The Relationship between T1DM and the Polymorphisms of IL2RA (Rs2104286) and PTPN22 (Rs2476601) Genes among Children

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ABSTRACT

Background: Type 1 diabetes mellitus (T1DM) is an autoimmune condition caused by T lymphocytes that results in the death of pancreatic cells. Protein tyrosine phosphate non-receptor type 22 (PTPN22) and interleukin 2 receptor alpha (IL2RA) polymorphisms have been discovered to have a connection to a number of autoimmune illnesses, including T1D.

Objective: The purpose of this study is to clarify the significance of the polymorphisms PTPN22 (rs2476601) and IL2RA (rs2104286) in the susceptibility to T1DM in young Iraqi children.

Methods: The study included 60 Iraqi children diagnosed with T1DM within the past three years and a control group of 30 healthy individuals without diabetes or autoimmune diseases. In order to conduct a molecular investigation. Five ml of venous blood from 90 participants (60 patients and 30 controls) was collected for nucleic acid extraction.

Results: The PTPN22 (rs2476601) and IL2RA (rs2104286) polymorphisms were genotyped using the amplification refractory mutation system (ARMS) technique and specific primers. According to the results, PTPN22 (rs2476601) has a wild-type homozygous C/C genotype and C allele frequency of 90%, a mutant C/T genotype frequency of 10%, and no T allele. Following were the genotype frequencies for IL2RA (rs2104286): AA, AG, and GG in T1DM patients were 79%, 16%, and 4%, respectively, compared to 83%, 13%, and 3% in controls.

Conclusion: The polymorphisms of PTPN22 rs2476601 and IL2RA rs2104286 did not significantly differ in their connection with type 1 diabetes. It does not appear to affect the susceptibility of Asian Iraqis to T1D.

KEYWORDS: Protein tyrosine phosphates non-receptor type 22 (PTPN22), Interleukin 2 receptor alpha (IL2RA), polymorphisms, Type 1 diabetes mellitus (T1D).

INTRODUCTION

Type 1 diabetes (T1D) is an autoimmune condition caused by autoreactive T lymphocytes specifically destroying pancreatic (β-cells) ¹. Only 5–10% of people with diabetes are thought to have T1DM, according to estimates ². Although type 1 diabetes has traditionally been thought of as having a youthful onset and significant familial patterns ³. It is now thought that 90% of people with type 1 diabetes do not have a family history of the condition. Teenagers are often the first to notice patients with type 1 diabetes, and having first-degree relatives with the disease raises the risk. Contrary to other autoimmune diseases, type 1 diabetes has a roughly similar risk for both men and women ⁴. As a result of T-cell mediated autoimmune destruction of pancreatic β-cells of the islets of Langerhans, type 1 diabetes often presents as inadequate absorption of glucose from the bloodstream, which causes tiredness, polyuria, polydipsia, and hyperglycemia ⁵. Before Banting and Best's 1921 discovery of insulin, the condition proved lethal. The only treatments now available are exogenous insulin administration and islet transplantation ⁶. T1D is a multigenic autoimmune disease that affects a particular organ and is characterized by the T-cell-dependent
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death of pancreatic β-cells. This includes the defective function of various populations of regulatory T cells (Tregs). Numerous investigations have shown that newly diagnosed T1D patients had much lower T cell suppression than was reported in control persons. Although there are uncommon monogenic variants of T1D, which are thought to result from the actions and potential interactions of numerous genetic and environmental risk factors, the typical variety is considered to be genetically complex. Molecular, clinical, and epidemiological research all point to a common genetic predisposition to T1D. The genes PTPN22 and IL2AR are part of the system that regulates T cell activation and tolerance to self-antigens. PTPN22 resides on chromosome 1p13.2 and comprises 24 exons. Exon 14 of the PTPN22 gene has the polymorphism rs2476601, which is linked to T1D and other autoimmune illnesses. Genetic association studies were used to evaluate PTPN22’s early-stage functional impact on autoimmune disorders. The missense single nucleotide polymorphism (SNP) C1858T, rs2476601, was found to be particularly associated with type 1 diabetes mellitus, systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA).

IL2RA, found on the 10p15 chromosome, codes for the beta-chain of the (IL-2R) complex (also known as CD25). IL2RA Regulatory T cells need to express IL2RA in order to block T cell immune responses to pathogen-derived antigens, tumor antigens, autoantigens, and alloantigens. This study is aimed to investigate the relationship between T1DM in patients from the Iraqi population and the IL2RA rs2104286 polymorphism and the (PTPN22) C1858T polymorphism.

MATERIALS AND METHODS

The study designs

This study was carried out from the beginning of November 2022 to the end of March 2023. A total of 60 blood samples were collected from diagnosed T1DM children who attended the Thi-Qar Specialized Center for Diabetes and Endocrinology hospital in Iraq.

Inclusion criteria

Type 1-diabetic patients’ ages ranged between 3 and 15 years. They had a similar drug protocol that included insulin. In addition, they had a disease period of less than 3 years.

Exclusion criteria

People were not allowed to participate in the study if they had concurrent immune-mediated diseases, were receiving chemotherapy, had type 2 diabetes, or had the disease for more than three years. People with type 1 diabetes who were younger than three years old or older than 15 years old were also not allowed to participate.

Ethical Considerations

Basra Health Institute’s Ethics Research Committee granted authorization for the study to be carried out at Thi-Qar province and the Specialized Center for Diabetes and Endocrinology with permission number 2022/11/1. The parents or guardians gave informed consent before the potential subjects were included, indicating that they understood and agreed to take part in the study.

Statistical Analysis

The data in this investigation was shown as a mean standard deviation. A combination of SPSS and GraphPad Prism® (version 9.5.1) was used to conduct the statistical analyses. t-test that is normally distributed and independent (two-tailed). *P<0.05, *P<0.01 and *P<0.001 denote statistical significance used the Chi-Square test as well.

Participants and blood collection

A case-control study involved a total of 60 Iraqi children (27 males and 33 females) who were diagnosed with type 1 diabetes (T1D) at Thi-Qar Specialized Center for Diabetes and Endocrinology. The children included in the study were aged from 3 to 15 years, and they were all given T1D diagnoses before turning 15 years old. They were also dependent on insulin to manage their condition. The diagnosis of T1D was based on the World Health Organization’s (WHO) diagnostic criteria, which involved evaluating blood glucose levels. The participants provided information regarding their demographic details, clinical symptoms, the existence of other autoimmune diseases, and hemoglobin A1C levels. For the control group, 30 healthy individuals without T1D, autoimmune conditions, or a T1D family history were taken into account. In order to conduct a molecular investigation, five ml of venous blood from 90 participants (60 patients and 30 controls) was collected for further nucleic acid extraction.

Laboratory Methods

DNA extraction:

Using the Geneaid Genomic DNA Extraction Kit (Taiwan), genomic DNA was extracted using a conventional procedure from EDTA whole blood in accordance with the manufacturer’s instructions.

Genotyping of PTPN22 and IL2RA polymorphism

Genotyping for the PTPN22 C1858T SNP (rs2476601) and SNP was done from patients and a control sample (rs2104286). SNPs can be genotyped easily and affordably by employing the tetra-primer amplification refractory mutation system-polymerase chain (ARMS-PCR). In a single PCR, four primers are used in a final volume of 25 liters. The PCR reaction mixture contained 13 liters of master mix, 2 liters each of forward and reverse primers (outer and inner), 4 liters of nuclease-free water, and 4 liters of template DNA. Conditions included initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 62 °C for 60 s, and 72 °C for 60 s, and a final extension at 72 °C for 5 min, followed by gel electrophoresis.
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Table 1: IL2RA and PTPN22 primers and PCR conditions, including the PCR program utilized in the thermo-cycler

<table>
<thead>
<tr>
<th>SNP &amp; Gene</th>
<th>Primer Name</th>
<th>Primer Sequence</th>
<th>Annealing Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP: rs2104286</td>
<td>IL2RA-outerF</td>
<td>ATCTATCCAATATCTCTCATGCCCCTTT</td>
<td></td>
</tr>
<tr>
<td>Gene: IL2RA</td>
<td>IL2RA-outer R</td>
<td>CCCATGCTCAGTAGATCTTACCACAGAC</td>
<td>64 °C</td>
</tr>
<tr>
<td></td>
<td>IL2RA-inner F</td>
<td>AAAATTCTACACAAGCAAAACACAGC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL2RA-inner R</td>
<td>GCAATAGATATAGTCATGGTAACACAGGCA</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td>Reference 13</td>
<td></td>
</tr>
<tr>
<td>SNP: rs2476601</td>
<td>PTPN22-outer F</td>
<td>CTCACACATCAGCTTCCAAAAAGTG</td>
<td></td>
</tr>
<tr>
<td>Gene: PTPN22</td>
<td>PTPN22-outer R</td>
<td>CAACTTTACTGATAATGTGTCTTTACAACAGGA</td>
<td>62.5 °C</td>
</tr>
<tr>
<td></td>
<td>PTPN22-inner F</td>
<td>CAACCCCAATAAAATGATTCAGGTGTACG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PTPN22-inner R</td>
<td>ATCCCCCCTCCACTTCCCTGGAT</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td>Reference 14</td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

Characteristics of T1D patients and controls' clinical and laboratory

Regarding sex and age, there was no statistically significant difference between the control and patient groups. Comparing the sick group to the control group, the levels are noticeably greater in the patient group. This distinction was present in. With a $P$-value of 0.001, the patient group's HbA1C level is substantially greater than that of the control groups. With a $P$-value of 0.001, the sick group's fasting blood sugar (FBS) levels are also considerably higher than those of the control group (Table 2).

Table 2. Characteristics of T1D patients and controls' clinical and laboratory.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient group n = 60</th>
<th>Control group n = 60</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean ±SD</td>
<td>9.97 ± 3.56</td>
<td>8.61 ± 3.18</td>
</tr>
<tr>
<td>HbA1C</td>
<td>Mean ±SD</td>
<td>10.2 ± 2.30</td>
<td>5.21 ± 0.258</td>
</tr>
<tr>
<td>FBS (mg/dl)</td>
<td>Mean ±SD</td>
<td>237 ± 140</td>
<td>95.2 ± 12.7</td>
</tr>
<tr>
<td>Sex</td>
<td>Male, n (%)</td>
<td>27 (45 %)</td>
<td>30 (50 %)</td>
</tr>
<tr>
<td></td>
<td>Female, n (%)</td>
<td>33 (55 %)</td>
<td>30 (50 %)</td>
</tr>
</tbody>
</table>

Distribution of T1D patients' and controls' PTPN22 C1858T and IL2RA genotypes and alleles

Table (3) shows that the IL2-RA and PTPN22 genes in both a patient group and a control group and used a Chi-Square test to compare the frequencies of the genotypes and alleles between the two groups. There was no statistically significant difference between the patient and control groups (Figure 1 and 2). Due to our financial inability, we did not examine complete IL2-RA samples (Table 3).
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Table 3. Distribution of the PTPN22 C1858T and IL2RA genotypes and alleles in T1D patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Control (N30)</th>
<th>T1D (N 60)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PTPN22</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC genotype</td>
<td>26</td>
<td>54</td>
<td>0.73 (0.204 to 2.44)</td>
<td>0.635</td>
</tr>
<tr>
<td>CT genotype</td>
<td>4</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C Allele</td>
<td>56</td>
<td>114</td>
<td>0.74 (0.187 to 2.39)</td>
<td>0.645</td>
</tr>
<tr>
<td>T Allele</td>
<td>4</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>IL2-RA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA genotype</td>
<td>25</td>
<td>34</td>
<td>1.28 (0.34 to 4.26)</td>
<td>0.710</td>
</tr>
<tr>
<td>AG genotype</td>
<td>4</td>
<td>7</td>
<td>1.32 (0.44 to 3.18)</td>
<td>0.606</td>
</tr>
<tr>
<td>GG genotype</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure (1) Identified bands after UV transillumination are displayed: The C allele is indicated by the 213 bp bands. The T allele is shown by the 151 bp bands.

Figure (2) After UV transillumination, identifying bands are displayed: The A allele is indicated by the bands of 242 bp. The G allele is shown by the 164 bp.

DISCUSSION
Type 1 diabetes mellitus is a complex and polygenic disease due to gene-environment interactions. There has been a great deal of research done on the genetic basis of diabetes. Final judgments have not yet been drawn, though.

The missense mutation (c.1858T > C) in the P1 domain of the polymorphism rs2476601, also known as R620W (Trp → Arg) or (c.1858T > C), decreases the ability of LYP to bind to the tyrosine kinase Csk. However, it is unclear exactly how this mutation affects the PTPN22 gene's function and increases one's risk of developing an autoimmune illness.

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The context of the current study provides the opportunity to examine and further develop the evidence for this polymorphism in childhood-onset T1DM. It is claimed that rs2476601 of the PTPN22 gene is not connected with children-onset T1DM in the Iraqi population since there was no statistically significant difference between genotypes ($P = 0.635$) and allelic frequencies ($P = 0.645$). Given the conflicting results in other populations, the absence of a significant connection in this study does not necessarily imply that there is no association. Acknowledging the limitation of the sample size, the wild type homozygous C/C genotype and C allele were common (90%) while the mutant C/T genotype was uncommon (only 10%) and the T allele was absent. Results of past studies have been contradictory as regard the association between a PTPN22 C1858T SNP and T1DM in different ethnicity; an association has been found in US 15–17, German 18, Estonian 19, Italian 20, Croatian 21, Emiratis 22 and Russian populations 23. In contrast, studies conducted in Asia found no correlation between the SNP 1858C > T and T1D in any of the Asian countries, including Japan, Korea, China, Indians, and Iranians 24–27 and Egyptians in Africa, they demonstrated a feeble connection between the T allele of the PTPN22 (rs2476601). However, no difference that was statistically significant was discovered 8. However, the C1858T SNP appears to be an uncommon mutation and to have no effect on Asian Iraqi people's susceptibility to T1D. Similarly, other Asians, such as Koreans and Japanese, did not exhibit a relationship between the C1858T SNP and T1D susceptibility. As they did not find the +1858T allele, previously identified PTPN22 risk variant 16 in the screening of SNPs in PTPN22, this SNP was further genotyped in 1,698 participants of Asian descent, including 732 patients with type 1 diabetes, 141 patients with autoimmune thyroid illness, and 825 healthy controls using PCR-RFLP with restriction enzyme XcmI. The wild-type homozygous genotype (C/C) was present in all patients. Direct sequencing of the PCR-amplified product and/or PCR-RFLP with the restriction enzyme Rsai was used to validate the absence of the +1858T allele 24.

Since protein tyrosine phosphatases are crucial for TCR signaling, PTPN22 is a strong candidate gene for T1D from a functional standpoint. Four of the fifteen genotyped markers underwent TDT analysis, and it was discovered that they were positively related to type 1 diabetes 20. It appears plausible to assume that certain other SNPs with potential functional roles could be causing the Asian Iraqi population's increased susceptibility to T1D. The main goal of studying T1D's genetics is to gain knowledge that will help researchers fully comprehend the pathophysiology of the illness and pave the way for the creation of more traditional immunological therapies to stop β-cell death. Rs2104286 are located at the 5-proximal intron 1 region of the IL2RA. The IL-2/IL-2RA pathway is crucial for controlling immunological reactions. High amounts of soluble IL-2RA are present in healthy individuals and are elevated in those with autoimmune illnesses, infections, and inflammation. IL-2 is essential for both the growth and death of T cells. Furthermore, it showed that individuals with the rs2104286 risk haplotype had higher CD25 expression in naïve Treg and reduced pSTAT5 expression 28.

The results of the present study revealed the following genotype frequencies: AA, AG, and GG were present in 79 percent, 16 percent, and 4 percent of T1DM patients, compared to 83 percent, 13 percent, and 3 percent of controls. However, $P = 0.710$ indicated that these results were not statistically significant. The same was clear with respect to the IL2RA rs2104286 polymorphism's low frequency of the A allele ($P = 0.606$). Therefore, in the population of Iraq, it is hypothesized that the IL2RA gene variant rs2104286 is not connected to child-onset T1DM (Table 3). This result was discovered to be compatible with some related research investigations that indicated there was no discernible link between the studied polymorphism and type 1 diabetes 29–31, contrary to other studies that showed a connection between T1D and rs2104286 28,31–33. Although it is possible that this gap is the consequence of population differences and a variety of genetic and environmental factors, we cannot rule out the possibility that these negative results are the result of a small sample size.

It appears plausible to assume that certain other SNPs with potential functional roles could be causing the Asian Iraqi population's increased susceptibility to T1D. rs41295061 has been shown to be connected to glutamate decarboxylase antibody positivity in T1D patients 34. The circulating concentration of these SNPs was found to independently correlate with rs11594656, rs2104286, and rs41295061. These findings may indicate distinct biological processes that affect illness vulnerability, such as the levels of surface IL-2RA expression and the regulation of IL-2RA transcription 32. Although there is significant functional support for these SNPs' relationship with immunological state in T1D, the findings of genetic association studies of T1D are still ambiguous. To assess how these SNPs would affect a person's vulnerability to T1D.

CONCLUSIONS
This study showed that connections between the genotype and allele distribution of the PTPN22, IL-2RA, and T1D SNPs in Iraq, an Asian nation, are more comparable to those of Asian people than of European populations. These results demonstrate that the polymorphisms of PTPN22 rs2476601 and IL2RA rs2104286 did not significantly differ in their association with T1D and do not appear to contribute to T1D susceptibility in Asian Iraqis.

Conflicts of interest
The authors declare there are no conflicts of interest.
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