Association of rs9939609 FTO gene variant with obesity among Karachi adolescent

Muhammad Haris Lucky, Saeeda Baig, Zil-e-Rubab, Hassan Danish, Farah Ahmed

Abstract

Obesity results due to the interaction of inherited susceptibility and environmental exposures. Genetic susceptibility plays a critical part in increasing the risk of obesity. It is clear that genetic variations in the fat mass and obesity-associated (FTO) gene affect body mass index and the risk of obesity. The objective of this study was to find out the association of FTO gene rs9939609 polymorphism with BMI status in Karachi adolescents. One hundred and fifty subjects 18-35 years old were recruited from different location of Karachi between February to September 2014. Anthropometric measurements were taken from subjects and categorized into normal, overweight and obese based on the BMI Scale. Oral rinse samples were collected and genomic DNA was extracted from PureLink® Genomic DNA kits (Lifetechnologies, USA). PCR was performed using rs9939609 FTO gene forward and reverse primers. PCR-RFLP was then performed by using restriction enzyme ScaI (Thermo Scientific, Fermentas USA). Out of 150 samples 89 (59.3%) were normal (TT), whereas, 49 (32.6%) were heterozygous (AT) and 11 (7.3%) were homozygous (AA) mutant. The result showed that subjects with the AA genotype had increased risk of obesity compared with TA or TT genotype. As BMI increased it was observed that the number of participants with AA gene escalated. Obese adults are largely unsuccessful in long-term weight reduction; thus, early prevention of obesity reduction. This study confirmed the role of rs9939609 FTO gene polymorphism with risk of obesity among Karachi adolescent.

Keywords: BMI, FTO gene, Oral Rinse, SNP and PCR-RFLP

1. Introduction

Overweight and obesity, defined as body mass index (BMI) >25 and >30, respectively is associated with many chronic diseases and cancers. Mainly habits like food preferences and sedentary lifestyle set early in life are the foundations of the physique outcome of an individual in later years. Obesity is a major international public health threat causing economic burden due to fastest increase in different diseases in the modern society. It has no distinction of gender, age, socioeconomic strata, or ethnic groups. It was estimated that the number of overweight adults has reached more than 1.1 billion worldwide [2-3]. It was first assumed that the rapid increase in the prevalence of obesity is largely due to social, environmental and behavioral changes rather than changes in hereditary [4-5]. Although environmental factors have driven the recent rise in the number of people who are overweight or obese, a genetic factor is estimated to account for 40-90% of the population difference in BMI [6-7]. According to Neel et al in 1962 proposed the thrifty gene hypothesis suggesting that populations whose inherited environments were characterized by periods of feast and famine, experienced positive selection for thrifty alleles that support the storage of fat and energy [8]. But in genome wide association studies, a number of common genetic variants linked to body mass index (BMI) and obesity have been identified [9]. The strongest association has been found for a single nucleotide polymorphism (SNP) rs9939609 in the FTO gene (fat mass and obesity-associated gene), located on chromosome 16 [10]. The study of this association in different populations has shown diversified results [11-13]. Several large studies have verified that the A-allele of rs9939609 associates with higher weight and BMI [14-15]. In human studies, FTO genotypes influence appetite regulation and food intake, especially in children and adolescents [16-18].
Amongst the several single nucleotide polymorphisms (SNPs) in this gene, T-to-A change in intron 1 (rs9939609) is the most widely explored. Subsequently, the association with BMI and obesity was unequivocally replicated in European, American, Hispanic, Chinese population in both children and adults. The objective of this study was to perform genotyping of the FTO rs9939609 SNP among subjects from Karachi, Pakistan, to determine the prevalence of the variant genotypes and alleles and to investigate if there is any association between this SNP with obesity and the related anthropometric measurements.

2. Materials and Methods

2.1 Subject Recruitment and Anthropometric Measurements

One hundred and fifty subjects 18-35 years old were recruited from different location of Karachi between February to September 2014. The subjects were then categorized into normal, overweight and obese according to the BMI scale. This study protocol was approved by the Ethics Review Committee of Ziauddin University Clifton Karachi. All study participants provided written informed consents prior to giving oral rinse samples. Subject’s demographic information including age, gender and ethnicity were obtained. Anthropometric measurements included height, waist circumference (WC) and hip circumference (HC) of subjects were measured and their waist-to-hip ratio (WHR) was calculated by dividing the WC by HC. All anthropometric measurements were taken in accordance with WHO standards. Body mass index (BMI) was obtained by using the weighing scale; height was recorded in meters and weight in kilograms with BMI calculated by using the formula weight/height². Subjects with the BMI cutoff point of ≥ 18.5 to 24.5 kg/m² were considered as normal, whereas with the BMI cutoff point of ≥ 24.5 to 29.9 kg/m² were considered as overweight and with the BMI cutoff point of ≥ of 30 kg/m² or higher were considered as obese.

2.2 Sample Collection and DNA Extraction

Oral rinse was taken according to Lucky MH 24. The subjects, after collection of 40 ml oral rinse, were asked to swipe the bristle on the oral mucosa of cheeks to gather a good number of mucosal cells. Genomic DNA was extracted according to PureLink® Genomic DNA kit (Lifetechnologies, USA).

2.3 PCR

Partial amplification of the FTO gene was conducted using the forward primer 5’-AACTGGCTCTTGAAATAGGATTAGA-3’and reverse primer 5’-AGAGTAACAGAGACTTCCAAGTGCA-3’ and polymerase chain reaction (PCR) conditions as described 25. The PCR reaction was carried out in 50µl volume, containing 25 µl of GoTaq® Green master mix (GoTaq® DNA Polymerase is supplied in 2X Green GoTaq® Reaction Buffer pH 8.5, 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP and 3 mM MgCl2, Promega, USA) 4 µl of 1 µM of each primer (Genelink, USA), 5 µl (100-200 ng) of DNA template and 12 µl of PCR graded water (Promega, USA). After gel electrophoresis 182bp bands were visualized.

2.4 PCR-RFLP

Genotypes for FTO rs9939609 were then determined by restriction enzyme length polymorphism (RFLP) with restriction enzyme ScaI. The 0.2µl of PCR product was digested with 1 µL of Fast Digest ScaI restriction enzyme (Fast Digest Scal Thermo Scientific Ferrmentas, USA) and the 1X FastDigest buffer and incubated at 37°C for 5 minutes. The RFLP products were resolved by performing electrophoresis on 2.5% agarose gel, where the homozygous wild-type TT genotypes has 182bp band only, heterozygous TA genotype has the 182,154 and 28bp bands only, while homozygous mutated AA genotypes has the 154 and 28bp bands.

2.5 Statistical Analysis

The statistical analysis of sample data was obtained by using SPSS version 20.0. The descriptive statistics were used to analyze demographic characteristics of the subjects. Allele frequencies of FTO rs9939609 with respect to BMI status and gender 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.

3. Results

The demographic data for total 150 participants is shown in Table 1. All participants were categorized into three groups according to their BMI as normal, overweight and obese, including 65 (normal), 36 (overweight) and 48 (obese) subjects respectively (Table 2). With relation to BMI the majority of the participants [89 (59.3%)] had the normal WT genotype. The polymorphic genotype AA gene was overall found in 11 (7.3%). As BMI increased it was observed that the number of participants with AA gene escalated.

Table 1: Demographic profile of all subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean and standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>26.32±3.362</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.68±6.689</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.71±13.723</td>
</tr>
<tr>
<td>BMI</td>
<td>27.33±5.084</td>
</tr>
<tr>
<td>Waist (inches)</td>
<td>34.74±4.079</td>
</tr>
<tr>
<td>Hip circumference</td>
<td>38.21±4.159</td>
</tr>
<tr>
<td>Waist hip ratio</td>
<td>0.9057±0.0254</td>
</tr>
</tbody>
</table>

Table 2: Separate analysis of FTO rs9939609 allele frequency distribution according to BMI status.

<table>
<thead>
<tr>
<th>FTO rs9939609 genotypes</th>
<th>According to BMI status</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal weight adolescents (n=65)</td>
<td>Overweight adolescent (n=36)</td>
<td>Obese adolescent (n=48)</td>
</tr>
<tr>
<td>TT</td>
<td>48(73.8%)</td>
<td>19(52.8%)</td>
<td>22(44.9%)</td>
</tr>
<tr>
<td>TA</td>
<td>15(23.8%)</td>
<td>15(41.7%)</td>
<td>19(38.8%)</td>
</tr>
<tr>
<td>AA</td>
<td>02(3.1%)</td>
<td>02(5.6%)</td>
<td>07(14.3%)</td>
</tr>
</tbody>
</table>
European Journal of Biotechnology and Bioscience

FTO is found in the brain, which is a key controller of energy of the current obesity epidemic. The highest expression of obesity. The increase in energy intake is a major determinant behaviors stand out as a clear association of FTO SNPs and diverse food selection among individuals and these dietary effects of genetic features and lifestyle behaviors lead to a humans, obesity is a common complex disease which results from the interaction of gene and environment. The combined effects of genetic features and lifestyle behaviors lead to a diverse food selection among individuals and these dietary behaviors stand out as a clear association of FTO SNPs and obesity. The increase in energy intake is a major determinant of the current obesity epidemic. The highest expression of FTO is found in the brain, which is a key controller of energy balance revealed by recent studies in fetal and adult tissues. Although FTO has emerged as major obesity-related factor in populations of European descent, results in Asian populations to date, is inconclusive. Li H, et al did not find any significant association between FTO polymorphism and obesity in Chinese Han populations. The association between FTO and BMI among Pakistani populations, was first studied in obese females only and the researchers found an association of obesity with FTO variants (rs9939609). Our study was designed for both males and females (age range from 18-35 years) including adolescents and adults. With relation to their BMI the majority of the participants [89 (59.3%)] had the normal TT gene, which included 48 (73.8%) normal individuals, 19 (52.8%) overweight and 22 (44.9%) obese. The 19 overweight and 22 obese had normal genetic makeup, but their lifestyle could be the reason for their being overweight. In a biomedical cohort study in Australia, the cumulative incidence of obesity was found associated with Physical inactivity (RR 1.48, 95% CI 1.14-1.90 according to the South Australian Monitoring and Surveillance System and RR 1.41, 95% CI 1.03-1.93 according to North West Adelaide Health Study). The polymorphic AA genotype was found in 11 (7.3%) individuals out of which 7 were obese (in early 20s) whereas, 2 overweight and 2 normal. The normal individuals, both male teenagers (age 18, 19), though they were categorized as normal according to BMI calculation but they were big for their age and had a tendency to compulsive eating. According to Speakman, et al, Tanofofsky-kraff M, et al and Cecil, et al AA genotype carriers have diminished satiety and are compulsive eaters with frequent loss of control over eating. The two overweight individuals were a male and a female (age 24, 25) with similar habits of overeating and less physical activities. Cecil and colleagues found that the A allele of FTO rs9939609, is not only associated with the excessive food intake and craving for energy-rich, fatty food in subjects carrying this allele compared to those who carry homozygote wild type (TT) gene. This suggests a link to a hyperphagic phenotype which consequently is connected with obesity. Obesity-related traits and their association with SNPs in FTO gene polymorphism and Obesity in both children and adults, has been reported in studies around the world. From a public health perspective, early prevention of childhood overweight and obesity seems to be especially important among children of parents having a high BMI. Obesity should be checked from the childhood which mainly is the time when eating habits are developing. This study confirms that subjects carrying at least one rs9939609 FTO risk allele are heavier having excessive eating habit than subjects with no risk allele. Seven individuals who had polymorphic AA were obese (2 female and 5 male) with an age range of 19 to 29 years having a sedentary lifestyle. The two normal category individuals who had mutant AA polymorphism, both were male with ages 19 and 25 years, had a family history of diabetes, were unmarried and had a routine of work out one hour every day. In overweight category two individuals, one male and one female with ages 18 and 24 had AA polymorphism, were counseled about their tendency towards obesity and to change their lifestyles accordingly. Overall the common FTO SNP rs9939609 was not found significantly associated with the risk of obesity and obesity-related traits among the study subjects. Likewise, few studies failed to associate FTO polymorphism with obesity, suggesting the need for more studies in different populations for better recognition of the role of FTO gene in Obesity. In our population, people are now becoming more aware of this polymorphism and more studies in the near future are anticipated. We also found an association of FTO SNPs with the utilization of a greater proportion of fatty food in their diet. The finding also identified that those subjects with at least one A allele (47 AT heterozygous) are likely to eat more and that was probably the reason 23 (49%) of heterozygous persons were obese, which may lead further to complication. A high-fat diet leads to higher expression of FTO, phosphorylation of FoxO1, and decreased phosphorylation of activated protein kinase AMPK. These results suggest that the interactions between FTO and FoxO1 (forkhead transcription factor O1) are closely related to the pathogenesis of NAFLD Non-alcoholic fatty liver disease. This obesity is found associated with High Blood Pressure when variables like BMI and socioeconomic status were distributed over normal, overweight and obese individuals. Overweight and obese individuals tend to have lower central Systolic Blood Pressure compared to normal or lean people. A strong association between DHEAS levels and obesity...
related metabolic and cardiovascular risk factors has also been found. Obesity induced in rats showed that childhood/adolescent obesity and insulin resistance cause severe deficits on cognitive function in later life through irreversible epigenetic modifications in the brain leading to brain synaptic dysfunction during aging even after the normal metabolic homeostasis is restored. The limitation of this study is that there is the possibility several other genetic variants that have been shown to predispose to obesity. In Greek obese and control subjects FTO polymorphisms were studied along with VDR Taq1 polymorphism was the reason for an elevated BMI of 3 kg/m² per risk allele in 82 obese subjects.

5. Conclusion
From a public health perspective, early prevention of childhood overweight and obesity should be emphasized among children of parents having a high BMI. Obese adults are largely unsuccessful in long-term weight reduction; thus, early prevention of obesity reduction This study confirmed the role of rs9939609 FTO gene polymorphism with risk of obesity among Karachi adolescent.

6. References
34. Kristiansen AL, Bjelland M, Brantsæter AL et al. Tracking of body size from birth to 7 years of age and factors associated with maintenance of a high body size from birth to 7 years of age - the Norwegian Mother and Child Cohort study (MoBa). Public Health Nutr. 2014 Nov 10:1-10