Al-Kindy College Medical Journal (KCMJ)

Research Article

The Association of HLA-DRB1/DQB1 Genes with Type 1 Diabetes Mellitus among Sudanese Children and Adolescents

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Article history:
Received 14 March 2023
Accepted 9 May 2023
Available online 30 August 2023

https://doi.org/10.47723/kcmj.v19i2.980

Keywords: Type 1 Diabetes, HLA, DRB1/DQB1, Single nucleotide polymorphism.

ABSTRACT

Objective: This study was conducted to identify the association of HLA-DRB1/DQB1 genes with the susceptibility or resistance to type 1 diabetes mellitus (T1D) among patients between the ages of five and eighteen.

Subjects and Methods: The study included 200 Sudanese participants, ages ranging from 5 to 18. One hundred participants were healthy non-diabetic as the control group and 100 with T1D as the case group. The investigation was carried out in Khartoum state. The selection of patients with T1D was from diabetic centers and 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-value0.001).

Conclusion: This study found that patients' genotypes and allele frequencies are significantly correlated when compared to those of healthy participants.

Introduction

Type 1 diabetes (T1D) is a multifactorial disease in which cells of the immune system destroy pancreatic beta cells that produce insulin in the body (1). T1D is a condition that is a major public health concern(2). In the world, there are currently an estimated 366 million people who have T1D, and by 2030, that number is anticipated to increase to 552 million(3). As with other autoimmune diseases, the cause of T1D is currently unknown. People with a genetic predisposition to T1D experience chronic autoimmune disease. It is brought on by environmental triggers such germs, viruses, and certain foods. Over 60 unique genes have been shown to play critical roles 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). Human Leukocyte Antigen (HLA), a locus on chromosome 6p21.31, is involved in 40% to 50% of T1D familial aggregations. This gene causes peptide antigens to be 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. Susceptibility to T1D is markedly increased when these alleles are in linkage disequilibrium with the HLA-DRB1*03(DR3) or HLA-DRB1*04(DR4)(7-9). A large number of loci have been recognized by genome-wide association studies (GWAS) across an infinite range of single nucleotide polymorphisms (SNPs) that are located throughout the genome(10). Numerous polymorphisms in the HLA genes have been linked to diabetes.

. In this study, we assess the frequency of the rs3104413 polymorphism between HLA-DRB1 and HLA-DQA1 between HLA-DRB1 and HLA-DQA1 in the HLA vicinity in the people with diabetes in contrast to healthy subjects was evaluated. The effects of polymorphism have been studied in many groups, but no studies of a similar nature have been conducted in Sudan. Prominent breeds have a large impact on genomic polymorphisms, so it is very important to study the association between polymorphisms and T1D(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).

Subjects and method:

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 (NDDG), there are 100 instances of T1D, and 100 (non-diabetic) individuals who had neither clinical indications of T1D nor a family history of the disease. The study was conducted during the period from December 2020 until November 2022. All participants gave written informed consent 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. For DNA extraction from blood cells, we used commercially available kits (G-DEXTM11b Genomic DNA Extraction Kit (Blood) 200T catalog numbers 17241).

Genotyping

Amplification- refractory Mutation System Polymerase Chain Reaction (ARMS-PCR) was used to detect the rs3104413 (C/G) mutation. These methods are straightforward, speedy, and touchy to discover the mutations16. The HLA gene sequences were retrieved from the National Center for Biotechnology Information, (NCBI). Polymerase chain Reaction (PCR) was performed concurring with the manufacturer's information utilizing commercially accessible PCR premixes (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 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 the final extension for position rs3104413 (C/G) for 5 min at 72°C. PCR products were examined using gel electrophoresis. The length of the PCR products was 372 bp, shown in Fig. 1 for rs3104413 (C/G) polymorphism.

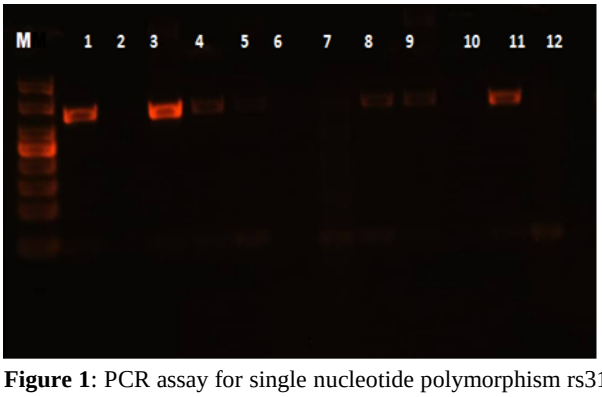

Figure 1: PCR assay for single nucleotide polymorphism rs3104413 in HALA agarose gel electrophoresis showing 327 bp .M: Molecular marker 5o bp, Lane 1 C. Lane 3 G, Lane 11 G

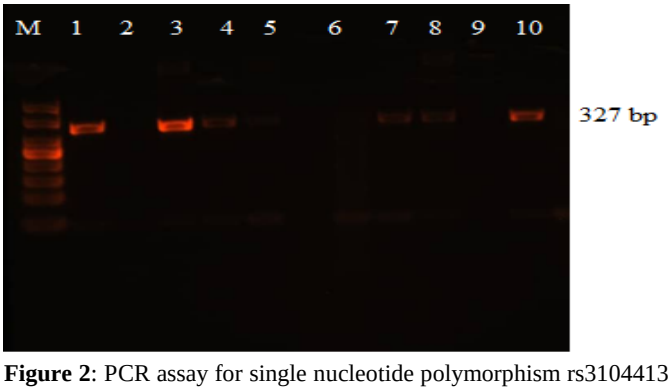

Figure 2: PCR assay for single nucleotide polymorphism rs3104413 in HALA agarose gel electrophoresis showing 327 bp M: Molecular marker 50 bp, Lane 1 C. Lane 3 G, Lane 10 G

Statistical Analysis

The statistical package for social science (IBMSPSS version 26.0) for Windows was used to analyze the data. Statistical significance was defined as a p-value of 0.05 or lower. Using Pearson's Chi-square test, categorical variables, allele frequency, and genotype frequency were all examined. 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-value (adjusted p-value= 0.05/number of new adjusted residuals or cells), the intensity of significance was determined.

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

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

Results

Two hundred Sudanese participants, ranging in age from 5 to 18, were engaged in the research. 100 patients with T1D and 100 healthy as control. In 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 ±3.7) and the controls' was (12.25 ±3.6) (Table 2).

Genotypes and Alleles Frequency

Comparison of Genotypes between Groups:

According to the gene presence, HLA-DRB1/DQB1 was detected in 87 T1D. 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-value0.001) (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).

Comparison of allele frequency between the Study Groups:

The C genotype was much more prevalent in patients (Chi-square, p-value= 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).

Discussion

HLA class II genes have the biggest influence on a person's susceptibility to T1D out of the more than 50 known genes associated with T1D risk17. Combining the HLA genotype with non-HLA SNP has been shown to improve disease prediction, even though other loci have little effect on T1D risk(18-19).

Table 2: Descriptive Statistics

Groups			Frequency
Control	Gender	Females	51.0(%)
		Males	49.0(%)
	Age (mean ±S.D.)		12.25±3.686
Patients	Gender	Females	51.7(%)
		Males	48.3(%)
	Age (mean ±S.D.)		12.00±3.735

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

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

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%

According to previous reports from different studies, it can be concluded that HLA plays an important role in the susceptibility or protection from T1D20-22. To date, several HLA gene polymorphisms are associated with diabetes. This study aimed to examine the association of the rs310441 polymorphism (in the HLA area) with the susceptibility of T1D or resistance. In the intergenic space between HLA-DRB1 and HLA-DQA1, there is a polymorphism called rs3104413.

The polymorphism investigated in this study is one of several reported genetic studies associated with autoimmune diseases. Compared to healthy participants, the current study found a strong correlation between allele frequency and genotype 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 T1D.

Cao Nguyen et al(17) studied the prevalence of high-risk HLA haplotypes. 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 research.

Another study done by Jamehbozorg et al.(23) found, between patients and healthy controls, there were statistically significant differences in the frequencies of alleles and genotypes.

We found that individuals at the highest risk for T1D were heterozygous for three single-nucleotide polymorphisms (SNP) in regions of the major histocompatibility complex (rs3104413, rs2854275, and rs9273363), known to be associated with high-risk 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 to DRB1 and DQB1 alleles and haplotypes in populations of Emiratis.

Janelle and Ana(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(26) demonstrated that IDDM patients had considerably higher frequencies of both DR3 and DR4 (respectively, p-value= 0.02, p-value= 0.01).

A high-risk HLA-DR4/DQ8 haplotype is associated with a higher incidence of T1D in that group, According to Duarte et al.(27) studies, 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 as 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. Genetic risk factors for susceptibility include HLA polymorphism (C/G) and (C/C) genotypes, whereas (G/G) genotypes are associated with protection against T1D.

Acknowledgment

The authors of this study acknowledge the participants of the study and the co-patients that they agree to be included. Also, the authors acknowledge the staff at the diabetes center for their help and support.

Funding

This research did not receive any specific fund.

Conflict of Interest

Authors declare no conflict of interest

Data availability

Data are available upon reasonable request

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[13] https://gnomad.broadinstitute.org (Genome Aggregation Database (gnomAD) is a resource developed by an international coalition of investigators

[14] https://www.nhlbi.nih.gov/science/trans-omics-precision-medicine-topmed-program) is the most comprehensive and longest-running translational research study in amyotrophic lateral sclerosis (ALS.(

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To cite this article: Hussein, H. O. A., Abdalla, S. S., Salih, S. N., Abdrabo, A., & Mahdi, M. A. Association of HLA-DRB1/DQB1 Alleles and Genetic Susceptibility to Type 1 Diabetes Mellitus. AL-Kindy College Medical Journal, 2023;19(2), 202–206.