Book Chapter

Combined Effect of Glutamine at Position 70 of HLA-DRB1 and Alaline at Position 57 of HLA-DQB1 in Type 1 Diabetes: An Epitope Analysis

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Abstract

The contribution of specific HLA Class II alleles in type 1 diabetes is determined by polymorphic amino acid epitopes that direct antigen binding therefore, along with conventional allele frequency analysis, epitope analysis can provide important insights into disease susceptibility. Within our highly genetically heterogeneous patient cohort we identified a subgroup that did not carry the DRB1*03:01-DQA1*05:01-DQB1*02:01 and DRB1*04:xx-DQA1*03:01-DQB1*03:02 risk haplotypes but a novel recombinant one, DRB1*04:XX-DQA1*03:01-DQB1*02:01 designated DR4-DQ2.3. Through epitope analysis we identified established susceptibility (DQB1 A^{57}, DRB1 H^{13}) and resistance (DQB1 D^{57}) epitopes as well as other novel

susceptibility epitopes DRB1 Q^{70}, DQB1 L^{26} and resistance epitopes DRB1 D^{70}, R^{70} and DQB1 Y^{47}. Prevalence of susceptibility epitopes was higher in patients and was not exclusively a result of linkage disequilibrium. Epitopes DRB1 Q^{70}, DQB1 L^{26} and A^{57} and a 10 amino acid epitope of DQA1 were the most significant in discriminating risk alleles. An extended haplotype containing these epitopes was carried by 92% of our patient cohort. Sharing of susceptibility epitopes could also explain the absence of risk haplotypes in patients. Finally, many significant epitopes were non-pocket residues suggesting that critical immune functions exist spanning further from the binding pockets.

Key words

HLA; Type 1 Diabetes; Epitopes; Susceptibility; Resistance

Introduction

Type 1 diabetes is an organ-specific autoimmune disease affecting the insulin producing β cells of the pancreas, leading to absolute insulin deficiency [1]. Although more than 40 diabetes-associated genetic linkages have been reported to date [2], numerous studies show that the strongest association involves the inheritance of specific HLA alleles [3]. In particular, a number of HLA class II alleles namely DRB1*03:01, *04:01, *04:02, *04:05 and DQB1*02:01, *03:02 are strongly linked. A set of class II haplotypes DRB1*03:01-DQA1*05:01-DQB1*02:01 (known as DR3-DQ2.5) and DRB1*04:01/02/04/05/08-DQA1*03:01-DQB1*03:02 (known as DR4-DQ8), confer the highest risk with up to 50% of patients carrying both haplotypes, whilst haplotype DRB1*15:01-DQA1*01:02-DQB1*06:02 contributes to protection [4]. Independent associations with HLA class I alleles have also been suggested and shown to potentially affect age of onset [5-10]. More recent data have described a novel association of the less polymorphic non-classical HLA class I allele HLA-G [11-13].

HLA molecules function to present antigenic peptides to T cells and thus have a central role in immune cell activation and

autoimmune disease. The peptide-binding grooves of HLA molecules are made of amino acids arranged in pockets; these amino acids are highly polymorphic and, either as single or groups of continuous or non-continuous residues create millions of possible epitopes. These epitopes determine the repertoire of peptides a given HLA allele can present. Class I molecules (HLA-A, -B and -C) have binding grooves made of six pockets. Class II molecules (HLA-DR, -DQ, -DP) are heterodimers comprised of α and β chains creating binding grooves with four major pockets. In the HLA-DR heterodimer polymorphism is found only on the β chains whereas both α and β chains of the HLA-DQ and HLA-DP heterodimers are polymorphic.

In addition to conventional allele frequency studies, the systematic analysis of HLA epitopes has recently been highlighted as a critical component in providing a better understanding of genetic susceptibility to type 1 diabetes [14]. Indeed, even in the absence of statistically significant HLA allele association, disease susceptibility may be determined by the independent contribution of polymorphic residues participating in the formation of a functional arrangement within the binding cleft of an HLA molecule, a concept first proposed by Zerva et al. in 1996 [15]. Epitope analysis can also uncover allele associations that are missed due to their low frequency in the population or disparate alleles that share peptide-binding motifs, known as shared epitopes. In addition, single amino acid polymorphisms in the same allele have been shown to alter disease susceptibility, for example aspartic acid in position 57 of the HLA-DQB1 is protective whereas substitution with alanine is associated with susceptibility [14,16]. Amino acid differences have been shown to discriminate even closely related alleles and alter the binding avidity for insulin peptides [17]. Finally, specific epitopes may explain disease susceptibility in patients that do not carry the established risk alleles.

In the current study we investigated the HLA frequencies in a cohort of Cypriot type 1 diabetes patients. We propose that studying genetically heterogeneous populations such as the Cypriot population can be critically informative in allowing further validation of established risk alleles and epitopes or

uncovering new ones. Our study revealed a new recombinant risk haplotype DR4-DQ2.3. Furthermore, we identified previously reported HLA class II susceptibility epitopes including DQB1 L^{26} and A^{57} but also susceptibility epitope DRB1 Q^{70} and alternative protective R^{70} not previously reported to be associated with type 1 diabetes. Finally, we showed that an extended risk haplotype of HLA class II susceptibility epitopes identified in this study DRβQ^{70}-DQβ$L^{26}A^{57}$ DQα$Y^{11}R^{52}R^{55}F^{61}T^{64}I^{66}L^{69}$V/$L^{76}$ H^{129}E/K^{175} could account for 92% of our patient cohort.

Research Design and Methods

Study Population

A dataset of previously enrolled consented case (type 1 diabetes patients) and control (individuals with no history of diabetes) subjects was used. The case cohort consisted of 170 Greek Cypriot patients with a cut off age of disease onset set at 39 years and the mean age being 11 years. Information regarding demographics, gender, ethnicity and age of onset was also collected for each patient. The control group consisted of 192 healthy individuals of the same ethnic descent. The study was reviewed and approved by the Cyprus National Bioethics Committee.

Genotyping of HLA class II Loci

Genomic DNA was extracted from whole blood with the use of commercially available QIAGEN® genomic DNA extraction kit. High resolution HLA genotyping was performed by HistoGenetics at 4 classical major histocompatibility complex loci DRB1, DQA1, DQB1 and DPB1 using Next Generation Sequencing (NGS), as previously described [18].

HLA Allele Frequency and Epitope Analysis

For allele frequency and epitope analysis the SKDM HLA Tool beta was used [19], which can test for HLA allele differences between two populations and perform amino acid analysis by retrieving amino acid sequences. Highest polymorphism is found

amongst residues lining the binding pockets of HLA molecules however in this analysis both pocket and non-pocket amino acid epitopes were investigated. Once primary associations are identified other parameters are determined such as zygosity, interaction and linkage disequilibrium.

SKDM output includes the difference (Delta) in frequency between case and control alleles for a particular locus. A corresponding odds ratio (OR) and a corrected p-value are also supplied. P-values are corrected by the number of distinct alleles present in cases and controls. For each zygosity comparison, the OR is calculated by Haldane's modification of Woolf's method: $OR = [(a + ½)(d + ½)]/[(b + ½)(c + ½)]$, and the significance of derivation from unity is estimated by Fisher's exact test. HLA epitopes with a corrected P value ≤ 0.001 were defined as statistically significant. In addition, odds ratio (OR) ≥ 1.5 determined susceptibility while $OR \leq 0.5$ determined resistance.

Results

HLA class II allele frequencies in Type 1 Diabetes

We used a dataset of 170 type 1 diabetes patients and 192 control subjects for whom high-resolution HLA genotyping was performed at 4 classical major histocompatibility complex class II loci DRB1, DQA1, DQB1 and DPB1. Allele frequency analysis (Figure 1 and Supplemental Tables 1-4) showed that consistent with previous studies the alleles most significantly associated with disease were HLA-DRB1*03:01 and *04:05, HLA-DQB1*02:01 and 03:02, HLA-DQA1*03:01 and HLA-DPB1*03:01. In contrast, the alleles most commonly found in the control population, and therefore deemed protective, were HLA-DRB1*14:01, *11:04, *10:01 and *16:02, HLA-DQB1*03:01 and *05:03, HLA-DQA1*01:01 and HLA-DPB1*04:02.

HLA class II Epitopes Associated with Susceptibility and Resistance

The high-resolution HLA genotyping dataset of patients and control subjects was imported into the SKDM HLA Tool for

analysis to identify epitopes associated with type 1 diabetes. Both pocket and non-pocket residues were investigated for each allele and a number of them were found significantly associated. The susceptibility epitopes with the lowest p values and highest OR and the resistance epitopes with the lowest p values and lowest OR are summarized in Table 1. No statistically significant associations were found for HLA-DPB1 epitopes.

The strongest association was shown for the previously reported HLA-DQB1 epitope A^{57} ($p= 1.3x10^{-27}$, OR 22.3). This epitope is found in HLA-DQB1*02:01 and *03:02 alleles which were significantly more common in patients according to the allele frequency analysis (Supplementary Table 2). Aspartic acid in the same position (D^{57}) was identified as the epitope most strongly associated with resistance ($p= 4.5x10^{-25}$, OR 0.07). Two of the HLA-DQB1 alleles (*05:03, *03:01) sharing this epitope were more frequent in controls. Similarly, the presence of leucine (L) in position 26 was the second strongest epitope associated with susceptibility ($p= 1.4x10^{-22}$, OR 18.47), whilst tyrosine (Y) ($p= 1.1x10^{-12}$, OR 0.14) or glycine (G) ($p= 1.9x10^{-5}$, OR 0.32) at the same position confer resistance. L^{26} is found in HLA-DQB1*02:01 and *03:02 alleles which are more common in patients. HLA-DQB1 susceptibility epitopes F^{47} ($p= 1.6x10^{-14}$, OR 6.2) and R^{70} ($p= 1.6x10^{-8}$, OR 12.1) were also found in the same position as resistance epitopes Y^{47} ($p= 4x10^{-6}$, OR 0.09) and G^{70} ($p= 1.8x10^{-7}$, OR 0.26). In contrast, resistance epitopes A^{13} ($p= 1.1x10^{-12}$, OR 0.14), Y^{37} ($p= 1.9x10^{-6}$, OR 0.08), T^{28} ($p= 4x10^{-6}$, OR 0.09) were not in the same position as susceptibility epitopes.

The DRB1 locus contained the next most significant HLA epitope associated with susceptibility to disease; HLA-DRB1 Q^{70} had the strongest association ($p= 3.6x10^{-18}$, OR 12.42). This epitope is shared between most high risk alleles including DRB1*03:01, *04:05 and *04:01, which were all significantly more common in patients (Supplementary Table 1). In addition, previously reported HLA-DRB1 epitopes H^{13} ($p= 8.8x10^{-17}$, OR 7.57) and K^{71} ($p= 3.x10^{-11}$, OR 5.48) were also significantly associated with susceptibility. HLA-DRB1 H^{13} is shared by a number of HLA-DRB1 *04:xx alleles that confer high risk.

HLA-DRB1 *04:01 which shares all three of these epitopes had the highest OR 10.07. HLA-DRB1 resistance epitopes R^{70} (p= $8.2x10^{-12}$, OR 0.04) or D^{70} (p= $7.4x10^{-9}$, OR 0.22) and R^{71} (p= $5.12x10^{-6}$, OR 0.14) are at the same positions as susceptibility epitopes. Resistance epitope R^{70} in particular had the lowest OR and is shared among two protective alleles HLA-DRB1*10:01 and *14:01 which were significantly less common in patients. The presence of arginine (R) at position 74 is associated with susceptibility (p=$4.2x10^{-12}$, OR 6.6), whereas glutamic acid (E) at the same position is significantly associated with resistance (p=$1.4x10^{-5}$, OR 0.17). Finally, epitopes R^{30} and A^{38} were also significantly associated with resistance and had the next lowest OR after R^{70} (p= 0.0003, OR 0.07).

A number of DQA1 epitopes were strongly associated with diabetes; Y^{11}, R^{55}, I^{66} and L^{69} (p= $4.2x10^{-9}$, OR 11) and the previously reported R^{56} and V^{76} (p= $1.6x10^{-15}$, OR 6.8 (Supplementary Table 7). All these susceptibility epitopes are present in DQA1*03:01 (R^{56} and V^{76} are only found in this allele) most frequently observed in patients (Supplementary Table 3). Furthermore, all resistance HLA-DQA1 epitopes G^{55}, C^{11}, M^{66}, A^{69}, G^{56} and M^{76} (p= $3x10^{-8}$, OR 0.24) are at the same positions as susceptibility epitopes and are shared between HLA-DQA1* 01:03 and *01:01 alleles, which were significantly more frequent in controls.

HLA Epitopes Associated with Susceptibility and Resistance Show Gene-Dose Effect

The effect of homozygosity or heterozygosity of class II epitopes identified to be associated with susceptibility or resistance was analyzed. Homozygosity of susceptibility epitope DQB1 A^{57} had the strongest association with disease (p= $1.25x10^{-24}$, OR 100.6) (Table 2), whilst inheritance of two copies of the resistance epitope at the same position, DQB1 D^{57}, had the strongest negative association (p= $3.94x10^{-9}$, OR 0.03). A gene dose effect was apparent since inheritance of one copy of the A^{57} epitope decreased the probability of disease as shown by the lower OR (p= $5.61x10^{-23}$, OR 17.5), in the same way inheritance of one copy of the susceptibility epitope D^{57} (p= $4.8x10^{-22}$, OR 0.08)

was not as protective as inheritance of two copies but still lowered probability of disease compared to inheritance of one copy of A^{57}. However, resistance appears to be dominant since inheritance of only one copy of D^{57} still conferred a lower probability of diabetes (OR 0.08). A similar effect was observed for DQB1 L^{26}, inheritance of two copies is strongly associated with diabetes ($p= 4.3x10^{-21}$, OR 45.8), inheritance of one copy greatly decreased the probability of disease ($p= 3.9x10^{-19}$, OR 14.9) and two copies of G^{26} is protective ($p= 8.68x10^{-6}$, OR 0.11). Inheritance of two copies of the HLA-DRB1 Q^{70} susceptibility epitope was also strongly associated with diabetes ($p= 1.4x10^{-17}$, OR 20.1) and probability decreased with inheritance of one copy ($p= 5x10^{-15}$, OR 10.2). Among the two alternative resistance epitopes identified for this position, inheritance of R^{70} appears to be more protective than inheritance of D^{70}; one copy of R^{70} conferred lower probability of disease ($p= 9.44x10^{-13}$, OR 0.04) than one ($p= 1.8x10^{-7}$, OR 0.27) or two copies of D^{70} ($p= 6.1x10^{-10}$, OR 0.11).

Linkage Disequilibrium between DQ and DR Susceptibility Epitopes

The prevalence of having both the highest susceptibility epitopes DRβ Q^{70} and the DQβ A^{57} was compared between patients and control subjects (Table 3). The majority of patients (90%) had both epitopes in contrast to 29.7% of the control subjects ($p \leq 0.0001$, OR 21.3). A very small percentage of patients had only the DRβ Q^{70} epitope (2.9%) or only the DQβ A^{57} epitope (4.1%) or none of the two epitopes (2.9%). Among control subjects the highest percentage (38.5%) had none of these susceptibility epitopes ($p \leq 0.0001$, OR 0.05). These findings suggest that our observations are not only due to linkage disequilibrium between the two loci.

Shared Susceptibility Epitopes Account for type 1 Diabetes in Patients Lacking HLA Class II Risk Haplotypes

Having identified a number of susceptibility and resistance epitopes associated with type 1 diabetes we aimed to further

dissect our patient cohort with regards to their HLA genotypes aiming to explain disease susceptibility by the presence of shared susceptibility epitopes. More specifically, among our patients the majority (135 of 170 or 79%) carried at least one or both of the susceptibility alleles DR3-DQ2.5 and DR4-DQ8 (Table 4). However, a significant number of patients (35 of 170 or 21%) did not carry any copies of either haplotype. We observed a new recombinant haplotype DRB1*04:XX-DQA1*03:01-DQB1*02:01, henceforth named DR4-DQ2.3, dominant within this subgroup; 22 (63%) of 35 patients carrying non-risk haplotypes and 13% of all patients carried one copy of the DR4-DQ2.3 haplotype in contrast to only 9 of 192 control subjects (4.7%). Interestingly, no DR4-DQ2.3 homozygous individuals were identified. The DR4-DQ2.3 haplotype contains the HLA-DQB1*02:01 allele instead of the HLA-DQB1*03:02, however both alleles share a number of DQB1 susceptibility epitopes such as L^{26}, R^{70}, L^{85}, E^{86}, T^{89}, but more importantly the most significant susceptibility epitope A^{57}. Hence the presence of either HLA-DQB1*02:01 or HLA-DQB1*03:02 makes the patient homozygous for the A^{57} epitope, associated with the highest OR in the zygosity analysis ($p=1.25x10^{-24}$ OR 100.6).

Carrying at least one copy of any of the risk haplotypes DR3-DQ2.5, DR4-DQ8 or DR4-DQ2.3 could account for 92% (157 of 170) of patients (Table 4). However, an additional 8% of our patients (13 of 170) do not carry any of the risk haplotypes. We were able to verify that all these patients carried susceptibility associated epitopes identified in this study on the DRB1 locus and one or more in the DQA–DQB loci.

HLA Epitopes Associated with Susceptibility and Resistance and their Potential Function

To investigate whether epitopes differentiate associated alleles, allele sequences were retrieved and aligned using the IMGT/HLA database of the European Bioinformatics Institute. Within these sequences we noted all the susceptibility and resistance epitopes, both pocket and non-pocket, identified in this study and also found to have a proposed function according to literature **[20-22]**. The DQA, DQB and DRB domains show a

considerable number of polymorphisms that are mainly involved in antigen binding by the anchoring pockets, the heterodimer formation by salt bridges, T-cell receptor (TCR) or CD4 co-receptor binding and in the formation of the dimer of heterodimers.

Epitopes of HLA DRB1 E^{9}, V^{11}, H^{13}, Y^{26}, N^{37} and R^{74} that are associated with diabetes are amino acids that are part of binding pockets (Table 5). The residue at position 57 is involved in pocket 9 but also participates in hydrogen bond formation to the peptide. The amino acids at positions 67, 70 and 71 were also part of the pocket formation but are also sites for TCR contact. Lastly, position 112 has a potential function in the homodimer of heterodimers and the amino acid at position 140 is a potential contact side for the CD4 co-receptor. HLA DQB1 epitopes involved in the formation of the peptide pocket included positions 13, 26, 28, 30, 37, 47, 57, 67, 70, 71, 74, 85, 86, 89 and 90 (Table 6). Amino acids at position 30 and 57 are also involved in the formation of a hydrogen bond to the peptide while residues at position 67, 70 and 71 are also a potential TCR contact site. Amino acids 52, 53 and 55 act as a homodimerization patch in the dimer formation. Pocket epitopes of the HLA DQA1 molecule include residues at positions 11, 52, 66, 69 and 76 (Table 7). Amino acids at positions 69 and 76 also form a hydrogen bond to the peptide. Important amino acids at positions 55-64 are potential TCR contact sites while amino acid at position 129 is a potential CD4 contact site. The residue at position 175 upholds a function in the formation of the homodimer of heterodimers.

Overall, all risk associated HLA alleles contain more susceptibility epitopes and protective alleles contain more protective alleles. Some epitopes, however, might not be as critical as others. For example, HLA-DRB1 susceptibility epitopes E^{9} and L^{67} and resistance epitope T^{77} are found in both risk and protective alleles. In contrast, all risk alleles contain the susceptibility epitope Q^{70} which is absent from the protective alleles. Epitopes also alter the susceptibility of closely related alleles. For example, HLA-DQB1*03:01 and *03:02 have very similar amino acid sequences but differ at critical position 57,

risk associated HLA-DQB1*03:02 contains alanine (A), whereas protective HLA-DRB1*03:01 contains aspartic acid (D) in the same position. In addition, risk associated HLA-DQB1*03:02 also contains the susceptibility epitope L^{26}. Finally, a sequence of 10 amino acid residues could differentiate risk and protective HLA-DQA1 alleles. Epitope $Y^{11}R^{52}R^{55}F^{61}T^{64}I^{66}L^{69}V/L^{76}H^{129}E/K^{175}$ was exclusive to risk associated alleles and was not observed in any protective alleles.

Extended Risk Haplotype of HLA class II Susceptibility Epitopes Accounts for 92% of Patients

We counted the number of individuals carrying an extended haplotype containing all the HLA class II risk associated epitopes in our patient-control cohort (Table 8). The epitopes we included were the ones that differentiated risk and protective alleles as described above. The vast majority of patients (167, 92%) carried at least one copy of the $DR\beta Q^{70}$-$DQ\beta L^{26}A^{57}DQ\alpha Y^{11}R^{52}R^{55}F^{61}T^{64}I^{66}L^{69}V/L^{76}$ $H^{129}E/K^{175}$ epitope haplotype compared with only 27% of the control subjects. A very small percentage of patients had only the $DR\beta$ Q^{70} epitope (2.9%) or only the $DQ\beta$ A^{57} epitope (4.1%) or none of the two epitopes (2.9%). Among control subjects the highest percentage (73%) had none of these susceptibility epitopes compared to only 8% of patients ($p \leq 0.0001$, OR 0.03).

Discussion

The association of class II alleles with type 1 diabetes susceptibility is well documented even though the exact mechanism that confers the disease risk is yet to be fully understood. The allele frequencies of Cypriot type 1 diabetes patients had not been previously reported. The vast majority of patients (79%) carried the established risk haplotypes DR3 - DQ2.5 and DR4 - DQ8 either in heterozygous or homozygous, or carried both haplotypes, while only 20% of the control population were carriers ($p<0.001$). A significant percentage (21%) of our diabetic cohort did not carry the risk haplotypes probably because these do not present at high frequency in the Cypriot population especially as compared to European

Caucasians where they represent the first and second most common haplotypes respectively in contrast to ranking 87th and 91st respectively in Cypriots. We thus believe that the Cypriot population represents an excellent study sample that can allow further dissection of disease susceptibility. Using our highly diverse cohort of Cypriot patients we were able to identify a new recombinant predisposing haplotype DR4 – DQ2.3 carried by 13% of our patients, but only 4.7% of control subjects. This haplotype failed to reach significance in a large type 1 diabetes Genetics Consortium investigating HLA-DR-DQ haplotypes in families of mostly European descent (23). Further verification of the significance of this haplotype in disease susceptibility should be pursued in a larger patient cohort.

Recent studies have supported the significance of epitope analysis as an additional piece to the complex puzzle of deciphering autoimmune disease susceptibility (Roark et al., 2008; Freed et al., 2011), we thus attempted a similar analysis in our own cohort. The polymorphic residues of the HLA class II molecules are important not only for peptide binding but also interaction with the T cell receptor and CD4 as well as dimerization and stability of the heterodimer therefore in our study we also included residues outside of the binding pockets unlike previous studies. Using the SKDM HLA Tool, an independent tool from ones used in previous studies, we were able to confirm the significance of a number of previously reported susceptibility and resistance epitopes. Susceptibility epitope HLA-DQB A^{57} and protective epitope consisting of aspartic acid (D) in the same position were found to have the strongest association in agreement with previous reports (14; 16). This aspartic acid forms a salt bridge with a conserved arginine (R) at position 76 of HLA-DQA (21) and has been correlated with protection whereas the presence of a non-charged amino acid at position 57, likely incapable of forming a salt bridge, predisposes to type 1 diabetes (24; 25). In addition, absence of HLA-DRB D^{57} in combination with HLA-DQA R^{52} has been associated with susceptibility (26); the proximity of these residues to the interface of the dimer may affect the stability or structure of the dimer of heterodimers (21).

The most significantly associated HLA-DRB1 susceptibility epitope identified in our study was Q^{70} not previously associated with type 1 diabetes. We were able to show that this residue alone discriminated between resistance and susceptibility HLA-DRB1 alleles. In addition to Q^{70}, we found epitopes HLA-DRβ V^{11}, H^{13} and L^{67} that were previously reported to have the highest association with RA susceptibility, whereas D^{70} strongly correlated with resistance (27). The same study identified a two amino acid epitope $QA^{70,74}$ associated with RA susceptibility. In addition to resistance epitope HLA-DRβ D^{70}, we identified an alternative epitope in the same position of the HLA-DRB1 allele, R^{70}, which was actually more protective.

The importance of the epitope analysis becomes apparent when considering closely related haplotypes or alleles with different risk determined by the presence of certain epitopes. For example, the closely related haplotypes DRB1*04:01-DQA1*03:01-DQB1*03:02 and DRB1*04:04-DQA1*03:01-DQB*03:02 differ only at amino acid positions 71 (lysine vs. arginine) and 86 (glycine vs. valine) of DRB1; however, the former is highly predisposing whereas the latter haplotype is neutral (23). Similarly, we showed that closely related alleles HLA-DQB1*03:02, which is risk associated, and HLA-DQB1*3:01, which is protective, differ at critical positions 26 and 57. In addition, sharing of epitopes by disparate alleles may explain disease association but also disease susceptibility in the absence of high risk alleles. For example, two distinct HLA molecules not closely related but both risk associated, HLA-DQA1 *03:01 and *05:01, shared an extended haplotype of 10 amino acid residues (DQA $Y^{11}R^{52}R^{55}F^{61}T^{64}I^{66}L^{69}V/L^{76}H^{129}E/K^{175}$) all found to be significantly associated with disease susceptibility and all entirely different from all other DQA alleles suggesting that this constitutes a shared epitope for type 1 diabetes. Finally, we observed that whilst the majority of our patient cohort carried the established DR3 - DQ2.5 and DR4 - DQ8 risk haplotypes, 13% carried one copy of the DR4 - DQ2.3 haplotype and a small number of patients did not carry any of the known risk alleles. We were able to find that all patients however, even those that did not carry susceptibility haplotypes, carried susceptibility

epitopes that we have identified in their DRB1 locus and one or more in the DQA1 and DQB1 loci.

Our study reports for the first time a new haplotype, DR4 – DQ2.3 in type 1 diabetes patients. In addition, our study lends further support to the significant role of HLA risk epitopes. HLA epitopes DRB Q^{70}, DQB L^{26} and A^{57} and a 10 amino acid epitope of DQA were identified to be the most significant in discriminating risk alleles. Of our patient cohort 92% were carriers of the DQA $Y^{11}R^{52}R^{55}F^{61}T^{64}I^{66}L^{69}V/L^{76}H^{129}E/K^{175}$ with DRβ Q^{70} and DQβ $L^{26}A^{57}$, in contrast to 25% of our controls, suggesting that this extended HLA class II epitope haplotype is involved in the disease pathogenesis while other genetic factors may act as disease modifiers. Since these amino acids are implicated in functions other than antigen binding this suggests that the importance of the HLA molecule spans further from the peptide groove and its binding affinity to auto-antigens, to other allosteric sites that also hold important immune functions.

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Supplementary Materials

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