Genetic Association Study of *STAT4* Polymorphisms and Type 1 Diabetes in Pakistani Children

(Penyelidikan Perkaitan Genetik Polimorfisme *STAT4* dan Diabetes Jenis 1 pada Kanak-kanak di Pakistan)

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ABSTRACT

The present study investigated the relationship of *STAT4* single nucleotide polymorphisms (SNPs) rs7574685, rs10181656, and rs3821236 with T1D susceptibility visiting tertiary care hospital in Lahore, Punjab, Pakistan. One hundred and fifty-five T1D patients and one hundred and five healthy individuals were enrolled. An expert endocrinologist collected the clinical data of T1D patients. The genotyping of three potential *STAT4* SNPs was performed through Tetra ARMS-PCR assay. The relationship between SNPs and T1D susceptibility under several genetic models, including dominant, recessive, and codominant models, was assessed by regression analysis. All clinical features of T1D demonstrate a significant difference from control groups (P<0.01) except blindness. The characteristic biochemical analysis determined that participants with T1D had significantly higher fasting blood glucose levels and glycated hemoglobin (HbA1c) levels than the control group (P<0.01). Genetic analysis of rs7574685 depicts GT genotype was found to be the risk allele for the development of T1D when compared to the control group. For rs10181656 and rs3821236, the GC genotype and GA genotype were observed to be the risk alleles in the T1D cases as compared to the control group (P=0.04, P<0.01, respectively). Genetic models showed that the *STAT4* GG genotype of rs7574685 in the dominant model (OR=1.73, 95% CI=1.05-2.86), GC genotype of rs10181656 in the codominant model (OR=2.079, 95 % CI=1.16-3.71), and AA genotype of rs3821236 showed significant risk association with T1D (OR=3.486, 95% CI=1.72-7.03). It is concluded that the risk of T1D is highly correlated with the *STAT4* variants of rs7574685 and rs10181656 among children of the Pakistani population.

Keywords: Polymorphism; *STAT4*; T1D; variants

ABSTRAK

Penyelidikan ini mengkaji hubungan polimorfisme nukleotida tunggal *STAT4* (SNP) rs7574685, rs10181656 dan rs3821236 dengan kerentanan T1D yang melawat hospital penjagaan tertiari di Lahore, Punjab, Pakistan. Seratus lima puluh lima pesakit T1D dan seratus lima individu yang sihat telah didaftarkan. Pakar endokrinologi mengumpul data klinikal pesakit T1D. Genotip tiga SNP *STAT4* yang berpotensi dilakukan melalui ujian Tetra ARMS-PCR. Hubungan antara SNP dan kerentanan T1D di bawah beberapa model genetik, termasuk model dominan, resesif dan kodominan, telah dinilai oleh analisis regresi. Semua ciri klinikal T1D menunjukkan perbezaan yang ketara daripada kumpulan kawalan (P<0.01) kecuali buta. Analisis biokimia ciri menentukan bahawa peserta dengan T1D mempunyai paras glukosa darah puasa dan paras hemoglobin terglikasi (HbA1c) yang jauh lebih tinggi daripada kumpulan kawalan (P<0.01). Analisis genetik rs7574685 menggambarkan genotip GT didapati sebagai alel risiko untuk pembangunan T1D jika dibandingkan dengan kumpulan kawalan. Untuk rs10181656 dan rs3821236, genotip GC dan genotip GA diperhatikan sebagai alel risiko dalam kes T1D berbanding kumpulan kawalan. (P = 0.04, P <0.01, masing-masing). Model genetik menunjukkan bahawa genotip *STAT4* GG rs7574685 dalam model dominan (OR=1.73, 95% CI=1.05-2.86), genotip GC rs10181656 dalam model kodominan (OR=2.079, 95% CI=1.16),-3 dan genotip AA rs3821236 menunjukkan perkaitan risiko yang ketara dengan T1D (OR=3.486, 95% CI=1.72-7.03). Disimpulkan bahawa risiko T1D sangat berkorelasi dengan varian *STAT4* rs7574685 dan rs10181656 dalam kalangan populasi kanak-kanak Pakistan.

Kata kunci: Polimorfisme; *STAT4*; T1D; varian

INTRODUCTION

Type 1 diabetes (T1D) is a multigenic disorder developed due to T cell-mediated autoimmune devastation of pancreatic beta cells (Abdelmajed et al. 2021). As a result of islet cell destruction, various signs and symptoms of T1D appear suddenly, including frequent thirst and urination, blurry vision, fatigue, muscle cramps, and candidiasis (Grayson et al. 2010). Persistent symptoms lead to microvascular complications such as diabetic nephropathy, retinopathy, and neuropathy (Baynes 2015). T1D is most common in children and adolescents (Fichna et al. 2020). International Diabetes Federation (IDF) estimated that 5% to 10% of type 1 diabetes mellitus patients worldwide (Goyal et al. 2020). The incidence of T1D has been reported as 1.02 per 100,000 per year in Pakistan, and the prevalence is less than 2% in the total diabetic population (Shera et al. 2008).

Genetic and environmental factors contribute to T1D etiology. Over 60 genes and loci are associated with T1D (Xie et al. 2020). Genetic approaches, including Genome-wide association studies (GWAS) and meta-analyses, identified genetic risk factors and susceptibility loci of T1D (Storling et al. 2017). The most commonly reported genes associated with T1D are *STAT4, CTLA-4, STAT3, PTPN22,* and *IFIH1* (Kiani et al. 2015). STAT4 is an important transcription factor that plays a fundamental role in regulating T-cell activation differentiation and promoting proinflammatory cytokine signaling. Moreover, STAT4 differentiates T helper cells (type 1 and 17), leading to T1D pathogenesis (Lee et al. 2008).

The signal transducer and activator of transcription (*STAT4*) gene is located on chromosome 2q32.2-32.3 and encodes for the STAT4 protein responsible for activating multiple proinflammatory cytokines (Martinez et al. 2008). STAT4 is one of the most important cytokines-responsive transcription factors that mediate response to interleukin-12 (IL-12) signaling and regulates type 1 helper (Th1) cell differentiation (OMIM# 600558). STAT4 also facilitates the establishment of adaptive immunity in intracellular infection by activating several cytokines in the IL-12 and alpha-interferon receptor (IFNAR) signaling pathways (Bi et al. 2013). The activation of Th1 cells and IL-12 contribute to the progression of several autoimmune illnesses, including T1D (Cui et al. 2009).

Several *STAT4* SNPs, including rs11889341, rs7574865, rs8179673, rs3821236, and rs10181656, have been linked to T1D (Shimura et al. 2018). *STAT4* polymorphism (SNP) such as rs7574685 is reported to be significantly associated with increased risk of T1D in the Egyptians (Abdelmajed et al. 2021), Crete (Zervou et al.

2008), Polish (Fichna et al. 2020) and Chinese population (Yan et al. 2014). This association is also confirmed in other autoimmune disorders, such as autoimmune thyroid disorder (AITD) reported in the Chinese Han (Yan et al. 2014) and Asian population (Gao, Wang & Yu 2019), as well as in the colonic Crohn's disease (Glas et al. 2010), and systemic sclerosis (Dieude et al. 2009). A similar relationship of rs7574685 with rheumatoid arthritis had been reported in Spanish, Swedish, and Dutch populations (Settin, Salama & Elshazli 2014).

In addition, *STAT4* SNP, including rs10181656, showed a significant connection with T1D (Lee et al. 2008) and with other autoimmune disorders such as neuromyelitis optica spectrum disorder (NMOSD) (Shi et al. 2017), rheumatoid arthritis (Seddighzadeh et al. 2012) and systemic lupus erythematosus (SLE) (Hellquist et al. 2010). Furthermore, rs3821236 correlates significantly with type 2 diabetes (T2D) (Cui et al. 2021). However, no relationship between T1D (Shimura et al. 2018), systemic sclerosis (Yi et al. 2013), and antiphospholipid syndrome (APS) (Yin et al. 2009) has been reported. The study of genetic association analysis of *STAT4* polymorphisms is important as it will be helpful to identify or investigate the genetic determinants of T1D in the Pakistani population.

In Pakistan, the incidence of T1D has been increasing, doubling to previously reported values from 1990-1999, comparatively lower than in other countries (Condie, Allen & Ogle 2020). In addition, prevalence (< 2%) and clinical presentation of T1D, including the age of diagnosis (<19 years) and disease onset (<16 years), diabetic ketoacidosis (DKA) (2%), and other diabetes-related complications of nephropathy (5.5%), retinopathy (7.7%) and neuropathy (2.9%) had been assessed for the first time among the children of Pakistan (Shera et al. 2008). Furthermore, it has been reported that a higher frequency of thyroid dysfunction (TSH) is observed in T1D patients in Pakistan (Sajid et al. 2019).

In Pakistan, a genetic study was conducted to identify 32 T1D gene loci already reported in the European population. The results showed 10 SNPs of genes such as *GLIS3, ERBB3, SIRPG, HLA-DQA1, IL2-KIAA1109, CD226, BACH2, IKZF1, C6orf173*, and *PTPN2,* had been observed to be significantly associated with increased risk of T1D pathogenesis (Kiani et al. 2015). Similarly, another study investigated seven genes such as *SKAP2, GSDMB, GLIS3, C6orf173, PRKCQ/DKFZp667, DLK1*, and *BACH2* showed a significant relationship with rheumatoid arthritis and T1D (Kiani et al. 2015). Strong evidence has supported the polymorphisms at RsaI on rs2476601

and BsmI on rs1544410 on the *PTPN22* and *VDR* genes with the risk association of T1D (Batool et al. 2016). In addition, Vitamin D receptor (VDR) polymorphism of rs2228570 (FokI), rs7975232 (ApaI), and rs731236 (TaqI) were also identified that contributes to the T1D progression in the Pakistani population (Mukhtar et al. 2017). However, the effect of *STAT4* polymorphism on T1D patients in the Pakistani population has not been explored yet. Therefore, the present study was designed for the first time to examine the impact of *STAT4* variants (rs7574685, rs10181656, and rs3821236) on the susceptibility of T1D among the children of the Pakistani population.

MATERIALS AND METHODS

STUDY PARTICIPANTS

This study was approved by the Ethical Review Board (ERB) of Lahore College for Women University (LCWU) (ORIC/LCWU/22/07) and also attained ethical approval from the Children Hospital and University of Child Health Sciences, Lahore, Pakistan (2021-270-CHICH). In this study, participants and parents were provided with written informed consent as per the declaration of Helsinki on human experimentations guidelines. The onset of T1D is highly prevalent among children and adolescents aged 4 to 17 years (Abdelmajed et al. 2021). The study subjects were enrolled according to the American Diabetes Association (ADA) guidelines. The inclusion criteria of the study were that participants who were insulin therapy dependent and positive for one of the following autoantibody detections in serum such as glutamic acid decarboxylase (GAD), protein tyrosine phosphatase antibody (IA-2A), and zinc transporter 8 antibodies (ZnT8A) were included in the study. The exclusion criteria were that the study participants with other autoimmune disorders and cancers were excluded. Healthy control criteria for enrollment were the absence of any chronic diseases, including hepatic liver disorder, renal, cardiovascular disorder, and autoimmune disease were included.

The sample size was calculated by the method of Gauderman (2002) and by using Raosoft® software by keeping a margin of error at 5% with a confidence interval of 95% http://www.raosoft.com/samplesize.html. In this case-control study, one hundred and fifty-five unrelated participants with T1D and one hundred and five healthy individuals of the same age group and ethnicity were recruited from the Department of Endocrinology of The Children Hospital and The Institute of Child Health Sciences, Lahore, Pakistan.

INVESTIGATION OF CLINICAL CHARACTERISTICS

Data on age, sex, and duration of disease onset were collected. The clinical phenotypes, including frequent thirst and urination, blurry vision, dizziness, joint pain, blindness, weight loss, ketoacidosis, and hypertension, were also recorded. The concentration of fasting blood glucose (FBG) and HbA1c levels was measured by the automated chemiluminescence method (Alinity C, Abbott, USA).

DNA EXTRACTION

Blood samples of all subjects were obtained in EDTA (ethylenediaminetetraacetic acid used as an anticoagulant) tubes. The total DNA isolation was performed using a non-organic method (Grimberg et al. 1989). To assure the quality and quantity of the extracted DNA, the concentrations of DNA were determined and measured with NanoDrop (NanoDrop 2000/2000c, Thermo Scientific™). DNA samples were stored at -20 °C after extraction.

SNP SELECTION

The three SNPs in the *STAT4* gene (rs7574685, rs10181656, and rs3821236) were chosen based on the functional effect, minor allele frequency (MAF), and previously reported associations with autoimmune disorders. The SNP (dbSNP) database https://www.ncbi.nlm.nih.gov/snp and the University of California Santa Cruz (UCSC) genome browser https://genome.ucsc.edu/ were used to determine the sequences of the three SNPs in the *STAT4* gene.

GENOTYPING ASSESSMENT

Genotyping was performed by Tetra ARMS-PCR assay. Primers were designed through software, http://primer1.soton.ac.uk/primer1.html (available upon request). Genomic DNA (2 uL) was added, and the outer primer fragment of the three SNPs was amplified in a total volume of 10 uL master mix consisting of 50 ng of DNA template, 10 µM of outer primer, 200 µM dNTPs (Invitrogen, CA, USA), 2 mM MgCl2 and 0.05 U of Taq DNA polymerase (Thermo Fisher Scientific, USA). The PCR cycles included: initial denaturation at 95 °C for 3 min, 35 cycles of denaturation at 95 °C for 45 s, annealing at 55 °C (rs7574685), 62 °C (rs10181656), 63 °C (rs3821236) for 40 s, and extension at 72 °C for 45 s, followed by a final extension step at 72 °C for 7 min.

Statistical analysis was performed by the Statistical Package for Social Sciences (SPSS, IBM Statistics, version 22, Armonk, NY). Independent sample T-tests for continuous data were used to evaluate differences between the case and control groups. Using a chi-squared goodness-of-fit test, the genotype distributions for the controls were examined for Hardy-Weinberg equilibrium (HWE). Based on the genotype frequencies of each locus, three genetic models such as dominant, recessive, and codominant models were applied to assess the variations in genotype distributions further. Using binary logistic regression analysis, each genetic model's genotype and allele frequencies were statistically compared between the T1D patients and control groups. A p-value of less than 0.05 (95% CI) was considered statistically significant in all statistical analyses.

RESULTS

BASELINE CHARACTERISTICS

The baseline characteristics of the T1D and control group are summarized in Table 1. The clinical features of T1D cases have been presented in Figure 1. The present study's mean age of T1D cases and control groups was 11.80±6.46 vs. 11.58±3.06; P=0.90, respectively. The characteristic biochemical analysis displayed those participants with T1D had significantly higher concentrations of fasting blood glucose than the control group (258.86±130.86 vs. 87.12±7.30; P<0.001), and the glycated hemoglobin (HbA1c) levels also presented significant difference than the control group (11.51 ± 7.33 vs. 4.89 ± 0.39; P<0.001), respectively. The clinical data analysis showed that 70 of 155 T1D patients belonged to the consanguineous family (34%), and 144 out of 155 individuals received intensive insulin therapy (70%). Moreover, other clinical findings of T1D include polyuria (39%), polydipsia (49%), blurred vision (18%), joint pain (36%), body itching (19%), weight loss (26%), dizziness (34%), hypertension (11%), and ketoacidosis (3%) except for blindness (2%), (P=0.06), showing a significant difference from control groups, (P<0.001).

DISTRIBUTIONS OF GENOTYPE FREQUENCIES AND ASSOCIATION ANALYSIS OF THE *STAT4* VARIANTS rs7574685, rs1018656, rs3821236

The results of genotype and allele distributions of rs7574865, rs10181656, and rs3821236 in *STAT4,* were presented in Table 2. The genotype distribution in controls was consistent with HWE for the three SNPs.

For rs7574865, lower GT genotypes and higher TT genotypes frequencies were found in the T1D cases than in controls (GT:26.7% vs. 31.4%, P=0.020, TT:18% vs. 14.3%. P=0.290, respectively). The T allele frequency was present in 40.2% of cases and 26.4% in the control group. The results showed that the T allele and T carrier state of rs7574685 seemed to be risk alleles for developing T1D susceptibility (OR=1.87, 95% CI=1.23-2.83 and OR=2.23, 95%CI=1.10-4.48, respectively). All results exhibited that rs7574685 was correlated with T1D susceptibility, and the T allele seemed to be the risk allele compared to the G allele.

For rs10181656, the frequencies of GG, GC, and CC genotypes were 28%, 14%, and 33% in cases, respectively, and 38%, 36%, and 26% in controls, respectively. The allele frequency of C was present in 66% of cases and 42% of controls. The regression analysis showed a positive association between GC heterozygous and T1D at rs10181656 (OR=1.83, 95%CI=1.01-3.32; P=0.04). Moreover, a strong association was identified that the C allele was related to T1D (OR=2.60, 95%CI=1.80-3.77; P<0.001).

Lower frequencies of rs3821236 GA and AA genotypes were observed in the cases than in controls (GA:21% vs. 45%, P<0.001, AA:7% vs. 19%, P=0.02). The A allele was present in 19% of the cases and 42.4% of the control group. The results demonstrated that the A allele and A allele carrier state of rs3821236 seemed to be the protective allele against T1D susceptibility (OR=0.31, 95% CI=0.20-0.48, P=0.001). In addition, binary logistic regression analysis showed that rs3821236 was not significantly associated with T1D susceptibility because the A allele seemed to be the protective allele compared to the G allele.

Genetic models were performed to determine the function of the three *STAT4* polymorphisms in T1D predisposition (Table 2). Comparing the genotype distributions between T1D and controls cases showed that the GG genotype for rs7574685 was significantly associated with T1D (OR=1.73, 95% CI=1.05-2.86) under the dominant model (GG vs. GT+TT). GG genotype for both rs10181656 and rs3821236 showed no significant risk association with T1D (OR=1.02, 95% CI=0.61-1.71 and OR=0.378, 95% CI=0.22-0.63, respectively) under the dominant model (GG vs. GC+CC and GG vs. GA+AA, respectively).

In the recessive model (AA vs. GG+GA), the AA genotype distribution of rs3821236 showed a significant association with T1D susceptibility (OR=3.486, 95% CI=1.72-7.03). However, the TT genotype of rs7574685 and the CC genotype of rs10181656 showed no significant

risk association (OR=0.532, 95% CI=0.27-1.02, and OR=0.443, 95% CI=0.25-0.76) with T1D in the recessive model (TT vs. GG+GT and CC vs. GG+GC, respectively).

Moreover, the results demonstrated that the presence of the GT genotype for the rs7574685 showed lower risk towards T1D susceptibility (OR=0.833, 95%CI=0.49-1.41) under a codominant model (GT vs. GG+TT), as well as the GA genotype for rs3821236 showed no significant association for the T1D susceptibility under a codominant model (OR=1.431, 95% CI=0.84-2.43). The GC genotype of rs10181656 showed a higher risk for T1D susceptibility (OR=2.079, 95% CI=1.16-3.71) under a codominant model (GC vs. GG+CC).

DISCUSSION

T1D is a pediatric endocrine disorder, and its incidence has constantly increased among children and adolescents worldwide (Mayer et al. 2017). In a research study, the mean age of diagnosis of T1D is 14 years among children, which increases by three to four percent annually in the United States (Farsani et al. 2017). In a Chinese study, the peak incidence of T1D was 46.35% in the older age group from 10 to 14 years (Xin et al. 2010). In Pakistan, 24.4% of T1D patients were diagnosed between the age of 10 to 14 years (Condie, Allen & Ogle 2020). However, in the present study, the mean age of T1D patients was 11.80 ± 6.46 years, with equal sex distribution, representing 37% in both males and females.

The most common symptoms presenting T1D among children were polyuria (39%), polydipsia (49%), and weight loss (26%); however, in some populations where cousin marriage is more common Asian, and Middle East populations, the frequency of polyuria, dipsia,

and weight loss is high which may be due to increased severity of the disease (Al-Yaarubi et al. 2014). DKA is a serious, lethal complication that may be the leading cause of mortality among children with T1D. It has been shown that 42% of T1D patients presented with DKA complications, with a higher incidence of infections (Xin et al. 2010). DKA is less frequently present in the T1D patients of the Omani population (31%) as compared to other diabetic centers in the Middle East (Al-Yaarubi et al. 2014). However, this study determined a lower incidence (3%) rate of DKA in T1D patients of Pakistan.

Higher HbA1c variability in T1D adolescents predicts retinopathy, early nephropathy, and other known risk factors (Virk et al. 2016). In another study, the risk of retinopathy and nephropathy was the same, but it increased the severe hypoglycemia as the HbA1c rose from 6.5 to 6.9 percent. However, the risk for milder consequences of retinopathy and nephropathy increased when HbA1c levels increased to 7.0 percent, and the risk for severe complications of retinopathy and nephropathy mostly occurred at 8.6 percent HbA1c levels (Lind et al. 2019). On the contrary, the results of this study report that T1D patients had elevated levels of HbA1c (11.51± 7.33) compared to control (4.89 ±0.39), which may be related to the enhanced risk of diabetes complications.

To the best of our knowledge, this is the first study that investigated the relationship between rs7574685, rs10181656, and rs3821236 in *STAT4* and T1D susceptibility in the children of Pakistan. It was found that *STAT4* rs7574865 polymorphism is significantly associated with T1D patients, indicating that the locus may work as a crucial agent of the disease. In addition, putative functional variants, or haplotypes tagged by rs7574865, could be responsible for a biological effect on intragenic RNA or other factors. The finding of this study

TABLE 1. Baseline characteristics of Type 1 diabetes cases and control groups

Parameters	Case	Control	P-value
Sample Size (n)	155	105	NA
Male/Female (n (%))	78 (38)/77 (37)	53(51)/52 (50)	NA
Age (Year) (Mean ± SD)	11.80 ± 6.46	11.58 ± 3.06	0.90
Age of onset of disease (Year) (Mean ± SD)	5.46 ± 3.89	NA	NA
HbA1c (%) (Mean ± SD)	11.51 ± 7.33	4.89 ± 0.39	<0.01*
Fasting Blood Sugar (mg/dl) (Mean ± SD)	258.86 ± 130.86	87.12 ± 7.30	<0.01*
Consanguinity n (%)	70 (34)	NA	NA
Family History of Diabetes n (%)	72 (35)	NA	NA
Insulin Dose n (%)	144(69.9)	NA	NA

*Independent T-test was applied for continuous data and P<0.05 means a significant difference between T1D cases and control the group. NA=Not Applicable

TABLE 2. Genotypic and allelic distributions of *STAT4* polymorphisms (rs7574685, rs10181656, rs3821236)

Model	Genotype/Allele	Case n (%)	Control n (%)	OR (95%CI)	P-value
General	**rs7574865**				
	GG	63 (30.6%)	57 (54.3%)	-	-
	GT	55 (26.7%)	33 (31.4%)	2.23 (1.10-4.48)	0.02*
	TT	37 (18%)	15 (14.3%)	1.48 (0.70-3.09)	0.29
Additive	Allele				
	T	102 (40.2%)	47 (26.4%)	1.87 (1.23-2.83)	0.003*
	G	152 (59.8%)	131(73.6%)	-	-
Dominant	GG/GT+TT	62(44.3)/92(65.7)	57(40.7)/48(34.3)	1.73 (1.05-2.86)	0.03*
Recessive	TT/GG+GT	37(26.4)/117(83.6)	15(10.7)/90(64.3)	0.532 (0.27-1.02)	0.06
Co-dominant	GT/GG+TT	55(39.3)/99(70.7)	33(23.6)/72(51.4)	0.833 (0.49-1.41)	0.49
General	**rs10181656**				
	GG	59 (28.6%)	40 (38.1%)	-	-
	GC	28 (13.6%)	38 (36.2%)	1.83 (1.01-3.32)	0.04*
	CC	68 (33.0%)	27 (25.7%)	3.14 (1.61-6.13)	0.001*
Additive	Allele				
	C	250 (65.8%)	73 (42.4%)	2.60 (1.80-3.77)	0.001*
	G	130 (34.2%)	99 (57.6%)	-	-
Dominant	GG/GC+CC	58(41.4)/96(68.6)	36(25.7)/69(49.3)	1.029 (0.61-1.71)	0.91
Recessive	CC/GG+GC	68(48.6)/86(61.4)	43(30.7)/62(44.3)	0.443 (0.25-0.76)	0.01*
Co-dominant	GC/GG+CC	28(20.0)/126(90.0)	26(18.6)/79(56.4)	2.079 (1.16-3.71)	0.01*
General	**rs3821236**				
	GG	97 (47.1%)	30 (28.6%)	-	-
	GA	44 (21.4%)	75 (71.4%)	0.23 (0.11-0.48)	0.000*
	AA	14 (6.8%)	-	0.41 (0.18-0.90)	0.027*
Additive	Allele				
	A	50 (18.9%)	73 (42.4%)	0.31(0.20-0.48)	0.001*
	G	214 (81.1%)	99 (57.6%)	-	-
Dominant	GG/GA+AA	96(68.6)/58(41.4)	35(25.0)/70(50.0)	0.378 (0.22-0.63)	<0.01*
Recessive	AA/GG+GA	14(10.0)/140(100)	38(27.1)/67(47.9)	3.486 (1.72-7.03)	<0.01*
Co-dominant	GA/GG+AA	44(31.4)/110(78.6)	32(22.9)/73(52.1)	1.431 (0.84-2.43)	0.18

*The binary logistic regression test was applied, P<0.05 means significant association with type 1 diabetes. For rs7574865: Dominant model (GG vs GT+TT), Recessive model (TT vs GG+GT), Co-dominant model (GT vs GG+TT); *rs10181656*: Dominant model (GG vs GC+CC), Recessive model (CC vs GG+GC), Co-dominant model (GC vs GG+CC); *rs3821236*: Dominant model (GG vs GA+AA), Recessive model (AA vs GG+GA), Co-dominant model (GA vs GG+AA)

might help understand the mechanism of T1D pathogenesis (Bi et al. 2013). Similarly, a research study has shown that the polymorphism rs7574865 is related to the prevalence of T1D among Egyptians (Abdelmajed et al. 2021) and Asian and European populations (Liang et al. 2012). Additionally, a meta-analysis study on Caucasians and Asian participants showed that rs7574865 was found to have a relationship with the risk of diabetes development (Yi et al. 2015). Also, another study showed that the *STAT4* rs7574865 was overexpressed in T1D Polish patients (Fichna et al. 2020).

In autoimmune disorders like rheumatoid arthritis (RA)*, STAT4* rs7574865 displayed significant association with that might be due to an alternative splicing or regulatory effect of SNPs in strong disequilibrium with rs7574865 on the *STAT4* gene may occur in RA, which is also confirmed in Spanish, Swedish (Settin, Salama & Elshazli 2014), Mexico (Ramirez et al. 2016), Crete (Zervou et al. 2008), and Dutch patients (Orozco et al. 2008). Additionally, the relationship between *rs7574865* had been demonstrated with autoimmune thyroid disorders in the Chinese Han population (Yan et al. 2014). Asian population's predisposition to AITD, but not the African population (Gao, Wang & Yu 2019). Another study reported a similar association between colonic Crohn's disease (Glas et al. 2010) and systemic sclerosis (Dieude et al. 2009).

This study has confirmed the significant association of rs10181656 polymorphism with increased risk of T1D.

The findings are supported by previous research suggesting that *STAT4* rs10181656 polymorphism is involved in the development of T1D (Lee et al. 2008). Similarly, minor alleles of two SNPs, rs7574865 and rs10181656*,* showed a significant correlation with susceptibility to T1D in the early-onset subgroup of the Korean population (Lee et al. 2008). Moreover, *STAT4* rs10181656 polymorphism has also been studied in various autoimmune disorders. An earlier investigation found a strong correlation of rs10181656 with NOSD in the Chinese population (Shi et al. 2017), rheumatoid arthritis patients in the Swedish population (Seddighzadeh et al. 2012), SLE in the Caucasians Finnish population (Hellquist et al. 2010) and psoriatic arthritis in the Greek population (Myrthianou et al. 2017).

The rs3821236 SNP of *STAT4* is not significantly associated with T1D, which might be due to the heterogeneity in the genetic background of the studied groups. Conversely, rs3821236 is strongly related to T2D risk across several subgroup studies in the Chinese Han population (Cui et al. 2021). The genetic diversity of the Pakistani population in comparison to other ethnic populations must be considered because it could have a significant impact on the complex trait of T1D.
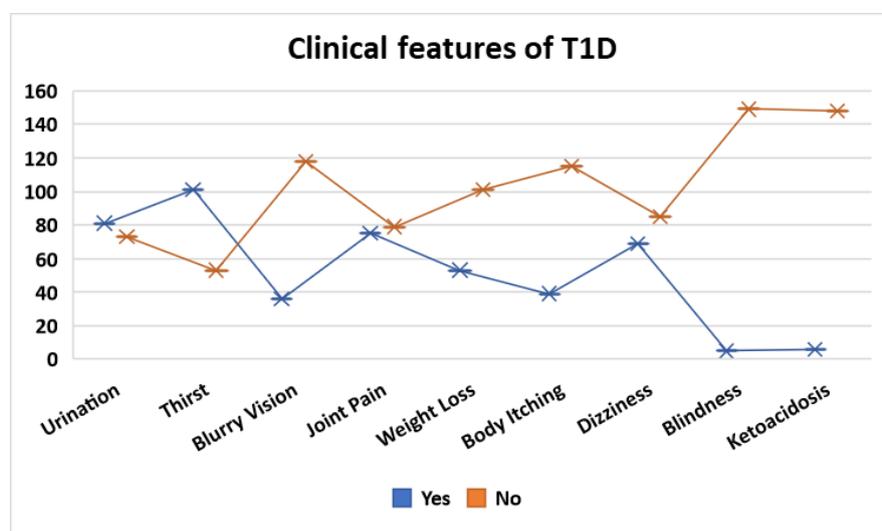

FIGURE 1. Clinical features of T1D cases including urination (39%), Thirst (49%), blurry vision (18%), joint pain (36%), weight loss (26%), body itching (19%), dizziness (34%), blindness (2%) and ketoacidosis (3%) among them

Conclusion

In conclusion, this is the first report of genetic association studies of *STAT4* polymorphisms (rs7574685, rs10181656, and rs3821236) in Pakistani children with type 1 diabetes. Results of this study showed that the frequency of the T allele in rs7574685 and the C allele in rs10181656 of the *STAT4* gene are significantly associated and have high potential with the risk of T1D children. These results are consistent with previous reports in other ethnic populations, suggesting that polymorphisms of *STAT4* may play an important role in T1D susceptibility. This is a multifactorial disease; therefore, further studies should be conducted to determine the genetic factors associated with T1D.

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