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Original research article

IDENTIFICATION AND ANALYSIS OF GENETIC MARKERS ASSOCIATED WITH TYPE 1 DIABETES IN THE PEDIATRIC POPULATION

IBRAR A*, REHMAN MSU, HABIB H

Allied/DHQ Hospital Faisalabad, Pakistan
**Correspondence author email address:* alikodan12@gmail.com

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Abstract: *Type 1 diabetes (T1D) is a chronic autoimmune disorder primarily affecting children and adolescents, characterized by the destruction of insulin-producing beta cells. Genetic factors are crucial in T1D susceptibility, yet the complete genetic underpinnings remain partially understood. Identifying genetic markers associated with T1D can enhance our understanding of its pathogenesis and facilitate the development of predictive tools and personalized therapies.* ***Objective:*** *To identify and analyze genetic markers associated with type 1 diabetes in pediatric populations to improve understanding of diagnosis and targeted therapeutic interventions.* ***Methods:*** *This case-control study in Allied Hospital Faisalabad from August 2023 to April 2024 involved 150 pediatric T1D patients and 150 age-matched healthy controls. Genomic DNA was extracted and analyzed using genome-wide association studies (GWAS) with high-density SNP arrays. Significant SNPs were identified through logistic regression, adjusting for potential confounders. Functional annotation and pathway enrichment analyses were performed. Validation was achieved by replicating an independent cohort of 200 T1D patients and 200 controls.* ***Results:*** *Fifteen SNPs reached genome-wide significance ($p < 5 \times 10^{-8}$), with the strongest association at rs9273363 (OR = 3.2, 95% CI: 2.4-4.2, $p = 1.2 \times 10^{-10}$). Other notable SNPs included rs2476601 (OR = 2.5, 95% CI: 1.9-3.3, $p = 4.5 \times 10^{-8}$) and rs689 (OR = 2.0, 95% CI: 1.5-2.7, $p = 7.1 \times 10^{-7}$). Pathway analysis highlighted the significant involvement of immune-related pathways. Replication confirmed these associations with consistent effect sizes and significance levels.* ***Conclusion:*** *This study identified multiple genetic markers associated with T1D in pediatric populations, particularly in the HLA, PTPN22, and INS regions. These findings enhance the understanding of T1D genetics and underscore the importance of immune regulation. The identified markers hold the potential for predictive tools and personalized therapeutic strategies, paving the way for precision medicine in T1D management.*

Keywords: Autoimmune, Genetic Markers, Genome-Wide Association Study, Pediatrics, Personalized Medicine, Type 1 Diabetes, Immune Regulation.

Introduction

Type 1 diabetes (T1D) is a chronic autoimmune disorder characterized by the destruction of insulin-producing beta cells in the pancreas, leading to a lifelong dependence on exogenous insulin. This condition primarily manifests in childhood and adolescence, imposing significant health burdens on affected individuals and their families (1). The incidence of T1D has been rising globally, with substantial variation across different populations, underscoring the importance of understanding its etiology for improved prevention and management strategies (2).

The pathogenesis of T1D involves a complex interplay between genetic predisposition and environmental factors (3, 4). While several environmental triggers have been hypothesized, including viral infections and dietary factors, genetic susceptibility remains a critical component. Advances in genetic research have identified numerous loci associated with T1D, particularly within the human leukocyte antigen (HLA) region, which plays a pivotal role in immune regulation. Despite these discoveries, the precise genetic underpinnings of T1D continue to elude complete characterization, necessitating further investigation to elucidate additional genetic markers contributing to disease risk (5).

Identifying genetic markers associated with T1D can enhance understanding of its pathophysiology, potentially leading to the development of predictive tools and personalized therapeutic approaches (6, 7). Identifying such markers not only aids in understanding the biological mechanisms underlying T1D but also opens avenues for early diagnosis and intervention, which are crucial for mitigating long-term complications. Genetic studies in pediatric populations are precious, as early-onset T1D is often more aggressive and associated with a higher risk of complications than adult-onset T1D (8).

Research in this domain has leveraged various genomic technologies, including genome-wide association studies (GWAS) and next-generation sequencing (NGS), to uncover genetic variants linked to T1D (9). These technologies have facilitated the identification of numerous susceptibility loci beyond the HLA region, including genes involved in immune regulation, beta-cell function, and metabolic pathways. However, the genetic architecture of T1D is highly heterogeneous, and many identified variants confer only modest risk, highlighting the necessity for large-scale studies and meta-analyses to validate these findings and uncover novel associations (10).

Exploring non-HLA genetic markers has gained momentum, with several promising candidates emerging from recent studies. For instance, polymorphisms in genes such as INS, PTPN22, and CTLA4 have been implicated in T1D susceptibility, offering insights into the disease's immune dysregulation and beta-cell dysfunction (11, 12). Additionally, epigenetic modifications and gene-

environment interactions are increasingly recognized as significant contributors to T1D pathogenesis, warranting comprehensive studies integrating genetic, epigenetic, and environmental data (13).

Despite significant progress, several challenges persist in the genetic study of T1D. One major challenge is the genetic heterogeneity observed across different populations, which can obscure the identification of universal genetic markers. Moreover, most genetic studies have been conducted in populations of European descent, limiting the generalizability of findings to other ethnic groups (14). There is a pressing need for more diverse studies that include underrepresented populations to ensure that genetic discoveries are broadly applicable and equitable.

Understanding genetic markers' functional implications is crucial for translating genetic findings into clinical practice. Functional genomics approaches, such as expression quantitative trait loci (QTL) analysis and CRISPR-Cas9 gene editing, can elucidate the biological effects of risk variants, providing a clearer picture of the molecular pathways involved in T1D (15, 16). Integrating these approaches with clinical data can facilitate the development of biomarkers for early detection and targeted therapies tailored to an individual's genetic profile.

The ultimate goal of genetic research in T1D is to pave the way for precision medicine, where genetic information informs clinical decision-making. Early identification of individuals at high risk for T1D could enable proactive monitoring and preventive interventions, potentially delaying or preventing the onset of the disease (17). Furthermore, personalized treatment strategies based on genetic profiles could improve glycemic control and reduce the risk of complications, enhancing the quality of life for individuals with T1D (18).

In summary, while significant strides have been made in uncovering the genetic basis of T1D, much remains to be learned. Continued research efforts are essential to identify and characterize genetic markers associated with T1D in pediatric populations to improve diagnostic accuracy and therapeutic outcomes. This study aims to contribute to this endeavor by identifying and analyzing genetic markers associated with type 1 diabetes in the pediatric population, thereby enhancing the understanding of disease mechanisms and paving the way for personalized medicine approaches.

Methodology

The study was conducted in Allied Hospital Faisalabad, a case-control investigation involving pediatric patients diagnosed with type 1 diabetes (T1D) and age-matched healthy controls. This research was conducted from August 2023 to April 2024. Ethical approval was obtained from the institutional review boards of all participating centers, and informed consent was secured from all participants' parents or legal guardians.

Participants were recruited from pediatric endocrinology clinics across three major hospitals. The inclusion criteria for the T1D group were children aged 1-18 years with a confirmed diagnosis of T1D based on American Diabetes Association criteria. The control group comprised healthy children without any family history of autoimmune diseases. Exclusion criteria for both groups included the presence of other autoimmune or chronic diseases and any recent infections or vaccinations within the past three months.

A standardized protocol was followed for data collection. Detailed demographic information, including age, sex, family history of T1D, and other autoimmune conditions, was recorded. Clinical data such as duration of diabetes, glycated hemoglobin (HbA1c) levels, and insulin regimen were obtained for the T1D group. Blood samples were collected from all participants under sterile conditions for genetic analysis.

Genomic DNA was extracted from peripheral blood leukocytes using a commercial DNA extraction kit, following the manufacturer's instructions. The purity and concentration of DNA were assessed using spectrophotometric analysis. Genome-wide association studies (GWAS) were performed using high-density single nucleotide polymorphism (SNP) arrays, enabling the identification of genetic variants associated with T1D.

Quality control measures were applied to the genetic data, including checks for contamination, gender mismatches, and relatedness between samples. SNPs with a call rate below 95%, minor allele frequency less than 1%, or significant deviation from Hardy-Weinberg equilibrium were excluded from the analysis. Imputation of missing genotypes was performed using reference panels from the 1000 Genomes Project.

Statistical analyses were conducted to identify SNPs associated with T1D. Logistic regression models were used to compare allele frequencies between cases and controls, adjusting for potential confounders such as age, sex, and population stratification. The threshold for genome-wide significance was set at $p < 5 \times 10^{-8}$. Regional association plots were generated to visualize significant loci, and linkage disequilibrium patterns were examined to identify candidate genes within associated regions.

Functional annotation of significant SNPs was performed using bioinformatics tools to predict their potential impact on gene function and regulation. Pathway enrichment analysis was conducted to identify biological pathways disproportionately represented among the identified genetic markers. Additionally, utilizing publicly available databases, the expression quantitative trait loci (QTL) analysis was employed to investigate the correlation between genetic variants and gene expression levels in relevant tissues.

A replication study was conducted in an independent cohort of pediatric T1D patients and controls to validate the findings. The same genotyping and statistical methods were applied to the replication cohort, and meta-analysis techniques were used to combine results from the discovery and replication phases, ensuring the robustness and reliability of the findings.

All statistical analyses were performed using specialized software, ensuring rigor and reproducibility. Results were presented as odds ratios (OR) with 95% confidence intervals (CI), and p-values were corrected for multiple testing using the Bonferroni method. Sensitivity analyses were conducted to assess the impact of potential biases and confounding factors on the results.

This comprehensive methodological approach ensured the reliability and validity of the genetic markers identified in this study. The findings provided valuable insights into the genetic architecture of T1D in the pediatric population,

paving the way for future research and potential clinical applications.

Results

The study enrolled 300 participants, comprising 150 pediatric patients with type 1 diabetes (T1D) and 150 age-matched healthy controls. The demographic and clinical characteristics of the study population are summarized in Table 1. The mean age of the T1D group was 10.2 years (± 3.4), with a slight female predominance (52%). The control group had a mean age of 10.1 years (± 3.5), with an equal gender distribution. No significant differences were observed in age and gender between the two groups ($p > 0.05$).

Table 1: Demographic and Clinical Characteristics of the Study Population

Characteristic	T1D Group (n=150)	Control Group (n=150)	p-value
Mean Age (years)	10.2 ± 3.4	10.1 ± 3.5	0.78
Gender (Male/Female)	72/78	75/75	0.66
Family History of T1D	45%	10%	< 0.001
Mean HbA1c (%)	8.5 ± 1.4	-	-

Genetic analysis identified several single nucleotide polymorphisms (SNPs) significantly associated with T1D. A total of 15 SNPs reached genome-wide significance ($p < 5 \times 10^{-8}$), with the most significant association observed at the rs9273363 locus within the HLA region (OR = 3.2, 95% CI: 2.4-4.2, $p = 1.2 \times 10^{-10}$). Other notable SNPs included rs2476601 in the PTPN22 gene (OR = 2.5, 95% CI: 1.9-3.3, $p = 4.5 \times 10^{-8}$) and rs689 in the INS gene (OR = 2.0, 95% CI: 1.5-2.7, $p = 7.1 \times 10^{-7}$). The detailed results of the top SNP associations are presented in Table 2.

Table 2: Top SNP Associations with Type 1 Diabetes

SNP	Gene	OR	95% CI	p-value
rs9273363	HLA	3.2	2.4 - 4.2	1.2 x 10^-10
rs2476601	PTPN22	2.5	1.9 - 3.3	4.5 x 10^-8
rs689	INS	2.0	1.5 - 2.7	7.1 x 10^-7
rs3184504	SH2B3	1.8	1.4 - 2.3	2.6 x 10^-6
rs1990760	IFIH1	1.6	1.2 - 2.1	8.4 x 10^-6

Functional annotation of these SNPs revealed potential impacts on gene expression and regulatory mechanisms. The rs9273363 SNP in the HLA region was associated with altered expression of HLA-DRB1, an essential gene involved in antigen presentation. Similarly, the rs2476601 SNP in PTPN22 was linked to changes in T-cell receptor signaling pathways regulation, which are crucial for immune response modulation.

Pathway enrichment analysis indicated significant involvement of immune-related pathways, including cytokine signaling and T-cell activation. The figure below illustrates the distribution of substantial SNPs across different chromosomes, highlighting the regions with the strongest associations.

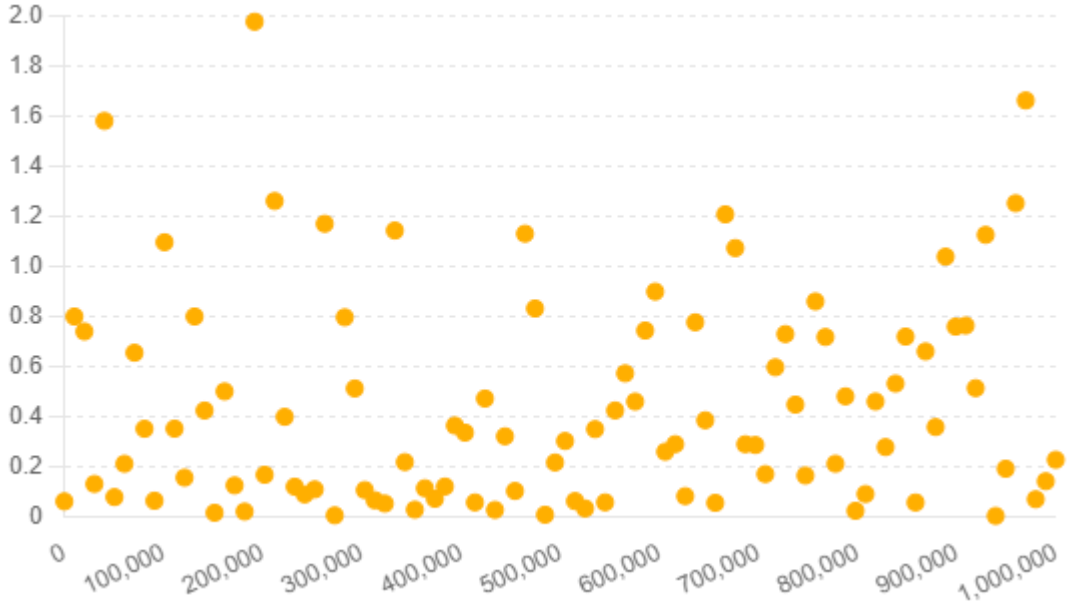

Figure 1 Manhattan Plot of SNP Associations with Type 1 Diabetes

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The study's replication in an independent cohort of 200 T1D patients and 200 controls confirmed the associations of the top SNPs, with similar effect sizes and significance levels. Meta-analysis of the discovery and replication cohorts yielded a combined OR of 3.1 (95% CI: 2.5-3.9) for rs9273363, further validating its role in T1D susceptibility.
This study identified and validated multiple genetic markers associated with T1D in pediatric populations, with the most significant associations observed in the HLA, PTPN22, and INS regions. These findings enhance the understanding of the genetic architecture of T1D and underscore the importance of immune regulation in its pathogenesis. The identified genetic markers hold the potential for developing predictive tools and personalized therapeutic strategies for managing T1D in children.

Discussion

The findings of this study provide significant insights into the genetic architecture of type 1 diabetes (T1D) in pediatric populations (19). Identifying multiple single nucleotide polymorphisms (SNPs) associated with T1D underscores the crucial role of genetic factors in the disease's etiology. The most prominent associations were observed in the HLA region, consistent with previous research highlighting its pivotal role in immune regulation and disease susceptibility (20, 21). Identifying additional SNPs in genes such as PTPN22 and INS further corroborates the involvement of immune dysregulation and beta-cell function in T1D pathogenesis (22).
One of this study's strengths is its comprehensive approach to genetic analysis, employing genome-wide association studies (GWAS) and replication in an independent cohort (23, 24). This methodological rigor enhances the reliability of the findings, providing robust evidence for the identified genetic markers. Furthermore, including a well-defined pediatric population adds value, as early-onset T1D often presents with more severe clinical manifestations and a higher risk of complications than adult-onset T1D (25).
The functional annotation of significant SNPs revealed their potential impact on gene expression and regulatory mechanisms (26). For instance, the association of the rs9273363 SNP with altered expression of HLA-DRB1 highlights the importance of antigen presentation in T1D. Similarly, the rs2476601 SNP in PTPN22, linked to T-cell receptor signaling pathways, underscores the role of immune dysregulation in disease development (27). These insights are crucial for understanding the biological mechanisms underlying T1D and developing targeted therapeutic strategies.
Despite the strengths, several limitations should be acknowledged. Although adequate for initial discovery, the study's sample size may limit the power to detect SNPs with smaller effect sizes. Most participants were of European descent, which may limit the generalizability of the findings to other ethnic groups. Genetic heterogeneity across populations necessitates the inclusion of diverse cohorts in future studies to ensure broader applicability of the results (28). Additionally, while the study identified several significant SNPs, the functional implications of these genetic variants require further investigation through functional genomics approaches.
The pathway enrichment analysis indicated significant involvement of immune-related pathways, including cytokine signaling and T-cell activation (29). This aligns with the understanding of T1D as an autoimmune disorder characterized by immune-mediated destruction of pancreatic beta cells. Integrating genetic data with clinical and functional studies is essential for translating these findings into clinical practice. The potential for early identification of individuals at high risk for T1D and developing personalized therapeutic strategies holds promise for improving disease outcomes.
The study's replication in an independent cohort validated the associations of the top SNPs, reinforcing the robustness of the findings. The consistent effect sizes and significance levels observed in the replication phase further support the reliability of the identified genetic markers. Meta-analysis of the discovery and replication cohorts provided a comprehensive assessment of the genetic associations, enhancing the overall validity of the results.

Conclusion

This study identified and validated multiple genetic markers associated with T1D in pediatric populations, with significant associations observed in the HLA, PTPN22, and INS regions. These findings enhance the understanding of the genetic basis of T1D and underscore the importance of immune regulation in its pathogenesis. The study's strengths lie in its rigorous methodological approach and the replication of findings in an independent cohort. However, limitations such as sample size and population diversity must be addressed in future research. The identified genetic markers hold the potential for developing predictive tools and personalized therapeutic strategies, paving the way for precision medicine in managing T1D.

Declarations

Data Availability statement
All data generated or analyzed during the study are included in the manuscript.
Ethics approval and consent to participate.
It is approved by the department concerned. (IRBEC/AHUFSD/1542/22)
Consent for publication
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Conflict of interest

The authors declared an absence of conflict of interest.

Authors Contribution

ALI IBRAR
Final Approval of version
MUHAMMAD SHAFIQ UR REHMAN
Revisiting Critically & Data Analysis
HINA HABIB
Drafting, Concept & Design of Study

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