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Original article

Genetic association of PTPN22 polymorphisms with Type 1 diabetes in Pakistani children

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ABSTRACT

Objective: Type 1 diabetes, a multigenic autoimmune disorder, is caused by the destruction of pancreatic beta-cells leads to insufficient insulin production and hyperglycemia, resulting in early morbidities and mortality. This study was designed to explore the genetic association of *PTPN22* gene polymorphisms with T1D susceptibility among Pakistani children.

Methods: Blood samples of T1D patients were obtained from the Department of Diabetes and Endocrinology of Children Hospital & University of Child Health Sciences, Lahore. Genotyping of rs2476601, rs1310182, and rs1217414 of the *PTPN22* gene was performed by Tetra ARMS-PCR assay. Statistically, binary logistic regression was applied to determine variation in genotype distribution and association of *PTPN22* gene polymorphism with T1D.

Results: Genetic analysis showed that the A allele of rs2476601 (OR = 0.53, 95 % CI = 0.31–0.90; P < 0.02) and T allele of rs1310182 was found to be risk allele for T1D development (OR = 0.51, 95 % CI = 0.36–0.76; P < 0.01) while the A allele of rs1217414 was a protective allele against T1D (OR = 1.19, 95 % CI = 0.80–1.77; P = 0.36). Genetic models revealed that GG genotypes of rs2476601 (OR = 2.01, 95 % CI = 1.13–3.58; P < 0.01), and AA genotypes of rs1310182 in the dominant model (OR = 1.83, 95 % CI = 1.03–3.24; P < 0.03) showed significant risk association with T1D.

Conclusion: From the results, it is concluded that PTPN22 gene has a strong genetic association with SNP rs2476601 and rs1310182 with T1D in Pakistani children.

1. Introduction

Type 1 Diabetes (T1D) is a chronic immune-mediated disease and is characterized by the damage of the endocrine pancreatic beta-cells, which results in insulin insufficiency and may induce early morbidities and mortality in children (Kiani et al., 2015). According to the International Diabetes Federation, the prevalence of T1D has increased from 5 % to 10 % of all cases of diabetes (Goyal et al., 2020). In Pakistan, less than 2 % of the T1D prevalence has been reported (Shera et al., 2008).

Environmental and genetic factors both have an impact on the development of T1D (Fung et al., 2009). There are more than 60 genes and loci have been linked to T1D (Xie et al., 2020). Genetic risk factors and susceptibility loci of T1D have been discovered by Genome-wide association studies (Storling et al., 2017) including *INS*, *STAT4*, *CTLA-4*, *IL-2RA*, *PTPN22*, and *IFIH1* (Kiani et al., 2015). The *PTPN22* gene is one of the strongest genetic candidate genesthat has been identified for T1D susceptibility and revealed a possible relationship with the clinical heterogeneity of the disease which makes it a potential therapeutic target (Prezioso et al., 2017).

The protein tyrosine phosphatase, non-receptor type 22 (PTPN22) is a powerful inhibitor of T-cell activation and negatively regulates the signaling of T or B-cell receptors (Welter et al., 2018). PTPN22 protein is expressed in many immune cells, such as T and B lymphocytes, dendritic, and natural killer cells. It is known as the third important genetic locus that increases the likelihood of developing T1D (Hasegawa et al.,

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2004) and other autoimmune disorders such as rheumatoid arthritis, Type 1 diabetes, and systemic lupus erythematosus, etc. (Namjou et al., 2013).

Several *PTPN22* polymorphisms including rs1310182, rs1217414, rs33996649, rs1217419, rs2476601, and rs12760457 have been reported to be associated with T1D (Prezioso et al., 2017; kumar et al., 2014). Many studies have revealed that rs2476601 is found to be a significant SNP for T1D risk in the European (Smigoc Schweiger et al., 2019), Middle East (Sharma et al., 2021), and Asian populations (Pei et al., 2013). The rs2476601 SNP is a risk factor for various autoimmune diseases too (Dieude et al., 2008).

In Pakistan, the incidence of T1D doubled from the previous report (Condie et al., 2020). A genetic study was conducted in Pakistan to identify 32 T1D gene loci that had previously been reported in European populations (Kiani et al., 2015). However, the role of *PTPN22* polymorphisms has not yet been investigated for their effects on T1D patients in the Pakistani population. Therefore, this study was designed to explore the role of PTPN*22* polymorphisms of rs2476601, rs1310182, and rs1217414, and the susceptibility of T1D in Pakistani children.

2. Materials and methods

2.1. Ethical approval

Ethical approval was obtained from the Ethical Review Board (ERB) of Lahore College for Women University (ORIC/LCWU/22/07), and Department of Endocrinology and Diabetes of Children Hospital, and the University of Child Health Sciences in Lahore, Pakistan (2021–270-CHICH). In compliance with the Declaration of Helsinki's rules governing human testing, written informed consent from participants and their parents was obtained.

2.2. Inclusion and exclusion criteria

In this case-control study, one hundred and fifty-five unrelated T1D participants and one hundred and five apparently healthy individuals were included. Inclusion criteria for T1D patients were insulin therapy dependent and had positive autoantibodies (glutamic acid decarboxylase, protein tyrosine phosphatase antibody, or zinc transporter 8 antibody) detected in their serum. Exclusion criteria were that participants who had cancer or any other autoimmune illness were also excluded.

2.3. Demographic data and clinical investigation

Demographic information regarding age, sex, and duration of disease onset was recorded. The clinical characteristics encompassing polyuria, polydipsia, blurry vision, lightheadedness, joint discomfort, blindness, weight reduction, and ketoacidosis, were also documented. The levels of fasting blood glucose (FBG) and glycated hemoglobin (HbA1c) were assessed using an automated chemiluminescence technique (Alinity C, Abbott, USA).

2.4. Blood sample collection, DNA isolation, and quantification

A five-milliliter of venous blood sample was drawn using purple caps vacutainer® tubes (Becton Dickinson NJ, USA), which serve as an anticoagulant for collecting the blood samples. Total DNA was isolated by a non-organic method (Grimberg et al., 1989). The concentrations of DNA were assessed and analyzed with NanoDrop (NanoDrop 2000/2000c, Thermo ScientificTM).

2.5. SNPs selection and genotype assessment

Three SNPs (rs2476601, rs1310182, and rs1217414) were selected and Tetra ARMS (allele-specific) primers were designed by primer 1 software, http://primer1.soton.ac.uk/primer1.html (available upon request). For genotype assessment, Tetra ARMS-PCR assay was used, followed by allele calling 2 % agarose gel electrophoresis (Medrano and De Oliveira 2014; Muhammad et al., 2021).

2.6. Statistical analysis

T-test was used to determine the mean difference between T1D cases and the control group. Using a chi-squared goodness-of-fit test, genotype distributions for the controls were examined for Hardy-Weinberg equilibrium (HWE). To further investigate the variations in genotype distributions, three genetic models: dominant, recessive, and codominant models were applied depending on the genotype frequencies of each locus. Using binary logistic regression analysis, the genotype and allele frequencies for each genetic model were statistically compared between the T1D patients and control groups. In all statistical assessments, a p-value below 0.05 (with a 95 % confidence interval) was considered statistically significant.

3. Results

In the current study, the average age of diagnosis of the T1D cases and control groups was 11.80 ± 6.46 and 11.58 ± 3.06, respectively (Table 1). The biochemical analysis revealed that participants with T1D had remarkably higher levels of fasting blood glucose (258.86 ± 130.86 vs. 87.12 ± 7.30; $P < 0.001$) and glycated hemoglobin (HbA1c) (11.51 ± 7.33 vs. 4.89 ± 0.39; $P < 0.001$) when compared to the control group. The analysis of clinical data was depicted in Fig. 1.

3.1. Allelic and genotypic frequencies of PTPN22 polymorphisms

In rs2476601, higher GG (65 %) and AG (30 %) genotype frequency were observed in T1D patients compared to control (15 % and 6 %, respectively) while lower AA (5 %) genotype frequency was observed in T1D cases when compared to control (100 %). According to the regression analysis, the GG genotype of rs2476601 was set as a reference category, found that the homozygous AA genotype was discovered to be a protective allele against T1D (OR = 0.39, 95 % CI = 0.11–1.35; P = 0.94), while the risky A allele exhibited a high connection with T1D (OR = 0.95, 95 % CI = 0.31–2.96; $P < 0.02$).

For rs1310182, the frequency of the AA genotype was lower (20 %) in T1D than in controls (31 %). In addition, lower AT genotype frequency (31 %) and higher TT genotype frequency (49 %) were found in the T1D cases compared to controls (40 % and 29 %) indicating AT and TT allele was risky for T1D pathogenesis. The heterozygous AT and homozygous TT genotype of rs1310182 showed a significant relationship with an increased risk of T1D (OR = 2.69, 95 % CI = 1.41–5.15; P <

Table 1
Demographic Data and Clinical Characteristics of T1D 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

Data was expressed as frequency and percentage. *Independent T-test was applied for continuous data and $P < 0.05$ means a significant difference between T1D cases and the control group. NA: Not applicable (see Table 1).

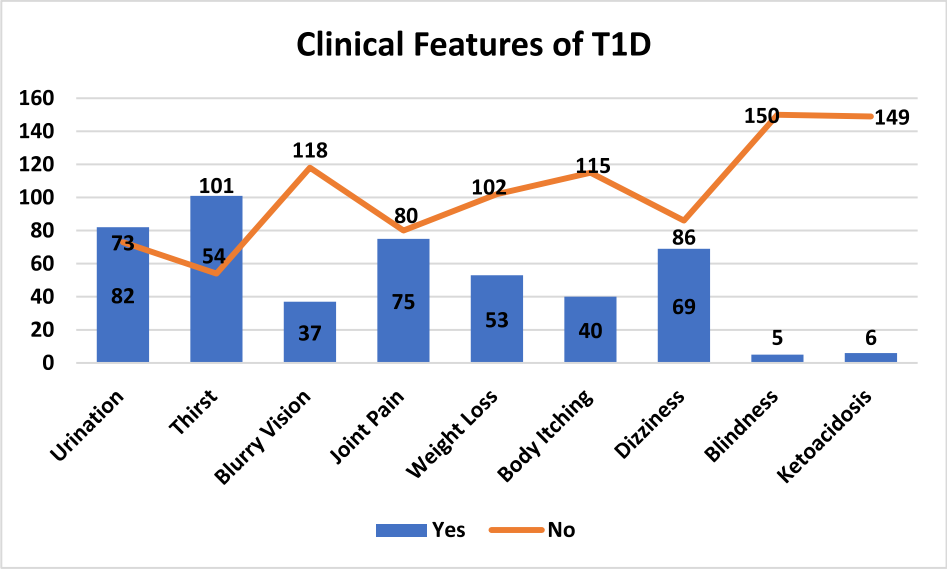

Fig. 1. Clinical characteristics of T1D cases.

0.01; OR = 2.21, 95 % CI = 1.22–4.00; P < 0.01).

In rs1217414, the GG (61 %) and AA (12 %) genotypes were slightly lower in T1D cases than in control (57 % and 15 %, respectively). All the genotypes of rs1217414 did not have significant differences in their frequencies in cases and the healthy control group. In contrast, the heterozygous AT genotype of rs1217414 was protective against T1D (Table 2).

3.2. Association analysis of PTPN22 polymorphisms and statistical models

The GG genotype of rs2476601 (OR = 2.01, 95 % CI = 1.13–3.58; P < 0.01) exhibited a significant connection with T1D in the dominant model. However, the AG and AA genotype was not significantly associated with T1D risk under the recessive (OR = 0.41, 95 % CI = 0.21—0.77, P = 0.01) and co-dominant models (OR = 1.28, 95 % CI = 0.41–3.92, P = 0.66). For rs1310182, the dominant model demonstrated a significant relationship between the AA genotype and T1D susceptibility (OR = 1.83, 95 % CI = 1.03–3.24; P < 0.03). However, the recessive (OR = 0.41, 95 % CI = 0.24–0.70; P < 0.01) and co-dominant models did not show such association with T1D (OR = 1.48, 95 % CI = 0.88–2.49; P = 0.13). For rs1217414, the GG, AG, and AA genotypes did not exhibit any significant link with T1D susceptibility, according to dominant (OR = 0.86, 95 % CI = 0.52–1.43; P = 0.57), recessive (OR = 1.36, 95 % CI = 0.66–2.82; P = 0.39), and co-dominant models (OR = 0.99, 95 % CI = 0.57–1.72; P = 0.98).

4. Discussion

Type 1 Diabetes is a heterogeneous genetic disorder, multiple genes and their interactions are responsible for disrupting the mechanism of glucose metabolism. Since the *PTPN22* gene is involved in immune cell signaling and regulation, consequently, genetic variation of the *PTPN22* gene may contribute to the T1D pathogenesis (Blasetti et al., 2017). Previously, it is reported that polymorphisms of the *PTPN22* gene are genetically associated with type 1 diabetes (Abbasi et al., 2017; Haider et al., 2018; Smigoc Schweiger et al., 2019). Therefore, this study was designed to investigate whether these polymorphisms (rs2476601, rs1217414, and rs1310182) were involved in increasing the risk of T1D or not among children.

In the present study, polyuria, polydipsia, joint pain, dizziness, and weight loss are the most common clinical features observed in affected children, and these symptoms are predominant in Asian and Middle Eastern populations, possibly due to a higher rate of consanguinity (Al-Yaarubi et al. 2014). Diabetic Ketoacidosis (DKA) is another severe and life-threatening complication that can result in death among children. The prevalence of DKA has been reported to vary widely, ranging from 10 % to 80 % in different populations (Shera et al., 2008; Al-Yaarubi et al., 2014). However, in Pakistani children, the frequency of ketoacidosis is comparatively low (3 %). One reason might be the deprived diagnostic facility and the unawareness of Pakistani parents.

This study showed that the AG genotype of rs2476601 of the *PTPN22* gene showed a statistically significant (OR (95 % CI) = 0.95 (0.31–2.96); P < 0.02) association among T1D Pakistani children that might be involved with an increased risk of developing disease. A allele is found to be a risky allele (OR (95 % CI) = 0.53 (0.31–0.90); P < 0.02) for T1D development, which may be a consequence of dysregulation of the immune response, making beta cells more susceptible to the autoimmune attack (Blasetti et al., 2017). rs2476601 SNP has been identified as a pathogenic mutation (c.1858C > T) that encodes an amino acid substitution from arginine to tryptophan (R620W). This substitution disrupts the Lyp-Csk interaction, leading to reduced T-cell signaling and ultimately diminishing T-cell activation, which results in early-onset diabetes (Sharma et al., 2021). In addition, linkage disequilibrium analysis showed that rs2476601 SNP is the best candidate for T1D susceptibility in the European population (Smyth et al., 2008). However, no significant association is reported in China, and Japan, which may be due to heterogeneity between ethnic backgrounds (Pei et al., 2014; Taniyama et al., 2010). Additionally, several research studies have shown that rs2476601 plays an essential role in other autoimmune diseases (Piotrowski et al., 2008; Cinek et al., 2007).

TT genotypes of *PTPN22* rs1310182 were significantly associated with increased T1D susceptibility (OR (95 % CI) = 0.53 (0.31–0.90); P < 0.02) that might affect its splicing mechanisms, leading to unusual *PTPN22* expression contribute to T1D susceptibility. In the same way, the haplotypes of rs1310182 SNP are associated with T1D risk reported in the Japanese population (Taniyama et al., 2010) andother autoimmune disorders (Aflatounian et al., 2017).

AG and AA genotypes of rs1217414 were found to be aprotective variant against T1D risk. The findings of this study suggest that the intronic SNP (rs1217414) within the *PTPN22* gene might potentially impact the splicing processes of PTPN22 leading to abnormal PTPN22 expression, potentially influencing the vulnerability to T1D. Although our data showed a significant association between the *PTPN22*

Table 2
Allelic and genotypic distributions, and association analysis of *PTPN22* gene polymorphisms (*rs2476601, rs1310182,* and *rs1217414*) in T1D cases and control group.

Model	Genotype/ Allele	T1D Cases	Control	OR (95 % CI)	P-value
	rs2476601				
General	GG	101(65)	16(15)	–	–
	AG	47(30)	6(6)	0.95 (0.31–2.96)	0.020*
	AA	7(5)	105(100)	0.39 (0.11–1.35)	0.940
Additive	G	202(77)	142(86)	–	–
	A	61(23)	23(14)	0.53 (0.31–0.90)	0.020*
Dominant	GG/AG + AA	101 (65)/54 (35)	83(79)/ 22(21)	2.01 (1.13–3.58)	0.010*
Recessive	AA/AG + GG	7(5)/ 148(96)	6(6)/99 (94)	1.28 (0.41–3.92)	0.660
Codominant	AG/AA + GG	47(30)/ 108(70)	16(15)/ 89(85)	0.41 (0.21–0.77)	0.006*
	rs1310182				
General	AA	31(20)	33(31)	–	–
	AT	48(31)	42(40)	2.69 (1.41–5.15)	0.004*
	TT	76(49)	30(29)	2.21 (1.22–4.00)	0.003*
Additive	A	110(36)	108(51)	–	–
	T	200(65)	102(49)	0.51 (0.36–0.74)	0.000*
Dominant	AA/AT + TT	31(20)/ 124(80)	33 (31)/ 72(69)	1.83 (1.03–3.24)	0.037*
Recessive	TT/AT + AA	76(49)/ 79(51)	30(29)/ 75(71)	0.41 (0.24–0.70)	0.001*
Codominant	AT/TT + AA	48(31)/ 107(69)	42(40)/ 63(60)	1.48 (0.88–2.49)	0.134
	rs1217414				
General	GG	94(61)	60(57)	–	–
	AG	43(28)	29(28)	0.71 (0.34–1.51)	0.686
	AA	18(12)	16(15)	0.75 (0.33–1.72)	0.385
Additive	G	231(75)	149(71)	–	–
	A	79(26)	61(29)	1.19 (0.80–1.77)	0.369
Dominant	GG/AG + AA	94(61)/ 61(39)	60(57)/ 45(43)	0.86 (0.52–1.43)	0.573
Recessive	AA/AG + GG	12(18)/ 137(88)	16(15)/ 89(85)	1.36 (0.66–2.82)	0.396
Codominant	AG/AA + GG	28(43)/ 112 (72)	29(28)/ 76(72)	0.99 (0.57–1.72)	0.983

Data was expressed as frequency (percentage). OR = Odds Ratio. CI = Confidence Interval. *The binary logistic regression test was applied, $P < 0.05$ means a significant association with type 1 diabetes. For rs2476601: Dominant model (GG vs AG + AA), Recessive model (AA vs GG + AG), Co-dominant model (AG vs GG + AA); rs1310182: Dominant model (AA vs AT + TT), Recessive model (TT vs AA + AT), Co-dominant model (AT vs TT + AA); rs1217414: Dominant model (GG vs AG + AA), Recessive model (AA vs AG + GG), Co-dominant model (AG vs AA + GG). The dashed line (-) indicates a reference category.

polymorphisms and T1D risk, extensive studies with large sample sizes of different ethnicities residing in different areas of Pakistan are required to detect the genetic variants impacting multifactorial traits of T1D in Pakistan.

5. Conclusion

The findings of this study revealed that the A allele of rs2476601 and the T allele of rs1310182 of the *PTPN22* gene are significantly associated and have a high potential with the risk of T1D children. Results of this studymay help in a better understanding of T1D and will pave the way for improved detection and treatment to prevent complications related to T1D in children.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2023.102967.

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