 0.001	0.99 \pm 0.07

Data presented as mean \pm standard deviation for genotypic classes, GG, GT and TT for rs17817449 and AA, AT and TT for s9939609; FBS, fasting blood sugar; BMI, body mass index; BMI Z-score, body mass index standard deviation score; WC, waist circumference; HC, hip circumference; WHC, waist-to-hip circumference. HDL-C: high-density Lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; BMI: body mass index.

measures of adiposity and metabolic consequences in South Indians with an enhanced effect associated with urban living³⁵ and Omori confirmed its association with Japanese.³⁶ Few studies have been done on the populations of African origin, and the data resulted from those studies have been largely negative, as in the first study which included African Americans by¹⁰ did not find an association of the FTO with obesity in a sample of 1100 African Americans, neither did two other studies that targeted Africans as well.^{37,38} Also another study targeted different African population as his studies of a sub-Saharan African population and Gambians were negative.³⁸ In parallel to these findings a study on South African adolescents showed a very little association between the FTO SNP rs17817449 and BMI.³⁹

Moreover there was no association with rs9939609 and BMI in between a subset of 1100 African–American women.⁴⁰ Although, in a separate family-based study, it was found that there was an association between rs9939609 ($P = 0.01$) with BMI using variance components analysis in a sample of 581 African–American subjects.¹⁷ Consequently, the effect of FTO during growth and development follow a complex trajectory that fluctuates throughout childhood and puberty, peaking in early adulthood.^{41,42}

In conclusion, although our study didn't show an association between the polymorphisms rs9939609 and rs17817449 with obesity, but our study support the idea that those two variants can be a determinant of obesity due to their effect on the lipid profile which is one of the major caused of obesity, and detecting associations between SNPs in FTO and BMI/obesity in children may require larger sample sizes with greater statistical power.

And of course, the differences of ethnicity also play an important role of the genetic component of subjects and their prevalence to obesity. As per prior studies, showed that a prevalence of 35% or less in Caucasian, Asian populations to 50% or more and less strong or no convincing association in African population and obesity which was confirmed by our study on Egyptian population.

Conflicts of interest

No conflict of interests.

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