



DISTRIBUTION OF FTO RS9939609 POLYMORPHISM AND ITS ASSOCIATION WITH OBESITY IN SOUTH INDIAN WOMEN - A PILOT STUDY

Medical Science

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ABSTRACT

Aim: The present study was performed to understand the extent of FTO rs9939609 gene polymorphism and its association with obesity in South Indian Women of TamilNadu

Methods and results : Concentration of glucose ,insulin was determined and Insulin Resistance and BMI was calculated in 24 obese and 30 normal subjects . Genotyping was done by the polymerase chain reaction-restriction fragment length polymorphism method. There were statistically significant differences in the means of BMI , Fasting serum Insulin and HOMA-IR but not in the levels of fasting plasma glucose between both the groups. Interestingly both groups showed only wild type FTO T/T.

Conclusion: The rs9939609 of FTO gene was not found to be associated with obesity. Looking into a multiple SNPs of the FTO gene could provide us with a better insight

KEYWORDS

FTO rs9939609, obesity, Indian Population, BMI

Introduction

Obesity is recognized as a growing epidemic and has serious implications on health. It poses as an increased risk factor for type 2 diabetes, hypertension and cardiovascular disorders and even several types of cancer⁽¹⁾. The distribution of overweight and obesity vary widely among the different population groups across the globe and even between the states of India.

The National Family Health Survey -4 (NFHS-4) showed the prevalence of overweight and obesity (BMI $\geq 25\text{kg/m}^2$) in India to be 20.6. % in women and 18.9 % in men⁽²⁾. This is a gross increase from the NFHS-3 survey which showed overweight and obesity present in only 12.6% and 9.3 % in Indian women and men respectively. The NFHS-4 survey also showed high prevalence of Obesity in the state of Tamilnadu . 30.9% of women and 28.2 % of men belonging to Tamilnadu were found to be obese⁽³⁾. These alarming rates of increase and also the difference in distribution of obesity even amongst Indian population cannot be explained only by lifestyle differences such as dietary habits or physical activity , genetic predisposition also would be a major contributory factor.

Genome-wide association studies of T2DM discovered a novel fat mass and obesity-associated (FTO) gene on chromosome 16q12.2 that predisposes to T2DM through effects on obesity as assessed by body mass index (BMI)⁽⁴⁻⁶⁾. Ever since the discovery of the FTO gene various studies have been performed on many loci in search of variants predisposing mainly to obesity and diabetes making it the most widely studied gene in relation to obesity.

The rs9939609 polymorphism of the FTO gene is frequently reported to be associated with obesity in most of the European population. Individual studies performed in European population showed FTO variants to be associated with diabetes and this was explained entirely by the association with BMI⁽⁷⁻⁹⁾. However inconsistent results were reported when using non - European populations especially Asian population

A study using Chinese subjects showed that FTO variants were not associated with BMI or T2DM⁽¹⁰⁾ whereas, a population study in Japanese subjects showed weak association with BMI⁽¹¹⁾ and also with severe obesity⁽¹²⁾. Another Study performed by Ng et al found association with both T2DM and BMI in east Asians⁽¹³⁾. But this was weaker than the association of BMI in European population .This raises suspicion that FTO gene variants association with BMI might be different among the Asian population itself Similarly, the discrepancies of the association of FTO rs9939609 with obesity between north and south Indian population would seem to be present owing to ancestral differences . Not many studies have been performed on Indian population using FTO rs9939609 especially in the South

Indian population . Studies using North Indian Sikhs have reported a weak association of the FTO gene variant rs9939609 and rs7193144 with obesity as defined by BMI⁽¹⁴⁾. Also a recent study in Tamilnadu where 3 variants of FTO gene were studied showed association with obesity. However the rs9939609 variant was not included in this⁽¹⁶⁾. The present study attempts to understand the extent of FTO rs9939609 gene polymorphism and its association with obesity in South Indian Women of TamilNadu

Research design and methods :

The case – control pilot study was conducted among 54 randomly selected nondiabetic unrelated South Indian women (24 women with obesity [BMI $\geq 25.0\text{ Kg/m}^2$] and 30 controls) with the mean age of 26 . All 54 women were home makers whose daily routine involves household chores with no other extra activities . Since the prevalence of obesity was larger in female population of the state we decided to involve only women into the study

The study was performed after receiving ethical clearance and after getting informed consent from each participant . Height and height, was measured using standardized techniques and BMI was calculated as weight (in kg) divided by the square of height (in m). Overweight and obesity was defined according to the World Health Organization Asia Pacific Guidelines for Asians as non-obese (BMI $<25\text{ kg/m}^2$) and obese (BMI $\geq 25\text{ kg/m}^2$).

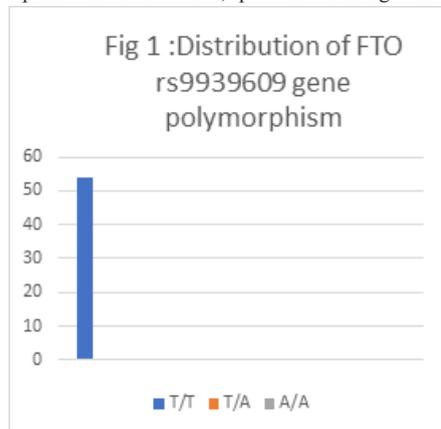
Peripheral venous blood samples were drawn after 8 to 12 hour overnight fast in every subject enrolled in the study. Concentration of plasma glucose was determined by glucose oxidase peroxidase method using Siemens Advia1800 and serum insulin was determined using DRG Insulin ELISA kit .Insulin Resistance was calculated using the Homeostasis Model of Insulin resistance (HOMA-IR) {fasting insulin ($\mu\text{U/ml}$) x fasting glucose (mg/dl)}/405.

About 3 ml of peripheral whole blood was drawn from each subject in a purple top vacutainer containing EDTA as anticoagulant and DNA was isolated from fresh samples using salting out protocol . The rs9939609 of FTO gene was amplified by polymerase chain reaction (PCR) using Eppendorf Mastercycler with the following primers (designed by Bangalore Genei) forward 5' -AACTGGCTCTT GAATGAAATA G G A T T C A G A - 3 ' and reverse 5 ' - AGAGTAACAGAGACTATCCAAGTGCAGTAC-3'⁽¹⁶⁾. Amplified products were digested overnight at 37 °C with ScaI restriction enzyme to obtain fragment lengths of 182 bp (TT genotype), 154 bp (TA genotype) and 28 bp (AA genotype). Products were visualised using Gel DocIt Imager Statistical Package for Social Sciences (SPSS) version 19 by IBM was used for statistical analysis. Comparison of means between both groups were analysed by Mann Whitney U test. Allele frequencies were estimated by gene counting. A p value of <0.05 was considered as statistically significant

Results:**Table 1: distribution of variables among the study population**

| Variabes | Control | Case | p-Value |
|------------------------|--------------|--------------|---------|
| Age | 25.10 ± 2.83 | 27.13 ± 3.89 | 0.036 |
| BMI | 21.64 ± 2.11 | 28.85 ± 3.64 | <0.001 |
| Fasting plasma glucose | 82.67 ± 8.02 | 86.50 ± 7.77 | 0.219 |
| Insulin | 10.95 ± 5.29 | 16.80 ± 7.71 | 0.003 |
| HOMA -IR | 2.27 ± 1.19 | 3.66 ± 1.85 | 0.004 |

Data are represented as mean ± SD;*p-Value = 0.05 significant.



The mean age of control group was 25.10 ± 2.83 whereas the mean age of those in the case group 27.13 ± 3.89. On comparing the mean of different variables between both the groups, we found a statistically significant difference in the means of BMI, Fasting serum Insulin and HOMA-IR^(table1). There was no significant difference in the levels of fasting plasma glucose.

Interestingly the study population showed similar distribution in the FTO rs9939609 polymorphism though they were statistically different in distribution according to BMI. None of the subjects were polymorphic and all of them showed only wild type FTO T/T^(fig1) genotype irrespective of BMI or insulin resistance.

Discussion :

The human FTO gene was first discovered by Genome Wide Association studies (GWAS) while searching for a gene variation implicated in obesity development. Multiple SNPs in the FTO gene have been associated with obesity and diabetes. However, the polymorphic sites were not consistent in different nationalities and ethnic groups.

The FTO mRNA is expressed mainly in the hypothalamus and thus linked to obesity as it controls eating habits and energy balance^(17,18). FTO gene polymorphisms are now among the strongest known genetic risk factors for obesity. The first report linking FTO gene and obesity came from a study linking FTO variants with type 2 diabetes in a European population⁽⁴⁾. The association between FTO SNPs and diabetes disappeared after adjustment for BMI⁽⁴⁾ suggesting that these SNPs were actually associated with BMI and increased weight and thus to obesity.

Following this many studies were done in different populations and ethnic groups to study the pattern of SNPs and its association with obesity. To the best of our knowledge this is the first study performed in South Indian Women subjects who were obese but not diabetic. Both fasting serum Insulin and insulin resistance (calculated by HOMA-IR) was statistically significant in the case group. This is of no surprise as insulin hypersecretion and insulin resistance is a key feature of obesity⁽¹⁹⁾ as seen in the case group (BMI ≥ 25). This increase in insulin could be the reason that the obese and overweight subjects had normal fasting glucose which was not significantly different from the control group. Interestingly, both the groups did not show any polymorphism in the FTO rs9939609.

According to Cheung et al The FTO SNP rs9939609 has the strongest known effect on increased BMI. It was stated that individuals homozygous for the A "risk" allele weigh approximately 3 kg more and have a 1.67-fold increased risk of developing obesity whereas,

heterozygous carriers display intermediate weight gain of 1.5 kg after confirming in studies replicated in both children and adults of multiple ethnic groups⁽²⁰⁾. This finding is contradictory to our study as even those in the obese and overweight category did not show either heterozygous or homozygous A risk allele.

A study performed by Munoz-Yanez C et al in Turkish population explained that rs9939609 influence BMI through various factors, such as dietary behaviour, physical activity, food intake, and mental health⁽²¹⁾. This contradiction in the present study could be due to the fact that both the groups were matched to physical activity and diet. But taking into consideration the effect of ethnicity and the variations of FTO that were found in different populations we started looking into studies that were performed using FTO variants in the Indian population.

S.S. Ningombam et al conducted a study in two tribal populations in India showed association with obesity and the A allele of FTO⁽²²⁾. Although a correlation was made, the percentage of A allele was found to be lesser than the European population. The study concluded that the difference could be due to migration of tribes and different genetic diversity.

Another study performed in south India, Chennai⁽¹⁵⁾ showed good associations between FTO gene and development of Diabetes and obesity. However, this study was done using multiple FTO SNPs (rs9940128, rs7193144, and rs8050136, rs918031, rs1588413, rs11076023). What was to be noted was that the rs9939609 of the present study was not analysed.

In conclusion the rs9939609 of FTO gene was not found to be associated with obesity in South Indian Women Population. This is not a feasible study to perform on a large scale as other FTO variants seem to be more strongly associated with obesity. The main drawback of this study would be the small sample size which was chosen as this was a pilot study. Also, looking into a multiple SNPs of the FTO gene could have provided us with a better insight.

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