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The association of Human Leukocyte Antigens Complex with Type 1 Diabetes in Omanis

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Abstract

Background: Identifying the human leukocyte antigens (HLA) high risk alleles, genotypes and haplotypes in different populations is beneficial for understanding their roles in type 1 diabetes (T1D) pathogenesis and intervention practices. ***Objective:*** The aim of this study was to identify T1D associated HLA gene alleles in the Omani population. ***Methods:*** Our case-control study included 73 diabetic seropositive children (mean age 9.08±3.27 years) and 110 healthy controls. *HLA–A, -B, -C, -DRB1,* and *-DQB1* genes were genotyped using sequence specific primer polymerase chain reaction (SSP-PCR). ***Results:*** Two HLA class I alleles (*B*08, B*58*) and three class II alleles (*DQB1*02, DRB1*03* and *DRB1*04*) were associated with T1D susceptibility, while one class I (*B*51*) and three class II (*DQB1*05, DQB1*06,* and *DRB1*16*) alleles were associated with T1D protection. *HLA- DRB1*03* and *DQB1*02* alleles showed the strongest risk association among all alleles. Six DRB1 residues (E^{9}, S^{11}, S^{13}, Y^{30}, V^{70} and K^{71}) were significantly associated with T1D susceptibility. Heterozygous genotypes, *HLA-DRB1*03/*04* and *DQB1*02/*03* were significantly associated with T1D susceptibility (*P*=4.29E-07, OR=63.2 and

P=0.02, OR=3.6, respectively). Furthermore, we detected a significant combined action of *DRB1*03-DQB1*02* haplotype in T1D risk (*P*=1.76E-05, OR=15), and *DRB1*16-DQB1*05* haplotype in protection (*P*=3.12E-2, OR=0.48). ***Conclusion:*** Known HLA class II gene alleles are associated with T1D in Omani children.

***Keywords*:** Type 1 diabetes; human leukocytes antigens; zygosity; alleles; residues; haplotypes, case-control study; Oman

Advances in Knowledge

- HLA class II alleles (*DQB1*02*, *DRB1*03* and *DRB1*04*) are the major genetic risk factors for T1D in Omanis.
- Combined action in *DRB1*16-DQB1*05* haplotype is associated with T1D protection.
- Combined action in *DRB1*03-DQB1*02* haplotype is associated with T1D risk.

Application to Patient Care

- The associated gene alleles can be used for disease prediction and intervention.

Introduction

Type 1 diabetes (T1D) is a common incurable chronic autoimmune disease of childhood, with an estimated incidence increase of 9.5% globally.[1] It is a complex disease that develops from collective contribution from genetic, epigenetic, and environmental factors.[2]

Both the cellular and humoral adaptive immune mechanisms are implicated in T1D. The destruction of β-cells driven by self-reactive CD8+ and CD4+ T cells leads to total insulin deficiency.[3] Autoantibodies to pancreatic islet β-cell autoantigens are detected prior to disease development and are used as biomarkers for β-cells dysfunction and T1D progression.[4]

Determining the associated environmental triggers, autoimmune-mechanisms and predisposing genetic background hold potentials for interventions through prediction, prevention or slowing down the rate of disease progression.

T1D estimated heritability is high (0.53 to 0.92) and familial and population based genetic studies identified more than 60 genes, responsible for about 80% of the disease heritability.[5] Most of the

T1D genetic predisposition (60%) is attributed to the human leukocytes antigen (HLA) class I and class II genes, in the major histocompatibility complex (MHC) region, which encode for proteins that present antigenic peptides for CD8+ and CD4+ T cells, respectively.[6]

Markedly, 45% of the genetic predisposition is attributed to HLA class II genes[7], thus, it is considered as a major genetic risk determinant for T1D. The strongest T1D risk is associated with the *DRB1*, *DQA1*, *DQB1* gene alleles and there is a cumulative supporting evidence for the role of *DRB1* and *DQB1* genes in combination as a haplotype.[8] In European, more than 95% of T1D cases have DR3 (*HLA-DRB1*0301-DQB1*0201*) or DR4 (*HLA-DRB1*04-DQB1*0302*). [7] The same HLA susceptibility and protection gene alleles and haplotypes were reported in Arabs.[9]

With the current knowledge about autoantigens, genetic risk alleles and biomarkers, disease interventions are more informed and can be considered at three stages: prior to the development of autoimmunity (primary prevention), after autoimmunity is recognized (secondary prevention) or after diagnosis, if significant numbers of β-cells are left (tertiary prevention). [4]

In a study conducted over two years on Omani children with T1D (9 months -14 years), reported incidence rates of 2.45 and 2.62 per 100, 1000 P-Y in 1993 and 1994, respectively.[10] The reported gender-specific incidence rates among boys and girls were 3.23 and 1.99 per 100,000 P-Y in 1993 and 2.91 and 1.95 per 100,000 P-Y in 1994, respectively. During the two years, they found higher age-specific incidence rates in the 10–14year old group children compared to the younger age group. Furthermore, a retrospective (June 2006 to May 2013) analysis of 144 T1D Omani children reported that the disease is highly prevalent in the family history of these patients (22%).[11]

In Oman, the incidence of T1D is comparatively less than other Arabs, and also, ketoacidosis reported to be less in the Omani cases[11]. Although the Omani population is genetically related to Mediterranean and West-Asian populations[12,13], the high frequency of *HLA-DR2* and *-DQ1* alleles (*DRB1*15* and DRB1**16,* and *DQB1*05 and DQB1*06,* respectively) were suggested as genetic protection factor against T1D in the Omani population.[14] However, it remains to be elucidated whether this is true or attributed to low frequency of risk alleles.

To identify the potential HLA gene alleles associated with T1D risk and/or protection in Omanis, we genotyped T1D patients, attending the pediatric clinic at Sultan Qaboos University Hospital (SQUH) in Muscat for HLA class I (*A*, *B* and *C*) and class II (*DRB1* and *DQB1*) alleles and compared them to healthy Omani controls.

Materials and methods

Statement on Ethics

The study was approved by the Ethics Research Committee in the College of Medicine and Health Science. A written informed consent was obtained from all participants guardians enrolled in the study to use their blood sample for research purpose.

Cases and Controls

One hundred Omani diabetic patients attending the pediatric clinic at SQUH were included based on their medical records (mean age 9.31±3.27 years, 47% male and 53% female). All patients did not have another autoimmune disease or syndrome and the diagnosis of T1D was confirmed by the presence of diabetes autoantibodies to islet cell (ICA) and glutamic acid decarboxylase (GADA). Family history of T1D and T2D in cases was recorded.

Peripheral venous blood samples (5 ml) were collected in EDTA – anticoagulated vacutainer tubes and stored at -20 ˚C. HLA data for 110 healthy potential bone marrow stem cell donors (mean age 10.77±3.36 years, 51% male and 49% female) from the national HLA database was used as the healthy population control.

DNA was extracted from whole blood samples using QIAamp® DNA Medi Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). DNA concentration and purity was measured using Nano Drop spectrophotometer (ND 2000; Thermo Scientific, Germany). The extracted DNA (20-35 ng/µl) was HLA genotyped for *HLA-A, -B, -C, -DRB1*, and *-DQB1* loci using a commercial sequence specific primer polymerase chain reaction (SSP-PCR) following the manufacturer's protocol (Olerup SSP). The generated genotypes data are at low resolution.

Agarose gel (1.3 %) electrophoresis was used to detect the amplified PCR product. The gel was visualized using the gel documentation system INGENIUS 3 (Syngene) with GeneSys software. The appearance of the internal control bands in all lanes indicated successful amplification of the studied DNA. Negative control wells were checked for contamination. HLA genotypes for each locus were identified using the Olerup SSP score software (version 5.00.72.5T).

Statistical analysis

Hardy-Weinberg equilibrium tests were conducted for each locus using the Basic statistics tool (One locus summary) available at HLA-net (https://hla-net.eu/tools/basic-statistics). Alleles at each locus were considered in Hardy-Weinberg equilibrium if the observed and expected (estimated) frequencies did not differ significantly ($P > 0.05$).

Tests for allele associations, zygosity, as well as tests for, independence, difference in association, combined action, interaction, and linkage disequilibrium were conducted using PyHLA.[15]

The comparison of allele frequencies was performed using Fisher's exact test. The *P* value for each test was corrected for multiple comparisons by FDR. Adjusted *P* values less than 0.05 was considered statistically significant. The strength of the association between HLA antigens and T1D was determined by odds ratio (OR). An OR ≥ 1.5 was associated with susceptibility or ≤ 0.5 with resistance.

In addition, tests for pockets with significant residues association were conducted using SKDM human leukocyte antigen tool.[16]

Results

Out of the initially screened 100 T1D patients, 73 (73%, mean age 9.08±3.27 years, 41.1% male and 58.9% female) were included in the study because they were seropositive for GADA and/or ICA autoantibodies. Twenty-six patients (26%) were seronegative (mean age 9.77±3.25 years, 61.5% male and 38.5% female), out of which three patients (two males and one female) were heterozygous for mutations in different genes (*KLF11*, *WFS1* and *HNF1A*). About 23% of the seropositive cases have family history of T1D and 59% of T2D. About 19% of the seronegative

cases have family history of T1D and 54% of T2D. One patient was excluded as no antibodies test results were reported.

All tested loci were in Hardy-Weinberg equilibrium in cases but not in controls (Supplementary data). However, as our single center project is considered as a preliminary study, we did conduct the association tests to detect any potential associations.

HLA Class I and II Loci are Associated with Risk and Protection of T1D

Association test results indicated that the risk and protection of T1D in seropositive cases are associated with alleles belonging to the HLA class I (*HLA-B*) and class II, (*HLA-DRB1* and *HLA-DQB1*) genes [Table 1].

The strongest significant susceptibility alleles are the *HLA-DRB1*03* (*P*=9.19E-11, OR= 5) and *DQB1*02* (*P*=9.76E-08, OR=3.5). We also observed that the seropositive cases for GADA (98.6%), ICAs (23.3%) or both autoantibodies (21.9%) have more *DRB1*03* or *DRB1*04* alleles (95.8%), than the seronegative cases (65.2%) and healthy controls (39%). However, the presence of risk alleles did not correlate with higher GADA autoantibody levels and the presence of protection alleles did not correlate with lower levels.

Seronegative cases also, showed significant risk association with *HLA-DRB1*03* and *-DQB1*02* alleles but to a lesser extent (*P*=1.74E-3, OR= 5.6 and *P*=1.20E-2, OR= 4.4).

The most significant resistance alleles are *HLA-DQB1*06* (*P*=6.40E-05, OR=0.05) and *HLA-DQB1*05* (*P*=9.59E-05, OR=0.4).

Zygosity at HLA Class II Loci is Associated with Risk and Protection of TID

The zygosity tests were performed to investigate homozygous, heterozygous, and zygosity associations based on the genotype frequency differences in cases and controls. The results indicated that *HLA-DRB1*03* and *DQB1*02* zygosity is associated with disease susceptibility (*P*=2.3E-05, OR=8.2 and *P*=6.6E-07, OR=9.4, respectively), i.e., significantly higher frequency

of risk allele homozygous genotypes than risk allele absent genotypes in cases compared to in controls [Table 2].

Notably, heterozygous genotypes, *DRB1*03/04* and *DQB1*02/03* are associated with significant T1D risk (*P*=4.294e-07, OR= 63.2; *P*=0.02, OR =3.6, respectively).

However, heterozygosity, i.e., higher frequency of risk alleles (*B*08*, *B*58*, *DRB1*03 DQB1*02* and *DRB1*04*) heterozygous genotypes than risk allele absent genotypes in cases compared to in controls, is associated with disease protection (*P*=0.03, OR=0.46; *P*=1.0E-12, OR=0.08; *P*=3.5E-06, OR=0.17; and *P*=0.01, OR=0.33, respectively).

T1D protection is associated with zygosity of protective alleles, *DRB1*16* (*P*=1.3E-3, OR=0.10) and *DQB1*05* (*P*=4.5E-05, OR= 0.11) and susceptibility is associated with *DQB1*06* heterozygosity (*P*=4.14E-04, OR=10.77).

Pocket residues of HLA Class II DRB1 chain are Associated with increased risk of T1D

As the HLA genotypes dictate the affinity to the presented peptides, the T1D associated HLA alleles are implicated in the selective presentation of self-peptides. Therefore, we investigated the potentially associated residues in the HLA chains using the pocket test. The results showed that six residues (Glu-9, E^{9}; Ser-11, S^{11}; Ser-13, S^{13}; Tyr-30, Y^{30}; Val-70, V^{70}; and Lys-71, K^{71}) in pockets 4, 6, 7 and 9 of HLA class II DRB1 chain are significantly associated with T1D susceptibility [Table 3] [Figure 1].

The zygosity analysis for five associated residues showed that only the heterozygotes are associated with T1D susceptibility (E^{9} *P*=1.547E-7, 6.04; S^{11} *P*=3.13E-12, 10.43, S^{13} *P*=3.13E-12, 10.43, V^{70} *P*=7.357E-13, 11.68, and K^{71} *P*=3.13E-12, 10.43). In contrast, residue Y^{30} homozygotes (*P*=1.199E-7, 33.65), heterozygotes (*P*=0.02305, 6.7) and zygosity (*P*=8.753E-6, 5.02) are all associated with T1D susceptibility.

Interactions between T1D associated alleles

Since T1D association with HLA alleles reported at the haplotypic context as well as the genotypic context, we also analyzed the associated allele interactions. Two haplotypes found to be associated with risk (*HLA-B*08-DRB1*03*, *P*8=1.57E-08, OR=12.71 and *HLA-DRB1*03-DQ*02*, *P*8=1.66E-12, OR=14.99) [Table 4] [Figure 2]. However, the interaction analysis indicated that *DRB1*03* association with T1D is independent of B*08 (*P*5=8.23E-04, *P*6=1.95E-09), while B*08 association is dependent (*P*3=0.64, *P*4=1) and that both alleles have a combined effect in disease (*P*8=1.57E -08) [Table 4]. Also, our data indicated that a combined -dependent effect of the *HLA-DRB1*03-DQ*02* haplotype results in T1D susceptibility, while a combined -dependent effect of the *DRB1*16-DQB1*05* haplotype results in protection [Table 4] [Figure 2].

Discussion

The risk and protection to T1D in Omani are associated with alleles belonging to the *HLA-B, HLA-DRB1* and *HLA-DQB1* genes [Table 1], which were reported in other populations.[8] This was expected as the Omani population is genetically related to Arab, Mediterranean and West-Asian populations.[12,13,17]

The HLA class I alleles associated with T1D susceptibility are *B*08* (*P*=1.82E-02, OR= 2.51), *B*58, P*=2.86E-02, OR=2.47) and with protection is *B*51* (*P*=1.82E-02, OR=0.41). These associated were reported in previous studies.[18] *B*08* association with autoimmune diseases was attributed to its presence in linkage disequilibrium (LD) with *DRB1*03*,[18] which we observed in both cases and controls [Table 4]. Furthermore, results indicated that *B*08* association is dependent on *DRB1*03.* Also, *B*58* is part of a significantly associated haplotype in North Indians and Han Chinese and results from both populations suggested that the association is not attributed to the allele itself.[19,20]

As predicted by a past study, T1D protection in Omanis was found to be associated with HLA-DR2 (*DRB1*16*) and DQ1 (*DQB1*05* and *DQB1*06*) alleles.[14] The highest significant resistance alleles are *HLA-DQB1*06* (*P*=6.40E-05, OR=0.05) and *HLA-DQB1*05* (*P*=9.59E-05, OR=0.4). However, despite the high frequency of the *DRB1*16* allele in the Omanis compared to other populations[21], its significant association with protection is relatively weaker (*P*= 0.02, OR=0.5).

This is likely due to the presence of different alleles (DRB1*16:01:01, 16:02:01 and 16*64, personal communication) in the Omani population and not all are not protective.

Notably, about 96% of the seropositive cases have either *DRB1*03* or *DRB1*04* allele but the presence of these alleles did not associate with higher GADA autoantibody levels. Also, no association was detected between GADA autoantibody levels and risk or protection genotypes.

The zygosity test showed that the HLA-*DRB1*03* and *DQB1*02* zygosity are associated with risk, while heterozygosity is associated with protection (*P*=1.0E-12, OR=0.08 and *P*=3.5E-06, OR=0.17, respectively), indicating that the risk associated with both alleles is recessive, as suggested by others.[7] Also, we detected that heterozygous genotypes, *DRB1*03/04* (*P*=4.294e-07, OR= 63.2) and *DQB1*02/03* (*P*=0.02, OR =3.6), are associated with significant T1D risk.

In contrast, the protection associated with heterozygosity of the same risk associated alleles may be attributed to the presence of protection alleles in the genotypes. Twenty-seven of the *HLA-DRB1*03* heterozygous cases (44) have one of the HLA-DR2 protection associated alleles (five cases with *DRB1*15* and 22 with *DRB1*16*) and thirty of the *HLA-DQB1*02* heterozygous cases (39) have one of the HLA-DQ1 protection associated alleles (29 cases with *DQB1*05* and one with *DQB1*06*).

Also, the zygosity test showed that the protection associated with *DQB1*05* and *DRB1*16* are significant in homozygosity, suggesting that the protection associated with both alleles is recessive.

The side chains of self-peptide residues interaction with the binding groove pockets, stabilize the peptide–HLA-class II complex and therefore they are known as the anchor residues. The binding grooves of HLA class II chains are characterized by the properties of the P1, P4, P6 and P9 pockets that specificity the anchor residues.[22]T1D associated residues 9, 11, 13 and 30 are located in the β-sheet floor and their side chains are in the peptide-binding groove, while residues 70 and 71 are in the α-helix but their side chains are close to residue 13 [Figure 1]. DRB1 S^{13} is in pocket 4, K^{71} in pockets 4 and 7, V^{70} in pocket 4, S^{11} in pocket 6, E^{9} in pockets 6 and 9 and Y^{30} in pocket 6. As

S^{13}, V^{70} and K^{71} were associated with the strongest disease risk based on the *P* values and OR values, they might be the major contributors from pocket 4.

S^{13} and K^{71} association with T1D susceptibility was reported by others[23,24] and they were implicated in joint susceptibility to both T1D and autoimmune thyroid disease.[25] S^{11}, S^{13} and K^{71} residues were also associated with risk to rheumatoid arthritis.[26] This suggests common disease mechanisms that operate irrespective of the presented self-peptides.

Transgenic mice expressing TID human class II susceptibility alleles, showed that MHC class II molecules present specific autoantigenic peptides, such as GAD65 peptides[27], which can potentially activate autoreactive CD4+ T cells that is known to assist in targeting β cells by cytotoxic CD8+ and autoantibody producing B cells.

Interaction tests suggested that the association of *HLA-DRB1*03* and *-DQB1*02* haplotype with T1D risk is resulting from a combined -dependent effect [Table 4]. Notably, 78% of cases with this haplotype were GADA positive, as reported by others.[28] This suggested that both susceptibility HLA alleles and anti GAD are risk factors for T1DM. However, we did not detect an association between risk alleles and higher GADA levels. This may indicate that GADA autoantibody level, which is implicated in the destructive process in the islets, is not genetically driven.

Also, the analysis indicated that the association with T1D is resulting from a combined -dependent effect of the *DRB1*16-DQB1*05* haplotype [Table 4]. This haplotype thought to have a protective role, but its rare occurrence in Caucasians and east -Asians, could not prove its effect in T1D resistance. Furthermore, we also believe that *DRB1*16-DQB1*05* haplotype in Omanis could potentially protect autoantibody seropositive first-degree relatives from T1D, like the *HLA-DRB1*15:01-DQB1*06:02* haplotype in other populations.[6]

Although other T1D associated haplotypes were reported in the Omani population, such as *DRB1*04-DQB1*03* (7.7%), *DRB1*07-DQB1*02* (6.4%) and *DRB1*15-DQB1*06* (1%) [12], we did not detect significant LD in the investigated group of cases and controls, which is likely due to small sample size.

Notably, the frequency of seronegative cases (26%) is higher than what was reported from other ethnic groups (20%).[29] However, a relatively weaker association of T1D with *HLA-DRB1* and -*DQB1* alleles in seronegative cases, may reflect the fact that some of the cases may be positive for other autoantibodies associated with T1D that where not tested for in this study or they may show positive on repeat testing, as reported by Hameed et al. .[30]

A major limitation of the study was the sample size, because it was based on a single center. Therefore, we recommend conducting a larger size multi-center study to at least double the cases sample sizes and increase the controls to cases ratio (at least 3:1) to reach acceptable power (≥80%) for verifying our preliminary study results. In addition, sequencing of the associated risk and protection allele should be considered.

Conclusion

The majority of the seropositive T1D cases (71%) have family history of T1D and/or T2D. Despite the study small sample size, we identified *DQB1*02, DRB1*03* and *DRB1*04* as potential risk alleles in GADA and/or ICA seropositive T1D in Omani children. In addition, we detected an association of the *DRB1*16-DQB1*05* haplotype with T1D protection in a combined -dependent manner.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Authors' contributions

MA-B, AA-J, SH and ES developed the proposal. MA-B, SA-B, AA-S, SA-H and AA collected the data. MA-B and HA-R ordered the required materials. MA-B and SA-B conducted the laboratory work. SA-Y reviewed the clinical and family histories. SA-B and AA-A analysed the data. AA-A drafted the manuscript. MA-B and SA-Y revised the manuscript. All authors approved the final version of the manuscript.

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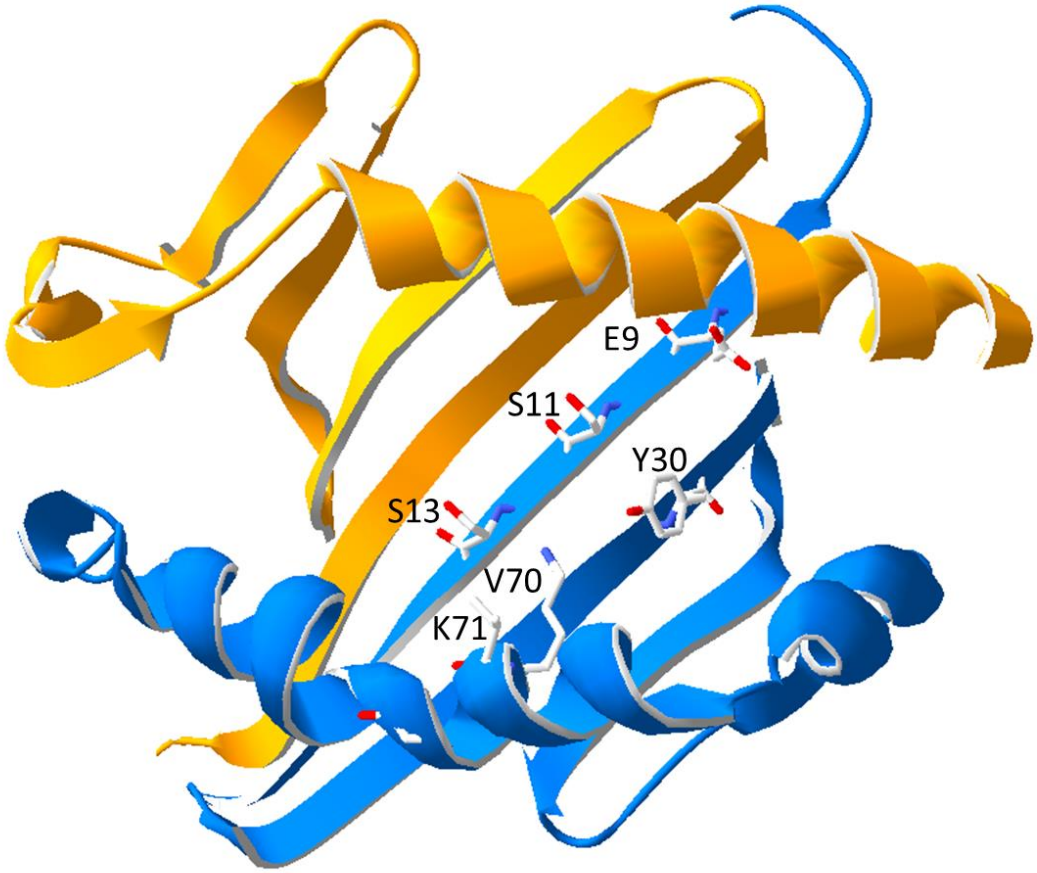

Figure 1. Ribbon model of an HLA-DR molecule peptide-binding groove, showing the position and the side-chain of significantly associated residues. The model was based on 3pdo entry from Protein Data Bank and the figure was prepared using Swiss-PdbViewer (http://spdbv.vital-it.ch/).

	HLA-B		HLA-DRB1		HLA-DQB1
Susceptibility	B*08 1.82E-02	1.57E-08	DRB1*03 9.19E-11	1.66E-12	DQB1*02 9.76E-08

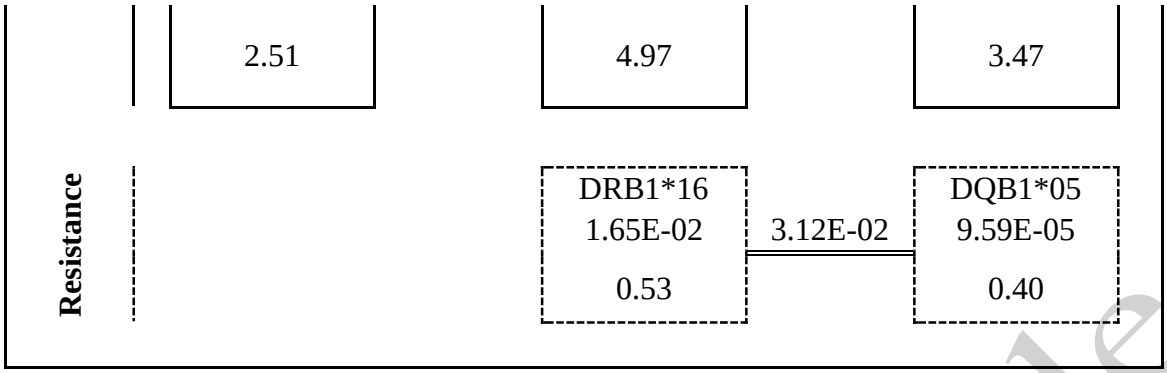

Figure 2. A representation of detected combined actions between T1D susceptibility and resistance alleles of HLA genes.

Top corrected P values and bottom odds ratios. The lines connecting gene alleles represent combined actions with P values on top.

Table 1. Distribution of significantly associated HLA alleles in T1D cases and controls

Allele	Cases %	Ctrl %	*P* value	OR	L95	U95	Adjusted *P*
Susceptibility							
DRB1*03	49.32	16.36	2.30E-11	4.97	3.07	8.06	**9.19E-11**
DQB1*02	59.59	29.82	2.44E-08	3.47	2.24	5.39	**9.76E-08**
DRB1*04	19.86	8.18	1.40E-03	2.78	1.48	5.23	**2.70E-03**
B*08	19.18	8.64	4.00E-03	2.51	1.34	4.69	**1.82E-02**
B*58	14.38	6.36	1.72E-02	2.47	1.21	5.04	**2.86E-02**
Resistance							
DQB1*06	0.68	11.47	3.20E-05	0.05	0.01	0.40	**6.40E-05**
DQB1*05	26.03	46.79	7.19E-05	0.40	0.25	0.63	**9.59E-05**
DRB1*16	20.55	32.73	1.24E-02	0.53	0.33	0.87	**1.65E-02**
B*51	8.90	19.09	7.30E-03	0.41	0.21	0.80	**1.82E-02**
DRB1*15	3. 42	8.64	5.38E-02	0.38	0.14	1.03	5.38E-02

Association test was performed using PyHLA program

Table 2. Zygosity test results for the associated HLA alleles

Allele	Hom_*P*	Hom_OR	Het_*P*	Het_OR	Zyg_*P*	Zyg_OR
DRB1*03	0.43	0.63	**1.05E-12**	**0.07**	**2.27E-05**	**8.22**
DQB1*02	0.32	1.60	**3.51E-06**	**0.17**	**6.59E-07**	**9.41**

DRB1*04	1.00	1.21	**0.01**	**0.35**	0.18	3.50
B*08	0.37	2.56	**0.04**	**0.46**	0.06	5. 61
B*58	0.63	0.6	**0.01**	**0.33**	0.62	1.81
DQB1*06	1.00	1.86	**4.14E-04**	**10.77**	0.25	0.17
DQB1*05	**0.00**	**0.19**	0.14	1.66	**4.51E-05**	0.11
DRB1*16	**0.00**	**0.10**	1.00	1.01	**0.00**	0.10
B*51	0.45	0.47	0.22	1.67	0.07	0.27

Abbreviations: Hom, homozygous test (homozygous compared to absent); Het, heterozygous test (heterozygous compared to absent); Zyg, zygosity test (homozygous compared to heterozygous); OR, odds ratio. Zygosity test was performed using PyHLA program.

Table 3. Significant residue associations in the HLA-DRB1 pockets

Position	Amino acid	Association	*P* value	Corrected *P*	Odds Ratio
Pocket 4 [13,71,78,70,74,26]					
13	S	+	2.19E-13	**1.69E-11**	11.46
71	K	+	2.19E-13	**1.69E-11**	11.46
70	V	+	3.41E-13	**2.63E-11**	11.31
Pocket 6 [9,11,30]					
9	E	+	1.98E-7	**1.37E-5**	5.43
11	S	+	1.04E-12	**7.20E-11**	10.43
30	Y	+	6.92E-05	**4.77E-03**	12.29
Pocket 7 [28,61,71,47,67]					
71	K	+	2.19E-13	**1.51E-11**	11.46
Pocket 9 [9,60,57,37,38]					
9	E	+	1.98E-7	**1.37E-5**	5.43

Residue association test was performed using SKDM program

Table 4. Significant interaction tests including independent association, Difference, action, and linkage disequilibrium (LD)

Alleles		A independent of B				B independent of A				Difference		Combined action		LD in cases		LD in controls	
Allele A	**Allele B**	***P*3**	**OR3**	***P*4**	**OR4**	***P*5**	**OR5**	***P*6**	**OR6**	***P*7**	**OR7**	***P*8**	**OR8**	***P*9**	**OR9**	***P*10**	**OR10**
Susceptibility																	
B*08	**DRB1*03**	0.64	1.29	1	0.81	**8.23E-04**	15.67	**1.95E-09**	9.86	**0.00**	0.08	**1.57E-08**	12.71	**0.02**	6.4	**0.01**	4.03
DQB1*02	**DRB1*03**	0.59	2.22	0.25	1.91	**3.43E-06**	7.83	0.10	6.76	0.24	0.28	**1.66E-12**	14.99	**1.76E-05**	25.61	**1.32E-08**	22.13
Resistance																	
DQB1*05	**DRB1*16**	0.61	0.52	**0.02**	0.33	0.50	1.45	1	0.92	0.56	0.36	**0.03**	0.48	**1.76E-10**	47.24	**8.83E-11**	29.94

If both *P*3 and *P*4 are significant, then A is associated with T1D independently of B.

If *P*5 and *P*6 are significant, then B is associated with T1D independently of A.

If both *P*3 and *P*5 are significant, then A and B show interaction in T1D.

If *P*7 is significant, then Difference between A and B is associated with T1D.

If *P*8 is significant, then A and B have combined action.

If *P*9 is significant, then A and B are in LD in cases.

If *P*10 is significant, then A and B are in LD in controls.

Interaction tests was performed using PyHLA program