

A study of association between presence or absence of *GSTT1* and *GSTM1* and/or single nucleotide polymorphism in *FABP2* and *GSTP1* with incidence of diabetes type 2: A case-control study

Hira Jamil,¹ Adeela Awan,² Atif Akbar,³ Muhammad Babar,⁴ Sana Akhtar,⁵ Rana Khalid Iqbal,⁶ Furhan Iqbal⁷

Abstract

Objective: To assess the association of single nucleotide polymorphisms in fatty acid binding protein-2 (rs1799883) and glutathione S-transferase pi (rs1695) genes with presence/absence of glutathione S-transferase mu and glutathione S-transferase theta genes in type 2 diabetes.

Methods: The cross-sectional case-control study was conducted at Institute of Molecular Biology and Biotechnology during March till September 2019 and comprised type 2 diabetes patients and non-diabetic controls from two districts in southern Punjab. Polymerase chain reaction, polymerase chain reaction-restriction fragment length polymorphism and tetra-primer amplification refractory mutation system-polymerase chain reaction were applied to investigate glutathione S-transferase theta, mu and pi genes as well as fatty acid binding protein-2, as appropriate. The association of single nucleotide polymorphisms in all genes with the disease were studied either individually or in various combinations. Data was analysed using Minitab 18.

Results: Of the 448 subjects, 248(55.4%) were patients and 200(44.6%) were controls. Overall there were 213(47.5%) males and 235(52.5%) were females, and 141(31.5%) were aged 30-46 years. The presence of rs1799883 in fatty acid binding protein-2 ($p=0.03$) and the absence of glutathione S-transferase mu gene ($p<0.001$) had significant association with type 2 diabetes, while the presence of glutathione S-transferase theta and rs1695 in glutathione S-transferase pi genes were not associated with the disease. Individuals with glutathione S-transferase mu gene null and Ileu/Ileu or Ileu/Val genotype of rs1695 in glutathione S-transferase pi gene have potential to develop type 2 diabetes in their lifetime ($p<0.05$).

Conclusion: The presence of rs1799883 in fatty acid binding protein-2 and the absence of glutathione S-transferase mu gene were found to play significantly in the development of type 2 diabetes.

Keywords: Type 2 diabetes, Single nucleotide polymorphisms, *FABP2*, *GSTM1*, *GSTM1*, *GSTP1*. (JPMA 72: 714; 2022)

DOI: <https://doi.org/10.47391/JPMA.1337>

Introduction

Type 2 diabetes mellitus (T2DM) is the most prevalent type of diabetes mellitus (DM) in which elevated blood glucose (BG) levels are detected due to insulin resistance (IR) and deficiency.¹ It has become a global health issue and it has extended to epidemic proportions in several developing countries.² Both environmental and genetic factors contribute to the development of T2DM.³ In Pakistan, 15% people aged 60-70 years, are suffering from T2DM, while the prevalence is even higher (15-20%) aged >70 years. Globally, Pakistan is ranked 7th regarding T2DM prevalence and there is a trend of increase in morbidity and mortality rates due to this disease that also enhances the economic burden on families having T2DM patients.⁴

There are various genetic and inherited factors that are

.....
^{1,2,4,6}Institute of Molecular Biology and Biotechnology, ³Department of Statistics, ^{5,7}Institute of Pure and Applied Biology, Bahauddin Zakaria University, Multan, Pakistan.

Correspondence: Furhan Iqbal. Email: furhan.iqbal@bzu.edu.pk

involved in the aetiology of T2DM. Family of fatty acid-binding protein (FABP) genes have several members, including fatty acid binding protein 2 (FABP2),⁵ having affinity for saturated and unsaturated fatty acids.⁶ That is why this protein plays a significant role in fatty acid uptake, transport and regulation of associated metabolic processes.⁷ The FABP2 gene is a 15-kDa molecule, composed of 3.4 kilobase (kb) pairs positioned at chromosomal location 4q28-4q31 and consists of three intron and four exon.⁸ It has been reported that G to A single nucleotide polymorphism (SNP) in exon 2 of FABP2 gene in codon 54 leads to threonine (Thr) for alanine (Ala) substitution, and modifies the structure as well as the function of FABP2, and consequently alters the translation product.⁹ Ala54Thr variant in FABP2 gene leads to increased fatty acid uptake in the intestinal lumen as protein with this variant binds to fatty acids with two-fold affinity compared to the wild-type (WT) protein.¹⁰

Glutathione S-Transferase (GST) is isoenzymes phase II family and this family is important to protect cells against oxidative stress (OS). GSTs are composed of seven classes;

omega, mu, alpha, zeta, theta, sigma and pi.¹¹ The GST gene is a 25-kDa molecule. The glutathione S-transferase mu (GSTM1) gene is 3.4kb positioned at 1p13.3. The glutathione S-transferase theta (GSTT1) gene is 18kb positioned at 22q11.2. The glutathione S-transferase pi (GSTP1) gene is 2.8kb positioned at 11q13.¹² GSTs catalyse reduced glutathiones (GSH) conjugation to the numerous electrophilic compounds in the detoxification pathways. The redox form of GSH is extremely essential for numerous biological pathways. Any alterations in metabolism of GSH and GST activity could affect different signalling pathways (SPs) and increase the cells' sensitivity to the OSs, leading to progression of numerous diseases, including DM.¹³

The association of single nucleotide polymorphisms (SNPs) in FABP2¹⁴⁻¹⁷ or GSTs with DM has been reported in different populations,¹⁸⁻²⁰ but no such data has been reported from Multan and Layyah districts in southern Punjab area of Pakistan. The current study was planned to fill the gap by assessing whether or not the presence or absence of GSTM1, GSTT1, rs1799883 in FABP2 and rs1695 in GSTP1 are related to T2DM.

Subjects and Methods

The cross-sectional case-control study was conducted at Institute of Molecular Biology and Biotechnology during March till September 2019 and comprised of clinically-confirmed T2DM patients and healthy controls from two districts in southern Punjab. The cases of either gender were enrolled from various public and private hospitals in Multan and Layyah districts. The controls were age-matched to the cases and were not suffering from T2DM or any systemic illness. All the experimental protocols were approved by the ethical committee of Institute of Molecular Biology and Biotechnology at Bahauddin Zakariya University Multan Pakistan via letter number IMBB/Ethics/106/2019.

We used Solvin's formula to estimate sample size during random sample collection during present study. Solvin's formula was computed as;

$$n = N / (1 + N * e^2)^{21}$$

Whereas: n = no. of samples, N = total population, e = margin of error. This information has been updated in revised manuscript. Following informed consent, all the subjects were interviewed using a questionnaire to collect epidemiological data, including age, gender, marital status, family history, smoking habit, exercise performance and diet preferences. During physical evaluation, height and body-weight of each subject was recorded and body mass index (BMI) was calculated as per

the universal standard. Blood pressure (BP) and waist circumference (WC) were also recorded for each subject. During biochemical evaluation, the blood was sampled from the subjects following overnight fast to evaluate BG levels by using P500 biochemistry analyzer (Diatron, Hungary). Blood samples were also collected after 2 hours of meals to determine random BG (RBG) levels. Glycated haemoglobin (HbA1C) was also measured using A1C Glycohaemoglobin Analysis System (BioHermes, China).

The blood sample (3-5ml) from every participant was collected and stored at -4°C in vials coated with ethylenediaminetetraacetic acid (EDTA) until they were further examined. Deoxyribonucleic acid (DNA) was extracted from whole blood following inorganic DNA extraction protocol.²²

A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach was used to genotype Ala54Thr SNP (rs1799883) in FABP2 gene. A pair of oligonucleotide primers was used for amplification.²³: forward primer: 5'ACAGGTGTTAATATAGTGAAG3'; and reverse primer: 5'TACCCTGAGTTCAGTTCGTC3'. A 20µl master mixture was prepared that consisted of 3µl of extracted DNA, 1µl of DNA polymerase (Thermo Scientific, United States), 2µl of deoxyribonucleotide triphosphate (dNTP) mixture, 1µl of each primer (12 pmol), 3µl of PCR buffer containing ammonium sulphate, 7.8µl double-distilled water, and 1.2µl (25mM) magnesium chloride (MgCl₂).²³

A thermal cycler (Gene Amp® PCR system 2700, Applied Biosystems Inc., United Kingdom) was used to amplify DNA. Thermo-profile consisted of initial denaturation at 95°C for 3min which was followed by 30 cycles of denaturation at 95°C for 30s, annealing for 30s at 55°C, elongation for 45s at 72°C and the final extension was carried out for 3min at 72°C.¹⁷ Restriction of amplified product was carried out by using Hha1 [a restriction enzyme that is isolated from *Haemophilus haemolyticus* bacteria] (New England BioLabs, USA) in a 20µl reaction mixture that was composed of 10µl PCR product, 2µl of Cut smart buffer and 7µl of double-distilled water at 37°C for 1 hour.¹⁷ A 2% agarose gel was used to resolve the restriction products and were observed on an ultraviolet (UV) trans-illuminator (Biostep, Germany).

A multiplex PCR was carried out to document the presence and absence of GSTT1 and GSTM1 in the subjects.¹⁸ Cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1) gene (exon 7) was also amplified as the internal control. The oligo nucleotide primers used for the amplification of these genes were the following:

GSTM1 (Forward 5'-GAACTCCCTGAAAAGCTAAAGC-3'), reverse 5'-GTTGGGCTCAAATATACGGTGG-3'), GSTT1 (forward primer 5'-TTCCTTACTGGTCCTCACATCTC-3', reverse primer 5'-TCACGGGATCATGGCCAGCA-3'), CYP1A1 (forward 5'-GAACTGCCACTTCAGCTGTCT-3', reverse 5'-CAGCTGCATTGGAAGTGCTC-3').¹⁸

A 50µl master mixture was prepared for PCR that contained 5µl template DNA, 5µl PCR buffer, 3.5µl MgCl₂ (25 mM), 2µl of each primer (12 Pm), 2µl of dNTPs (2mM) and 1µl of DNA polymerase (Thermo Scientific, USA). Thermal profile for the amplification of GSTM1, GSTT1 and CYP1A1 was: initial denaturation for 5min at 94°C followed by 35 cycles of the denaturation for 2min at 94°C, annealing for 1min at 59°C and elongation for 1min at 72°C and final extension for 10min at 72°C.

A tetra primer amplification refractory mutation system PCR (T-ARMS-PCR) was conducted to genotype Ileu 105 Val of GSTP1 (rs1695 A/G) in exon 5 of GSTP1.²³ The primers used in this T-ARMS PCR were: outer forward 5' CAGGTGTCAGGTGAGCTCTGAGCAC 3', outer reverse 5' ATAAGGGTGCAGGTTGTGTCTTGTCCCA 3', inner forward 5' CGTGGAGGACCTCCGCTGCAAATCCA 3', inner reverse 5'-GCTCACATAGTTGGTGTAGATGAGGGATAC-3'.²⁴ A reaction mixture of 25µl was prepared comprising 3µl template DNA, 5µl PCR buffer, 2µl MgCl₂ (25mM), 2µl of each primer (12 Pm), 2µl dNTPs (2mM), and 1µl Taq DNA polymerase (Thermo Scientific, USA). Further, rs1695 in GSTP1 was amplified by using thermal conditions, like initial denaturation 5min at 95°C followed by 35 cycles of denaturation for 30s at 95°C, annealing for 30s at 59°C, elongation for 30s at 72°C and the final extension for 10min at 72°C.

Data was analysed using Minitab version 18. Chi-square test was applied to report the correlation between T2DM and the studied demographic, clinical and biochemical factors. Association of alleles with T2DM was estimated by calculating odd ratios (Ors) at 95% confidence interval (CI). Chi-square test was also used to compare the genotype and allelic frequency of SNPs in FABP2 and GSTP1 between the cases and the controls. Genotypic frequencies were determined by direct counting, while online calculator of Hardy Weinberg equilibrium 25 was used for allelic frequency count. Chi-square test was again used to study the correlation of diabetes with SNPs Ala54Thr and Ileu105Val in FABP2 and GSTP1 respectively. P <0.05 was taken as significant.

Results

Of the 448 subjects, 248(55.4%) were patients and 200(44.6%) were controls. Overall there were 213(47.5%) males and 235(52.5) were females, and 141(31.5%) were

aged 30-46 years. PCR amplified a fragment of 180bp from FABP2. Upon restriction with Hha1, homozygous Ala54Ala was presented by bands of 81bp and 99bp, heterozygous Ala54Thr produced bands of 81bp, 99bp and 180bp and homozygous mutant Thr54Thr remained uncut (180bp) (Figure-1 A-B).

Multiplex PCR amplified 480bp, 215bp and 312bp products for GSTT1, GSTM1 and CYP1A1 respectively (Figure 1C). During T-ARMS PCR, outer primers produced an amplicon of 467bp, homozygous WT (Ileu105Ileu)

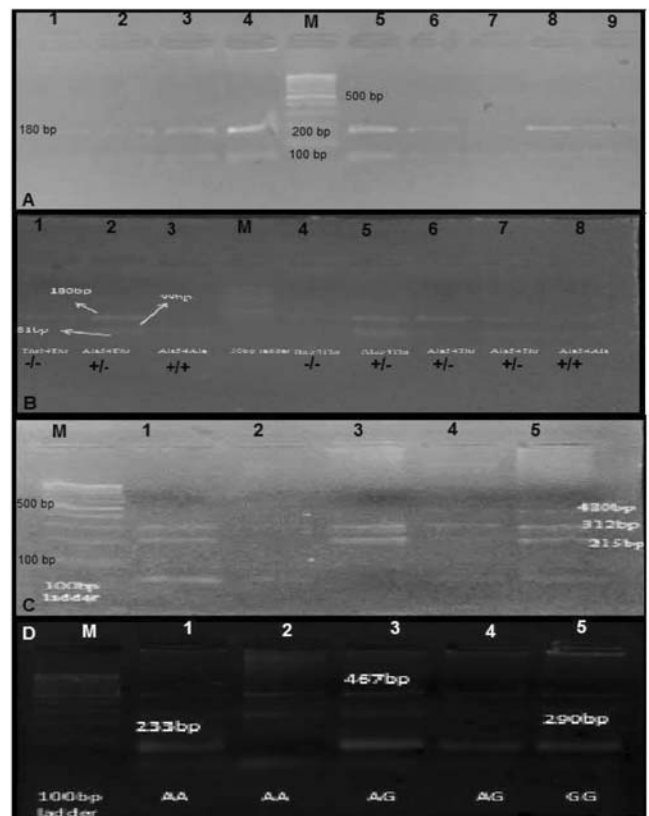


Figure: A. Agarose gel image showing 180bp amplicon from FABP2 (lane 1-9, lane M contain 100 bp DNA ladder). B. PCR-RFLP assay for the detection of genotype at codon 54 of FABP2. Lane 1, 4 homozygous mutant (Thr54Thr), lane 2, 5-7 heterozygous (Ala54Thr) genotype, lane 3 and 8, homozygous wild type (Ala54Ala) genotype, lane M contain 50 bp DNA ladder. C. PCR amplification of GSTM1, GSTT1 and CYP1A1 to report their presence or absence. Lane M 100 bp DNA ladder, lane 2 and 4 present GSTT1 null or GSTM1 present condition, lane 3 presents GSTM1 null or GSTT1 present, lane 5 and 6 represents presence of both GSTM1 and GSTT1. D. T-ARMS PCR assay to report the genotype at codon 105 of GSTP1. Lane 2, 3 homozygous wild (Ileu105Ileu), lane 3, 4 heterozygous (Ileu105Val) genotype, lane 5, homozygous mutant (Val105Val), lane M contain 100 bp DNA ladder.

FABP2: Fatty acid binding protein-2, GSTP1: Glutathione S-transferase pi, GSTM1: Glutathione S-transferase mu, GSTT1: Gglutathione S-transferase theta, PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism, T-ARMS PCR: Tetra-primer amplification refractory mutation system-polymerase chain reaction, DNA: Deoxyribonucleic acid, CYP1A1: Cytochrome P450, family 1, subfamily A, polypeptide 1.

Table-1: Analysis of the studied demographic, clinical and biochemical parameters and their association with the type 2 diabetes mellitus (T2DM).

Characteristics	Category	Case (N = 248)	Control (N = 200)	Odds Ratio 95% CI	P-value
Age (Years)	30-46	66	75	1.041 (1.02, 1.069)	0.002**
	47-56	76	57		
	57-66	61	44		
	Above 66	45	24		
Gender	Male=1	107	106	0.530 (0.29, 0.96)	0.04*
	Female=0	141	94		
Marital status	Single=0	27	23	0.49 (0.21, 1.20)	0.1
	Married=1	221	177		
Smoking	Yes=1	47	25	3.78 (1.73, 8.29)	P < 0.001***
	No=0	201	175		
Family History	Yes=1	146	29	7.91 (4.36, 14.35)	P < 0.001***
	No=0	102	171		
Exercise	Yes=1	139	147	0.51 (0.28, 0.91)	0.002**
	No=0	109	53		
Planned Diet	Yes=1	205	141	2.06 (1.32, 3.22)	P < 0.001**
	No=0	43	59		
Systolic Blood Pressure (mmHg)	120<=Normal 121 > = High	70	80	0.99 (0.97, 1.01)	0.3
	90 < = Low	91	97		
		79	32		
Diastolic Blood Pressure (mmHg)	80<=Normal 81 > = High	79	71	1.03 (0.99, 1.07)	0.1
	60 < = Low	89	98		
		78	39		
BMI (kg/m2)	30>=Obese 25-30= Overweight	88	9	1.28 (1.18, 1.37)	0.002**
	18.5-25 = Normal	88	48		
	18.5< = Underweight	69	141		
		3	1		
Waist diameter (Inches)	30 >= Obese	248	193	1.22 (1.14, 1.29)	0.005**
	18.5-25 = Normal	0	6		
Fasting blood glucose (mg/dL)	80-100 = Normal	1	81	-	P < 0.001**
	126 > = Diabetic	247	0		
Random blood glucose (mg/dL)	170-200= Normal	18	81	-	0.9
	200 > = Diabetic	230	0		
HbA1c	4-5.6% = Normal	4	81	-	0.002**
	5.7> = Diabetes	244	8		

P > 0.05= non-significant; P < 0.05(*) = least significant; P < 0.01(**) = significant; P < 0.001(***) = highly significant. CI: Confidence interval, BMI: Body mass index, HbA1c: Glycated haemoglobin.

Table-2: Genotype and allelic frequency distribution among cases and controls at rs1695 in GSTP1 and rs1799883 in FABP2 genes, and their possible association with type 2 diabetes mellitus (T2DM).

Gene and SNP	Parameters	Allelic Frequency					P-value
FABP2 rs1799883	Nucleotide/amino acid change	GG Ala54Ala	GA Ala54Thr	AA Thr54Thr	Ala	Thr	0.03*
	Controls	57 (28.14%)	96 (48.2%)	47 (23.6%)	0.52	0.47	
	Patients	76 (30.64%)	91 (36.7%)	81 (32.7%)	0.48	0.51	
GSTP1 rs1695	Nucleotide/amino acid change	AA Ileu105Ileu	AG Ileu105Val	GG Val105Val	Ileu	Val	0.07
	Controls	177 (88.5%)	19 (9.5%)	2 (1.0%)	0.952	0.048	
	Patients	218 (87.9%)	35 (14.1%)	1 (0.4%)	0.913	0.087	

P > 0.05= non-significant; P < 0.05(*) = least significant

SNP: Single nucleotide polymorphisms, FABP2: Fatty acid binding protein-2, GSTP1: Glutathione S-transferase pi.

generated 233bp, homozygous mutant (Val105Val) produced 290bp, while all three 467bp, 233bp and 290bp bands were generated by heterozygous (Ileu105 Val) subjects (Figure-1D).

When various risk factors were compared between the controls and T2DM patients, age, gender, smoking, family history, exercise and planned diet were significantly associated with T2DM (p<0.05). Also, BMI, WC, fasting BG

Table-3: Genotype and allelic frequency distribution among cases and controls for individual GSTs and/or their combinations and their possible association with type 2 diabetes mellitus (T2DM).

Genotype	Controls	Patients	Chi-Square	P-value
GSTM1				
Present	115	105	10.545	P < 0.001***
Null	84	143		
GSTT1				
Present	165	195	1.294	0.3
Null	34	53		
GSTP1				
Ileu/Ileu	179	205	1.171	0.3
Ileu/Val	19	37	1.779	0.2
Val/Val	0	3	2.424	0.1
GSTM1 and GSTT1				
Both present	86	80	0.217	0.6
Either Null	102	134	4.339	0.04*
Both Null	7	30	14.297	P < 0.0001***
GSTM1 and GSTP1				
M1(+/-) and P1(Ileu/Ileu)	107	88	1.851	0.2
M1(+/-) and P1(Ileu/Val)	8	12	0.8	0.4
M1(-/-) and P1(Ileu/Ileu)	70	110	10.522	P < 0.001***
M1(-/-) and P1(Ileu/Val)	12	28	6.4	0.01**
GSTT1 and GSTP1				
T1(+/-) and P1(Ileu/Ileu)	150	156	0.118	0.7
T1(+/-) and P1(Ileu/Val)	14	35	9	0.002**
T1(-/-) and P1(Ileu/Ileu)	28	47	4.813	0.03*
T1(-/-) and P1(Ileu/Val)	6	4	0.4	0.5
GSTT1, GSTM1 and GSTP1				
T1andM1(+/-) and P1 (Ileu/Ileu)	81	67	0.658	0.4
T1andM1(+/-) and P1 (Ileu/Val)	4	12	4	0.04*
T1(+/-), M1(-/-) and P1 (Ileu/Ileu)	58	86	5.111	0.01**
T1(+/-), M1(-/-) and P1 (Ileu/Val)	12	22	2.941	0.09
T1(-/-),M1(+/-) and P1 (Ileu/Ileu)	24	21	0.2	0.7
T1(-/-), M1(+/-) and P1 (Ileu/Val)	3	1	1	0.3
T1(-/-), M1(-/-) and P1 (Ileu/Ileu)	3	26	18.241	P < 0.001***
T1(-/-), M1(-/-) and P1 (Ileu/Val)	3	4	0.143	0.7

P > 0.05 = non-significant; P < 0.05 (*) = least significant; P < 0.01 (**) = Significant; P < 0.001 (***) = Highly significant

+/- represents that two copies specific gene present

-/- represents that two copies specific gene present

GSTP1: Glutathione S-transferase pi, GSTM1: Glutathione S-transferase mu, GSTT1: Glutathione S-transferase theta.

(FBG) and HbA1c were significantly different between the groups (Table-1).

For rs1799883 in FABP2, frequency of both WT (GG) and mutant (AA) alleles were significantly higher in the cases than the controls, while for rs1695 in GSTP1, none of the studied genotypes was associated with T2DM (Table-2).

All the analysed demographic, clinical and biochemical parameters varied with all the three genotypes of rs1799883 in FABP2 and with rs1695 in GSTP1, but the difference was not significant (p > 0.05).

Analysis of genotypic and allelic frequencies of GSTT1 and GSTM1 either individually or in combinations indicated that absence of GSTM1, absence of either GSTM1 or GSTT1, or the absence of both genes among the cases increased the risk of T2DM (p < 0.05). The subjects with missing GSTM1 and homozygous WT (Ileu/Ileu) or heterozygous (Ileu/Val) genotype of rs1695 in GSTP1 had higher incidence of disease (p < 0.05). Those either with missing GSTT1 and homozygous WT (Ileu/Ileu) at rs1695 in GSTP1 or with GSTT1 and heterozygous (Ileu/Val) genotype at rs1695 in GSTP1 had higher incidence of developing T2DM (p < 0.05). Patients with three combinations had significantly more association with T2DM: both GSTT1 and GSTM1 present and heterozygous (Ileu/Val) for GSTP1; GSTT1 present but GSTM1 absent and homozygous WT (Ileu/Ileu) for GSTP1; and both GSTT1 and GSTM1 absent and homozygous WT (Ileu/Ileu) for GSTP1 (Table-3).

All allelic combinations having significantly higher frequency among the controls were protected combinations, while those having higher frequency among the cases were at higher T2DM risk (Tables-4).

Discussion

T2DM is the most frequent subtype of diabetes characterized by high blood glucose levels due to defective insulin secretion or compromised insulin action. T2DM leads to long-term problems, like failure of different organs, including kidneys, eyes, heart, nerves and damage to the blood vessels.³ As the incidence of T2DM and its related complications are alarmingly increasing, Pakistan is facing a huge healthcare burden.⁴ It has been an established fact

Table-4: Distribution of various allelic combinations of rs1799883 in FABP2 and rs1695 in GSTP1 among cases and controls, and their association with type 2 diabetes mellitus (T2DM).

GSTP1 (Ileu105 Val) A/G	FABP2 (Ala54Thr) G/A	Haplotype	Case	Control	Proportion Test (P-value)	Chi-square value	P-value
A	G	GA	383	154	0.000	523.416	P < 0.001 ***
G	G	GG	60	33	0.007		
A	A	AA	3	443	0.000		

P < 0.001 = Highly significant (***)

FABP2: Fatty acid binding protein-2, GSTP1: Glutathione S-transferase theta gene.

now that environmental factors play a significant role in the onset of diabetes; in particular, physical inactivity and obesity.¹⁸ Analysis of biochemical parameters during the present study revealed that BMI, WC, FBG and HbA1c levels were significantly associated with the incidence of diabetes. The results are in support of Bid et al.¹⁸

FABP2 is involved in the absorption and intracellular transport of long-chain fatty acids. It has been documented that individuals having Thr at codon 54 in exon 2 of FABP2 gene, instead of the usual Ala, have more affinity for long-chain fatty acids and they can be obese and can develop insulin resistant.²² The results of the current are in agreement with the above-mentioned findings. The current results are also confirmation of those reporting that rs1799883 in FABP2 was significantly associated with T2DM.^{17,14,24,26} Contrasting results were reported by two studies.^{16,27}

GST is a family of detoxifying enzymes that protect human cells from ROS and polymorphisms/mutations in these genes can lead to the production of unwanted reactive oxygen species (ROS) associated with a variety of pathological conditions, including DM.²⁸ GSTM1 can detoxify genotoxins, GSTT1s are involved in the utilisation of oxidised lipids and DNA, while GSTP1 is responsible for detoxifying the products that result from DNA oxidation, and is also involved in the utilisation of smoke-derived carcinogens.¹⁸ The current study explored the presence or absence of GSTT1 and GSTM1 and polymorphism rs1695 in GSTP1 in subjects enrolled from southern Punjab comprising both healthy controls and T2DM patients. No association was observed between rs1695 in GSTP1 with DM. It was observed that the presence or absence of GSTT1 was not associated with T2DM, but the absence of GSTM1 had significant association with the disease. The findings are in agreement with those of Moasser et al.¹⁹ However, the frequency of GSTT1 and GSTP1 genotypes did not vary significantly between the groups. Bid et al.¹⁸ had observed a significant association of GSTM1 null, similar to the current findings, GSTP1 null allele with T2DM and no significant association of GSTT1 with T2DM, which, again, was similar to the current findings. Bid et al.¹⁸ postulated that GSTM1 null seemed to enhance the risk of developing DM almost two-fold due to OS. Mastana et al.²⁹ reported higher proportion of GSTT1 and GSTM1 null genotypes in diabetics when compared with healthy controls. Banerjee et al.³⁰ had documented that the null/null GSTM1 increased the DM risk up to 1.7-fold. Stoian et al.³¹ had documented that polymorphism in GSTP1 may lead to the development of T2DM in the Romanian population. In Iran, Akbari et al.²⁰ reported no effect of Ile105Val in GSTP1 on incidence of type one DM. They also documented that individuals with either double-null GSTP1 or having Ile/Val genotype were at

increased risk of DM. The presence of this Ile/Val allele affects the active site of GSTP1 led to reduced enzymatic activity.²⁰ This effect was not observed in the present study although it was observed that GSTM1 null and GSTP1 Ile/Val genotype was more susceptible to developing T2DM. We believe that odds ratio (OR) of the disease risk rises as more and more genes are interacting, indicating that gene-gene interaction and genotypes at specific SNPs play a significant role in the aetiology of T2DM.

The present investigation did not observe association of rs1799883 in FABP2 with the studied demographic, clinical and biochemical parameters, and the results are in line with earlier studies^{10,15}. In contrast, Martinez-Lopez et al.³² reported that carbohydrate intake and resting metabolic rate were higher in subjects with Ala54Thr/Thr54Thr genotype in FABP2.

The present study found that all the studied demographic, clinical and biochemical parameters varied non-significantly with all the genotypes of GSTP1 or with the presence/absence of GSTT1 and GSTM1. Bid et al.¹⁸ reported no association of any genotype of rs1695 in GSTP1 and the presence or absence of GSTT1 with the studied demographic and biochemical parameters in north Indian population, but they had found higher low-density lipoprotein (LDL) and cholesterol to be associated with GSTM1 allele. Nothing can be commented upon the matter as the current study did not determine serum cholesterol levels.

The results regarding FABP2 and GST genotypes and their association with T2DM in the current study were not in agreement with several studies that are cited above and that is probably due to the impact of ethnicity on the general characteristics of the study subjects.

The current study has several limitations. The sample size was small and it only analysed two SNPs. Despite the limitations, however, the current study is valuable as it the first such study from southern Punjab in Pakistan, and represents an advance in biomedical sciences, because it found that rs1799883 in FABP2 and the absence of GSTM1 had significant association with T2DM in the region. Individuals with GSTM1 null and Ileu/Ileu or Ileu/Val genotype of rs1695 in GSTP1 were found to at a very high risk of developing T2DM.

Conclusion

The presence of rs1799883 in FABP2 and the absence of GSTM1 gene were found to play significantly in the development of T2DM.

Disclaimer: The text is based on an M. Phil thesis.

Conflict of Interest: None.

Source of Funding: None.

References

- De Fronzo RA, Ferrannini E, Groop L, Henry RR, Herman WH, Holst JJ, et al. Type 2 diabetes mellitus. *Nat Rev Dis Primer*. 2015; 1:1-22.
- Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol*. 2018; 14:88-98.
- Dendup T, Feng X, Clingan S, Thomas Astell-Burt. Environmental Risk Factors for Developing Type 2 Diabetes Mellitus: A Systematic Review. *Int J Environ Res Public Health*. 2018; 15:78-84.
- Atif M, Saleem Q, Scahill S. Depression and mild cognitive impairment (MCI) among elderly patients with type 2 diabetes mellitus in Pakistan: possible determinants. *Int J Diabet Dev Count*. 2018; 38:312-20.
- Tsai IT, Wu CC, Hung WC, Lee TL, Hsuan CF, Wei CT, et al. FABP1 and FABP2 as markers of diabetic nephropathy. *Int J Med Sci*. 2020; 17:2338-45.
- Han T, So W. Effects of FABP2 Ala54Thr gene polymorphism on obesity and metabolic syndrome in middle-aged Korean women with abdominal obesity. *Cent Eur J Public Health*. 2019; 27:37-43.
- Liu Y, Wu G, Han L, Zhao K, Qu Y, Xu A, et al. Association of the FABP2 Ala54Thr polymorphism with type 2 diabetes, obesity, and metabolic syndrome: a population-based case-control study and a systematic meta-analysis. *Genet Mol Res*. 2015; 14:1155-68.
- Qiu CJ, Ye XZ, Yu XJ, Peng XR, Li TH. Association between FABP2 Ala54Thr polymorphisms and type 2 diabetes mellitus risk: a Huge Review and Meta-Analysis. *J Cell Mol Med*. 2014; 18:2530-5.
- Larifla L, Rambhajan C, Joannes MO. Gene Polymorphisms of FABP2, ADIPOQ and ANP and Risk of Hypertriglyceridemia and Metabolic Syndrome in Afro-Caribbeans. *PLoS One*. 2016; 11:e0163421.
- Liu PJ, Liu YP, Qin HK, Xing T, Li SS, Bao YY. Effects of polymorphism in FABP2 Ala54Thr on serum lipids and glycemic control in low glycemic index diets are associated with gender among Han Chinese with type 2 diabetes mellitus. *Diabetes Metab Syndr Obes*. 2019; 12:413-21.
- Gábor G, Tamas K, Lóránt K, Xing T, Li SS, Bao YY. Glutathione S-Transferase Enzymes in Plant-Pathogen Interactions. *Front Plant Sci*. 2018; 9:1836-42.
- Barseem N, Elsamalehy M. Gene Polymorphisms of Glutathione S-Transferase T1/M1 in Egyptian Children and Adolescents with Type 1 Diabetes Mellitus. *J Clin Res Pediatr Endocrinol*. 2017; 9:138-43.
- Saadat M. Evaluation of glutathione S-transferase P1 (GSTP1) Ile105Val polymorphism and susceptibility to type 2 diabetes mellitus, a meta-analysis. *EXCLI J*. 2017; 16:1188-97.
- Duarte NL, Colagiuri S, Palu T, Wang XL, Wilcken DEL. Obesity, Type II diabetes and the Ala54Thr polymorphism of fatty acid binding protein 2 in the Tongan population. *Mol Genet Metabol*. 2003; 79:183-8.
- Almeida JC, Gross JL, Canani LH, Zelmanovitz T, Perassolo MS, Azevedo MJ. The Ala54Thr polymorphism of the FABP2 gene influences the postprandial fatty acids in patients with type 2 diabetes. *J Clin Endocrinol Metabol*. 2010; 95:3909-17.
- Alharbi KK, Khan IA, Bazzi MD, Al-Daghri NM, Hasan TN, Alnabaheen MS, et al. A54T polymorphism in the fatty acid binding protein 2 studies in a Saudi population with type 2 diabetes mellitus. *Lipids Health Dis*. 2014; 13:61.
- Ganah H, Behl S, Bano H, Bensaheb E, Mohamed O, Al Molki M, et al. FABP-2 And PPAR-Y Genes as Risk Factors for Dyslipidemia In Type 2 Diabetes Mellitus In Residents Of United Arab Emirates. *Int J Biol Pharm Res*. 2015; 6:965-74.
- Bid HK, Konwar R, Saxena M, Chaudhari P, Agrawal CG, Banerjee M. Association of glutathione S-transferase (GSTM1, T1 and P1) gene polymorphisms with type 2 diabetes mellitus in north Indian population. *J Postgrad Med*. 2010; 56:176-81.
- Moasser E, Kazemi-Nezhad SR, Saadat M, Azarpira N. Study of the association between glutathione S-transferase (GSTM1, GSTT1, GSTP1) polymorphisms with type II diabetes mellitus in southern of Iran. *Mol Biol Rep*. 2012; 39:10187-92.
- Akbari A, Salehi Z, Koohmanai S. Significant difference between the frequency of glutathione-S-transferase M1, glutathione-S-transferase T1 and glutathione-S-transferase P1 polymorphisms in type 1 diabetes patients and controls. *Ann Trop Med PH*. 2017; 10:1479.
- Black TR. Doing quantitative research in the social sciences. In: Black TR, eds. *An integrated approach to research design, measurement, and statistics*. Thousand Oaks, CA: SAGE Publication Inc, 2017; pp-118.
- Aslam S, Khosa T, Akbar A, Latif M, Chaudary MJ, Shaikh RS, et al. Single Nucleotide Polymorphism (rs7543472) in EPHA2 gene is associated with Age-related Cataract in subjects enrolled from Multan in Southern Punjab: a case-control study. *J Pak Med Assoc*. 2020; 70:583-90.
- Baier LJ, Sacchettini JC, Knowler WC, Eads J, Paolisso G, Tataranni PA, et al. An amino acid substitution in the human intestinal fatty acid binding protein is associated with increased fatty acid binding, increased fat oxidation, and insulin resistance. *J Clin Invest*. 1995; 95:1281-7.
- Hashemi M, Eskandari-Nasab E, Fazaeli A, Bahari A, Hashemzahi NA, Shafiepour S, et al. Association of genetic polymorphisms of glutathione-S-transferase genes (GSTT1, GSTM1, and GSTP1) and susceptibility to nonalcoholic fatty liver disease in Zahedan, Southeast Iran. *DNA Cell Biol*. 2012; 1:672-7.
- Hardy-Weinberg E. Calculator of Hardy-Weinberg equilibrium. [Online] 2021 [Cited 2021 October 27]. Available from: URL: <https://wpcalc.com/en/equilibrium-hardy-weinberg/>.
- Albala C, Villarroel A, Santos JL, Angel B, Lera L, Liberman C, et al. FABP2 Ala54Thr polymorphism and diabetes in Chilean elders. *Diabet Res Clin Pract*. 2007; 77:245-50.
- Ito K, Nakatani K, Fujii M, Katsuki A, Tsuchihashi K, Murata K, et al. Codon 54 polymorphism of the fatty acid binding protein gene and insulin resistance in the Japanese population. *Diabet Med*. 1999; 16:119-24.
- Kazemisafa F, Jadidian K, Rahimi Z, Nomani H, Ghanbari A, Saeb G, et al. The Relationship Between Polymorphisms of Glutathione S-Transferase (GSTM1, GSTT1) Gene and Type 2 Diabetes Mellitus in Western Iran. *J Kermanshah Univ Med Sci*. 2018; 22: e85833
- Mastana SS, Kaur A, Rachel H, Lindley MR. Influence of glutathione S-transferase polymorphisms (GSTT1, GSTM1, GSTP1) on type-2 diabetes mellitus (T2D) risk in an endogamous population from north India. *Mol Biol Rep*. 2013; 40:7103-10.
- Banerjee M, Vats P, Kushwah AS, Shrivastava N. Interaction of antioxidant gene variants and susceptibility to type 2 diabetes mellitus. *Brit J Biomed Sci*. 2019; 76:1-6.
- Stoian A, Banescu C, Balasa RI, Moțățăianu A, Stoian M, Moldovan VG, et al. Influence of GSTM1, GSTT1, and GSTP1 Polymorphisms on Type 2 Diabetes Mellitus and Diabetic Sensorimotor Peripheral Neuropathy Risk. *Dis Markers*. 2015; 638693.
- Martinez-Lopez E, Garcia-Garcia MR, Gonzalez-Avalos JM, Gonzalez MM, Madrigal BR, Vizmanos B, et al. Effect of Ala54Thr polymorphism of FABP2 on anthropometric and biochemical variables in response to a moderate-fat diet. *Nutrition*. 2013; 29:46-51.