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Association of *FTO* variant with parental history of type 2 diabetes mellitus in adults

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Abstract

Objectives: To find out the association between fat mass and obesity-associated gene polymorphism and risk factors frequently associated with type 2 diabetes mellitus.

Method: The case-control study was conducted January 2020 to March 2021 at the Ziauddin University, Karachi, and comprised deoxyribonucleic acid samples for fat mass and obesity-associated gene polymorphism from non-diabetic Pakistani population. Group A comprised non-diabetics with parental history of type 2 diabetes mellitus and Group B had controls without parental history of type 2 diabetes mellitus. Analysis was based on restriction fragment length polymorphism and polymerase chain reaction. Data was analysed using SPSS 25.

Results: Of the 150 subjects, 75(50%) each were in Group A and Group B. There were 53.3% males and 46.7% females in Group A compared to 46.7% males and 53.3% females in Group B. Overall, 48% subjects were single and 52 % were married. A difference in frequency of fat mass and obesity-associated gene (rs9939609) alleles, such as TT, AA TA, was noted between the groups (p>0.999). TA allele was found to be associated with Group A (33) 44% (p=0.40), while TT allele was associated with Group B (41) 54% (p=0.414). AA allele was equally distributed between the groups (6) 8% (p=1.00).

Conclusion: The TT allele of fat mass and obesity-associated gene was found to be an independent allele associated with the risk of developing type 2 diabetes mellitus.

Key Words: Type 2 diabetes mellitus, FTO gene, BMI, SNP, Multifactorial inheritance, Family history, Genetic variant PCR and RFLP.

Introduction

Diabetes mellitus (DM), a kind of metabolic disorder characterised by high blood sugar level, occurs directly due to insulin resistance, poor secretion of insulin or excessive secretion of glucagon [1]. About one-third of the world's population is obese or overweight and carries the risk of type 2 (T2DM). Safer and more effective therapies are urgently needed to alleviate this pandemic [2]. DM is classified among the utmost serious health issues of the 21st century. It was severely underestimated as a global health threat until the last century. Significant attempts have been made to recognise DM types [3]. Diabetic treatment recommendations include monitoring of diet and risk factors, like glucose, blood pressure and cholesterol, and routine scrutiny of complications. [4] According to the Diabetes Prevalence Survey of Pakistan (DPS-PAK), the incidence of prediabetes is 10.91%, and T2DM 16.98 % wherea, the mean incidence of glycated haemoglobin (HbA1c) is 5.62% and 8.56% among the newly-diagnosed. The incidence is highest at age 51-60 years (26.03%), whereas 35.09% were class 3 obese, and 31.29% had positive family background.[5] In 2017, it was reported that there were 451 million people with DM globally aged 18-99 years. These statistics are likely to grow to 693 million by 2045.The style of living, eating pattern, obesity, physical exercise, genetic factors and smoking are risk factors identified by numerous studies in the development of T2DM patients. [6] Along with many other persuasive evidence, adiposity is one of the most influential risk factors for T2DM/ Therefore, the hallmarks of diabetes prevention are maintaining a stable body weight and preventing excess weight-gain through adulthood. Diet consistency, regardless of body weight, will lead to diabetes prevention. A lower risk of T2DM is related to increased intake of coffee, whole wheat, fruits and nuts, nut greater risk includes excessive intake of refined grains, red and processed meats [7]

The fat mass and obesity-associated (FTO) gene-associated protein or alpha-ketoglutarate-dependent dioxygenase is an enzyme encoded by the FTO gene and is found on chromosome 16 of family AlkB (Alkane hydroxylase gene). Many variants of the FTO gene associated with obesity are known. But amongst them the most common is rs9939609 located in the first intron, which shows strong relation with obese individual. FTO gene has ribonucleic acid (RNA) demethylase that has a part in RNA oxidative demethylation, including the messenger RNAs (mRNAs), transfer RNAs (tRNAs) and small nuclear RNAs (snRNAs), which play a part in fat mass adipogenesis and energy homeostasis regulator, with demethylated n6-methyladenosine RNA still being the most commonly known alteration of mRNA in eukaryotes organism. [8] T2DM has also been correlated with such variants as rs17817449, rs1421085 and rs9939609. [9] [10] In our community, single nucleotide polymorphism (SNP) rs9939609 has been shown to be more prominent.[10]

The current study was planned to find out the association of FTO variant with parental history of T2DM.

Subjects and Methods

The case-control study was conducted January 2020 to March 2021 at the Ziauddin University, Karachi and consecutive sampling technique was used. After approval from the institutional ethics review committee, the sample size was determined using OpenEpi calculator with two-sided confidence interval 95% and power 80% [12]. Those included were non-diabetic adult subjects of either gender from the general population of Ziauddin University. Those with parental history of T2DM were placed in Group A, while those without parental history of T2DM were in Group B. Those with co-morbidities, such as T2DM and T1DM, were excluded. The assessment of T2DM was based on fasting blood glucose (FBG) 126mg/dl or 7.0mmol/L. A standardized questionnaire, including demographics and medical records, was filled by all participants after furnishing informed consent.

Fasting blood 5ml was collected from the subjects, and 3ml was stored in ethylenediaminetetraacetic acid (EDTA) tubes for molecular examination, while 2 ml was obtained in a grey top bottle for FBG level. Following deoxyribonucleic acid (DNA) molecule isolation and FBG evaluation, the samples were stored at -80 degrees C.

Clinical and physiological characteristics, including body mass index (BMI), blood type, race, ethnic background, matrimonial status, diabetes period, were recorded. Using a Glucose-Glucose oxidase-phenol amino phenazone GOD-PAP enzymatic colorimetric system, FBG was evaluated.

DNA was extracted from whole blood by using a DNA isolation kit (GeNet Bio Prime Prep™ Genomics, South Korea), according to the manufacturer's protocol.

Polymerase chain reaction (PCR) genotyping of FTO polymorphism for 187bp DNA was done using the following primers: [13]

Forward: (A>G) 5′-AACTGGCTCTTGAATGAAATAGGATTCAGA-3′

Reverse: 5′-AGAGTAACAGAGACTATCCAAGTGCAGTAC-3′

Protocol for amplification was initial denaturation at 95°C for 5min, followed by 40 denaturation cycles at 95°C for 30sec, annealing at 60°C for 30sec, and extension at 72°C for 40sec.

Restriction fragment length polymorphism (RFLP) was then performed using endonuclease restriction (Thermo Scientific #ER0431) 1UL ScaI to digest the 10µL PCR product. Further, 18ul nuclease-free water and 10x buffer G were combined softly, and TT allele (182bp), AA allele (154bp and 28bp) and TA allele (182bp, 154bp, 28bp) were incubated for 16h at 37°C. By using 1-2gm of agarose as desired in each gel, the material was visualized at voltage of 110 amps for 45min.

Data was analysed using SPSS 25. Data was expressed as mean ± standard deviation, and frequencies and percentages, as appropriate. Data was checked for normality. Hardy-Weinberg equilibrium and chi-square tests were used as the genotype frequency relevance measure for each SNP between the groups. Inter- and intra-group contrast of the genotype between the cases and the controls with age, BMI and FBG was done.

Analysis of variance (ANOVA) was applied as significance measure. Bivariate analysis was carried out for finding the association of genes with the cases and the controls, and was reported as odds ratio (OR) to predict the odds of being a case based on the values of the independent variables. P>0.999 was considered significant at 95% confidence interval (CI).

Results

Of the 150 subjects, 75(50%) each were in Group A and Group B. There were (40) 53.3% males and (35)46.7% females in Group A compared to (35) 46.7% males and (40) 53.3% females in Group B. The overall age range was 18-38 years. Those aged 18-23 years were (24) 32% in Group A and (33) 44% in Group B, in the 24-28 years group, (29)38.7% were in Group A and 26.7% in Group B, in the 29-33 years age group, (10)13.3% were in Group A and (14)18.7% in Group B, while in the 34-38-years group, there were (10)13% in Group A and (8)10.7% in Group B. Overall, 48% subjects were single and (35) 52 % were married.

The association between FTO (rs9939609) and T2DM risk factors were evaluated after controlling for confounders, like age, BMI and FBG, and these factors were not independent risk factors (Table 1).

Genotype distribution between the two groups was noted (Table 2). A difference was noted in the frequency of FTO gene (rs9939609) alleles, such as TT, AA TA, between the groups (p>0.999). TA allele was found to be associated with Group A (p=0.40), while TT allele was associated with Group B (p=0.414). AA allele was equally distributed between the groups (p=1.00).

Discussion

The first common variations reported to be linked with BMI and body fatness were a cluster of SNPs in the first intron of the FTO gene. Figure 1 Researchers discovered that the ‘at risk' alleles of these SNPs were linked to higher food intake and increased

hunger/lowered satiety in people, but were not linked to changed resting energy expenditure or reduced physical activity. [14]

In the current study, the association of FTO (9939609) genetic variants and growth of T2DM was determined specifically for the local population. Yang Y et al. took various SNPs of FTO (rs6639609, rs8050136, rs1421085, rs17817499) gene in different regions around the globe and provided an initial phase in the spatial information on genetic and regional variables in diabetes progression, concluding that rs9939609 was related with T2DM, while SNPs were not associated in South Asia. However, SNPs like rs1421085 and rs17817499 were more common in North America and North Africa[14]. Further work needs to be done so that we may appreciate the effect on T2DM of biology, climate, geography, BMI and fat distribution, and to know how such links can differ. [15] Younus LA et al. conducted a case-control study on 800 Iraqi individuals to discover the link of FTO variant with T2DM. After modification for age, gender and BMI, homozygous TT considerably raised the probability of T2DM by three times with respect to those of wild AA. In the control group, TT allele was prominent, whereas TA genotype was found to be more prominent in the cases (Figure 2).

Sabarneh A et al. found significant association between FTO variant and mean BMI. A trend of increasing mean BMI was observed, with individuals with AA allele having higher BMI compared to those with AT and TT alleles in the cases. This trend was not found among the controls [16].

To the best of our knowledge, the current study was the first to record a relationship between the FTO variant and parental history of T2DM. Despite the growing occurrence of T2DM, there is no resolution between the FTO (rs9939609) variant and the production of T2DM to reach any firm consensus. [17]

Bakhashab S et al. found no association rs9939609 FTO gene with T2DM risk in Saudi Arabia. [18]

Ghafarian-Alipour F et al. reported that the FTO variant rs9926289 had a good correlation with serum apelin and dehydroepiandrosterone-sulfate levels. Moreover,

T2DM was associated with apelin and androgenic hormones. A strong linkage imbalance was observed in rs9939609 and rs9926289 polymorphisms. [19]

The distribution of the FTO (rs9939609) varied between the cases and the controls in the current sample. The TA genotype was found more frequently in patients with a family history of T2DM (Figure 3), while the TT genotype was found more in the controls.

A study conducted on gestational diabetes mellitus (GDM) patients, rs1421085 in FTO allele was not found to be related in Brazilian population [20]. The current study did not included pregnant women, and, as such, the exact association between FTO with GDM was not explored in the present study.

The current found TA genotype in cases as opposed to the controls. In European population, genome-wide interaction studies (GWAS) initially recognised FTO as a predisposition gene for obesity and later as a BMI-based factor for T2DM identifying 32 loci associated with BMI.[21]

Naaz k et al. evaluated a subset of Indian population for rs9940128, and found the frequency of AG allele to be significantly greater in cases compared to controls [20].

In the sense that both the cases and the controls were non-diabetic, the current study was special asit pointed out the factors that increased the chances of T2DM.

A case-control study among Mexican-Mestizo subjects reported genetic model-dependent gender effects on the variants of FTO (rs1121980, rs17817449, rs3751812, rs9930506, and rs17817449) and increased BMI. The risk of obesity in female was greater with rs9930506 FTO. [22]. Latest research on FTO genes rs8050136, rs9939609 and rs1421085 indicates that in the analysed ethnic community, no significant genetic regulator was found in the aetiology of GDM. [23] However, these SNPs have been correlated in GDM subjects with serum adiponectin and tumour necrosis factor (TNF) alpha concentrations which are mediators of insulin. [24]

In terms of limitations, the current study was done at a single centre with a small sample size. As such, the findings are not generalisable. Further studies are required to recognise the FTO gene as a diagnostic and prognostic marker.

Conclusion

The genotypic variants of FTO rs9969309 were significant enough to determine individuals at risk of developing T2DM. Those with genotype TT were at an increased risk of developing T2DM in the future compared to those with genotypes AA and AT.

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Table 1: Analysis of quantitative variables in subjects with fat mass and obesity-associated gene (FTO) polymorphism.

FTO (rs9939609) AGE	TT	AA	TA	P.value
Control	25.80+-4.45	23.50+-1.73	26.23+-4.89	0.531
Cases	26.25+-4.28	26.12+-5.38	26.97+-4.19	
BMI				
Control	24.76+-4.20	75.50+-7.89	23.70+-4.71	0.233
Cases	23.48+-2.73	86.62+-11.85	27.93+-6.32	
FBS				
Control	81.44+-11.42	77.83+-11.09	77.83+-11.09	0.824
Cases	84.84+-11.98	86.62+-11.85	81.08+-11.03	

BMI: Body mass index, FBS: Fasting blood sugar.

Table 2: Genotype distribution of fat mass and obesity-associated gene (FTO) variant (rs9939609) in the cases and the controls.

FTO	**CONTROL n (%)**	**CASE n (%)**	**Odds ratio**	**p-value**	**Confidence/ Interval**	**BMI(Categories) n (%)**
TT	**41(54.7%)**	**36(48.2%)**	**0.76**	**0.4144**	**0.4-1.4**	**i)Underweight 13(8.7%) ii) Normal weight 64 (42.7%)**
AA	**6(8%)**	**6(8%)**	**1.00**	**1.0000**	**0.3-3.2**	**iii)Overweight**

						61(40.7%)
TA	**28(37.3%)**	**33(44%)**	**1.31**	**0.4063**	**0.6-2.5**	**iv)Obese 12 (8.8%)**

BMI: Body mass index.

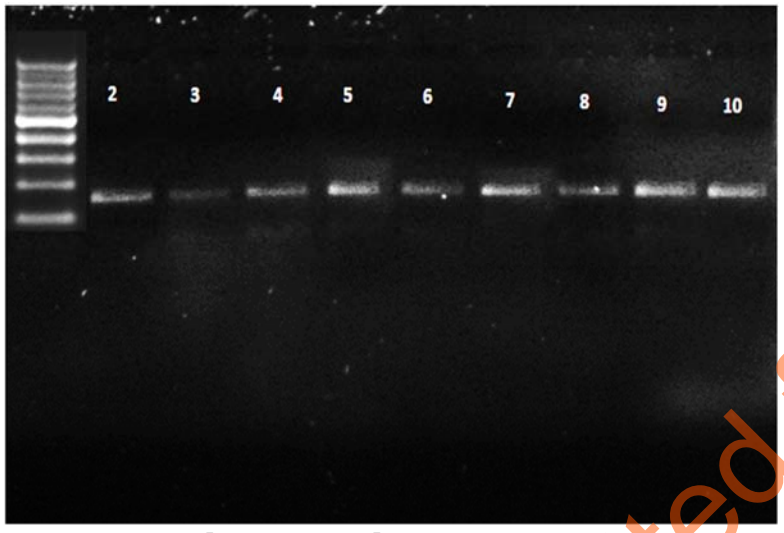

Figure 1: Polymerase chain reaction (PCR). Lane 2-10 shows 182bp band of rs3399609 fat mass and obesity-associated gene (FTO), while Lane 11 (left side) shows 100bp ladder.

Figure 2: Restriction fragment length polymorphism (RFLP) among the controls. Lanes 1, 3, 4, 7, 8, 9, 10 and 11 showing wild type TT genotype (182 bp); lanes 2 and 6 showing a homozygous mutant TA genotype (182bp, 154bp and 28bp); lane 5 showing a heterozygous AA genotype (154bp and 28bp). On the left side is seen a 50bp ladder.

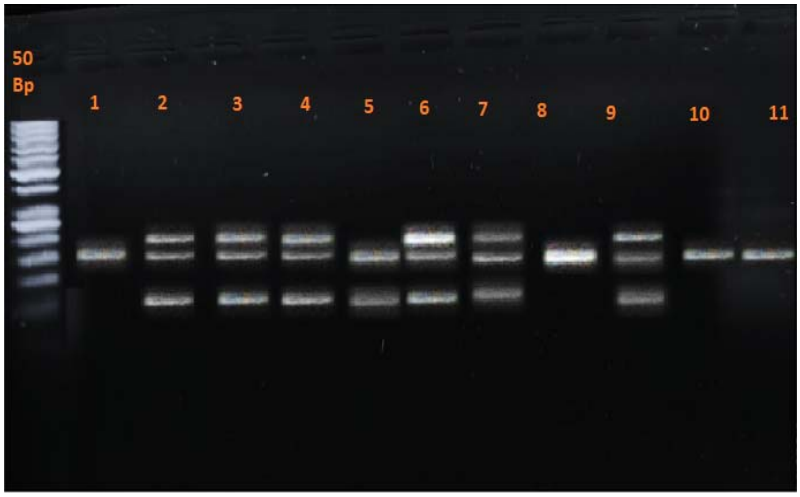

Figure 3: Restriction fragment length polymorphism (RFLP) among the cases. Lanes 1, 8, 10 and 11 showing wild type TT genotype (182bp); lanes 2, 3, 4, 6, 7 and 9 showing a homozygous mutant TA genotype (182bp, 154bp and 28bp); lane 5 showing AA genotype (154bp and 28bp). On the left side is seen a 50bp ladder.