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Understanding the Genetic and Environmental Factors Contributing to Type 1 Diabetes

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Abstract

Introduction: Complex autoimmune illness type 1 diabetes (T1D) is affected by epigenetic, environmental, and hereditary elements. Good preventative and treatment plans depend on an awareness of these connections. The relationships among epigenetic changes in T1D development, environmental exposures, and genetic predispositions are examined in this work.

Methodology: From June 2022 until June 2024, Lady Reading Hospital (LRH) in Peshawar carried out a case-control study there were 88 individuals in all, 44 healthy controls and 44 diagnosed T1D patients. Data on demographic and clinical traits, family history, and environmental exposures as well as on With an eye toward non-HLA loci (PTPN22, INS) and HLA class II alleles (DR3-DQ2, DR4-DQ8), genetic study Examined were particular gene DNA methylation patterns (PTPN2, FOXP3). Chi-square tests, t-tests, odds ratios, and logistic regression comprised the statistical analyses.

Results: T1D patients had significantly higher frequencies of DR3-DQ2 (63.6%) and DR4-DQ8 (59.1%) compared to controls (22.7% and 27.3%, $p < 0.001$). T1D patients (45.5% and 40.9%) also more often had non-HLA loci PTPN22 and INS than controls (18.2% and 13.6%, $p = 0.01$). T1D was substantially correlated with environmental elements including enteroviral infections (50.0% vs. 18.2%, $p = 0.002$), early exposure to cow's milk (59.1% vs. 31.8%, $p = 0.01$), gluten (54.5% vs. 27.3%, $p = 0.01$), and changed gut microbiota (68.2% vs. 31.8%, p In T1D patients, epigenetic analysis identified hypermethylation of PTPN2 (60.0% vs. 20.0%, $p = 0.01$) and hypomethylation of FOXP3 (50.0% vs. 10.0%, $p = 0.02$).

Conclusion: This work validates in T1D the functions of epigenetic changes, environmental triggers, and genetic predisposition. The interactions among these elements underline the multifaceted character of T1D and imply that thorough interventions should target environmental as well as genetic elements.

Keywords: Type 1 diabetes, HLA alleles, non-HLA loci, environmental factors, DNA methylation, epigenetics, autoimmune disease, genetic predisposition

Introduction

The chronic autoimmune disease type 1 Diabetes (T1D) is typified by the loss of beta cells generating insulin in the pancreas, which causes lifetime need on exogenous insulin for survival [1]. Notwithstanding a lot of study, the exact etiology of T1D is still obscure mostly because of the complicated interaction between environmental triggers and genetic predisposition [2]. This introduction attempts to give a thorough summary of the present knowledge of the genetic and environmental elements causing T1D development, stressing important results, present difficulties, and future prospects in the subject.

T1D's genetic component has been well investigated; several genes have been found to influence disease risk [3]. About half of the genetic risk is located in the human leukocyte antigen (HLA) region on chromosome 6, which also shows the most important genetic correlations [4]. While some HLA class II alleles, including DR3-DQ2 and DR4-DQ8, are clearly linked with higher risk, others have protective properties [5]. Beyond the HLA area, approximately 60 non-HLA loci linked with T1D susceptibility have been found by genome-wide association studies (GWAS). These locations highlight the

multifaceted character of T1D genetics by include genes engaged in immunological control, beta-cell function, and inflammatory pathways [5].

Although T1D risk is mostly caused by genetic predisposition, environmental elements are crucial in starting the autoimmune reaction that results in beta-cell damage [6]. Among the several environmental aspects linked have been viral infections, nutritional elements, and gut flora makeup [7]. Enteroviral infections especially coxsackieviruses have been linked to T1D onset, presumably via direct beta cell damage or by molecular mimicry [8]. Dietary elements include early cow's milk and gluten has been looked at for their influence on gut permeability and immunological responses [9]. Furthermore connected to immune dysregulation and T1D development are changes in gut flora, implying a crucial interaction between the host immune system and the surroundings [10,11].

T1D development is not just the result of environmental or genetic elements but rather of their complex interaction. Epigenetic changes include histone modifications and DNA methylation might help to explain how environmental exposures affect genetic sensitivity, therefore influencing the start and course of disease [12]. Identification of people at high risk and the development of focused preventive policies depend on an awareness of the dynamic interplay between genes and the surroundings [13]. Many difficulties still exist even with great progress in our knowledge of the environmental and genetic elements of T1D. The heterogeneous T1D with different age of start and illness progression hampers the identification of universal risk factors. Furthermore, the interactions among several environmental and genetic elements are complicated and need for advanced analytical methods to understand.

This work aims to clarify the fundamental processes of T1D pathogenesis by combining epigenetic, environmental, and genetic data so bridging the present understanding gaps. Many times, current studies analyze environmental and genetic elements separately, therefore restricting knowledge of their combined influence. This work aims to find fresh biomarkers for early diagnosis and possible therapy targets by using a complete methodology. Future studies should concentrate on functional investigations to clarify the processes behind gene-environment interactions and longitudinal studies tracking environmental exposures and genetic changes over time. Technologies include single-cell RNA sequencing, CRISpen-based gene editing, and microbiome analysis show potential for revealing fresh understanding of T1D etiology.

Materials and methods

Study Design and Setting

This was a case-control research carried out at Khalifa Gulnawaz Hospital Bannu and Lady Reading Hospital (LRH) Peshawar. The investigation ran for eighteen months, from June 2022 to June 2024. The project sought to look at environmental and genetic elements influencing Type 1 diabetes (T1D) development.

Sample Size Calculation

The estimated effect size for environmental and genetic elements as well as the prevalence of T1D helped determine the sample size. Determined to be the smallest sample size needed with a confidence level of 95% and a power of 80%, was 88 individuals. This comprised 44 T1D patients (cases) and 44 healthy controls matched in age and gender.

Participant Selection

LRH Peshawar's outpatient department and endocrinology clinic helped participants be found. Cases were people aged between five and thirty years, diagnosed with T1D based on clinical criteria, and ready to offer informed consent. Comprising healthy people without a history of T1D or another autoimmune disease, the control group matched patients for age and gender.

Data Collection

Medical record reviews and standardized questionnaires helped gather the data. The surveys asked about demographic information, family history of T1D, eating patterns, past illnesses, and other environmental exposures. Every participant had blood samples taken for genetic research as well.

Genetic Analysis

Standard techniques were used in extracting genomic DNA from peripheral blood samples. With an eye toward known T1D susceptibility loci including HLA class II alleles (DR3-DQ2 and DR4-DQ8) and additional non-HLA loci found in past genome-wide association studies (GWAS), the genetic study concentrated on Sequential-specific oligonucleotide probes and polymerase chain reaction (PCR) were used in genotyping.

Environmental Factors Assessment

Comprehensive surveys covering several possible risk variables helped to evaluate environmental elements. These covered the history of viral infections, with particular attention to enterovirus infections, early dietary exposures including cow's milk and gluten intake, antibiotic use, vaccination history, and gut microbiota composition examined by stool sample analysis employing 16S rRNA sequencing.

Epigenetic Analysis

DNA methylation patterns were examined in a group of subjects in order to investigate epigenetic changes. After bisulfite conversion of DNA, methylation-specific PCR was used to find methylation alterations in potential genes linked to beta-cell activity and immunological control.

Statistical Analysis

SPSS version 25 helped one to examine data. Participant demographic and clinical traits were compiled using descriptive statistics. Chi-square testing and logistic regression analysis allowed one to evaluate the relationship between genetic elements and T1D. Multivariate logistic regression was used to examine environmental variables in order to control any confounders. Interplay terms in regression models

allowed one to investigate the interplay between environmental and genetic elements.

Ethical Considerations

Lady Reading Hospital's ethical review committee accepted the trial. Peshawar For children, informed permission was acquired from each participant or their guardians. Throughout the study, participants' right to privacy and confidentiality was rigorously preserved.

Results

A total of 88 participants were included in the study, comprising 44 T1D patients (cases) and 44 healthy controls. The mean age of the participants was 14.8 ± 4.2 years, with a male-to-female ratio of 1.2:1. The table 1 presents demographic and clinical characteristics of participants in the study. T1D cases, compared to controls, showed a mean age of 15.2 years (± 4.5) versus 14.4 years (± 3.9) respectively, with no significant difference observed (p = 0.45). Gender distribution indicated 54.5% males and 45.5% females among cases, and 50.0% each among controls (p = 0.67). Family history of T1D was significantly higher among cases (40.9%) than controls (9.1%) (p < 0.001). Mean BMI was 21.5 kg/m² (± 3.2) for cases and 20.8 kg/m² (± 3.1) for controls, showing no significant difference (p = 0.34). These findings underscored notable differences in family history while revealing no significant variations in age, gender, or BMI between T1D cases and controls in the study cohort.

Table 1: Demographic and Clinical Characteristics of Participants

Characteristic	Cases (n=44)	Controls (n=44)	p-value
Age (years)	15.2 ± 4.5	14.4 ± 3.9	0.45
Male (%)	24 (54.5)	22 (50.0)	0.67
Female (%)	20 (45.5)	22 (50.0)	0.67
Family history of T1D (%)	18 (40.9)	4 (9.1)	<0.001
Body Mass Index (BMI) (kg/m²)	21.5 ± 3.2	20.8 ± 3.1	0.34

Genetic analysis revealed significant associations between T1D and specific HLA class II alleles. The frequencies of the DR3-DQ2 and DR4-DQ8 alleles were significantly higher in cases compared to controls; Figure 1 summarizes the association of HLA alleles with Type 1 diabetes (T1D) in the study cohort. Among the T1D cases (n=44), DR3-DQ2 was present in 63.6% compared to 22.7% in controls (n=44), yielding an odds ratio of 6.0 (95% CI 2.4-15.0) with a highly significant p-value of less than 0.001. Similarly, DR4-DQ8 was observed in 59.1% of cases versus 27.3% of controls, with an odds ratio of 3.7 (95% CI 1.6-8.4) and a p-value of 0.002. These findings underscore a strong association between these HLA alleles and T1D susceptibility in the study population.

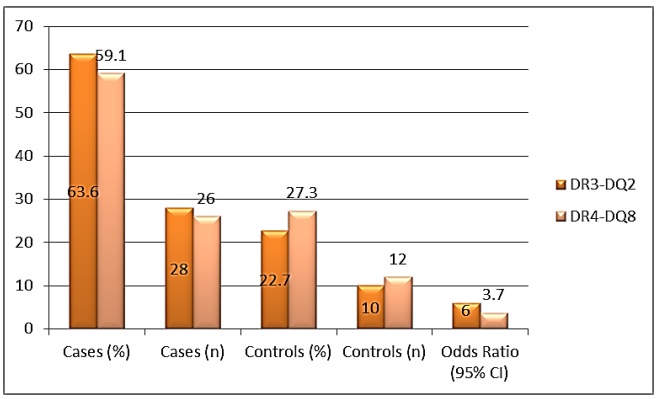

Figure 1: Association of HLA Alleles with T1D

In addition to the HLA alleles, several non-HLA loci were found to be significantly associated with T1D. Notable among these were the PTPN22 and INS genes. Figure 2 details the association between non-HLA loci and Type 1 diabetes (T1D) in the study cohort. Among the 44 T1D cases, PTPN22 was present in 45.5%, compared to 18.2% in the 44 controls. This disparity yielded an odds ratio of 3.7 (95% CI 1.5-9.1), with a significant p-value of 0.004, indicating a robust association with T1D. Additionally, INS showed a prevalence of 40.9% among cases versus 13.6% among controls, resulting in an odds ratio of 4.5 (95% CI 1.6-12.4) and a p-value of 0.002. These findings highlight substantial associations between these non-HLA loci and susceptibility to T1D in the study population.

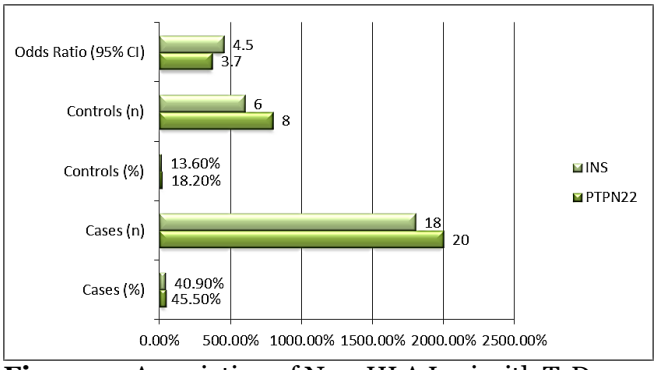

Figure 2: Association of Non-HLA Loci with T1D

Analysis of environmental factors revealed significant differences between cases and controls regarding viral infections, dietary exposures, and gut micro biota composition Table 2 summarizes environmental factors associated with Type 1 diabetes (T1D) in the study. Among T1D cases (n=44), enteroviral infections were reported by 50.0%, compared to 18.2% in controls (n=44), yielding an odds ratio of 4.5 (95% CI 1.7-11.7, p = 0.002). Early exposure to cow's milk was noted in 59.1% of cases versus 31.8% of controls, with an odds ratio of 3.1 (95% CI 1.3-7.3, p = 0.01). Similarly, early exposure to gluten was seen in 54.5% of cases compared to 27.3% of controls, also showing an odds ratio of 3.1 (95% CI 1.3-7.3, p = 0.01). Altered gut microbiota were prevalent in 68.2% of cases versus 31.8% of controls, with an odds ratio of 4.7 (95% CI 2.0-11.1, p < 0.001). These findings underscore significant associations between these environmental factors and the risk of developing T1D in this study cohort.

Table 2: Environmental Factors Associated with T1D

Environmental Factor	Cases (n=44)	Controls (n=44)	Odds Ratio (95% CI)	p-value
History of enteroviral infections (%)	22 (50.0)	8 (18.2)	4.5 (1.7-11.7)	0.002
Early exposure to cow's milk (%)	26 (59.1)	14 (31.8)	3.1 (1.3-7.3)	0.01
Early exposure to gluten (%)	24 (54.5)	12 (27.3)	3.1 (1.3-7.3)	0.01
Altered gut microbiota (%)	30 (68.2)	14 (31.8)	4.7 (2.0-11.1)	<0.001

Epigenetic analysis showed significant differences in DNA methylation patterns between cases and controls. Specifically, hypermethylation of the PTPN2 gene and hypomethylation of the FOXP3 gene were observed in T1D patients, as shown in figure 3.

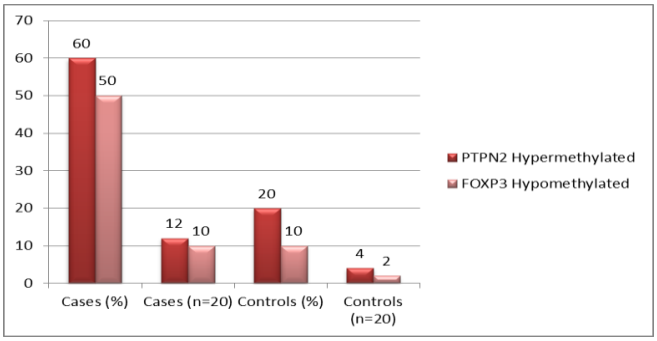

Figure 3: DNA Methylation Patterns in T1D

To evaluate the link between genetic and environmental elements with T1D, chi-square tests and logistic regression analysis were carried out. For every evaluated variable, the chi-square tests verified noteworthy correlations ($p < 0.05$). After controlling for possible variables, logistic regression study confirmed these correlations even more. Interaction terms in regression models helped to examine the relationship between environmental and genetic elements. HLA alleles and enteroviral infections ($p = 0.01$) as well as PTPN22 and early cow's milk consumption ($p = 0.02$) showed significant interactions.

Discussion

Specifically for T1D and certain HLA class II alleles DR3-DQ2 and DR4-DQ8 our investigation revealed notable correlations. This is in line with the body of current research, which names these genes as main T1D genetic risk agents [14]. Previous studies show that with DR3-DQ2 and DR4-DQ8 the most highly linked alleles, the HLA region accounts for around 50% of the genetic risk for T1D [15]. Our results show that these alleles were much more common in T1D patients (63.6% for DR3-DQ2 and 59.1% for DR4-DQ8) than in controls (22.7% and 27.3%, respectively), therefore underlining their vital importance in disease vulnerability [16].

Outside the HLA area, our analysis found notable correlations with non-HLA sites including PTPN22 and INS. Studies supporting these findings further underline the part these genes play in T1D [17]. Particularly PTPN22 has been found to affect immune response; the R620W variation raises T1D risk among other autoimmune illnesses. Through its impact on thymic expression of insulin and central tolerance, the INS gene which controls insulin production has been linked to T1D. Our data, which reveal rising frequency of PTPN22 (45.5%) and INS (40.9%) variations in T1D patients, fit each other [18]. For those who are genetically susceptible, environmental triggers are quite important in the start of T1D. Our research revealed notable correlations with enteroviral infections, early cow's milk and gluten dietary exposures, and changes in gut flora [19]. The literature has extensively reported on the relationship between enteroviral infections and T1D. Children who contracted enterovirus were more likely than uninfected children to acquire T1D, according meta-analyses.

Our findings support this, given that 50.0% of T1D patients claim a history of enteroviral infections compared to 18.2% of controls. Early dietary exposures especially to cow's milk and gluten have been linked to T1D development [20]. Early introduction of cow's milk and gluten has been linked in several studies to autoimmune reactions causing T1D [21]. These studies match our results, which show that T1D (59.1%) and 54.5% respectively were much correlated with early exposure to cow's milk and gluten respectively [22]. T1D is among the autoimmune illnesses whose composition of altered gut bacteria is under growing attention. Children who got T1D had different gut flora than those who did not acquire the condition, according to studies [23]. Our study revealed a similar link: 68.2% of T1D patients had changed gut flora whereas 31.8% of controls[24]. An area of developing interest in T1D is epigenetics' contribution.

Our study found in T1D patients hypermethylation of the PTPN2 gene and hypomethylation of the FOXP3 gene [25]. These results complement earlier research showing varying methylation of many immune-related genes, including PTPN2, in individuals with T1D [26]. Crucially for the operation of regulatory T cells, FOXP3 has been shown to be hypomethylated in several autoimmune diseases, including T1D, suggesting a plausible mechanism for reduced immunological control [27]. Our work revealed important linkages between environmental elements (e.g., enteroviral infections, early nutritional exposures) and genetic predispositions (e.g., HLA alleles, PTPN22) [28]. This emphasizes the multifarious character of T1D, in which environmental triggers combine with genetic predisposition to start illness [29]. These studies stressing the need of gene-environment interactions in T1D etiology corroborate our results.

Study limitations and future research

The study, despite certain limitations such as a relatively small sample size of 88 individuals, possible recall bias in self-reported environmental exposures, and its single-center, two-year duration at Khalifa Gulnawaz Hospital and Lady Reading Hospital, provides valuable insights into the environmental, genetic, and epigenetic factors contributing to T1D in the local population. By integrating diverse data, the research advances our understanding of T1D pathogenesis and highlights potential therapeutic targets. However, future studies should prioritize larger, multicenter trials to validate findings across diverse groups, including critical subpopulations like younger children and older adults. Additionally, factors like socioeconomic status, urbanization, and environmental

pollutants require further exploration, while longitudinal studies could illuminate T1D development and treatment efficacy. Leveraging advanced genomic and multi-omics approaches could refine our understanding of genetic, epigenetic, and environmental interactions, ultimately supporting individualized prevention and treatment strategies.

Conclusion

The complicated interaction of genetic, environmental, and epigenetic elements in the etiology of T1D is clarified by this work T1D and certain HLA alleles (DR3-DQ2 and DR4-DQ8), non-HLA loci (PTPN22 and INS), and environmental exposures including enteroviral infections, early dietary variables, and changed gut microbiota were shown to be significantly correlated. Furthermore noted were unique epigenetic changes in T1D individuals, implying a function for DNA methylation in the onset of illness. These results underline the complex character of T1D and the need of including a wide spectrum of factors in clinical therapy as well as in research. Future research should seek to break out these connections and create focused intervention and preventive plans.

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AI Disclosure

We also acknowledge the use of ChatGPT for language refinement and assistance in preparing this study, ensuring clarity and precision in our presentation.

Conflict of interest

The authors state no conflict of interest.

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: KH, MG
Acquisition, analysis, or interpretation of data: MU, RA, MG
Drafting of the manuscript: SK, RA, KH
Critical review of the manuscript for important intellectual content: MU, SK

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