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HLA CLASS II ALLELE AND HAPLOTYPE ASSOCIATIONS WITH TYPE 1 DIABETES IN JEDDAH, SAUDI ARABIA

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ABSTRACT

Abstract: Type 1 diabetes (T1D) is a chronic autoimmune disorder caused by the destruction of the β-cells of pancreas. Genetic and environmental factors are involved in the pathogenesis of T1D. This study aimed to determine the association between Human Leukocyte Antigens (HLA) Class II alleles encoding DR and DQ haplotypes and T1D in Saudi children. Human Leukocyte Antigens were analyzed by polymerase chain reaction sequence-specific oligonucleotide technique (PCR-SSO). The results indicated a significant association between T1D and DRB1*04:05 (*P* 0.001; OR = 3.14; 95% CI: 1.59-6.16) and DRB1*04:02 (*P* 0.010; OR = 2.87; 95% CI: 1.33-6.22) alleles. While DQB1*06:03 (*P* 0.013) and DRB1*13:01 (p 0.0067) alleles were significantly unassociated with T1D. The frequencies of the haplotypes DRB1*04:05-DQA1*03:01-DQB1*03:02 (*P* 0.004), DRB1*04:02-DQA1*03:01-DQB1*03:02 (*P* 0.005), and DRB1*04:02-DQA1*03:01-DQB1*02:02 (*p* 0.008) were significantly higher in T1D patients. On the contrary, the haplotype DRB1*13:01-DQA1*01:03-DQB1*06:03 (*P* 0.034; OR: 0.12; 95% CI: 0.014-0.94) was significant higher in control. Moreover, a significant association was observed in DRB1*04:05-DQA1*03:02 (*P* 0.006; OR: 3.47; 95% CI: 1.46-8.21) and DRB1*04:05-DQA1*05:01 (*P* 0.016; OR: 4.26; 95% CI: 1.36-13.35) haplotypes. In addition, thehaplotypeDRB1*04:05-DQB1*03:02 (*P*<0.001; OR: 8:54; 95% CI: 2.85-25.60) revealed the highest associated haplotype to T1D. DRB1*04:02-DQB1*03:02 haplotype was present in one fourth of T1D patients (n=24) as compared with a 10th control (*P* 0.022; OR: 2.71; 95% CI: 1.21-6.06). The results also showed that the haplotype DRB1*04:02-DQB1*02:01 was present in 13.54% T1D patients and 4.17 % of controls (*P* 0.042, OR: 3.60; 95 percent CI: 1.13-11.48). Our investigation showed that the studied alleles and haplotypes have a highly significant association with T1D. It could be considered that the susceptible risk factors for developing T1D in Saudi children would be DRB1*04:02 and DRB1*04:05 alleles as well as DRB1*05:05-DQA1*03:01-DQB1*03:02 haplotypes. On the other hand, the DQB1*06:03, DRB1*13:01, DRB1*10:01 alleles and DRB1*13:01-DQA1*01:03-DQB1*06:03 haplotype may be considered as protective factors for T1D.

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Introduction

Chronic diseases are essential health and therapy challenges of modern societies [1]. Diabetes is a common chronic metabolic disease and irreversible condition that is a growing global health concern. T1D is an insulin deficiency that is increasing 3–4% year by year. T1D was considered a death until the 1930s; animal insulin was successfully used as a treatment for human T1D patients. Animal insulin was first isolated from pigs and cows in 1923 [2, 3]. Diabetes is one of the eldest diseases known to humans. Over 3,000 years ago, it was first described by ancient Egyptians. Then, ancient India discovered the sweetness of urine and blood of T1D patients and described it by Honeysweet. Near 2000 years ago, an early Greek doctor "Aretaeus" used the word "diabetes" to name the disease that means "passing through" and may have been its earliest reference [4]. India and China during the 15th century differentiated the two types of diabetes; Type 1 diabetes and Type 2 diabetes. In the 17th century, scientists developed chemical tests to detect the existence of excess sugar in urine and blood and confirmed it as a cause of their sweetness. Since that time, a lot of attempts were made to find treatments, but T1D patients did not survive for a long time. In the third quarter of the 18th century, Paul Langerhans noted the development of

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diabetes in dogs after isolating their pancreas. It wasn't until 1922 that a scientist called Banting could reduce the sugar level in the blood after he extracted a substance from the pancreas which is known now as insulin. This was the first step to start the treatment of T1D patients. Then, diabetes was characterized by hypoglycemia that is following the defecting in insulin-secreting, insulin action, or both [5, 6]. The classification of different types of diabetes is today based on the World Health Organization report. Instabilities in glucose metabolism are separated into four types based on its etiology [5]. T1D is a widely studied autoimmune disorder caused by a complex combination of genetic and environmental factors [7]. The immune system districts β-cells in the pancreas that is producing insulin, which leads to insulin deficiency and fates individuals to require insulin treatment to survive [8]. T1D usually arises in childhood and adolescence, which accounts for about 10% of diabetes patients [9]. Type 2 diabetes (T2D) is caused by insulin insensitivity as a result of insulin resistance, decreasing insulin production, and eventual pancreatic β-cell failure. This leads to a decline in glucose passage into the liver, muscle cells, and fat cells; this type is typically diagnosed in adults [6]. The third type is gestational diabetes (GD) which is a well-characterized disease affecting a significant population of pregnant women worldwide [10]. It occurs when a woman's pancreatic function is not enough to overcome the diabetogenic environment of pregnancy. Pregnant women with a history of macrosomia, a strong family history of diabetes, or obesity have a high risk of the disease [11]. Pregnancies affected by this type impose a risk for both mother and child as the risk of cesarean and operative vaginal delivery, macrosomia, shoulder dystocia, neonatal hypoglycemia, and hyperbilirubinemia is increased [12]. Despite those common 3 types of diabetes, there are other specific types of diabetes including drugs or chemical-induced insulin deficiency, endocrinopathies, pancreatic destruction, and genetic defects. These unrelated forms of diabetes are included in the "Other Specific Types" and classified separately. The prevalence of T1D has been growing global by approximately 3-4% per year [13]. Diabetes is an increasing health concern, worldwide. In 2000, around 171 million individuals worldwide were identified by diabetes; by 2011 the estimation was reported to be more than 366 million, and studies predicted that it will exceed 552 million by 2030 [2]. According to the Diabetes Atlas (8th edition), Saudi Arabia is the 8th highest country for T1D in 20-year-old or younger patients, with a predicted around 35,000 affected children and adolescents [3]. T1D is a polygenic disorder whose main locus is the human leukocytes antigen (HLA) region [14]. Several studies pointed to the association between T1D and HLA, which were the first to report HLA associations with that type of diabetes currently considered T1D [15]. Later, using advanced techniques, other studies defined both positive and negative HLA associations with T1D [16]. The human leukocyte antigen (HLA) class II heterodimeric molecules consist of the alpha and beta chains and are encoded by numerous different alleles, which cause the high polymorphism characteristic of this locus. MHC class II molecules associated with T1D are encoded by three different loci are HLA-DR, HLA-DQ, and HLA-DP, which show high polymorphism. The polymorphism in these genes disturbs the ability to present antigens in the immune system, or even is made to detect which antigen is present in the population. HLA class II alleles are the main risk factors associated with several autoimmune diseases not only T1D [5]. Numbers of viruses have high similarity with peptide sequences in the insulin-producing beta cells. So, the immune system could misguidedly destroy the beta cells instead of virus-infected cells. The antibodies against these intracellular beta-cell proteins exist in 75% of newly diagnosed T1D patients, although it is not clear if this is a reason or a consequence of the attacked beta cells [17]. T1D affects between 0.5% and 1% of global population [18]. This study aimed to determine the association between Human Leukocyte Antigens (HLA) Class II alleles and haplotypes with T1D in Saudi children by describing the frequency of T1D genetic susceptibility and resistance conferred by HLA*DRB1, *DQA1, *DQB1.

Material and Methods

Study Subjects:

The study population was diagnosed in the King Abdulaziz University Hospital's pediatric endocrinology clinic with 96 T1D subjects with disease onset in 12 to 18 years of age. Of the T1D patients, 50 were female and 46 were male. While 96 participants in this study, 49 of them were males and 47 were females. The median and maximum age of both classes were 12-year-old. The 96 T1D patients and 96 Saudi healthy controls underwent blood testing and were screened for HLA-DRB1, DQA1, and DQB1 alleles. A written consent was obtained from all participants or their parents; the sample provider's proposal was approved by the Ethics Committee, Faculty of Medicine, KAU for the research title: (Vitamin D Receptor Gene Polymorphisms and Type 1 Diabetes Mellitus in Saudi Adolescents) by Khloud Kamel Alqudsi, a master student of Home Economics (food and nutrition).

HLA genotyping:

All patients were typed with polymerase chain reaction-sequence specific oligonucleotide (PCR-SSO) for HLA class II genomic polymorphisms at high to intermediate resolution levels. DNA was isolated from peripheral blood samples (Qiagen™ EZ1 ® DNA Blood 200 µl Kit (48)). HLA-DQA1, DQA1, and DQB1 gene polymorphisms were tested using commercial kits (LABType®SSO Typing Tests, One Lambda Inc., CA, USA) based on manufacturer's protocols using Recombinant Taq polymerase (OLI Cat. #TAQ75, One Lambda Inc. USA). Thermocycler (Veriti™ Thermal Cycler, Applied Biosystems, CA, USA) was used for amplification. PCR products were hybridized with microparticle beads-coated oligonucleotides and then visualized by a flow analyzer (LABScan 3D, One Lambda Inc.) by detecting fluorescent PE emissions. DNA and polymerase reaction-sequence specific oligonucleotide (PCR-SSP) extraction and amplification were

applied in laboratory B/5060 at King AbdulAziz University Hospital, Jeddah City, Saudi Arabia, under recommendations of laboratory supervisor Mr. Raid Alharbi.

Data analysis:

The odds ratio (OR) and confidence interval (95 % CI) were determined using the Woolf formula to determine the susceptible HLA DRB1, DQA1 and DQB1 alleles in this analysis using the SPSS and Vassar stats software. The Yates correction test (two-tailed) was used to determine the difference between patients and controls in the HLA haplotype distribution. Fisher's exact test was used when the expected frequency for one of the haplotypes was less than 5.

Results

Age and Gender

Results in Figure 1 showed that patients with T1D and controls were between 12 to 18 years old. The majority of participants were 12 years of age, accounting for 27% of patients and 39.6 percent of controls. In both patients and controls, the number and proportion of male and female participants were almost the same (Figure 2).

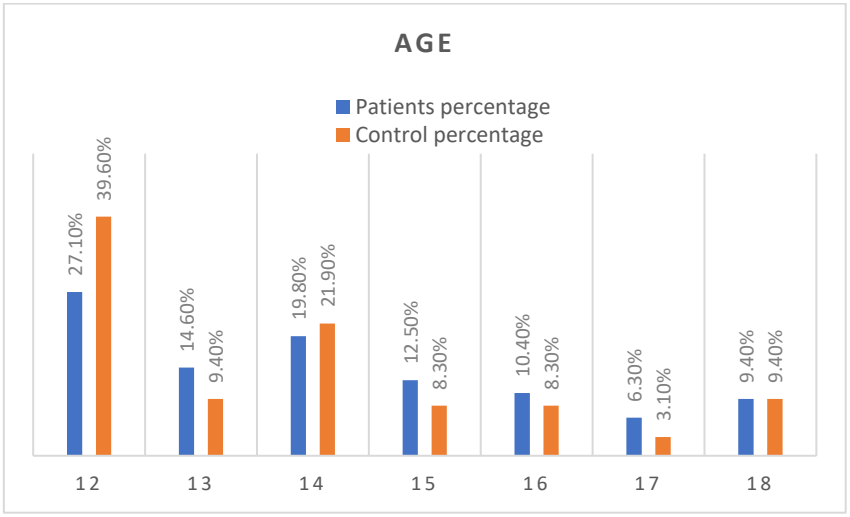

Figure 1: The age of T1D patients and control group.

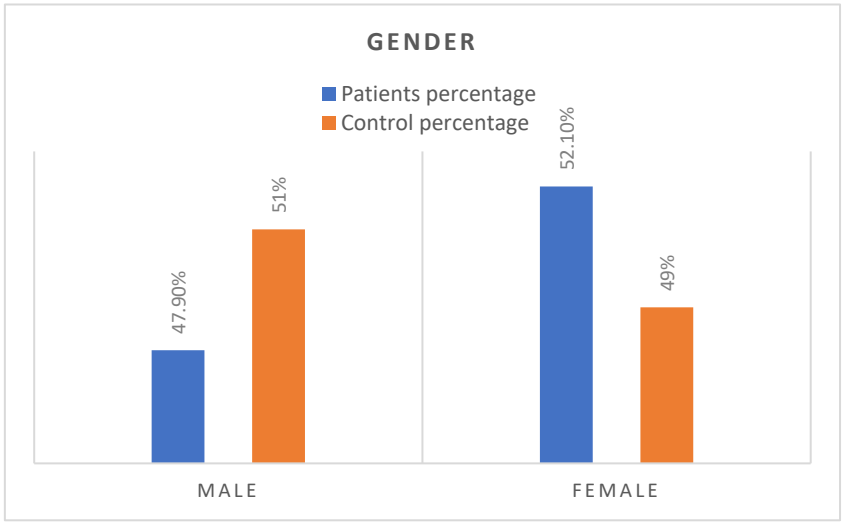

Figure 2: The gender of T1D patients and control group participating in the study.

Alleles:

DQA1 alleles:

Table 1 shows the frequencies of DQA1 alleles among T1D Saudi patients and controls. The results revealed higher allele frequencies of DQA1*03:01 and HLA-DQA1*05:01. All DQA1*03 patients (n=84) were DQA1*03:01 positive in high resolution-typing, whereas the DQA1*01 patients (n= 32) formed of 6 different sub-alleles were negatively associated with T1D. On the other hand, HLA-DQA1*05 reflected disparity in risk imposed by subtypes of DQA1*05. In both subtypes,

DQA1*05:01 patients (48 vs 37) were significantly higher in patients than in control. In comparison, DQA1* 05:05 was observed in lower-frequency patients compared to controls (13 vs 18) indicating a protective role against T1D. The remaining DQA1 alleles under DQA1* 02 and DQA1* 06 were normal.

Table 1: Frequencies of DQA1 alleles among T1D Saudi patients and controls.

DQA1	Patients N=96 (%)	Control N= 96 (%)	x^2	*P*	OR	(95% CI)
*01:01	9 (9.38)	10 (10.42)	0.00	1.000	0.8897	0.3446-2.2972
*01:02	17 (17.71)	23 (23.96)	0.79	0.374	0.683	0.3381-1.3796
*02:01	13 (13.54)	16 (16.67)	0.16	0.689	0.7831	0.3541-1.7319
*03:01	52 (54.17)	40 (41.67)	2.53	0.112	1.6545	0.9349-2.9281
*03:02	32 (33.33)	28 (29.17)	0.22	0.639	1.2143	0.6589-2.2379
*05:01	48 (50.00)	37 (38.54)	2.11	0.146	1.5946	0.8986-2.8298
*05:05	13 (13.54)	18 (18.75)	0.62	0.431	0.6787	0.3119-1.4769
*01:03	5 (5.21)	12 (12.50)	2.32	0.128	0.3846	0.13-1.1378

DQB1 alleles:

HLA-DQB1 typing revealed that 60.42% of patients had DQB1*03:02 which is almost significant compared to 46.88% of controls (*P* 0.082; OR: 1.73; 95% CI: 0.98-3.07). DQB1 typing showed that 54.17% of T1D patients were positive for DQB1*02:01 allele, while about 45.83% of healthy controls had this allele.In comparison, a substantial *P* value (*P* 0.013; OR: 0.15; 95% CI: 0.03-0.68) represented DQB1* 06:03 frequencies that decreased T1D patients relative to healthy controls (2.08% vs. 12.50%). In contrast, in comparison to T1D patients, DQB1* 05:01 allele was correlated with non-diabetics (healthy) (11.46% vs. 6.25%).DQB1*05:05,*03:21,*04:27,*05:12,*04:04, and*06:09 alleles were not present in T1D patients (0.00%), while a low percentage of controls were recorded (1.04 -4.17%). On the other side, DQB1*02:02 (30.21% vs. 28.13%) and DQB1*0502 alleles (11.46% vs. 10.42%) showed slight differences in both patients and control groups (Figure 2).

Table 2: Distribution of HLA-DQB1 alleles and haplotypes in T1D Saudi patients and control group.

DQB1	Patients N= 96 (%)	Control N= 96 (%)	x^2	*P*	OR	(95% CI)
*02:01	52 (54.17)	44 (45.83)	1.02	0.313	1.3967	0.7916-2.4642
*02:02	29 (30.21)	27 (28.13)	0.03	0.863	1.1061	0.5934-2.0618
*03:01	14 (14.58)	20 (20.83)	0.89	0.346	0.6488	0.3062-1.3747
*03:02	58 (60.42)	45 (46.88)	3.02	0.082	1.7298	0.9756-3.0672
*05:03	3 (3.13)	1 (1.04)	0.255	0.621	3.0645	0.3131-29.9968
*05:01	6 (6.25)	11 (11.4)	1.03	0.310	0.5152	0.1825-1.4545
*06:01	4 (4.17)	3 (3.13)	0.000	1.000	1.3478	0.2935-6.1901
*06:02	4 (4.17)	2 (2.08)	0.172	0.683	2.0435	0.3653-11.4299
*06:03	2 (2.08)	12 (12.50)	6.24	0.013*	0.1489	0.0324-0.6848
*03:10	1 (1.04)	1 (1.04)	0.000	1.000	1.000	0.062-16.223
*03:19	2 (2.08)	1 (1.04)	0.000	1.000	2.0213	0.1802-22.6714
*04:02	2 (2.08)	1 (1.04)	0.000	1.000	2.0213	0.1802-22.6714
*05:02	11 (11.46)	10 (10.42)	0.000	1.000	1.1129	0.4492-2.7576
*05:05	0 (0.00)	1 (1.04)	0.000	1.00	-	-
*06:04	2 (2.08)	2 (2.08)	0.000	1.000	1.000	0.138-7.2481
*06:24	1 (1.04)	3 (3.13)	0.255	0.621	0.3263	0.0333-3.1941
*02:82	1 (1.04)	0 (0.00)	0.00	1.000	-	-
*03:21	0 (0.00)	1 (1.04)	0.000	1.00	-	-
*04:27	0 (0.00)	1 (1.04)	0.000	1.00	-	-
*05:12	0 (0.00)	4 (4.17)	2.298	0.121	-	-
*04:04	0 (0.00)	1 (1.04)	0.000	1.00	-	-
*06:09	0 (0.00)	1 (1.04)	0.000	1.00	-	-

DRB1 alleles:

In Table 3, significant associations were observed between T1D and DRB1*04:05 alleles (*P* 0.001; OR= 3.14; 95% CI: 1.59-6.16), followed by DRB1*04:02 allele (*P* 0.010; OR= 2.87; 95% CI: 1.33-6.22), and DRB1*03:01 allele in the third rate (OR= 1.72; 95% CI: 0.92-3.20). In contrast, no significant associations were found between T1D and DRB1*0701 (OR = 0.80; 95% CI: 0.32-2.03) and DRB1*11:01 alleles (OR = 1.218; 95% CI: 0.374-3.962). Nevertheless, in the control group, DRB1*13:01 (11.46% vs. 2.08%) was significantly higher than in patients (*P* 0.022). There was no record for DRB1*10:01 allele in T1D patient, while it was reported in the control group explaining the high *P*-value (*P* 0.0067) (0.00% vs 8.33%). However, PCR-SSO typing showed that 23 alleles were identified in T1D, some of these alleles were present in the control group (1.04% -8.44%).On the other hand, 9 alleles appeared in PCR-SSO typing of T1D patients, 4 of which were identified once in 4 different patients and 2.08 % of the other 5 alleles. There was no report of those 9 alleles in the control group (Table 3).

Table 3: Distribution of HLA-DRB1 alleles and haplotypes in T1D Saudi patients and in the control group.

DB1	Patients N=96 (%)	Control N=96 (%)	x^2	*P*	OR	(95% CI)
*03:01	35 (36.46)	24 (25.00)	2.45	0.117525	1.7213	0.9247-3.2043
*03:05	2 (2.08)	5 (5.21)	0.593	0.4443	0.3872	0.0733-2.0468
*04:01	6 (6.25)	7 (7.29)	0.000	1.000	0.8476	0.274-2.6216
*04:02	26 (27.08)	11 (11.46)	6.56	0.010*	2.8701	1.3253-6.2156
*04:05	37 (38.54)	16 (16.67)	10.42	0.001*	3.1356	1.5948-6.1649
*07:01	9 (9.38)	11 (11.46)	0.06	0.807	0.7994	0.3153-2.0266
*11:01	6 (6.25)	7 (7.29)	0.000	1.000	0.8476	0.274- 2.6216
*04:03	1 (1.04)	6 (6.25)	2.372	0.11812	0.1579	0.0186-1.3374
*11:02	4 (4.17)	2 (2.08)	0.172	0.6825	2.0435	0.3653- 11.4299
*13:03	2 (2.08)	1 (1.04)	0.000	1.000	2.0213	0.1802-22.6714
*13:01	2 (2.08)	11 (11.46)	5.28	0.022*	0.1644	0.0354- 0.763
*13:02	1 (1.04)	4(4.17)	0.821	0.368	0.2421	0.0266-2.207
*01:01	3 (3.13)	0(0.00)	1.354	0.246	-	-
*01:02	1 (1.04)	5(5.21)	1.548	0.211	0.1916	0.022-1.6716
*04:07	1 (1.04)	2 (2.08)	0.000	1.000	0.4947	0.0441-5.5492
*08:04	2 (2.08)	4(4.17)	0.172	0.683	0.4894	0.0875-2.7372
*08:07	1 (1.04)	1(1.04)	0.000	1.000	1.000	0.062-16.223
*11:06	1 (1.04)	1(1.04)	0.000	1.000	1.000	0.062-16.223
*15:34	2 (2.08)	1(1.04)	0.000	1.000	2.0213	0.1802-22.6714
*03:06	1 (1.04)	0(0.00)	0.00	1.000	-	-
*04:06	2 (2.08)	0(0.00)	0.505	0.497	-	-
*04:11	2 (2.08)	0(0.00)	0.505	0.497	-	-
*07:05	1 (1.04)	2(2.08)	0.000	1.000	0.4947	0.0441-5.5492
*07:52	2 (2.08)	0(0.00)	0.505	0.497	-	-
*09:01	1 (1.04)	0(0.00)	0.00	1.000	-	-
*11:04	1 (1.04)	1(1.04)	0.000	1.000	1.000	0.062-16.223
*12:02	1 (1.04)	1(1.04)	0.000	1.000	1.000	0.062-16.223
*12:07	1 (1.04)	1(1.04)	0.000	1.000	1.000	0.062-16.223
*13:05	1 (1.04)	1(1.04)	0.000	1.000	1.000	0.062-16.223
*14:01	1 (1.04)	1(1.04)	0.000	1.000	1.000	0.062-16.223
*14:04	2 (2.08)	0(0.00)	0.505	0.497	-	-
*15:01	2(2.08)	2(2.08)	0.000	1.000	1.000	0.138-7.2481

*15:02	5(5.21)	4(4.17)	0.000	1.000	1.2637	0.3288-4.8571
*15:05	1(1.04)	0(0.00)	0.00	1.000	-	-
*16:01	7(7.29)	3(3.13)	0.95	0.330	2.4382	0.6113-9.7247
*16;02	3(3.13)	2(2.08)	0.000	1.000	1.5161	0.2476-9.2832
*02:01	0(0.00)	2(2.08)	0.505	0.497	-	-
*04:11	0(0.00)	2(2.08)	0.505	0.497	-	-
*05:05	0(0.00)	4(4.17)	2.298	0.121	-	-
*05:10	0(0.00)	2(2.08)	0.505	0.497	-	-
*08;05	0(0.00)	2(2.08)	0.505	0.497	-	-
*15:04	0(0.00)	2(2.08)	0.505	0.497	-	-
*01:05	0 (0.00)	1 (1.04)	0.000	1.00	-	-
*02:05	0 (0.00)	1 (1.04)	0.000	1.00	-	-
*03:32	0 (0.00)	3 (3.13)	1.354	0.246	-	-
*03:91	0 (0.00)	1 (1.04)	0.000	1.00	-	-
*04;10	0 (0.00)	1 (1.04)	0.000	1.00	-	-
*04;23	0 (0.00)	2 (2.08)	0.505	0.497	-	-
*05:01	0 (0.00)	1 (1.04)	0.000	1.00	-	-
*05:06	0 (0.00)	1 (1.04)	0.000	1.00	-	-
*06:03	0 (0.00)	1 (1.04)	0.000	1.00	-	-
*07:36	0 (0.00)	2 (2.08)	0.505	0.497	-	-
*08:01	0 (0.00)	1 (1.04)	0.000	1.00	-	-
*10:01	0 (0.00)	8 (8.33)	6.391	0.0067*	-	-
*10:05	0 (0.00)	1 (1.04)	0.000	1.00	-	-
*11:05	0 (0.00)	1 (1.04)	0.000	1.00	-	-
*12:01	0 (0.00)	2 (2.08)	0.505	0.497	-	-
*12:05	0 (0.00)	1 (1.04)	0.000	1.00	-	-
*15:03	0 (0.00)	3 (3.13)	1.354	0.246	-	-

Haplotypes

DRB1-DQA1-DQB1 haplotypes:

According to Table 4, a total of 96 cases and 96 controls analyzed for this study. Seven different DRB1-DQA1-DQB1 haplotypes were shown to be highly associated with T1D patients, while only one haplotype was significantly unrelated to those considered to be protective haplotypes. DRB1*13:01-DQA1*01:03-DQB1*0603 haplotypes were observed in 8 control participants and only one T1D patient (*P* 0.035; OR: 0.12; 95% CI: 0.01-0.94). Despite the protection of the allele, the most common haplotype with the highest significant *P*-value (0.0041) was DBR1*04:05-DQA1*03:01-DQB1*03:02. While the next two highly significant haplotypes were DBR1*04:02-DQA1*03:01-DQB1*03:02 (*P*0.0046; OR:3.40; 95% CI: 1.49-7.76) and DBR1*04:02-DQA1*03:01-DQB1*02:02 (*P*0.0067) followed by DBR1*04:05-DQA1*03:02-DQB1*03:02 (*P*0.0156; OR: 4.26; 59% CI: 1.36-13.35). 36.49% of patients had DBR1*03:01-DQA1*05:01-DQB1*02:01 haplotypes while the control group had 19.79% of those haplotypes. Furthermore, DBR1*04:02-DQB1*05:01-DQB1*02:01 (*P* 0.0314; OR:4.34; 95 % CI: 1.21-16.24) and DBR1*03:01-DQA1*05:01-DQB1*03:02 (*P* 0.032; OR: 2.93; 95 % CI: 1.16-7.40) haplotypes were of the high frequency haplotypes in T1D patients compared to control group.

Table 4: The frequencies of DRB1-DQA1-DQB1 haplotypes in T1D patients and control group.

DRB1-DQA1-DQB1	Patients N=96(%)	Control N=96(%)	x^2	*P*	OR	(95% CI)
*03:01-*01:02-*02:01	6 (6.25)	2 (2.08)	0.505	0.2786	3.1333	0.6162-15.9319
*03:01-*03:01-*02:01	9 (9.38)	4 (4.17)	1.32	0.251	2.3793	0.7069-8.0089

*03:01-*03:01-*03:02	9 (9.38)	6 (6.25)	0.29	0.590	1.5517	0.53-4.543
*03:01-*03:02-*02:01	12 (12.50)	6 (6.25)	1.53	0.2161	2.1429	0.7695-5.9671
*03:01-*03:02-*03:02	9 (9.38)	5 (5.21)	0.69	0.406	1.8828	0.6069-5.8403
*03:01-*05:01-*02:01	35 (36.46)	19 (19.79)	5.8	0.016*	2.3253	1.2117-4.4621
*03:01-*05:01-*03:02	18 (18.75)	7 (7.29)	4.6	0.032*	2.9341	1.1641-7.3954
*03:01-*05:01-*05:02	4 (4.17)	1 (1.04)	0.821	0.368	4.1304	0.4531-37.6527
*03:01-*05:05-*02;01	0 (0.00)	2 (2.08)	0.505	0.497	-	-
*03:01-*02:01-*02;01	5 (5.21)	2 (2.08)	0.593	0.444	2.5824	0.4886-13.6497
*03:01-*02:01-*02:02	4 (4.17)	2 (2.08)	0.172	0.6825	2.0435	0.3653- 11.4299
*04:05-*03:01-*02:01	5 (5.21)	0 (0.00)	3.286	0.059	-	-
*04:05-*03:01-*03:02	12 (12.50)	1 (1.04)	8.25	0.0041*	13.5714	1.728-106.592
*04:05-*03:01-*05:01	3 (3.13)	0 (0.00)	1.354	0.246	-	-
*04:05-*03:02-*02:01	15 (15.63)	7 (7.29)	2.52	0.112	2.3545	0.9139-6.0656
*04:05-*03:02-*02:02	2 (2.08)	5 (5.21)	0.593	0.444	0.3872	0.0733-2.0468
*04:05-*03:02-*03:01	1 (1.04)	3 (3.13)	0.255	0.621	0.3263	0.0333-3.1941
*04:05-*03:02-*03:02	15 (15.63)	4 (4.17)	5.84	0.0156*	4.2593	1.3586-13.3533
*04:05-*05:01-*02:01	15 (15.63)	7 (7.29)	2.52	0.112	2.3545	0.9139-6.0656
*04:05-*05:01-*03:01	10 (10.42)	3 (3.13)	2.97	0.085	3.6047	0.9599-13.536
*04:05-*05:05-*03:01	1 (1.04)	4 (4.17)	0.821	0.3684	0.2421	0.0266-2.207
*04:03-*03:01-*03:02	1 (1.04)	6 (6.25)	2.372	0.118	0.1579	0.0186-1.3374
*13:01-*01:03-*06:03	1 (1.04)	8 (8.33)	4.197	0.0347*	0.1158	0.0142-0.9446
*04:02-*03:01-*02:01	11 (11.46)	4 (4.17)	2.6	0.107	2.9765	0.913-9.7037
*04:02-*03:01-*02:02	8 (8.33)	0 (0.00)	6.391	0.0067*	-	-
*04:02-*03:01-*03:02	25 (26.04)	9 (9.38)	8.04	0.0046*	3.4038	1.4933-7.7582
*04:02-*05:01-*02:01	12 (12.50)	3 (3.13)	4.63	0.0314*	4.4286	1.208-16.2354
*04:02-*05:01-*03:02	9 (9.38)	3 (3.13)	2.22	0.1362	3.2069	0.8406-12.235
*04:02-*01:03-*03:02	4 (4.17)	0 (0.00)	2.298	0.121	-	-

DRB1-DQA1 haplotypes

Table 5 shows that four haplotypes of DQA1-DQB1 had higher frequency in T1D than control group. Two haplotypes of four accurately reflected high risk to T1D represented by *P*-value. 30% of T1D patients had DRB1*04:02-DQA1*03:01 haplotype (*P* 0.005; OR: 3.03; 95% CI: 1.44-6.39) followed by DRB1*04:02-DQA1*05:01 (*P* 0.031; OR: 4.43; 95% CI: 1.21-16.24) and DRB1*04:05-DQA1*03:01 (*P* 0.006; OR: 4.43; 95% CI: 1.60-15.32) haplotypes. In addition, significant association with T1D was present in DRB1*04:05-DQA1*03:02 haplotype (*P* 0.006; OR: 3.47; 95% CI: 1.46-8.21) followed by DRB1*04:05-DQA1*05:01 haplotype (*P* 0.016; OR: 4.26; 95% CI: 1.36-13.35).

Table 5: The frequencies of DRB1-DQA1 haplotypes in T1D patients and controls.

DRB1-DQA1	Patients N=96(%)	Control N=96(%)	x^2	*P*	OR	(95% CI)
*03:01-*05:01	31 (32.29)	20 (20.83)	2.67	0.102	1.8123	0.9438-3.48
*03:01-*03:01	14 (14.58)	8 (8.33)	1.28	0.258	1.878	0.749-4.709
*04:02-*03:01	29 (30.21)	12 (12.50)	7.94	0.005*	3.0299	1.4377-6.385

*04:02-*05:01	12 (12.50)	3 (3.13)	4.63	0.031*	4.4286	1.208-16.2354
*04:05-*0301	17 (17.71)	4 (4.1)	7.7	0.0055*	4.9494	1.5989-15.321
*04:05-*03:02	23 (23.96)	8 (8.33)	7.54	0.0060*	3.4658	1.4633-8.2087
*04:05-*05:01	15 (15.63)	4 (4.17)	5.84	0.0157*	4.2593	1.3586-13.353

DRB1-DQB1 haplotypes:

In table 6, DRB1-DQB1 haplotypes had a much higher association with T1D than of DRB1-DQA1 haplotypes. DRB1*0405-DQB1*0302 haplotype (*P*<0.001; OR: 8:54; 95% CI: 2.85-25.60) showed the highest significant *P*-value. 24% of patients had DRB1*04:02-DQB1*0302 haplotype (*P*0.022; OR: 2.71; 95% CI: 1.21-6.06) compared to 10% of the control group. In addition, the DRB1*04:02-DQB1*02:01 haplotype was found in 13.54% of patients and 4.17% of the control group resulting in a high *P*-value and high risk to T1D (*P*0.042, OR: 3.60; 95% CI: 1.13-11.48). Finally, DRB1*04:02-DQB1*02:02 haplotype was associated with T1D by the high significant *P*-value and OR (*P* 0.022: OR: 2.70 95% CI: 1.21-6.06).

Table 6: The frequencies of DRB1-DQA1 haplotypes in T1D patients and control group.

DRB1-DQB1	Patients N=96(%)	Control N=96(%)	x^2	*P*	OR	(95% CI)
*04:05-*03:02	26 (27.08)	4 (4.17)	17.42	<.0001*	8.5429	2.850-25.603
*04:05-*02:01	16 (16.67)	11 (11.46)	0.69	0.406	1.5455	0.677-3.531
*03:01-*02:01	34 (35.42)	20 (20.83)	4.35	0.0370*	2.0839	1.092-3.9766
*03:01-*03:02	19 (19.79)	10 (10.42)	2.6	0.107	2.1221	0.9298-4.8432
*04:02-*02:01	13 (13.54)	4 (4.17)	4.13	0.042*	3.6024	1.1301-11.483
*04:02-*03:02	23 (23.96)	10 (10.42)	5.27	0.022*	2.7096	1.2111-6.0622
*04:02-*02:02	4 (4.17)	0 (0.00)	2.298	0.121	-	-

DQA1-DQB1 haplotypes:

In table 7, there is no significant association between DQA1-DQB1 haplotypes and T1D neither as a risk nor a protective haplotype. On the other hand, DQA1*0501-DQB1*0201 showed high frequencies in T1D patients more than the control group (50% vs 39%) followed by DQA1*0302-DQB1*0302 haplotype (23% vs 16%) in patients and control group respectively. Other haplotypes had no sense in pointing out the relationship with T1D or not.

Table 7: The frequencies of DRB1-DQB1 haplotypes in T1D patients and controls.

DQA1-DQB1	Patients N=96(%)	Control N=96(%)	x^2	*P*	OR	(95% CI)
*03:02-*02:01	17 (17.71%)	15 (15.63%)	0.04	0.841	1.162	0.5432-2.4856
*05:01-*02:01	50 (52.08%)	39 (40.63%)	2.09	0.148	1.589	0.897-2.8124
*02:01-*02:02	13 (13.54%)	15 (15.63%)	0.04	0.841	0.8458	0.3788-1.8886
*05:05-*03:01	12 (12.50%)	16 (16.67%)	1.604	0.538	0.7143	0.3182-1.6036
*05:01-*03:02	23 (23.96%)	15 (15.63%)	1.61	0.204	1.7014	0.8254-3.507
*03:02-*03:02	18 (18.75%)	12 (12.50%)	0.99	0.320	1.6154	0.7311-3.5693
*03:01-*02:01	13 (13.54%)	10 (10.42%)	0.2	0.654	1.347	0.5599-3.2405
*03:01-*02:02	10 (10.42%)	3 (3.13%)	2.97	0.085	3.6047	0.9599-13.536
*03:01-*03:02	39 (40.63%)	32 (33.33%)	0.8	0.371	1.3684	0.7599-2.4642

Discussion

In the current study, DQA1, DQB1, and DRB1 alleles were investigated among diabetic Saudi children to determine the association between these alleles and T1D. The present study confirmed the associations between HLA class II (DRB1, DQA1, and DQB1) and T1D that already begun as documented 50 years ago in many societies, first by serology and later by using molecular tools. It is well known that DRB1, DQA1, and DQB1 alleles responsible for susceptibility and/or protection to T1D are well-identified in each community; therefore, the HLA susceptibility alleles to T1D are not the same in all populations [19]. Although HLA-DQA1 alleles were found at higher frequency in T1D patients compared to the control group. Only three alleles DQA1*03:01, *03:02, and *05:01 were commonly associated with T1D Saudi children. On the

other hand, the frequency of DQA1*01:02, *01:03, * 02:02 and *05:05 alleles were higher in the control group than in T1D patients and were identified as protective alleles. The results were consistent with the results obtained by an African American study [20]. Due to the low OR values < 1, DQB1 * 06:03, * 05:01, * 03:01 alleles were identified as protective alleles, which were consistent with those reported in other Saudi studies [21, 22]. However, in another study [23], the DQB1*06:03 allele was identified as a risk allele and was associated with T1D. On the other hand, three predisposing DQB1*03:02, *02:01, *02:02 alleles were associated with T1D and were identified as risk alleles (OR >1). These results were similar to those obtained by an Egyptian study [23, 24]. Our results showed a higher frequency of DRB1*10:01 and DRB1*13:01 alleles in the healthy control group than in T1D patients. This agreed well with previous studies that identified these alleles as protective alleles [25]. The present study found that DRB1*04:02 and* 04:05 alleles were extremely frequent in T1D patients and statistically highly significant. This was consistent with the results obtained from Arabian studies in which the DRB1*04:02 allele was highly frequent and associated with T1D [25], while in Japanese population DRB1* 04:05 was highly frequent and associated with T1D [8, 24]. There was a statistically significant risk among the associated DRB1-DQA1-DQB1 haplotypes, ranging from highly predisposing to highly protective. DRB1*04:05-DQA1*03:01-DQB1*03:02 combination was the most susceptible haplotype prevalent in the Saudi population, which agreed with previous reports [26-28]. Despite that DRB1*04:02-DQA1*03:01-DQB1*03:02 haplotype showed a high association with T1D in our results, it was identified as a risk haplotype in developing T1D in another study reported by [27]. Moreover, a significant positive association of DRB1*04:05-DQA1*03:02-DQB1*03:02 and DRB1*03:01-DQA1*05:01-DQB1*02:01 haplotypes with T1D were found. Such findings are in line with the previous study conducted in the United States [20].Nevertheless, the most negatively associated haplotype with T1D was DRB1*13:01-DQA1 01:03-DQB1*06:03. Such results were consistent with the research of African American studies [20, 29] and were consistent with the findings of Erlich and Noble [30]. Three novel haplotypes, DRB1*04:02-DQA1*05:01-DQB1*03:02, DRB1*04:02-DQA1*05:01-DQB1*02:01, and DRB1*03:01-DQA1*05:01-DQB1*03:02 were identified as T1D-associated high-risk haplotypes among Saudi children. These three haplotypes were not reported in previous studies and were believed to be novel high-risk T1D- associated haplotypes. The association of DRB1-DQA1 haplotypes with T1D was confirmed in our samples. Such haplotypes (DRB1*04:02-DQA1*03:01, DRB1*04:05-DQA1*03:01, and DRB1*04:05-DQA1*03:02) had a highly significant *P*-value that increased the risk of T1D susceptibility among Saudi populations. Moreover, significant associations of DRB1*04:02-DQA1*05:01 and DRB1*04:05-DQA1*05:01 haplotypes with T1D were also found to be significantly high. Those five highly significant DRB1-DQA1 haplotypes considered additional novel results that were not found in any previous studies.

The results clearly demonstrated that the contribution of DRB1-DQB1 haplotypes to the genetic susceptibility to T1D differs depending on the combination of DRB1-DQB1 haplotypes. The strongest susceptibility to T1D within all alleles and haplotypes that recorded in this study was DRB1*04:05-DQB1*03:02 haplotype ($P<0.0001$). A similar finding was obtained by a study performed in Finland (*P* 0.014) [31]. Similarly, DRB1*04:02-DQB1*03:02 and DRB1*03:01-DQB1*02:01 haplotypes had strong associations with T1D, which are directly compatible with previous Iranian findings [32]. To the best of our knowledge, the present study is the first article to establish a positive association between DRB1*04:02-DQB1*02:01 haplotype and T1D. There was no significant association of DQA1-DQB1 haplotypes with T1D. However, DQA1*05:01-DQB1*02:01 (52% vs 40%), DQA1*03:01-DQB1*05:02 (40% vs 33%), and DQA1*05:01-DQB1*03:02 (24% vs 15%) haplotypes were the most high-frequency haplotypes identified in this study that were higher in patients than in control group. Contrary to the above results, a study that was conducted in Spain found that, DQA1*01:01-DQB1*05:01 haplotype was strongly associated with T1D (*P* 0.007), followed by DQA1*01:03-DQB1*06:03 (*P* 0.03), and DQA1*05:01-DQB1*03:01 haplotypes (*P* 0.02) [33].

Our investigation indicated that there is a highly significant association between the studied alleles and haplotypes and type 1 diabetes, suggesting that DRB1*04:02 and DRB1*04:05 alleles and DRB1*04:05-DQA1*03:01-DQB1*03:02 and DRB1*04:02-DQA1*03:01-DQB1*03:02 haplotypes may be identified as susceptibility risk factors for developing T1D in Saudi children, while the DQB1*06:03, DRB1*13:01 DRB1*10:01 alleles and DRB1*13:01-DQA1*01:03-DQB1*06:03 haplotypes may be considered as protective to T1D.

In conclusion, there were nine novel haplotypes with high association with T1D. Three of these were haplotypes from DRB1-DQA1-DQB1 (DRB1*04:02-DQA1*05:01-DQB1*03:02, DRB1*04:02-DQA1*05:01-DQB1*02:01 and DRB1*03:01-DQA1*05:01-DQB1*03:02). The remaining five haplotypes were from DRB1-DQA1:(DRB1*04:02-DQA1*03:01, DRB1*04:05-DQA1*03:01, DRB1*04:05-DQA1*03:02, DRB1*04:02-DQA1*05:01, and DRB1*04:05-DQA1*05:01. The last novel haplotype was from DRB1-DQB1:(DRB1*04:02-DQB1*02:01).

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