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Association of HLA-DRB1/DQB1 Genes with Type1 Diabetes Mellitus among Sudanese Children and Adolescents

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Abstract: Background: *About 10% of diabetes cases are Type 1 diabetes mellitus (T1D), which is brought on by the autoimmune death of pancreatic beta-cells. This results in insulin insufficiency and condemns people with T1D to a life of insulin therapy. The interplay of environmental and genetic risk factors results in the activation of autoimmunity towards beta cells. The primary genetic causes of T1D, among the many loci linked to the condition, are variations in the class II HLA genes DQ and DR. The purpose of this study is to identify the impact of the HLA-DRB1/DQB1 genes in type 1 diabetes mellitus on the onset of susceptibility or resistance to the disease in patients between the ages of five and eighteen, in comparison to healthy controls.* ***Materials and Methods:*** *The study included 187 Sudanese participants, whose ages ranged from 5 to 18 on average. 100 non-diabetic patients and 87 cases of type 1 diabetes mellitus were used as the control group. The investigation was carried out in the state of Khartoum's diabetic central hospitals. The allele-specific-refractory mutation system-polymerase chain reaction (ARMS-PCR) technique was applied to identify the HLA gene polymorphism.* ***Results:*** *There was a significant difference in genotype frequency across the groups in the current investigation (Kruskal-Wallis, P value = 0.021). Whereas CG was not substantially different across groups (Chi square, P Value =0.116), the CC genotype was considerably greater (46.0%) in patients (Chi square Adjusted p value 0.001).* ***Conclusions:*** *This study found that patients' genotypes and allele frequencies are significantly correlated when compared to those of healthy participants.*

Keywords: Diabetes mellitus type 1, HALA, Single nucleotide poly morphisms

Introduction

Type 1 diabetes (T1D) is a multifactorial disease in which pancreatic beta-cells, which are responsible for manufacturing insulin in the body, are destroyed by immune system cells [1]. T1D is a condition that is a major public health concern [2]. According to estimates, 366 million individuals worldwide suffer with T1D, and by 2030, it's predicted that number would rise to 552 million [3]. The cause of type 1 diabetes is currently unclear, just like other autoimmune disorders. T1D is a chronic autoimmune condition that affects people who are genetically predisposed to it. It is brought on by environmental triggers such germs, viruses, and certain foods. More than 60 unique genes have been shown to have a significant role in T1D susceptibility [4]. Major histocompatibility complex genes account for between 30% and 50% of TID susceptibility, with DQ & amp; DR genes having the greatest influence [5]. For 40% to 50% of the family aggregation of T1D, the human leukocyte antigen (HLA), a genetic locus on chromosome 6p21.31, is to blame. This gene makes the peptide antigens visible on the cell surface, enabling the T-cells' awareness mechanism [6]. The occurrence of the alleles HLADRB1, DQA1*0301, DQB1*0302, and DQA1*0501, DQB1*0201 enhances the exposure to T1D, according to several research. The susceptibility to T1D rises noticeably if the aforementioned alleles are in linkage disequilibrium with the HLA-DRB1*03(DR3) or HLA-DRB1*04(DR4) [7-9]. A great amount of loci has been recognized through genome-wide association research (GWASs) which include infinite range of single nucleotide polymorphisms (*SNPs)* that are placed throughout the genome [10]. It is recognized that countless polymorphisms in *HLA* genes are related to diabetes. In this study, the frequency of *rs3104413* polymorphism, which is placed in the interagency place between HLA-DRB1 and HLA-DQA1 in the HLA vicinity in the people with diabetes in contrast to healthy subjects, was evaluated. Although the effects of polymorphism have been studied in a number of groups, no research of a similar nature has been done in Sudan. Studying the connection between polymorphism and diabetes is very important because of the significant impact that remarkable races have on the polymorphisms in the genome [11].
The allele frequencies of C/G are equivalent to 91%/9% routinely based entirely on records from Africa, according to task segment 3 of the 1000 genomes project. Ninety-one percent (1197) of persons have the C allele, while nine percent (125) have the G allele (ensemble facts base handy at [12]. According to (genome AD) genomes in Africa, the frequency of the C allele is 0.907 (7891), whereas the frequency of the G allele is 0.093 (809) [13]. The Trans-comics for Precision Medicine (TOPMED) tool estimates the allele frequencies of C and G to be 0.852 for C and 0.148 for the G alleles [14]. According to the UK10K program, the G allele frequency is 0.187 (719) while the C allele frequency is 0.813 (3135) [15].

Methods

Hundred Sudanese participants, whose ages ranged from 5 to 18, were the subjects of this study. Using the National Diabetes Data Group's diagnostic standards, there are 100 instances of type 1 diabetes mellitus (NDDG). And 100 (non-diabetic) individuals who had neither clinical indications of T1D nor a family history of the disease. December 2022 until November 2020.All participants gave written informed consents in accordance with the protocol that was authorized by the National University Research and Ethics Committee's ethics committee and all subjects provided informed consent as per the procedure. Using a commercially available kit (G-DEXTM11b Genomic DNA Extraction Kit (Blood) 200T catalog numbers 17241), the DNA was extracted from peripheral blood samples taken from the patients and controls.

Genotyping:

To identify the rs3104413 (C/G) mutation, we used an amplification refractory mutation system polymerase chain reaction (ARMS-PCR) (Table1). To find the most frequent mutations, these techniques are simple, quick, and sensitive [16]. From the National Center Amplification for Biotechnology Information, the HLA gene sequences were retrieved (NCBI). Following the manufacturer's instructions, polymerase chain reaction (PCR) was carried out using a commercially available PCR premix (AccuPower PCR Premix; BIONEER, Daejeon, Korea). In a nutshell, 15 mL of DNase-free water, 1 mL of each primer (10 pmol/mL), and 1 mL of template DNA (around 100 ng/mL) were added to the AccuPower PCR Premix. This was carried out in a reaction volume of 20 l using 100 ng of genomic DNA. The following thermal profiles were run: initial denaturation for 3 min at 95°C, 30 cycles of 95°C for 20 s, 60°C for 30 s, and 72°C for 40 s, and final extension for position rs3104413 (C/G) for 5 min at 72°C.The amplified PCR products were examined using 2% agarose gel electrophoresis and UV illumination. For the rs3104413 (C/G) polymorphism, the length of the anticipated PCR products was 372 bp (Fig. 1).

Table1: The primer sequences for the rs3104413 single-nucleotide polymorphism utilized in the study

Gene polymorphismHLA rs1304413	Primers Sequence (5′ to 3′)	Tm (˚C)	Product size
Reverse (C allele)	GGAGAAGCACGACAATAGGAC	59	C and G allele: 327 b p
Reverse (G allele)	GGAGAAGCAAGCCAATAGGAG	59	
Forward (common)	CTGCTTTTCACACCAACCTCT	60	

Statistical Analysis:

The statistical package for social science (IBMSPSS version 26.0) for Windows was used to analyze the data. Statistical significance was determined to have a P value of less than 0.05. Using a Pearson's Chi square test, categorical variables, allele frequency, and genotype frequency were all examined. In order to compare the groups, the Kruskal Wallis test was used. By calculating the contribution of each cell to the chi square using the adjusted residuals P values (adjusted P value = 0.05/number of new adjusted residuals or cells), the intensity of significance was determined.

Results

Two hundred Sudanese participants, ranging in age from 5 to 18, were engaged in the research. 100 cases of type 1 diabetes mellitus and 100 control cases. At the state of Khartoum, the study was carried out in hospitals with a focus on diabetes. The control group had a male frequency of (49%) and a female frequency of (51) whereas the patient group had a male frequency of (48.3%) and a female frequency of (51.7%), as shown in (Table 2). The patients' mean age was (12.00 3.735 and the controls' was (12.25 3.686. (Table2). According to Kruskal-Wallis, the frequency of genotypes varied significantly between the groups in the current study (P value = 0.021). (Table 3). While CG was not substantially different across groups (Chi square, P value =0.116), the CC genotype was considerably greater (46.0%) in patients (Chi square adjusted p value 0.001). (Table 3).

Individuals with the CC genotype were three times more at risk than those with the GG genotype (3.55 RR, 95% confidence interval [CI] 2.327-5.415); likewise, those with the CG genotype were two times more at risk (2.535 RR, 95% confidence interval [CI] 1.589-4.043); Contrarily, people with CC genotypes had a risk of only 40% more than people with CG genotypes (1.401 RR, 95% CI 1.054-1.862). The C genotype was much more prevalent in patients (Chi square, P 0.001) In addition, people with the C allele had a two-fold greater risk of developing the disease than people with the G allele (2.804 RR, 95% CI 2.173-3.618). (Table 4).

Table 2: Descriptive Statistics:

Groups			Frequency
Control	Gender	Females	51.0(%)
		Males	49.0(%)
	Age (mean ±Std.)		12.25±3.686
Patients	Gender	Females	51.7(%)
		Males	48.3(%)

Age (mean ±Std.)	12.00±3.735

Table 3: The cross tabulation of genotypes frequencies and the case and control group

			Groups		
			Control	Patients	
Genotypes	C/C	Count	11	40	p. value
		% within Genotypes	21.6%	78.4%	0.021
		% within Groups	11.0%	46.0%	
	G/G	Count	67	19	
		% within Genotypes	77.9%	22.1%	
		% within Groups	67.0%	21.8%	
	C/G	Count	22	28	
		% within Genotypes	44.0%	56.0%	
		% within Groups	22.0%	32.2%	

(Highlighted in yellow: significant at the P value ≤ 0.05 (post hoc for chi square)

Table 4: The Comparison of allele's frequency between the study Groups:

			Groups	
			Patients	Control
Alleles	C	Count	94	33
		% within Alleles	74.0%	26.0%
		% within Groups	64.4%	18.5%
	G	Count	52	145
		% within Alleles	26.4%	73.6%
		% within Groups	35.6%	81.5%

Figure 1: PCR assay for single nucleotide polymorphism rs3104413 in HALA agarose gel electrophoresis showing 327 bp .M: Molecular marker 5o bp, Lane 1 C. Lane 3 G, Lane 11 G
Lane 2, 4,5,6,7,8,9,10,12 SNPs rs3104413 not detected.

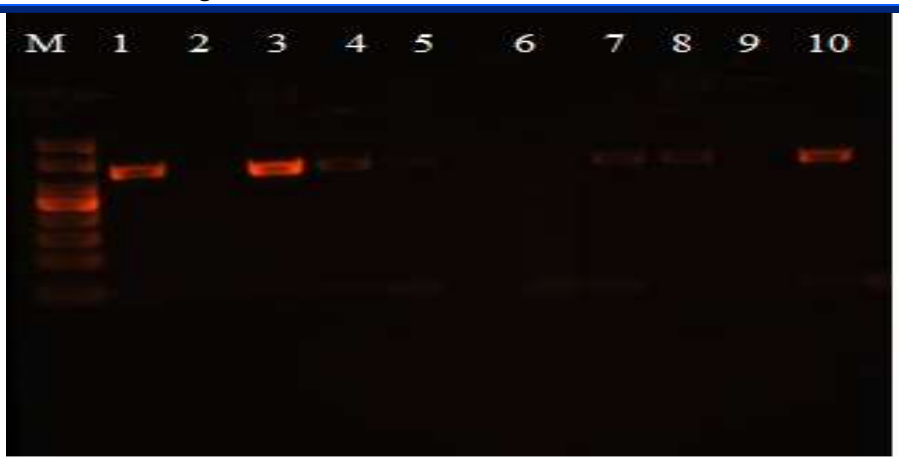

Figure 2: PCR assay for single nucleotide polymorphism rs3104413 in HALA agarose gel electrophoresis showing 327 bp
M: Molecular marker 50 bp, Lane 1 C. Lane 3 G, Lane 10 G
Lane 4,5,6,7,8,9, SNPs rs3104413 not detected.

Discussion

There are more than 50 known genes that can affect T1D risk, with HLA class II genes having the most influence on an individual's susceptibility [17]. Other loci have a negligible influence on T1D risk, however it has been demonstrated that the combination of HLA genotypes with non-HLA single nucleotide polymorphisms improves disease prediction [18 - 19]. The HLA has been shown to play a crucial part in determining whether a person is susceptible to or protected against T1D in a number of studies [20-22]. So far, it has been established that several HLA gene polymorphisms are connected to diabetes. This study has examined the impact of the rs310441 polymorphism in the HLA area on the emergence of T1D susceptibility or resistance in those who already have the disease. In the intergenic space between HLA-DRB1 and HLA-DQA1, there is a polymorphism called rs3104413. The polymorphisms studied in this inquiry are one of several reported genetic studies relating to widespread polymorphisms and autoimmune disorders. In comparison to healthy participants, the current investigation found that there is a strong correlation between the frequency of alleles and genotypes in patients; this supports the findings of Raha et al. (20) which show that the HLA class-II alleles have a significant role in the genetic underpinnings of T1DM. The prevalence of high-risk HLA haplotypes was also studied in the case and control groups by Cao Nguyen et al. (17). To do this, all of the samples were genotyped using a proprietary TaqMan genotyping assay 20x for three polymorphisms of the HLA class II loci (rs3104413, rs2854275, and rs9273363). These polymorphisms had 99% accuracy in predicting T1D-related HLA-DR/DQ haplotypes, according to a research.

Another research by Jamehbozorg et al. (23) found a statistically significant difference in the frequency of alleles and genotypes between patients and healthy controls. The highest risk of type 1 diabetes (T1D) was found in individuals heterozygous for three single-nucleotide polymorphisms in the major histocompatibility complex region (rs3104413, rs2854275, and rs9273363), which are known to be associated with high- and low-risk autoimmune diabetes (DR3/4, DR3/3, DR4/4, DR3/X, DR4/X, DR4-D.

According to Al Yafei et al. (24), T1D was related with DRB1 and DQB1 alleles and haplotypes in populations of Emiratis. Janelle A. and Ana M. (25) discovered that HLA class II DPB1 alleles, in particular DPB1*04:02, DPB1*03:01, and DPB1*02:02, can be associated with significant outcomes. The allele B*39:06 (OR =10.31; 95% CI, 4.21-25.1) confers the greatest vulnerability outside of the class II area.

OTTENHO et al. (26) demonstrate that IDDM patients had considerably higher frequencies of both DR3 and DR4 (respectively, p = 0.02, p = 0.01). The high-risk HLA-DR4/DQ8 haplotype is linked to a higher incidence of T1D in her group, according to Duarte et al. (27) the high-risk HLA-DR4/DQ8 haplotype is linked to a higher incidence of T1D in her group. It is now possible to accurately predict the genetic risk of non-HLA genes on T1D in Southern Brazil for various high-risk HLA-DR/DQ types.

Conclusion

This study showed that there is a substantial difference in allele and genotype frequency between sick and healthy people. The G/G genotype was demonstrated to be protective for T1D, while the C/C and C/G genotypes were more prevalent in patients than in controls. The G allelic frequency was significantly different between T1D patients and the control group. Between the two groups, there was a considerable difference in allelic frequency. According to our research, HLA polymorphism (C/G) and (C/C) genotypes are genetic risk factors linked to susceptibility, whereas (G/G) genotypes are linked to protection against T1D.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Hiba Omer AbdelRhman Hussein: PhD candidate, she selected the included patients, did all the laboratory steps and wrote the article.
Sababi Salih Abdalla: did statistical analysis and revision of the article.
Sakeena NourEldine Salih: diagnosis and selection of patients
Abdelkarim Abobaker Abdrabo: Revision according to PAMJ guidelines
Mohamed Abdelgadir Mahdi: the main supervisor.

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