

# Validation of Genome-Wide Association Studies (GWAS)-Identified Type 2 Diabetes Mellitus Risk Variants in Pakistani Pashtun Population

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## Abstract

**Objective.** Recent GWAS largely conducted in European populations have successfully identified multiple genetic risk variants associated with Type 2 Diabetes Mellitus (T2DM). However, the effects conferred by these variants in the Pakistani population have not yet been fully elucidated. The objective of this study was to examine European GWAS-identified T2DM risk variants in the Pakistani Pashtun population to better understand the shared genetic basis of T2DM in the European and Pakistani cohorts.

**Methodology.** A total of 100 T2DM patients and 100 healthy volunteers of Pashtun ethnicity were enrolled in this study. Both groups were genotyped for 8 selected single nucleotide polymorphisms (SNPs) using the Sequenom MassARRAY® platform. The association between selected SNPs and T2DM was determined by using appropriate statistical tests.

**Results.** Of the 8 studied SNPs, 5 SNPs, *SLC30A8*/ rs13266634 ( $p=0.031$ , OR=2.13), *IGF2BP2*/ rs4402960 ( $p=0.001$ , OR=3.01), *KCNJ11*/ rs5219 ( $p=0.042$ , OR=1.78), *PPARG*/ rs1801282 ( $p=0.042$ , OR=2.81) and *TCF7L2*/ rs7903146 ( $p=0.00006$ , 3.41) had a significant association with T2DM. SNP *GLIS3*/ rs7041847 ( $p=0.051$ , OR=2.01) showed no sufficient evidence of association. SNPs *KCNQ1*/ rs2237892 ( $p=0.140$ , OR=1.61) and *HHEX/IDE*/ s1111875 ( $p=0.112$ , OR=1.31) showed opposite allelic effects and were not validated for T2DM risk in the study population. Among the studied SNPs, *TCF7L2*/ rs7903146 showed the most significant association.

**Conclusion.** Our study finding indicates that selected genome-wide significant T2DM risk variants previously identified in European descent also increase the risk of developing T2DM in the Pakistani Pashtun population.

**Key words:** type 2 diabetes mellitus, European GWAS, SNPs validation, replication study, Pashtun population

## INTRODUCTION

Diabetes mellitus (DM) is a complex metabolic disorder with hyperglycemia as a hallmark. DM is one of the serious and common diseases of our time, causing disabling and life-threatening complications.<sup>1,2</sup> According to the American Diabetes Association (ADA), DM is classified into two broad forms: Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM). T2DM is the most frequent subtype of DM accounting for 90% of all diabetes cases.<sup>3</sup> It results from the combination of defective insulin secretion and peripheral insulin resistance.<sup>4</sup> However, these defects or alterations are not enough to explain the complex pathophysiology of this multifaceted metabolic disorder. Other factors like obesity,<sup>5</sup> physical inactivity,<sup>6</sup> environmental,<sup>7</sup> and genetic factors<sup>8</sup> are believed to have a key role in the development of T2DM. Identification of T2DM-causing genetic and non-genetic risk factors greatly helps in the assessment and prevention of this fatal and costly disease.

In recent decades, the global burden of diabetes has significantly increased.<sup>9</sup> Increased prevalence of diabetes caused unprecedented health<sup>10</sup> and economic challenges<sup>11</sup> worldwide. According to the latest epidemiological data from 10<sup>th</sup> edition of the International Diabetes Federation (IDF) Diabetes Atlas, approximately 537 million people (ages 20-79 years) are living with diabetes. This number is projected to soar to 643 million by 2030 and 784 million by 2045.<sup>12</sup> Around 81% of people with diabetes are living in low and middle-income countries.<sup>13</sup> The prevalence of diabetes is not similar among different ethnicities. South Asians (people living in China, India, Pakistan, Bhutan, Nepal, Sri Lanka, and the Maldives) are at high risk of developing diabetes compared to other ancestral groups.<sup>14,15</sup> In terms of diabetes prevalence, Pakistan ranks 3<sup>rd</sup> behind China and India.<sup>12</sup> The factors for an increased prevalence of diabetes in Pakistan include rapid transition in lifestyle and a high degree of urbanization.<sup>16,17</sup>

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The risk of developing T2DM is strongly heritable.<sup>18</sup> The chances of diabetes escalate by 40% if one parent has diabetes and by 70% if both parents have diabetes.<sup>19</sup> Genetic studies hold great promise in predicting an individual's disease risk, exploring disease molecular pathways, and selecting treatment therapy as per individual-specific biology.<sup>20</sup> To date, genetic studies have provided plentiful information on the pathogenesis of T2DM.<sup>21</sup> GWAS identified over 400 genetic signatures associated with T2DM.<sup>22</sup> However, most of these large-scale genetic studies disproportionately focused on individuals of European ancestry.<sup>23</sup> Currently few genetic studies reporting T2DM-associated risk variants are available in South Asians.<sup>24</sup> T2DM is a major public health care issue in Pakistan.<sup>25</sup> Since genetic susceptibility plays a key role in T2DM etiology, it is important to carry out genetic studies to assess the genetic risk of T2DM in the Pakistani population. Pakistan is a developing country with limited resources for comprehensive genomic research.<sup>26</sup> Despite limited resources, GWAS replication studies on previously reported risk loci will help to enhance our understanding of the genetics of T2DM in the Pakistani population. In this replication study, we attempted to investigate the 8 T2DM-associated SNPs previously identified by European GWAS in the Pashtun ethnic population of Pakistan to better understand the shared genetic basis of T2DM between Pakistanis and Europeans. The Pakistani Pashtun population was selected for this study because no comprehensive genomic research in this study group has been done. Secondly, this ethnic group has unique social values, traditions, lifestyles, and strict religious beliefs.

## METHODOLOGY

### Subject recruitment

A total of 200 unrelated individuals (persons with diabetes = 100 and without diabetes = 100) of Pashtun ethnicity belonging from different districts (Peshawar, Charsadda, Swat, Bannu, Kohat, Mardan, and Dir) of Khyber Pakhtunkhwa, Pakistan were included in the study. Persons with diabetes were recruited from endocrinology units of Hayatabad Medical Complex (HMC) Peshawar, Mardan Medical Complex (MMC) Mardan, and Khyber Teaching Hospital (KTH) Peshawar. Healthy volunteers that served as controls were recruited from free medical camps organized by Rehman Medical Institute (RMI) Peshawar and Khyber Medical College (KMC) Peshawar. Consent forms were taken from all the participants. Thorough demographic data, family history of diabetes, and clinical

profile of all the participants were noted on a carefully designed proforma. In the case of patients and volunteers who were illiterate and/or did not understand the English language, the consent forms were read and properly explained in the local Pashtu language and then signed on their behalf by their relatives. Inclusion and exclusion of study participants were according to the previously defined criteria used for Asian populations.<sup>27</sup>

Inclusion criteria were (i) T2DM diagnosed as per International Diabetes Federation-imposed etiologic classification, (ii) age above 30 years, and (iii) study subjects must be Pakistani & Pashtun ethnicity. Patients with chronic disorders like cancer and recent infections were excluded. Similarly, previously defined criteria were followed for control participants.<sup>28</sup> Controls were healthy volunteers from the general population with fasting blood sugars in the normal ranges. The ethical approval was obtained from the Ethical Committee of the Department of Pharmacy, University of Peshawar (Approval No. 907/PHAR). All procedures and experiments were carried out in conformity with the Helsinki declaration.

### Blood sampling

Three milliliters (ml) of venous blood from the mean cubital vein was collected from each study participant with the help of a trained nurse. The blood was placed in a properly labeled ethylenediaminetetraacetic acid (EDTA) tube and was stored at -10°C.

### DNA extraction

DNA was isolated from 200  $\mu$ l whole blood samples using an imported WizPrep DNA extraction kit (Product code No. W54100) which gives trusted and reliable results. Quantification of DNA was conducted with the help of the Qubit™ dsDNA HS Assay kit (Catalog No. Q32851) using Introgen Qubit™, and the final DNA concentration was normalized to 10 ng/ $\mu$ L for genotyping.

### SNPs selection

Eight T2DM-associated SNPs previously identified in different GWAS were selected to be investigated in the present study population. GWAS Catalog<sup>29,30</sup> which contains a high-quality curated collection of previously published large-scale genomic studies was used in the selection of SNPs. Table 1 shows detailed information on the selected SNPs

**Table 1.** Summary of selected T2DM risk variants curated from GWAS catalog

Variant	Allelic change	Variant description	Chromosomal position	Cytogenetic location	Mapped gene	Trait
rs13266634	C/T	Exonic	8:117172544	8q24.11	SLC30A8	T2DM
rs4402960	G/T	Intronic	3:185793899	3q27.2	IGF2BP2	T2DM
rs5219	C/T	Exonic	11:17388025	11p15.1	KCNJ11	T2DM
rs1801282	C/G	Exonic	3:12351626	3p25.2	PPARG	T2DM
rs7903146	C/T	Intronic	10:112998590	10q25.2	TCF7L2	T2DM
rs2237892	C/T	Intronic	11:2818521	11p15.4	KCNQ1	T2DM
rs1111875	T/C	Intergenic	10:94452862	10q23.33	HHEX/IDE	T2DM
rs7041847	A/G	Intronic	9:4287466	9p24.2	GLIS3	T2DM

**Table 2.** Socio-demographic features of cases and controls

Variables	Case n(f)	Control n(f)	p-value
Gender			0.061
Male	65 (65.0%)	77 (77.0%)	
Female	35 (35.0%)	23 (23.0%)	
Mean age (years)	58±12.40	56±13.43	0.951
Mean weight (kg)	62.64±6.07	59.55±8.32	0.104
Occupation			0.112
Labourer	20 (20.0%)	14 (14.0%)	
Government employee	13 (13.0%)	21 (21.0%)	
Businessman	16 (16.0%)	18 (18.0%)	
Farmer	7 (7.00%)	16 (16.0%)	
Housewife	34 (34.0%)	23 (23.0%)	
Driver	10 (10.0%)	8 (8.00%)	
Geographical area (District)			0.145
Peshawar	53 (53.0%)	19 (19.0%)	
Charsadda	13 (13.0%)	53 (53.0%)	
Swat	8 (8.00%)	7 (7.00%)	
Dir	5 (4.00%)	5 (5.00%)	
Mardan	12 (12.0%)	10 (10.0%)	
Kohat	6 (6.00%)	3 (3.00%)	
Bannu	3 (3.00%)	3 (3.00%)	
Family history of T2DM			0.012
Yes	94 (94.0%)	0 (0.0%)	
No	6 (6.00%)	100 (100%)	
Exercise			0.016
Non-exercising	85 (85.0%)	89 (89.0%)	
Walking	14 (14.0%)	4 (4.00%)	
Jogging	1 (1.00%)	5 (5.00%)	
Gym/Sports	0 (0.0%)	2 (2.00%)	
Smoking status			0.178
Cigarette smoker	19 (19.0%)	10 (10.0%)	
Snuff (smokeless tobacco)	21 (21.0%)	26 (26.0%)	
Non-smoker	60 (60.0%)	64 (64.0%)	
Diet control/compliance			0.031
Yes	50 (50.0%)	90 (90.0%)	
No	50 (50.0%)	10 (10.0%)	

kg: kilogram; T2DM: Type 2 Diabetes Mellitus; n(f): number (frequency)

retrieved from the GWAS Catalog. Selection of SNPs was based on their subsequent association with T2DM in many populations across the globe. For genotype distribution of selected SNPs in the study population, the co-dominant (additive) genetic model of inheritance was applied.

### Genotyping

Sequenom MassARRAY® platform (Agena Bioscience, San Diego, CA) available at the Centre of Genomics Rehman Medical Institute, Peshawar was used for genotyping carefully following the previously described protocols.<sup>31</sup> SNP-Genotyping by Agena MassARRAY® platform is not only cost-effective but also generates fast, sensitive, and

**Table 3.** Prevalence rates of co-morbidities among study subjects

Disease	Frequency		p-value
	Cases	Controls	
Hypertension	34.00%	10.00%	0.003
Ischemic heart disease	14.00%	0.00%	<0.001
Renal failure	5.00%	0.00%	<0.001
Retinopathy	61.00%	0.00%	<0.001
Hypercholesterolemia	6.00%	3.01%	0.005
Hepatitis C virus	1.00%	0.00%	<0.001
Hepatitis B virus	0.00%	0.00%	<0.001

highly accurate results. SNPs with genotyping call rates above 90% were considered for forward analysis.

### Statistical analysis

IBM SPSS (Statistical Package for Social Sciences version 26) was used for statistical analysis. The difference in the distribution of allelic and genotypic frequencies between cases and controls was analyzed using Chi-square ( $\chi^2$ ) test. Each SNP was tested for Hardy-Weinberg Equilibrium (HWE) using  $\chi^2$  test. Odds ratio (unadjusted) and 95% confidence interval were determined using logistic regression. The association analysis was performed using the Armitage trend test.<sup>32</sup> A *p*-value of <0.05 was considered statistically significant.

## RESULTS

### Subject description

The prevalence ratios of the study participants' demographics, general characteristics, and co-morbidities are described in Tables 2 and 3.<sup>33</sup> A greater ratio of co-morbid conditions like hypertension, renal failure, ischemic heart disease, hypercholesterolemia, nephropathy and retinopathy were observed in diabetic cases compared to controls. The majority of patients were obese and physically inactive. Most lived in the urban areas of Khyber Pakhtunkhwa. Diet compliance was recorded poor in the study subjects.

### Allele/genotype frequencies of the selected SNPs

All the selected 8 SNPs were successfully genotyped (call rate >90) and were found consistent with HWE (*p*>0.05) (Table 4). Allelic and genotypic frequency distribution

**Table 4.** Genotype call rate and HWE *p*-value information of the selected T2DM risk variants

Variant	Allelic change	Mapped gene	HWE p-value		Call rate
			Cases	Controls	
rs13266634	C/T	SLC30A8	1.00	0.24	98.2
rs4402960	G/T	GF2BP2	0.50	0.2	98.5
rs5219	C/T	KCNJ11	0.78	1.00	98.6
rs1801282	C/G	PPARG	1.00	0.81	99.1
rs7903146	C/T	TCF7L2	1.00	1.00	98.6
rs2237892	C/T	KCNQ1	0.71	0.47	99.3
rs1111875	T/C	HHEX/IDE	1.00	0.82	98.5
rs7041847	A/G	GLIS3	1.00	0.66	98.5

**Table 5.** Allele/genotype distribution and association results of eight selected SNPs in the Pakistani case-control sample

Gene/SNP	Allele/Genotype	T2DM patients	Healthy controls	Odds Ratio (95% CI)	p-value	Armitage trend test OR (p-value)
<i>SLC30A8</i> /rs13266634	C	147(73.5%)	159(79.5%)	1.73(1.04-2.86)	0.052	2.13 (0.031)
	T	53 (26.55)	41 (20.5%)			
	CC	50 (50%)	66 (66%)	Reference	—	
	CT	38 (38%)	23 (23%)	2.04 (1.11- 4.21)	0.011	
	TT	12 (12%)	11 (11%)	1.30 (0.09- 67.41)	0.771	
<i>IGF2BP2</i> /rs4402960	G	95 (47.5%)	137 (68.5%)	1.92 ( 1.93- 5.01)	0.010	3.01 (0.001)
	T	105 (52.5%)	63 (31.5%)			
	GG	17 (17%)	39 (39%)	Reference	—	
	GT	56 (56%)	41 (41%)	3.21 (2.64-12.89)	0.008	
	TT	27 (27%)	20 (20%)	2.23 (3.71-11.41)	0.042	
<i>KCNJ11</i> /rs5219	C	140 (70%)	161 (80.5%)	1.08 (0.48-0.27)	0.054	1.78 (0.042)
	T	60 (60%)	39 (19.5%)			
	CC	52 (52%)	63 (63%)	Reference	—	
	CT	34 (34%)	32 (32%)	1.43 (0.72-2.01)	0.320	
	TT	41 (41%)	05(05%)	4.48 (1.26-16.7)	0.012	
<i>PPARG</i> /rs1801282	C	132 (66%)	158 (79%)	1.12 (1.02–1.71)	0.013	2.81( 0.002)
	G	68 (34%)	42 (21%)			
	CC	47 (47%)	58 (58%)	Reference	—	
	CG	53 (53%)	42 (42%)	1.61 (0.36-2.91)	0.022	
	GG	0.0 (0.0%)	0.0 (0.0%)	1.01(0.02-54.91)	0.991	
<i>TCF7L2</i> /rs7903146	C	88 (44%)	139 (69.5)	2.88 (1.92-4.45)	0.001	3.41 (0.00006)
	T	112 (56%)	61 (30.5)			
	CC	11 (11%)	50 (50%)	Reference	—	
	CT	66 (66%)	39 (39%)	8.01 (2.64-16.51)	0.00001	
	TT	23 (23%)	11 (11%)	9.12 (3.71-26.61)	0.00002	
<i>KCNQ1</i> /rs2237892	C	147 (73.5%)	158 (79%)	1.32 (0.95-2.21)	0.192	1.61 (0.140)
	T	53 (26.5%)	42 (21%)			
	CC	48 (48%)	58 (58%)	Reference	—	
	CT	52 (52%)	42 (42%)	1.52 (0.77-2.71)	0.141	
	TT	0 (0.0%)	0 (0.0%)	1.24 (0.04-62.11)	0.980	
<i>HHEX/IDE</i> /s1111875	T	139 (69.5%)	129 (64.5%)	1.41 (0.85-2.01)	0.294	1.31 (0.112)
	C	61 (30.5%)	71 (35.5%)			
	TT	26 (26%)	29 (29%)	Reference	—	
	TC	41 (41%)	50 (50%)	0.72 (0.34-1.09)	0.313	
	CC	33 (33%)	21 (21%)	1.44 (1.05-3.11)	0.181	
<i>GLIS3</i> /rs7041847	A	195 (97.5%)	188 (94%)	5.12 (1.12-23.1)	0.032	2.01 (0.051)
	G	05 (2.5%)	12 (6%)			
	AA	96 (96%)	88 (88%)	Reference	—	
	AG	04 (4%)	12 (12%)	0.19 (0.05-0.96)	0.014	
	GG	0 (0.01%)	0 (0.0%)	0.82 (0.02-47.21)	0.981	

between diabetic cases and healthy controls, odds ratios (OD), 95% confidence intervals (95%CI), crude *p*-values, and Armitage trend test *p*-values of the selected SNPs are summarized in Table 5. Among the tested SNPs, five SNPs namely *SLC30A8*/rs13266634, *IGF2BP2*/rs4402960, *KCNJ11*/rs5219, *PPARG*/rs1801282 and *TCF7L2*/rs7903146 met the significant threshold (trend's test *p*-value <0.05) and showed strong association with T2DM in the study population. SNP *GLIS3*/rs7041847 showed no sufficient evidence of association with a *p*-value of 0.051. The remaining two SNPs (*KCNQ1*/rs2237892 and *HHEX/IDE*/s1111875) showed opposite allelic effects and were not validated for T2DM risk in the study population. The top three significant variants in our study were *TCF7L2*/rs7903146 (*p*=0.00006, OR=3.01) followed by *IGF2BP2*/rs4402960 (*p*=0.001, OR=3.01) and *PPARG*/rs1801282 (*p*=0.002, OR=2.81). *TCF7L2*/rs7903146 apart from our study was reported as a significant risk factor for T2DM by two previous replication studies in the Pakistani sub-population.<sup>34-36</sup>

## DISCUSSION

T2DM is a 21<sup>st</sup>-century epidemic.<sup>37</sup> It results from a complex interaction of genetic and environmental factors.<sup>38</sup> The genetic component of T2DM is well explored in European compared to other ancestral populations.<sup>39</sup> Despite significant progress in the genetics of T2DM around the world, limited genetic studies have been conducted in the Pakistani population where the incidence of T2DM is rapidly increasing.<sup>34,40</sup> To fill the gap of deficient genetic studies in Pakistan, this replication study examined European-originated genome-wide significant T2DM risk variants in the Pakistani Pashtun population to shed some light on the shared genetic basis of T2DM in European and Pakistani cohorts.

In this study, we tested the association of 8 previously GWAS implicated T2DM risk variants in the Pakistani Pashtun population and confirmed the association of 6 genetic risk variants with T2DM in the study population.

SNPs *SLC30A8*/ rs13266634, *GF2BP2*/ rs4402960, *KCNJ11*/ rs5219, *PPARG*/ rs1801282 and *TCF7L2*/ rs7903146 showed significant association ( $p < 0.05$ ) whereas the SNP, *GLIS3*/ rs7041847 showed no sufficient evidence of association ( $p = 0.051$ ). The aforementioned SNPs (*SLC30A8*/ rs13266634, *IGF2BP2*/ rs4402960, *KCNJ11*/ rs5219, *PPARG*/ rs1801282 and *TCF7L2*/ rs7903146) showed the same directional effects as reported in the earlier GWAS studies.<sup>41-44</sup> Two SNPs, *KCNQ1*/ rs2237892 and *HHEX/IDE*/ s1111875, showed opposite allelic effects and were not validated ( $p > 0.05$ ) for T2DM in the present study despite their well-documented role in the pathogenesis of T2DM in previous studies.<sup>45-49</sup> Conflicting results in our study population reflect the possible genetic heterogeneity that exists among different populations.

The *TCF7L2*/ rs7903146, a well-documented risk variant for T2DM<sup>44,50</sup>, showed a significant association with T2DM in the present Pashtun population. The same was reported by two earlier studies in the Punjabi population of Pakistan.<sup>34,35</sup> According to Lyssenko et al.,<sup>51</sup> the variant *TCF7L2*/ rs7903146 increases the risk for T2DM by enhancing hepatic glucose production and impaired secretion of insulin. *TCF7L2* is a transcription factor and an important component of the Wnt signaling pathway which has a key role in blood glucose regulation involving a complex mechanism. Genetic variations in *TCF7L2* disrupt the Wnt signaling pathway and impair glucose homeostasis leading to T2DM.<sup>52,53</sup> Of the studied variants, *IGF2BP2*/ rs4402960 also showed notable genetic association with T2DM in our investigated population. Significant association of *IGF2BP2*/ rs4402960 with T2DM has been reported in other populations.<sup>54,55</sup> According to the research finding of Chistiakov and colleagues, the genetic variants in *IGF2BP2* contribute to insulin resistance in persons with diabetes.<sup>56</sup> Among the studied SNPs, *PPARG*/ rs1801282 was the top third hit SNP validated for T2DM association in the present study population. Consistent with our study finding, the significant association of this variant has been documented in other populations.<sup>57,58</sup> Cumulative evidence suggests that variants in *PPARG* have an important role in glucose and lipid metabolism.<sup>59,60</sup> Among them, variant rs1801282 (also known as Pro12Ala) has been extensively reviewed in different studies and repeatedly documented for T2DM susceptibility.<sup>61,62</sup>

Likewise, the *SLC30A8*/ rs13266634 and *KCNJ11*/ rs5219 showed a strong association with T2DM in the present Pakistani Pashtun population. Both SNPs were previously reported in East Asian, South Asian, Caucasian, and European populations.<sup>42,63-65</sup> The *SLC30A8* located on chromosome 8q24.11 encodes Zinc transporter 8 (ZnT-8) which is highly expressed in beta cells of the pancreas. ZnT-8 carries zinc from the cytoplasm to insulin secretory vesicles. Non-synonymous variant *SLC30A8*/ rs13266634 disrupts insulin secretion from the vesicles and increases T2DM susceptibility.<sup>66</sup> The *KCNJ11* gene encodes Kir6.2 protein in the ATP-sensitive potassium (KATP) channel. The KATP channels are expressed throughout the body including

beta cells of the pancreas where they help in the release of insulin in response to increased blood glucose levels. The *KCNJ11*/ rs5219 polymorphism alters the normal function of KATP channels and increases the risk of developing T2DM.<sup>67,68</sup> Last but not least, variant *GLIS3*/ rs7041847 showed no sufficient evidence of association in our study population. *GLIS3*/ rs7041847 was previously marked as a risk factor for T2DM in the Chinese population.<sup>69</sup> Similar to our finding, one recent study<sup>36</sup> also replicated variant *GLIS3*/ rs7041847 in the Pakistani population.

## CONCLUSION

In conclusion, we investigated 8 selected T2DM-associated SNPs previously identified by European GWAS in the Pakistani Pashtun population. Of the selected variants, 6 genetic variants were replicated in the study population. Among the 6 replicated variants, 5 variants showed statistically significant association ( $p < 0.05$ ), 1 variant showed no sufficient evidence of association ( $p = 0.051$ ), while the remaining 2 variants showed no association with T2DM.

The strength of the present study is that it was conducted in an understudied and genetically unique Pakistani Pashtun population. The weakness of this study is the relatively small sample size. Future large-scale genomic studies are strongly suggested in Pakistani sub-populations to unmask the genetic architecture of T2DM and develop better management strategies for the control of this fatal and costly disease.

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## Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

## Author Contributions

AJ conceived the study, designed the methodology, analyzed the data and interpreted the results. Z wrote helped in designing the methodology, programmed and developed the software and supervised the research activity planning and execution. FK conducted the investigation process, provided the resources and administered the research. RA curated the data, wrote the initial draft, finalized results, did wet lab work and prepared the data presentation.

## Author Disclosure

The authors declared no conflict of interest.

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