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Research Article

Association of Fat, Mass and Obesity (FTO) Gene Polymorphism with Diabetes and Prediabetes in Pakistani Population

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Abstract

Obesity and Type 2 Diabetes Mellitus (T2DM) have become the most prevalent public health issue around the world. The etiology of obesity and T2DM is multifactorial; with genes like Fat, Mass and Obesity-associated or *FTO* gene add to the complexity of the disease and diagnosis. Pakistan has recently been reported the highest prevalence of T2DM in the world. Since Pakistanis have a unique genetic disposition to T2DM due to the influence of genetic variations like rs9939609 in the *FTO* gene, as it is likely to be quantifiably different against the global genetic backdrop. A total of 300 participants were enrolled in this study, belonging to Dera Ismail Khan and Peshawar districts. Blood samples were collected from the enrolled patients following ethical approval and informed consent. HbA1c was used as a criterion for classifying non-diabetic controls, prediabetic, and diabetic patients. Patients' anthropometric and demographic variables were recorded. Blood samples were subjected to both clinical chemistry and genetic analysis. Biochemically, samples were tested for HbA1c and lipid profile, whereas rs9939609 single nucleotide polymorphism (SNP) was assayed through direct sequencing. Demographic data showed that age and body mass index (BMI) were significantly higher in pre-diabetic and diabetic groups compared to the non-diabetic one. There was also statistically significant difference among the three groups in terms of their lipid profile parameters. Frequency of *FTO*-rs9939609 genotype distribution, however, was not significantly different in the three groups but patients with AA genotypes are 1.34 and 1.59 times more likely to develeop prediabetes and diabetes as compared to patients with TT genotypes. Lack of statistical association with FTO-rs9939609 SNP notwithstanding, our data reinforced the link between dyslipidemia, obesity and glycemic index.

Keywords: Type 2 diabetes mellitus, obesity, HbA1c, lipid profile, *FTO* gene

1. Introduction

Type 2 Diabetes Mellitus (T2DM) is a multifactorial disorder characterized by persistent hyperglycemia due to pancreatic beta cell dysfunction (Reaven et al. 1987). T2DM is a strong predictor of cardiovascular diseases (CVDs), neuropathies, nephropathies, retinopathies and other complex disorders (Faselis et al. 2020). According to a recent meta-analysis, the total prevalence of T2DM and prediabetes in Pakistan was 14.62% and 11.43% respectively (Akhtar et al. 2019). Furthermore,

Pakistan ranked 2nd in T2DM and 9th in obesity out of 21 countries (Adnan and Aasim 2020), an additional burden on already cash-strapped economy of the country. The fundamental drivers of T2DM are grouped into genetic and environmental factors (Huang, Chen, and Wang 2022), where genetics play an important role in the pathogenesis of T2DM (Ali 2013). The *FTO* gene (also called alpha-ketoglutarate dependent dioxygenase) is associated with increased basal metabolic rate (BMR) (Yajnik et al. 2009). The *FTO* gene is located on chromosome 16q12.2. This gene consists of 9 exons, exhibiting several SNPs (Shaikh et al. 2021). Polymorphisms in *FTO* gene have been linked to cancer (Lan et al. 2020), metabolic syndrome (Boiko et al. 2021), obesity (Zhao et al. 2019), and diabetes (Zhao et al. 2019) but its association with obesity and diabetes is distinctly strong in Asian population (Shaikh et al. 2021). The FTO gene is expressed in many tissue but its highest expression levels were found in hypothalamus in brain, where it is associated with body metabolic rate and food intake leading to obesity (Mehrdad et al. 2020), a strong predictor of T2DM that predisposes a person to prediabetes and diabetes (Golay and Ybarra 2005). The Genome wide association studies (GWAS) found the *FTO* to be associated with T2DM by the effect of obesity. However, the results in some other populations regarding this association with T2DM (Chauhan et al. 2011) remain inconclusive. The present study aims to determine the genotype frequency of *FTO*-rs9939609 and its association with prediabetes and diabetes in population of Khyber Pakhtunkhwa Pakistan.

2. Material and Methods

2.1. Study population

A case control study was designed to meet the objective of the study. Total 300 participants (100 healthy controls, 100 pre-diabetic and 100 diabetic) were enrolled in the study. Samples were enrolled in Mufti Mehmood Teaching Hospital, Dera Ismail Khan and PIA dispensary, Mall road, Peshawar. The glycated A1C levels were used for diagnosing non-diabetic (<5.7), prediabetes (5.7-6.4) and diabetic (>6.5) individuals following criteria given by the American Diabetes Association (ElSayed et al. 2023). Patients with other metabolic disorders like chronic liver disease, chronic kidney disease and CVDs were excluded from the study. Furthermore, patients not willing to participate in the study were also excluded.

2.2. Ethical Approval

The study was approved by the Advance Study Research Board (ASRB), Khyber Girls Medical College, and Khyber Medical University Peshawar Pakistan via letter no 3314/PGMED/KGMC. Written informed consent was also obtained from all the participant of the study prior to blood sample collection. 5cc blood sample was extracted from each patients and equally distributed in ethylenediamine tetraacetic acid (EDTA) and gel tube and store at 2-8°C.

2.3. Biochemical Analysis

The blood sample in gel tube was centrifuged at 4000 rpm for 10 minutes to extract the plasma and subsequently sent to the laboratory of Mufti Mehmood Teaching hospital for the determination of HbA1c and lipid profile.

2.4. DNA Extraction

The DNA was extracted using modified salting out procedure (Chacon-Cortes and Griffiths 2014). Briefly, 300µl blood and 600µl cell-lysis buffer (CLB) was added to presterilized 2ml eppendorf tube. The eppendorf tube was vortexed and centrifuged at 7000rpm for 7 minutes. The supernatant was discarded and the pellet was dispensed in 600µl CLB. This step was repeated until a clear white or yellowish white pellet is obtained. To the pellet, 400µl nucleus lysis buffer (NLB), 100µl 5M NaCl, and 600µl prechilled chloroform was added and vortexed until a clear homogenous mixture was obtained. The mixture was centrifuged at 5000rpm for 5 minutes to obtained two separate layers. The

supernatant (400µl) was transferred to a new 1.5ml eppendorf tube followed by addition of 1000µl absolute ethanol. The tube was chilled at -20°C for 10 minutes and then centrifuged at 14000rpm for 5 minutes. The supernatant was discarded and the

Table 1: Demographic and anthropometric data of study population

Characteristics	Non-diabetic controls	Prediabetics	Diabetics	p-value
Total Number	100	100	100	-
Gender (M/F)	63/37	48/52	54/46	-
Age (years)	47.23±8.48	40.19±8.44	56.29±8.66	< 0.001***
BMI*	20.41±4.6	25.7±4.1	30±6	< 0.001***
HbA1c (%)	5.505±0.32	6.22±0.15	9.2±2.2	< 0.001***
Total Cholesterol (mg/dl)	185.64±19.3	185±31	201±26.5	< 0.001***
HDL* (mg/dl)	54±13.5	47±14	57.9±18.1	< 0.001***
LDL* (mg/dl)	60±14.5	90± 31.7	74±18	< 0.001***
TG* (mg/dl)	88±32	128±41	88±34	< 0.001***
VLDL* (mg/dl)	43±22	20.2±7.5	21±16 5	< 0.001***

Legends: BMI-body mass index, HDL-high density lipoprotein, LDL- low density lipoprotein, TG-triglycerides, VLDL-very low density lipoprotein.

tube was placed at 65°C for 5-10 minutes until completely dry. To the dried tubes, 50µl Tris-EDTA (TE) buffer was added, vortexed, and stored at -20°C for further process.

2.5. Genotyping

The *FTO*-rs9939609 was genotyped using Sanger Sequencing method. The sequence were retrieved from online tool primer blast (Ye et al. 2012). Sequencing primers were designed with planks covering the SNP of interest. The forward primer 5' -TGGCTCTTGAATGAAATAGGATTCAGAA-3' and reverse primer 5'-AGCCTCTCTACCATCTTATGTCCAAACA-3' with product size 321bp were used. The amplification PCR reaction was performed in total 20µl including 10µl 2X master mix from thermoFisher with catalog no. K0171, 0.8µl each primer, 2µl DNA template and 5.6µl distill water. The reaction mixture was amplified using automatic thermal cycler with the following conditions; initial denaturation at 95°C for 5 minutes followed by 30 cycles of 94°C for 1 minute, 62°C for 40 seconds, 72°C for 1 min and final extension at 72°C for 7 minutes. The PCR product was run on 1.5% agarose gel stained with ethidium bromide (5µl/ml) at 90mA for 1 hour and visualized under gel documentation system. After confirmation of the specific band size, the cycle sequencing reaction was performed. Briefly, the reaction was performed in total 10 µl volume, with 2 µl BDT v3.1 (Thermofisher Scientifc USA Cat# 01109671), buffer 2 µl, purified PCR product 2 µl, and H_2O 4 ul. After proper mixing, the reaction was run in Proflex thermal cycler with the following conditions; initial denaturation at 96°C for 1 minute, then 25 cycles of 96°C for 10 seconds and 50°C for 30 seconds, followed by one cycle at 60°C for 4 minutes. Afterwards, the cycle sequence product was cleaned up using Bigdye Xterminator™ kit (Thermofisher scientific CA USA), as per manufacturer's protocol, and subsequently run on SeqStudio™ genetic analyzer (Applied Biosystems CA USA) on long run. The output AB1 files were analyzed via Finch TV v1.4.0 FinchTV 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com) to document the genotypes by alighning against the reference.

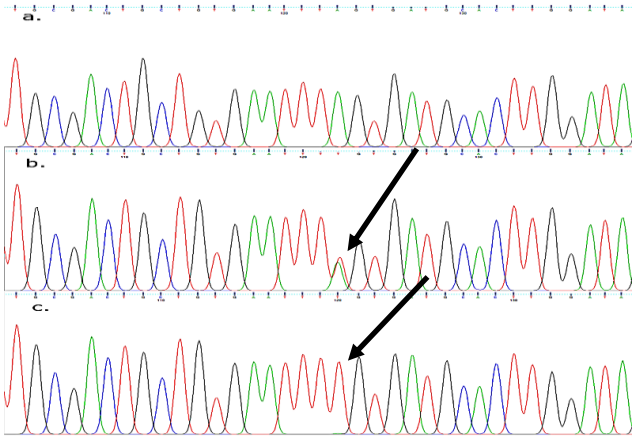

Figure 1: Chromatograms of three DNA samples showing AA homozygous (a), AT heterozygous (b) and TT homozygous (c). Arrow shows the polymorphism site.

2.6. Statistical Analysis

Descriptive statistical model was applied to determine the mean ±SD, frequencies and percentages of study variables. ANOVA was used to differentiate mean differences between groups.

Binary logistic regression was performed to find the possible association between FTO genotypes, prediabetes and diabetes. Odd ratio (OR) with 95% CI was used to determine the risk ratio. All the test values considered were two tailed and p-value <0.05 was considered significant. Graphpad prims version 6 was used to analyze all the data.

3. Results

There were 100 participants in each group. In non-diabetic control group, 63 male while 37 female were included. In prediabetic group, 48 participants were male and 52 were female. In diabetes group the distribution of males and females were 54 and 46 respectively. The mean age of patient in non-diabetic group was 47.23±8.48 years, BMI was 20.41±4.6, HbA1c was 5.505±0.32%, total cholesterol was 185.64±19.3 mg/dl, high density lipoprotein (HDL) was 54±13.5 mg/dl, low density lipoprotein (LDL) was 60±14.5 mg/dl, triglycerides (TG) was 88±32 mg/dl, and very-low density lipoprotein (VLDL) was 43±22 mg/dl. In prediabetics group, the mean age, BMI, HbA1c, total cholesterol, HDL, LDL, TG and VLDL was 40.19±8.44 years, 25.7±4.1, 6.22±0.15%, 185±3 mg/dl, 47±14 mg/dl, 90±31.7 mg/dl, 128±41 mg/dl and 20.2±7.5 mg/dl respectively. All the values are shown in **Table 1** and graphically shown in **Figure** 2. Statistical significant differences were observed between age, BMI, HIbA1c, total cholesterol, HDL, LDL, TG and VLDL with p-values <0.001.

The association of FTO genotypes with prediabetes and diabetes are shown in **Table 2** and **Table 3**. In non-diabetic controls the frequency of TT, AT and AA were 12, 15 and 3 respectively. In pre-diabetic group, TT genotype was found in 14 participants, AT was present 10 participants and AA was in 6 participants. Similarly, in diabetic group, 13 patients had TT genotypes, 12 had AT and 5 had AA genotypes. The chromatogram representing FTO-AA homozygous, AT heterozygous and TT homogyous are shown in **Figure** 1 while the genotype frequencies in all groups are graphically given in **Figure 3.** Binary logistic regression analysis reveals no association between healthy controls, prediabetes and diabtes with p-value >0.05. However, individuals with AA genotypes are 1.34 times and 1.59 times more likely to develop prediabetes and diabetes respectively.

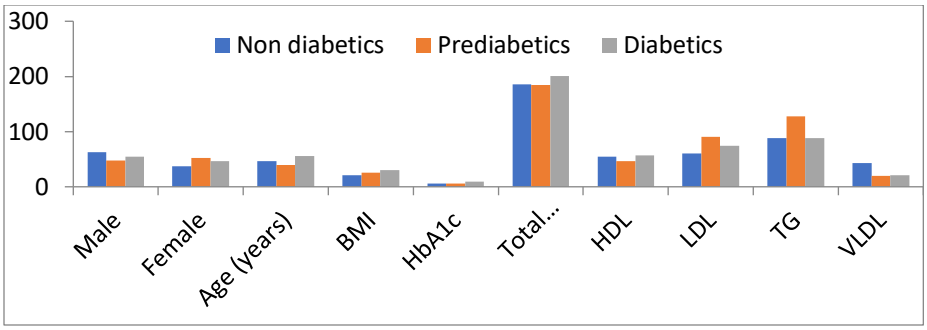

Figure 2: Differences in study variables among non-diabetics, prediabetic, and diabetic participants

Table 2: Association of FTO genotypes with Prediabetes

FTO genotypes	Healthy controls	Prediabetes	p-value	OR (95%CI)
TT	41	42	Reference	-
AT	44	35	0.43	0.77 (0.42-1.41)
AA	9	13	0.63	1.34 (0.53-3.28)

Table 3: Association of FTO genotypes with diabetes

FTO genotypes	Healthy controls	Diabetes	p-value	OR (95%CI)
TT	41	40	Reference	-
AT	44	39	0.99	0.95 (0.52-1.72)
AA	9	14	0.35	1.59 (0.64-3.86)

4. Discussion

T2DM is one of the most prevalent disorders in the world and is considered a global epidemic (Shaw, Sicree, and Zimmet 2010). Up to 95% of patients of diabetes suffer from T2DM (Cheekurhty, Rambabu, and Kumar 2015). This epidemic has gained momentum over the last century and is being labeled as one of the byproducts of the industrial revolution, which has made **'processed'** food more accessible, while the lifestyle has become more sedentary (Hu 2011). T2DM is marked by uncontrolled glucose level in the blood, mainly due to deregulation of insulin release and/or action (Association 2010).

Diabetes itself doesn't cause any serious health risks, however, chronicity and damaging effects of sustained high blood sugar cause severe and often irreparable damage to different organs of the body. Overwhelming rise in T2DM cases may also be attributed to factors like soaring population numbers, increase in life expectancy, urbanization, sedentary lifestyle leading to obesity (Wild et al. 2004). Obesity further aggravates the T2DM-induced complications. Obesity adds to multiple pathologies, which may lead to T2DM and cardiovascular events (Bernal-Lopez et al. 2011). Both the environment and genetics can contribute to obesity much like diabetes. Recently, researches have revealed a close association among genes, diabetes, and obesity (Bersoux et al. 2011, Zhang et al. 2010, Grundy 2012, Tabák et al. 2012) but only a few findings were replicated. In research on the genes discovered till now, the *FTO* gene polymorphism is among the strongest risk factors for developing obesity and diabetes. *FTO* gene was found to have functional relevance in metabolism. SNPs in intron 1 of *FTO* gene have been identified to

be linked with T2DM and obesity (Raji et al. 2001). *FTO* translates to an enzyme, which is responsible to remove methyl group from N6-methyl adenosine, which finally has a post-transcriptional regulatory function (Raji et al. 2001).

Asian people are especially at a higher risk (Gillett 2009). *FTO* was first discovered to be an obesity propensity gene (Frayling et al. 2007) but further researches identified that it was also linked with T2DM via BMI dependent association in Europeans (Scott et al. 2007, Frayling et al. 2007). Different conclusions came out in different studies in Asian population (Hotta et al. 2008, Chang et al. 2008, Horikoshi et al. 2007). A large scale analysis of frequent polymorphisms of *FTO* gene in East Asian population was analyzed to identify its nexus with obesity and T2DM (Liu et al. 2010). Interestingly, a Japanese (Organization 2011) and a Chinese research study failed to find association of *FTO* gene polymorphism with obesity. The environmental risks, body composition, and genetic makeup are different in Asians and Europeans. Prevalence of T2DM is higher in Asians even at lower BMI, may be due to their increased tendency for visceral adipose tissue deposition (Yoon et al. 2006) and their pancreatic B cells could potentially be less functional (Torréns et al. 2004). There are distinct regional influences on disease prevalence and

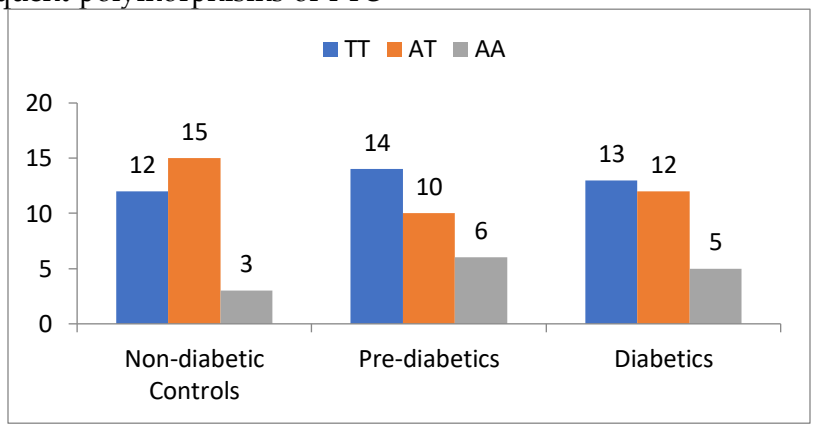

Figure 3: FTO-genotype distribution in different groups.

prognosis; the absence of replication in different studies is challenging to the researchers.

This research study is designed to study the role of *FTO* gene variants in prediabetes and diabetes. No study till date reports any findings regarding the association of FTO gene with prediabetes. Our results fails to find any possible association of FTO gene polymorphism with prediabetes. However, the odds ratio shows that individuals with AA genotypes are 1.34 times more likely to have prediabetes as compared to TT genotypes (OR,95%CI: 1.34, 0.53-3.28). Similarly our findings does not support the role of FTO gene in the development of diabetes (p-value >0.05). Similar study representing 3210 Han Chinese population also proposed no linkage of *FTO* genotypes with diabetes (Li et al. 2008). A research done in America, on 1517 post-menopausal diabetic women also revealed that the polymorphism of *FTO* gene is not associated with diabetes (Song et al. 2008). However, polymorphisms of *FTO* gene may augment future vulnerability to development of diabetes, especially the people with AA genotypes are at 1.59 times higher risk (OR,95%CI: 1.59;0.64-3.86). Consistent with our results, many studies representing different ethnic groups reported that *FTO* gene polymorphism exacerbates the risk for development of T2DM (Bressler et al. 2010, Sanghera et al. 2008).

This study failed to tie *FTO* gene polymorphism with obesity and T2DM. Yet, this study needs to be reconfirmed by replication studies. Diverse

genetic makeup and allele frequencies among Pakistanis may contribute to the difference in our data and earlier findings. Further study is needed to test the claim of polymorphisms of *FTO* gene and consequent obesity and T2DM.

There are some short-comings of the present research study. The outcome of association study greatly depends upon the design of the study, population being studied, and size of the sample. Other cofactors like diet and the lifestyle are also proven to have a modulating effect on the outcome i.e. obesity and T2DM along with *FTO* gene variation. The studies that relate genetic vulnerability to obesity and T2DM may be due to regional environmental factors and lifestyle. There is a need to study more SNP's to ascertain the similar outcome.

5. Conclusions

Our study does not find any possible association of FTO gene polymorphism with prediabetes and diabetes. However, individuals with mutant genotypes AA were 1.74 times and 1.53 times more likely to develop prediabetes and diabetes respectively as compared to individuals with TT genotpes.

Conflict of Interest

The authors declare that they have no competing interests.

Funding

NA.

Study Approval

The study was approved by the Advance Study Research Board (ASRB), Khyber Girls Medical College, and Khyber Medical University Peshawar Pakistan via letter no 3314/PGMED/KGMC.

Consent Forms

Consent forms are available with the authors.

Data Availability

All the raw data related to this study is available with the authors.

Authors Contributions

NM and WI conceptualized and organized the study, AB and IK did the literature search and analysis, MZ did the experiments, MH wrote the initial manuscript, AM wrote the final manuscript and supervised the project.

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