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ORIGINAL RESEARCH

Bioinformatics-Based Predictions of Peptide Binding to Disease-Associated HLA Proteins Suggest Explanation for Shared Autoimmunity

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Abstract:

Aim: This study was designed to examine the immunogenetic basis for shared autoimmunity, resulting in autoantigen presentation that leads to the production of two or more disease-specific autoantibodies.

Methods: A bioinformatics approach based on peptide binding predictions to disease-associated HLA determinants has been developed and tested here using 11 disease associations between autoimmune systemic and mucocutaneous blistering disorders. Various HLAs associated with antigens within a given "disease model" (set of HLA class II and protein sequences known to be associated with a specific autoimmune disease) were tested and ranked against the antigenic proteins, first with proteins they are known to associate with and then with proteins known to be implicated in a second disease model. In every case binding predictions were compared for different proteins binding to the same HLA. Subsequently, disease-related autoantigens have been tested for their binding affinity against each disease-specific HLA class II protein.

Results: For a single HLA haplotype, several binders have been generated from a related autoantigen with the variable binding score. In most cases, the binding score corresponding to the interactions between the autoantigen-derived epitope and the HLA associated with one disease was similar or lower than the interactions between the epitope from proteins associated with the second disease and the same HLA. Notably, there was no compelling promiscuity in peptide binding to each of the HLA molecules, in spite of the promiscuous nature of HLA class II binding.

Conclusions: The data suggest that, in susceptible individuals, shared autoimmunity might be initiated by two types of HLA/peptide interaction; first between an autoantigen-derived epitope and its disease-associated HLA molecules, and second, between a different peptide of the same autoantigen and HLA proteins specific for the second disease.

Keywords: autoantigenic epitopes, autoimmune blistering diseases, HLA determinants, peptide binding, Rankpep tool

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Introduction

Autoimmune mucocutaneous blistering diseases (AMBD) such as pemphigus vulgaris (PV), pemphigus foliaceus (PF), bullous pemphigoid (BP), ocular cicatricial pemphigoid (OCP), dermatitis herpetiformis (DH) and mucous membrane pemphigoid (MMP), are a group of rare organ-specific diseases that affect skin and multiple mucous membranes.[1–5] PV is a potentially fatal disease characterized by the loss of intercellular adhesion of keratinocytes, resulting in acantholysis.[6–8] In the serum of PV patients, high titers of circulating autoantibodies targeting the epidermal adhesion molecule desmoglein 3 (Dsg3)—keratinocyte transmembrane proteins localized in the desmosome, essential for maintaining the integrity of the epidermis—are believed to cause clinical disease by direct binding to and disruption of desmoglein proteins.[1,9] The association of HLA class II antigens with susceptibility to PV has been demonstrated in numerous studies.[10–14] In Ashkenazi Jews, PV appears tightly linked to the rare haplotype HLA-DR4 (DRB1*0402): DQB1*0302, while in non-Jewish patients, it is linked to the haplotype DRB1*404X: DQB1*0503.[15]

MMP affects mucous membranes of the body and is characterized by the presence of autoantibodies to human β4 integrin,[16,17] while BP which predominantly affects the skin and is associated with bullous pemphigoid antigen 1 (BPAg1) and 2 (BPAg2).[18] Both BP and MMP have been shown to have a strong linkage to DQB1*0301.[18,19] It has been demonstrated that the same patient may have antibodies against more than one autoantigen within the skin and mucous membrane, resulting in more than one autoimmune mucocutaneous disease. For example, patients with PF may develop BP,[20,21] patients with MMP may have PV (22), and some patients are affected with both PV and OCP.[23]

OCP is a systemic autoimmune pemphigoid disorder that has both ocular and non-ocular manifestations. OCP can cause bullous lesions of the skin and mucous membranes that result in scarring of the affected skin, conjunctiva (inner lining of the eye), and other mucous membranes.[24,25] Different epithelial membrane zone components have been recognized by antibodies in patients with OCP, ie, BPAg1 and BPAg2, laminin 5, laminin 6, type VII collagen, β4 integrin subunit, and antigens with unknown identities (a 45-kd protein, uncein, a 168-kd epithelial protein, and a 120-kd epithelial protein).[26,27] Among white patients in the United States, OCP is associated with the DQB1*0301 allele.

DH is an autoimmune blistering disorder associated with a gluten-sensitive enteropathy. It is characterized by grouped excoriations, erythematous, urticarial plaques, and papules with vesicles. DH is caused by the deposition of IgA in the papillary dermis, which triggers an immunologic cascade, resulting in neutrophil recruitment and complement activation.[28,29] DH is associated with an increased expression of HLA-A1, HLA-B8, HLA-DR3, and HLA-DQ2 haplotypes.[30,31] Evidence is mounting that epidermal transglutaminase 3 (TGM3), a cytosolic enzyme involved in cell envelope formation during keratinocyte differentiation, is the autoantigen of DH. Theoretically, DH is caused by dermal deposition of circulating immune complexes containing both IgA and TGM3.[32,33]

In contrast to these organ-specific diseases, connective tissue disorders, or systemic diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and systemic sclerosis (SSc), involve multiple tissues and organs.[34–36] Mixed connective tissue disease (MCTD) is also a systemic autoimmune syndrome that is characterized by the presence of high titers of serum antibodies against small nuclear ribonuclear proteins (U-snRNPs),[37,38] in particular against U1 small nuclear RNP polypeptide (U1 snRNP). It has been suggested that MCTD represents a distinct clinical entity, based on clinical manifestations that separate MCTD from other connective tissue diseases.[39] Various associations of HLA class II antigens with MCTD have been reported, including HLA-B7 and HLA-Dw1.[40] In another study, HLA-DR4 was found to be significantly increased in MCTD,[41] whereas others reported an association between HLA-DQw3 and anti-RNP antibodies in patients with MCTD.[42] Interestingly, MCTD patients with increased IgG autoantibodies against U1-70 kD polypeptide have an increased prevalence of HLA-DR4 compared with controls.[43] Furthermore, molecular biology studies have shown that most MCTD patients carrying HLA-DR4 or HLA-DR2 alleles share a region of homology consisting of seven amino acids in the HLA-DRB1 gene.[44] This "shared epitope" of HLA-DR molecules, in different alleles

and in different patients with MCTD, may be important for the modulation of the autoimmune response to the U1-70 kD antigen.[45]

Based on the accumulated evidence of shared autoimmunity, it has been intriguing to investigate the relationship between the genetic and immunological mechanisms for the simultaneous production of two or more autoantibodies in patients exhibiting more than one autoimmune disease. A hypothesis, which in part may explain some of the increased susceptibility to both autoimmune blistering and systemic connective tissue diseases, has recently been proposed.[46] This hypothesis is based on the unequivocal evidence that $CD4^+$ T cells play a critical role in autoantibody production, implying that existence of HLA class II-restricted T cell epitopes might trigger autoimmunity. Three possible scenarios for the immunopathogenic mechanisms leading to breakage of tolerance and induction of two distinct autoimmune diseases in the same individual have been discussed: 1) T cell epitopes of the two different autoantigens associate with each of the susceptible HLA molecules, resulting in dual autoimmunity; 2) a single epitope of an autoantigen binds to both HLA specificities, leading to the induction of both diseases by cross- reactivity; and 3) two distinct epitopes of the same autoantigen are able to bind two different HLA molecules that are associated with the two diseases, implying that both T cell epitopes originating from one autoantigen will activate immunopatogenic mechanisms by binding to two HLA molecules specific for two diseases. In order to test each of these hypotheses, bioinformatics-based predictions of peptide binding to disease-associated HLA determinants have been generated using multi-HLA II peptide binding analysis. This communication explores the nature of autoantigen-derived epitopes and their binding characteristics to HLA proteins in the context of shared autoimmunity.

Methods

Protein sequences

The protein sequences of autoantigens were collected from the Consensus Coding Sequence Database (CCDS), a collaborative effort between the NCBI and several other organizations which contains consistently annotated protein coding regions of human and mouse genes.[47] Only human sequences were used in this project, and alternate exons were included in the sequences. The autoantigens tested in this study are listed in Table 1, and in most cases consist of the entire protein sequence from which specific autoantigens (such as BP180 and BP230) are derived.

Peptide-HLA II binding predictions

The analysis of peptide binding to HLA II molecules known to be involved in autoimmune mucocutaneous blistering diseases was based on the use of the same methodology applied by the Rankpep web server.[48] Rankpep predicts peptide binding using position specific motif matrices—also known as motif profiles—that are derived from known peptide binders.[49,98] A program was written, cross-HLA II-binding analyzer, to perform multiple rankings of predicted binding potentials and classify the binding predictions into sets: predictions for protein-HLA II sets known to be involved in disease genesis. Results were organized by disease model—sets of HLA II and proteins known to be involved in the genesis of specific autoimmune diseases were used.

The cross-HLA II-binding analyzer performs three types of sorting based on the three scenarios proposed in this paper: 1) Single HLA II recognition, 2) Dual HLA II recognition with a single epitope, and 3) Dual HLA II recognition with dual epitopes. The analysis was designed to group the components of each disease model, the HLA IIs and proteins involved in known disease interactions—for example, HLA-DQB1*0301 with alpha 1 type XVII collagen (BP180) and dystonin (BP230) in the BP model—and sort results by within-model and cross-model HLA II-peptide binding pairings. Some details of the cited scenarios are as follows:

Scenario 1—Single HLA recognition: This scenario is assumed to be the standard case, since each disease model exists independently. The cross-HLA II-binding analyzer generates a series of ranked results within the known disease models described above (sets of HLA II and proteins known to contain antigenic sequences), showing all predicted binders for each HLA II-protein pairing—peptides scored above the binding threshold for that pairing. Because these interactions are within known disease models, they are used as reference values.

Scenario 2—Dual HLA II recognition with a single epitope: the cross-HLA II-binding analyzer has an intersection analysis function, which is used

Table 1. Autoantigens and HLA associations in autoimmune blistering and systemic diseases.

Disease	HLA II	Autoantigen	CCDS ID	Reference
Pemphigus vulgaris	HLA-DR4 (DRB1*0402) DQwB1*0302 HLA-DR4 (DRB1*0404) DQ5 (DQB1*0503)	Desmoglein 3	11898.1	10–15
Mucous membrane pemphigoid	HLA-DQ7 (DQB1*0301)	β4 integrin	11727.1	16,17
Bullous pemphigoid	HLA-DQ7 (DQB1*0301)	Alpha 1 type XVII collagen (BP180) Dystonin (BP230)	7554.1 4959.1	18,19
Ocular cicatricial pemphigoid	HLA-DQ7 (DQB1*0301) HLA-DR4 (DRB1*0401)	Alpha 1 type XVII collagen (BP180) β4 integrin	7554.1 11727.1	26,27
Mixed connective tissue disease	HLA-B7, HLA-Dw1 HLA-DQw3	U1 sn-RNP polypeptide	12756.1	38,39 41–43
Dermatitis herpetiformis	HLA-DQ8 (DQA1*0301/ DQB1*0302) HLA-DQ2 (DQA1*0501/ DQB1*0201)	Transglutaminase 3	33435.1	28–33 52,53
Pemphigus foliaceus	HLA-DRB1 (DRB1*0404, DRB1*1402, DRB1*1406, DRB1*1401, DRB1*0102)	Desmoglein 1	11896.1	20,21, 57

Abbreviation: CCDS, consensus coding sequence project.[47] (http://www.ncbi.nlm.nih.gov/projects/CCDS/).

to find promiscuous peptides in a series of two HLA II-peptide binding predictions. To identify promiscuous peptide-binding peptides, the analysis was limited to the set of the top 5% of peptides in each group of results. This analysis identifies promiscuous peptide binders across known disease models. When a common peptide was found in more than one autoantigen, it was included in an intersection analysis report, detailing the binding score of the common peptide for each matrix.

Scenario 3—Dual HLA recognition with dual epitopes: the cross-HLA II-binding analyzer produces results for cross-model pairings of HLA IIs and proteins. To evaluate the potential for the pairings to produce immunogenic reactions, binding predictions (binding affinity, number of predicted binders) across models are compared to the top predicted binding scores for known immunogenic reactions within known disease models (Scenario 1).

Results

Based on the published observations of shared autoimmunity and on theoretical predictions exploring the probability of its occurrence, the following disease associations have been tested: PV/MMP, PF/BP, PV/OCP, PV/BP, PV/MCTD, PF/MCTD, BP/MCTD, DH/MCTD, OCP/MCTD, MMP/MCTD and BP/DH. The list of autoantigens and HLA associations in autoimmune blistering and systemic diseases is presented in Table 1. For most diseases in this study, eg, PV, MMP, DH, PF and MCTD, a single autoantigen has been reported, with the exception of BP and OCP that are characterized by the presence of the same two autoantigens, BPAg1 (BP180), derived from alpha 1 type XVII collagen and BPAg2 (also known as BP230), part of the dystonin protein. It is noteworthy that MMP, BP and OCP are all associated with the prevalent HLA-DQB1*0301 allele, while PV, DH, PF and MCTD each carry distinct HLA haplotypes.

Three hypothetical models of peptide-HLA II interactions proposed by Fridkis-Hareli[46] were tested using HLA-specific binding matrices and autoantigen sequences listed in Table 1. For each HLA specificity, the autoantigen of interest was examined for the presence of potential binding epitopes and scored based on

peptide binding affinity. The results arranged by disease model are shown in Supplemental Tables 1–11. All potential epitopes (sequences predicted to bind above the binding threshold for the HLA matrix were retrieved from each analysis (raw data not shown). It is important to note that comparisons were made between HLA-peptide binding analyses against the same HLA molecule—all binding scores and values were relative to that specific HLA class II. It should also be noted that not all the HLA specificities known to associate with a particular disease were available from the database and thus were not included in the present report.

PV/MMP

Autoantigenic proteins associated with each of these diseases were tested for binding to the HLA haplotypes as described below:

PV Model: the HLA II-peptide biding analysis for desmoglein 3 preproprotein generated 991 sequences in total (Supplemental Table 1). Four Dsg3-derived peptides were predicted to bind to HLA-DQ8 (DQA1*0301/DQB1*0302) above the binding threshold, with a predicted range of 36.91%–25.22% (from high to low optimal binding). In contrast, binding of the same autoantigen to HLA-DR4 (DRB1*0402) determinants resulted in 11 predicted binders with a range of 40.33%–25.80% of the optimal score. Similarly, five predicted binders of Dsg3 to HLA-DR4 (DRB1*0404) were identified with a range of 53.46%–32.23% of the optimal score (Supplemental Table 1A). The sequences of Dsg3-derived binding epitopes are presented in Supplemental Table 1B.

MMP Model: Table 2 shows that the analysis for HLA-DQ7 (DQB1*0301) and β4 integrin generated 1814 sequences. Fourteen peptides were predicted to bind, at 44.19%–25.90% of the optimal binding score. For peptide sequences refer to Supplemental Table 1B.

PV/MMP: The cross-model analysis matches HLA-DQ7 (DQB1*0301) (from the MMP model) with Dsg3, the antigen-producing protein from the PV model. Four sequences from Dsg3 are predicted to bind, with scores between 37.32% and 29.70% of the optimal score for HLA-DQ7 (DQB1*0301). These sequences are within the range of binders predicted for the in-model interactions, but four of the predicted in-model binders have higher scores than the best cross-model prediction (Supplemental Table 1A).

Table 2. Predicted binding epitopes derived from autoantigens specific for blistering and systemic disease combinations.

HLA II/Protein	PV	MMP	PF	BP	OCP	DH	MCTD
PV	**20**	4		4	47		18
MMP	47	**14**					73
PF			**27**	6			22
BP	69		104	**26**		21	96
OCP	65				**188**		73
DH				6		**3**	18
MCTD	7	3	4	3	9	4	**5**

Notes: The number of predicted binders resulting from all MHC associated with a disease model (x-axis) and all protein sequences associated with another disease model (y-axis). Cells in bold are in-model results (predicted binders when testing all HLAs from one disease model against all protein sequences from the same disease model). Example: The total number of binding sequences predicted when analyzing all HLAs from the PV disease model against all protein sequences in the BP disease model is 69 (column one, row four).

Conversely, the cross-model analysis matches β4 integrin (MMP model) with HLA-DQ8 (DQA1*0301/DQB1*0302) (14 predicted binders), HLA-DR4 (DRB1*0402) (16 predicted binders) and HLA-DR4 (DRB1*0404) (17 predicted binders) from the PV model. Interestingly, the two highest scoring peptides from the HLA-DQ8 (DQA1*0301/DQB1*0302)—β4 integrin (DERCHLDTT and ANRCKKAPV), scored higher than all the predicted binders from the PV in-model analysis. In contrast, the binding of β4 integrin to HLA-DR4 (DRB1*0402) and HLA-DR4 (DRB1*0404) was within the binding range of Dsg3 to these molecules (Supplemental Table 1B).

PF/BP

In this case, three autoantigens, ie, Dsg1 for PF, alpha 1 type XVII collagen (BP180) and dystonin (BP230) for BP have been analyzed for their binding to several disease-associated HLA haplotypes, as shown in Supplemental Table 2. Analysis of Dsg1 binding to PF-associated molecules revealed 1041 sequences in total, whereas alpha 1 type XVII collagen (BP180) generated 1489 sequences and dystonin (BP230) generated 2641 sequences derived from their binding to BP-associated MHC molecules (Supplemental Table 2A).

PF Model: Peptide binding analysis for desmoglein 1 (Dsg1) to HLA-DR1 (DRB1*0101) showed 22 results with 53%–16% predicted optimal binding. In addition, analysis of Dsg1 and HLA-DR4 (DRB1*0404) identified five binders in the range of

48%–32% of predicted optimal values. In contrast, analysis for Dsg1 binding to HLA-DR4 (DRB1*0406) and HLA-DR1 (DRB1*0102) showed no binding epitopes above the binding threshold (Supplemental Table 2A). Peptide sequences are shown in Supplemental Table 2B.

BP Model: HLAII-peptide binding results for alpha 1 type XVII collagen peptides binding to HLADQ7 (DQB1*0301) showed 14 peptide sequences ranging between 43%–26% optimal binding (Supplemental Table 2A). Binding of dystonin to the same molecules resulted in 12 predicted binders with 31%–26% of optimal binding (Supplemental Table 2A).

PF/BP: Analysis of Dsg1 binding to BP-associated HLA-DQ7 (DQB1*0301) molecules resulted in six peptides with 39%–28% optimal binding (Supplemental Table 2A). Notably, these values were lower than the binding of Dsg1 to PF-associated MHC molecules. Predictions for alpha 1 type XVII collagen peptides binding to PF-associated HLA-DR1 (DRB1*0101) resulted in 30 binders with 40%–17% optimal binding (Supplemental Table 2A). In contrast, analysis of alpha 1 type XVII collagen/HLA-DR1 (DRB1*0102) interactions showed no detectable binding. On the other hand, the binding of alpha 1 type XVII collagen to HLA-DR4 (DRB1*0404) showed higher range of optimal binding (52%–35%) for six predicted epitopes (Supplemental Table 2A). Interestingly, predictions of alpha 1 type XVII collagen binding to HLA-DR4 (DRB1*0406) resulted in one peptide epitope with the binding score of 36.062 and optimal binding of 49.23% (Supplemental Table 2A). Peptide sequences are listed in Supplemental Table 2B.

Binding of dystonin to PF-associated HLA-DR1 (DRB1*0101), HLA-DR1 (DRB1*0102), HLA-DR4 (DRB1*0404), HLA-DR4 (DRB1*0406) was tested similarly to alpha 1 type XVII collagen. For HLA-DR1 (DRB1*0101) molecules, 56 predicted binders with 44%–16% optimal binding have been identified, and for HLA-DR4 (DRB1*0404), 11 dystonin-derived binders with 54%–32% optimal binding have been detected (Supplemental Table 2A). Of note is that these values are higher than the ones found for dystonin binding to BP-associated molecules. Interestingly, no predicted epitopes were found either for HLA-DR1 (DRB1*0102) or for HLA-DR4 (DRB1*0406) interactions with dystonin. In summary, these results suggest that here, as in the case of PV/MMP, epitopes derived from one disease-specific autoantigenic protein may bind both disease-specific HLA molecules.

PV/OCP

Analysis of Dsg-3-derived peptides to PV-associated HLA-DQ8 (DQA1*0301/DQB1*0302), HLA-DR4 (DRB1*0402) and HLA-DR4 (DRB1*0404) molecules has been described in *PV* section (Supplemental Table 1). Likewise, due to the fact that OCP shares the same autoantigens and HLA specificities with BP and MMP, ie, alpha 1 type XVII collagen, β4 integrin and HLA-DQ7 (DQB1*0301), the corresponding data have been provided in *BP* and *MMP* sections (Supplemental Tables 1 and 2). Analysis of β4 integrin binding to OCP-associated MHC molecules generated 1814 peptide sequences, whereas alpha 1 type XVII collagen generated 1489 sequences (Supplemental Table 3A).

PV/OCP: PV-specific desmoglein 3 preproprotein peptide binders examined for the OCP-associated HLA-DQ7 (DQB1*0301) revealed four binders with 37%–29% predicted optimal binding, as described for PV/MMP model (Supplemental Table 1A). The binding of Dsg3 to HLA-DR4 (DRB1*0401) resulted in 43 binders with 46%–11% predicted optimal values (Supplemental Table 3A). In the case of OCP-related autoantigen association with PV-specific MHC, eight binders derived from alpha 1 type XVII collagen (BP180) exhibited 36%–25% optimal predicted binding to HLA-DQ8 (DQA1*0301/DQB1*0302). The binding to HLA-DR4 (DRB1*0402) was between 38%–28% optimal values for four predicted binders, while the binding to HLA-DR4 (DRB1*0404) was even higher (52%–34% optimal binding). Another potential OCP-associated autoantigen, β4 integrin, bound to PV-specific HLA molecules with differential predicted scores as shown in Supplemental Table 3A. For HLA-DQ8 (DQA1*0301/DQB1*0302), 14 binders with 42%–25% optimal binding have been identified, whereas 16 binders were detected for HLA-DR4 (DRB1*0402) (37% optimal binding), and 17 peptides for HLA-DR4 (DRB1*0404) with 51%–34% optimal binding (Supplemental Table 3A). Interestingly, the binding of β4 integrin to PV-associated HLA-DR4 (DRB1*0404) was higher than to OCP-associated molecules (51% vs. 44%, respectively). Peptide sequences are shown in Supplemental Table 3B.

PV/BP

For this disease combination, individual parts of PV/Dsg3 and BP/alpha 1 type XVII collagen/dystonin analysis have been described in *PV* and *BP* sections, respectively (Supplemental Tables 1 and 2).

PV/BP: Analysis of the binding of Dsg3 to BP-associated HLA-DQ7 (DQB1*0301) resulted in four binders with 37%–29% optimal binding (Supplemental Table 4A). In the opposite direction, the binding of alpha 1 type XVII collagen to PV-associated HLA-DR4 (DRB1*0402), HLA-DR4 (DRB1*0404), and HLA-DQ8 (DQA1*0301/DQB1*0302) showed four binders (38%–28% optimal binding), six binders (52%–34% optimal binding) and eight binders (36%–25% optimal binding), respectively. For dystonin-derived peptides, 28 predicted binders were found with DRB1*0402 (37%–28% optimal binding), 11 peptides bound to DRB1*0404 (54%–32% optimal binding), and 12 predicted binders (47%–25% optimal binding) were detected for HLA-DQ8 molecules (Supplemental Table 4A). Of note is that the binding score for dystonin-derived peptides bound to PV-associated HLA-DQ8 molecules was higher than the one for dystonin binding to BP-associated HLA-DQ7 molecules (Supplemental Table 4A). Peptide sequences are presented in Supplemental Table 4B.

PV/MCTD

PV-related Dsg3 association with its HLA-DQ8 (DQA1*0301/DQB1*0302) receptors was already described in this report (Supplemental Tables 1, 3 and 4). Peptide binding analysis of MCTD-specific autoantigen UI-snRNP C [Homo sapiens] resulted in 429 sequences in total (Supplemental Table 5). Binding of UI-snRNP-derived peptides to HLA-DR1 (DRB1*0101) revealed three predicted epitopes with 37%–25% optimal binding, while interaction with the 9mer HLA-B07 resulted in two peptides with 63%–62% optimal binding (Supplemental Table 5A). PV-related Dsg3 association with its HLA-DQ8 (DQA1*0301/DQB1*0302) receptors was already described in this report (Supplemental Tables 1, 3 and 4).

PV/MCTD: The combination of PV and MCTD was tested using autoantigens UI-snRNP and Dsg-3, and HLA molecules associated with both diseases, ie, HLA-DR1 (DRB1*0101), HLA-DR4 (DRB1*0402), HLA-DR4 (DRB1*0404), HLA-DR4 (DRB1*0406), HLA-DQ8 (DQA1*0301/DQB1*0302) and HLA-B07 alleles. The binding of PV-specific Dsg3 to MCTD-associated HLA-DR1 (DRB1*0101) showed 18 binders with 44%–17% optimal binding, while analysis of Dsg3/9mer-HLA-B07 interactions showed no predicted binding epitopes (Supplemental Table 5A). Similarly, there was no binding of UI-snRNP to PV-associated HLA-DR4 (DRB1*0404), but three peptides (55%–26% optimal binding) were found to bind HLA-DR4 (DRB1*0402), and four peptides (40%–27% optimal binding) were detected for HLA-DQ8 (DQA1*0301/DQB1*0302) molecules (Supplemental Table 5A). Importantly, the optimal binding of UI-snRNP-derived peptides to PV-associated HLA-DR4 (DRB1*0402) was higher than its binding to MCTD-related HLA-DR1 (DRB1*0101) but lower than binding to the second susceptible MCTD allele, HLA-B07 (Supplemental Table 5). Peptide sequences are shown in Supplemental Table 5B.

PF/MCTD

Binding analysis of PF- and MCTD-related peptides to their disease-specific HLA molecules was described in the earlier sections of this report (Supplemental Tables 2 and 5). The largest number of Dsg1-derived epitopes was found to bind to HLA-DR1 (DRB1*0101) as compared to other PF-specific alleles (Supplemental Table 6). This disease combination is unusual since both diseases share the presence of HLA-DR1 (DRB1*0101), suggesting that PF- and MCTD-derived peptides might bind to the same molecule and thus trigger initiation of the second disease.

PF/MCTD: Binding analysis of Dsg1-derived peptides to MCTD-associated HLA-DR1 (DRB1*0101) molecules showed 22 epitopes (53%–16% optimal binding), while no binders were found for the 9mer-HLA-B07 (Supplemental Table 6A). Predictions for UI-snRNP-derived peptides bound to HLA-DR1 (DRB1*0101), HLA-DR1 (DRB1*0102), HLA-DR4 (DRB1*0404) and HLA-DR4 (DRB1*0406) molecules showed three (38%–25% optimal binding), zero, zero, and one binders (35% optimal binding), respectively (Supplemental Table 6A). Interestingly, the binding of Dsg1-derived peptides to HLA-DR1 (DRB1*0101) was higher than that of the UI-snRNP-derived peptides as expressed by the optimal binding values. Peptide sequences are shown in Supplemental Table 6B.

BP/MCTD

Binding of alpha 1 type XVII collagen and dystonin to BP-associated HLA-DQ7 (DQB1*0301) molecules, as well as the binding of UI-snRNP to MCTD-associated HLA-DR1 (DRB1*0101) and the 9mer-HLA-B07 has been described in the previous sections of this study and is shown in Supplemental Tables 2, 4, 5 and 6.

BP/MCTD: Binding analysis of alpha 1 type XVII collagen peptides to MCTD-specific HLA-DR1 (DRB1*0101) resulted in 30 predicted peptides (40%–17% optimal binding), while the binding to the HLA-B07 molecules showed nine binders (59%–45% optimal binding) (Table 7). Moreover, the binding of dystonin-derived peptides to HLA-DR1 (DRB1*0101) showed 56 peptides (44%–17% optimal binding), and only one peptide (53% optimal binding) for the 9mer-HLA-B07 molecules (Supplemental Table 7A). Interestingly, three UI-snRNP-derived peptides were shown to bind to BP-associated HLA-DQ7 (DQB1*0301) molecules (33%–26% optimal binding). Here, the binding of BP- and MCTD-associated autoantigens to their disease molecules was similar to the cross-disease combination. Peptide sequences are presented in Supplemental Table 7B.

DH/MCTD

Analysis of DH-specific TGM3 transglutaminase 3 peptide binders resulted in 685 peptide sequences (Supplemental Table 8). Binding predictions for TGM3-derived peptides to HLA-DQ8 (DQA1*0301/DQB1*0302) showed three binders (30%–28.5% optimal binding), whereas no binders were found for HLA-DQ2 (DQA1*0501/DQB1*0201) (Supplemental Table 8A). Studies of UI-snRNP/HLA-DR1 (DRB1*0101)/9mer-HLA-B07 have been described in the previous sections (Supplemental Tables 5–7).

DH/MCTD: Binding analysis of TGM3 to HLA-DR1 (DRB1*0101) alleles resulted in 17 binders (41%–17% optimal binding), but only one binder was found for the 9mer-HLA-B07 molecules (optimal binding 47.5%). In the opposite direction, the binding of UI-snRNP to HLA-DQ2 (DQA1*0501/DQB1*0201) showed four peptides (40%–27% optimal binding) and no binding to HLA-DQ8 (DQA1*0301/DQB1*0302) molecules (Supplemental Table 8A). Peptide sequences are shown in Supplemental Table 8B.

OCP/MCTD

For a single disease model, analysis of OCP- and MCTD-specific peptides bound to their associated HLA molecules has been shown earlier (Supplemental Tables 3, 5–8).

OCP/MCTD: Several UI-snRNP-derived peptide epitopes were shown to bind to OCP-associated HLA-DQ7 (DQB1*0301) and HLA-DR4 (DRB1*0401) molecules. Thus, three peptides bound to HLA-DQ7 (33%–26% optimal binding), and six epitopes bound to HLA-DR4 molecules (29%–15% optimal binding) (Supplemental Table 9A, B). On the other hand, 30 OCP-derived alpha 1 type XVII collagen peptides bound to MCTD-associated HLA-DR1 (DRB1*0101) (40%–17% optimal binding) and nine alpha 1 type XVII collagen peptides bound to the 9mer-HLA-B07 (59%–45% optimal binding). In the case of β4 integrin, 29 binders were detected for MCTD-associated HLA-DR1 (DRB1*0101) (37%–17% optimal binding) and five peptides were predicted to bind to the 9mer-HLA-B07 (62%–46% optimal binding). Peptide sequences are shown in Supplemental Table 9B.

MMP/MCTD

Analysis of MMP-specific β4 integrin binding to MMP-associated HLA-DQ7 (DQB1*0301) molecules, as well as the binding of MCTD-related UI-snRNP peptides to MCTD-specific HLA-DR1 (DRB1*0101) and the 9mer-HLA-B07 has been described in the previous sections of this report (Supplemental Tables 1, 5–9).

MMP/MCTD: In the dual disease model, the binding of β4 integrin to HLA-DR1 (DRB1*0101) resulted in 29 predicted epitopes (37%–17% optimal binding), while only five β4 integrin-derived peptides were found to bind to the 9mer-HLA-B07 (62%–46% optimal binding), as shown in Supplemental Table 10A. On the other hand, three UI-snRNP-derived peptides bound to HLA-DQ7 (DQB1*0301) molecules (33%–26% optimal binding). For peptide sequences refer to Supplemental Table 10B. Thus, based on similar values for peptide binding in these two disease models, it is possible that autoantigen-derived epitopes from one disease interact with the HLA alleles of the second disease.

BP/DH

Analysis of BP-specific autoantigens alpha 1 type XVII collagen and dystonin, as well as of DH-related

TGM3 binding to HLA molecules known to be associated with BP (within a known disease model) has been performed and described in the previous sections of this study (Supplemental Tables 2, 4, 7 and 8).

BP/DH: Binding of type XVII collagen-derived peptides to DH-associated HLA-DQ8 (DQA1*0301/DQB1*0302) resulted in eight top binders (36%–25% optimal binding), and in one binder for HLA-DQ2 (DQA1*0501/DQB1*0201) molecules (optimal binding 45.89%) (Table 11A). For dystonin-related peptides, 12 were found to bind HLA-DQ8 (DQA1*0301/DQB1*0302) (47%–25% optimal binding) but none to HLA-DQ2 (DQA1*0501/DQB1*0201) molecules (Supplemental Table 11A). In the opposite direction, six TGM3-derived peptides were predicted to bind BP-associated HLA-DQ7 (DQB1*0301) molecules (45%–26% optimal binding). Peptide sequences are shown in Supplemental Table 11B.

Based on the predicted optimal binding data, these results suggest that epitopes derived from the autoantigenic proteins specific for one disease may bind to one or more HLA alleles associated with the second disease with various affinities. Most importantly, these observations show that while one peptide is capable of binding to its disease-associated HLA, a different peptide of the same autoantigen may bind to the HLA related to the second disease. This phenomenon may produce T cell recognition with different signaling outcomes, leading to the production of several autoantibody specificities characteristic for each of the induced disease conditions.

Discussion

It has been well documented that autoimmune diseases may coexist in the same patient, either sequentially or concurrently.[50–64] PV, DH, BP, and SLE have all been reported in association with other autoimmune diseases as well as with each other. In particular, observations of dual autoimmunity in some patients who concurrently develop organ-specific and systemic disease have been reported.[52,54,55,58,60,62,63] Multiple factors, including those of immunological, genetic, endocrine and environmental origin, contribute to the above condition. The immunogenetic mechanisms of this phenomenon present an intriguing unresolved problem of autoimmune predisposition, calling for development of prospective approaches of prediction and ultimately prevention of the disease. As a matter of fact, the involvement of T cells in immunopathogenesis of MCTD, PV and MMP has been well established. In MCTD, the role of anti-RNP-reactive T cells in autoantibody production has been demonstrated.[65,66] In PV, it has been shown that B cells function as antigen-presenting cells stimulating Dsg3-specific $CD4^+$ T helper (Th) cells to secrete cytokines such as interleukin (IL)-4, IL-6 and IL-10 which are required for proliferation of memory B cells and differentiation into antibody-producing plasma cells.[67–69] Thus, the interplay between B and T cells seems to be critical, which is further supported by the finding that depletion of $CD4^+$ T cells prevents antibody production.[69] Moreover, a clinical study showed that the mean frequency of Th2 $CD4^+$ T cells was significantly elevated in PV patients with active disease, while no responses were detected for patients with disease in remission or controls.[71] Characterization of autoreactive T-cells has led to identification of immunodominant T-cell epitopes and the repertoire of Dsg3- or Dsg1-specific T-cells at the clonal level.[72,73] Lastly, the potential role for antigen-specific autoreactive T cells in the pathogenesis of MMP has also been addressed.[74,75] Collectively, these observations suggest that T cell epitopes of the respective autoantigens may bind to their HLA molecules and trigger the activation of autoreactive T cells, which in turn would induce production of pathogenic autoantibodies.

In the present study, a bioinformatics-based search for potential epitopes restricted by HLA molecules associated with autoimmune mucocutaneous blistering diseases and systemic diseases has been undertaken in order to address the immunogenetics of the phenomenon of shared autoimmunity. The data across different disease combinations obtained in this report suggest that coexistence of two autoimmune diseases in the same patient might be triggered by the following mechanisms: 1) binding of each of the autoantigenic peptides related to a specific disease, to its specific HLA-associated molecules, or 2) binding of two epitopes derived from the same autoantigen to two HLA class II molecules, each associated with one disease. This binding may potentially result in differential signaling leading to the generation of disease-specific antibodies which contribute to the pathogenesis of both disease conditions. The data on the number of predicted peptide binding epitopes for each disease combination described in this study is

presented in Table 2. The highest number of binders was found for OCP while the lowest was in the case of DH, BP/MCTD and MMP/MCTD. Figure 1 shows disease models arranged by the number of predicted binders from other diseases. In each case, the point of reference is the HLA class II molecule. An analysis of in-model binding between HLA II molecules and associated protein within a given disease model is compared to the binding predictions for those same HLA II molecules against the set of proteins associated with the second disease model (cross-model analysis).

It should be noted that there are certain limitations to the methodology employed in this study. The binding matrices used are based on motifs developed from sequences known to bind to the MHC for which the matrix is developed. There are inherent problems which limit the suitability of the matrices for prediction of novel binding sequences, particularly the variable number of known binding sequences available for the development of binding matrices, in some cases quite small. Another limitation is that laboratory confirmation of binding predictions has yet to be completed. However, a recent publication has identified a peptide epitope derived from BP180 associated with IgA dermatosis that was shown to interact with monoclonal antibodies[76]. Parts of BP180 mapped sequences SMDRIEKDRL and QEELWMFVRKKL in this publication were predicted by our Rankpep tool as follows: the WMFVRKKLM epitope (overlapping sequence underlined) is a predicted binder to HLA-DR1 (DRB1*0101) in the top 2% of ranked peptides.

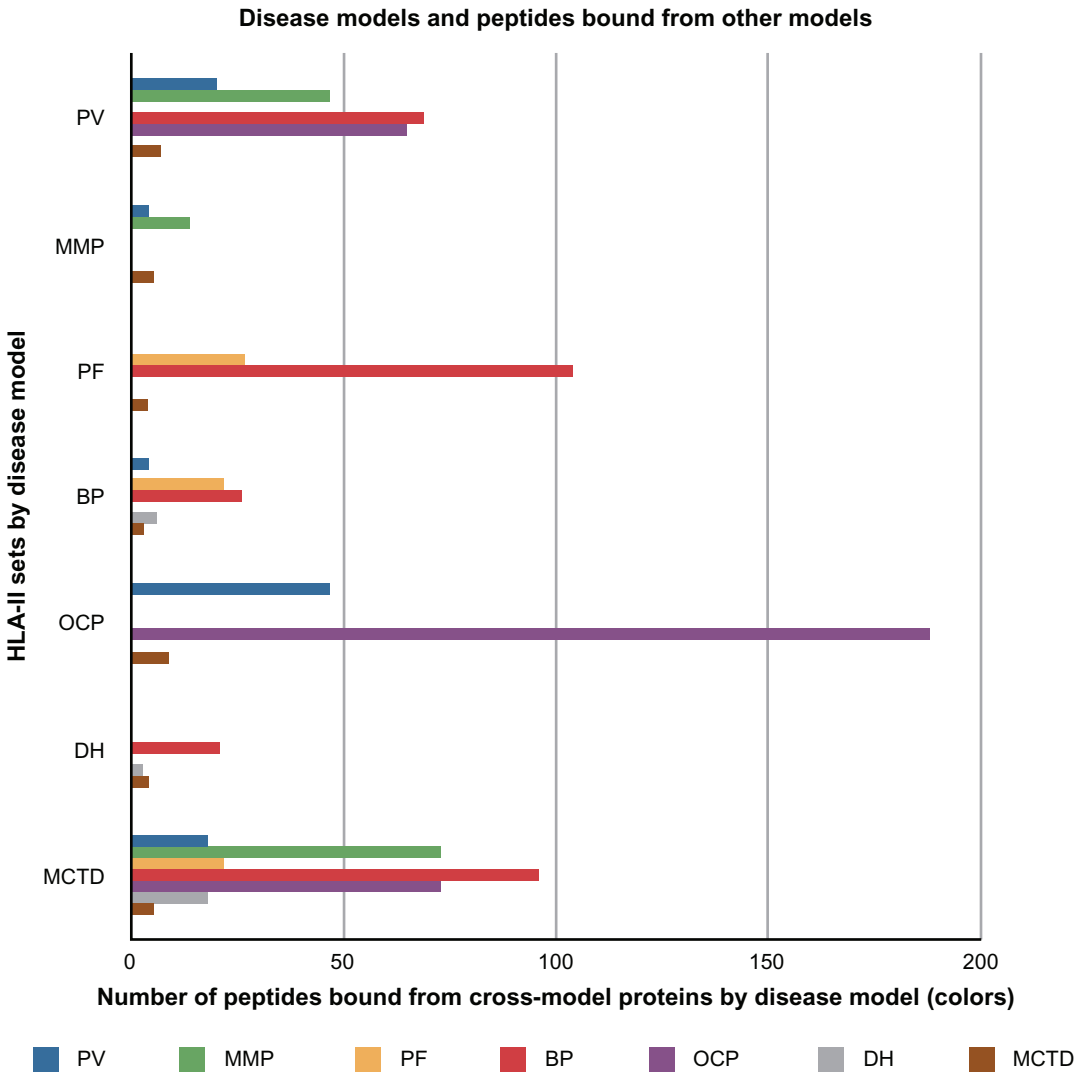

Figure 1. Disease models and peptides bound from other models.

The same epitope is also in the top 2% of ranked peptides for HLA-DR4 (DRB1*0402), where potential binders should be concentrated.[48,49] This makes WMFVRKKLM a potentially antigenic sequence for MCTD, PV and PF. The epitope IEKDRLQGM, although not predicted above the binding threshold, was also within the top 2% of predicted binders to HLA-DR4 (DRB1*0402).

Following pioneering biochemical studies which led to elucidation of peptide motifs associated with class I and class II MHC receptors,[77–80] numerous analyses on the nature of the peptide/MHC interactions in the context of autoimmune/inflammatory diseases have been performed showing promiscuity of peptide binding to class II MHC molecules.[81,82] In the present study, each autoantigen of interest was subjected to the cross-HLA II-binding analyzer tool resulting in multiple peptides which were ranked based on the probability of their binding to the respective HLA molecule. It is of interest that, in spite of the large number of sequences generated for each autoantigen (varying from 429 for UI-snRNP to 2641 for dystonin) the number of the actual binders above the threshold was specific for each peptide/HLA combination and resulted in either none, very few or multiple binders, as shown in Supplemental Tables 1–11. This is likely due to the polymorphism in the HLA pocket residues allowing specific peptide motifs to bind based on the size and hydrophobicity of the pockets, so that only the matching amino acids of the peptide would fit in.

Sequences of autoantigenic immunodominant epitopes for a number of blistering diseases have been previously reported.[83–93] In the most studied model, PV, extensive analysis of peptide motifs bound to the susceptible alleles showed sequence specificities and variability in the HLA binding pockets.[84–86] For example, at least nine previously identified stimulatory Dsg3 peptide sequences corresponding to the amino acid residues 96-112 (PFGIFVVDKNTGDINIT), 191-205 (NSKIAFKIVSQEPAG), 206-220 (TPMFLLSRNTGEVRT), 252-266 (ECNIKVKDVNDNFPM), 342-356 (SVKLSIAVKNKAEFH), 380-394 (GIAFRPASKTFTVQK), 763-777 (SGTMRTRHSTGGTNK), 810-824 (NDCLLIYDNEGADAT) and 963-977 (ERVICPISSVPGNLA) were shown to bind to PV-associated HLA-DR4 (DRB1*0402) and DQ5 (DQB1*0503) with a sliding window of up to three amino acids for the core residues.[84] In the present study, our binding analysis of Dsg3 epitope binding to DRB1*0402 revealed several sequences that were identical to the reported core and flanking residues within the peptide Dsg3 (flanking residues spaced, core underlined): NSK IAFKIVSQE PAG—191-205 (NSKIAFKIVSQEPAG); SGT MRTRHSTGG TNK-763-777 (SGTMRTRHSTGGTNK); and NDC LLIYDNEGA DAT—810-824 (NDCLLIYDNEGADAT) (ref. 82 and Supplemental Table 1B).

It is of interest to note that, according to Mouquet et al,[87] human Dsg1 was found to contain a T cell epitope capable of binding to PF-associated HLA Class II DRB1*0102 molecules. In contrast, no predicted binders derived from Dsg1 were detected for HLA-DR1 (DRB1*0102) in the present study (Supplemental Table 2). Also of interest, a recent study showed that the HLA-DR3 (DRB1*0301) molecule is linked to endemic PF in Tunisian patients.[92] It would be of interest to analyze the peptide binding predictions on Dsg1 to this PF-associated HLA molecule in order to identify potential epitopes.

Importantly, no similar sequences were found in two different autoantigens specific for the two diseases, suggesting that promiscuous binding of the same epitope to the two HLA alleles associated with these diseases is unlikely to be the cause of shared autoimmunity. Rather, the data presented in this study demonstrates the possibility that an additional epitope derived from the same autoantigen binds to the HLA specific for the second disease. To our knowledge, this is the first study suggesting the potential mechanism of the induction of dual autoimmunity mediated by epitopes derived from a single autoantigen. In support of this mechanism, it is noteworthy that the affinity of the binding between the autoantigenic peptide epitope, the susceptible HLA and the TCR play an important role in T cell activation. Due to certain degree of promiscuity and specificity in peptide recognition by the HLA receptors, not a single binding affinity, but rather a range of affinities would account for the productive interaction between the peptide epitopes, HLA and the TCR, leading to T cell-mediated B cell activation and antibody secretion. Modeling of the bound conformation of PV-associated peptides revealed the role of DRB1*0402 in the selection of specific self-epitopes.[84] Several studies suggest that autoantigenic peptides do not necessarily bind to disease-associated HLA molecules with high affinity,

but rather within the intermediate range, thus allowing for the rescue of autoreactive T cells by virtue of weaker HLA/peptide/TCR interactions. In contrast, protective HLA proteins are more efficient binders of self-antigens, which results in elimination of autoreactive T cells.[85]

It should be noted that peptide binding to HLA is facilitated by the interactions between the amino acid residues lining the groove of HLA molecules and the side chains of the bound peptide. The binding pockets of HLA class II, defined by the polymorphic β chain and the more conservative α chain of the αβ heterodimer, share homology between some alleles but may also differ from other alleles as defined by size, charge and hydrophobicity.[83] Thus, autoantigens may not share sequence homology, but still may encompass peptides able to bind different HLA due to the presence of certain amino acids which would fit to the binding pockets of the HLA molecules. In spite of this fact, the two peptides may share common binding motifs, dictated by structural requirements of the HLA pockets accommodating the peptides. Due to the degenerate nature of the HLA binding and TCR recognition, an observation which has been widely accepted for the past decade,[94] common binding motifs would be sufficient to allow peptide binding to different HLA molecules. However, in this case, it is possible that the recognition of the HLA/peptide complex by T cells will differ depending on the orientation of the TCR interacting with the amino acids facing away from the binding groove, and thus will result in differential activation by T cells.

Molecular and cellular mechanisms governing the concurrent or sequential presence of autoimmune blistering and systemic diseases in patients remain to be elucidated. Investigation of these mechanisms has been significantly delayed due to the lack of animal models in which both the systemic and organ-specific autoimmune diseases can be induced. To this end, only a small number of experimental models of susceptibility to a single disease have been developed with limited success.[91,95–97] Development of such animal models allowing investigation of the effects of the triggering factors on shared autoimmunity would require genetic manipulations enabling the introduction of elements of susceptibility, ie, human HLA and/or autoantigen-specific TCR/BCR. Thus, a transgenic mouse model expressing two disease-associated HLA and two TCR/BCR specific for each of the autoantigenic peptides would be most suitable for this purpose. In these mice, the experimental approach would include the administration of disease-inducing peptides, separately or concomitantly, and monitoring the animals for manifestations of each disease. In parallel, ex-vivo functional analysis including antigen-specific proliferation, cytokine secretion and antibody phenotyping, has to be performed. The in vitro binding studies employing purified HLA proteins and synthetic peptides, and the cellular assays with antigen-presenting cells and patient's lymphocytes would also be instrumental.

To our knowledge, this is the first study reporting extensive analysis of peptide binding predictions to a number of HLA alleles associated with autoimmune blistering and systemic diseases. Further studies of these patients, and especially of T and B lymphocytes administered into HLA-transgenic mice, will provide valuable information on cellular and molecular mechanisms critical for immunoregulation and production of pathogenic autoantibodies. Such studies have significant clinical ramifications and implications for the development of novel immune therapies targeting both autoimmune diseases. The elucidation of HLA-restricted immune recognition mechanisms prompting the production of two or more disease-specific autoantibodies holds significant clinical ramifications and implications for the development of more effective treatment protocols. Currently, blistering and systemic diseases are treated by a number of protocols, including administration of IV Ig regiments, bone marrow transplantation, steroids (prednisone, prednisolone, dapsone, clobetasol), adjuvant drugs (azathioprine, mycophenolate mofetil, cyclosporine, rituximab) and emerging treatments by gene therapy or stem cell transplantation. These treatments are aimed at suppression or replacement of affected proteins and cells, with no specificity of targeting individual components.

Our findings provide important information on the identity of potential epitopes implicated in pathogenesis of blistering and systemic diseases, and specifically, on autoantibody reactivity in these patients. Potential therapies for these conditions could include targeted strategies to eliminate these autoantibodies and/or combination therapy with agents directed against several such specificities.

Abbreviations

AMBD, autoimmune mucocutaneous blistering diseases; APC, antigen-presenting cells; BP, bullous pemphigoid; DH, dermatitis herpetiformis; Dsg, desmoglein; HLA, human leukocyte antigens; IL, interleukin; MCTD, mixed connective tissue disease; MMP, mucous membrane pemphigoid; OCP, ocular cicatricial pemphigoid; PF, pemphigus foliaceus; PV, pemphigus vulgaris; RNP, ribonucleoprotein antigen; TCR, T cell receptor; Th, T helper cells.

Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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Supplementary Material

Table S1A. Rankpep analysis of PV- and MMP-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease 1	MHC	Performance*	Disease 2	Protein	Total sequences
PV	HLA-DQ8 (DQA1*0301/DQB1*0302)	0.72 ± 0.06	PV	desmoglein 3 preproprotein	991
	HLA-DR4 (DRB1*0402)	0.72 ± 0.04		desmoglein 3 preproprotein	991
	HLA-DR4 (DRB1*0404)	0.61 ± 0.05		desmoglein 3 preproprotein	991
	HLA-DQ8 (DQA1*0301/DQB1*0302)	0.72 ± 0.06	MMP	β4 integrin	1814
	HLA-DR4 (DRB1*0402)	0.72 ± 0.04		β4 integrin	1814
	HLA-DR4 (DRB1*0404)	0.61 ± 0.05		β4 integrin	1814
MMP	HLA-DQ7 (DQB1*0301)	na	MMP	β4 integrin	1814
	HLA-DQ7 (DQB1*0301)	na	PV	desmoglein 3 preproprotein	991

Total predicted binders	Threshold	High binding score	Low binding score	% optimal high	% optimal low
4	12.635	18.963	12.956	36.91	25.22
11	11.444	17.99	11.507	40.33	25.8
5	12.059	20.331	12.258	53.46	32.23
14	12.635	**21.785**	13.172	42.4	25.64
16	11.444	16.62	–	37.04	–
17	12.059	19.434	12.808	51.1	33.68
14	11.701	20.182	11.829	44.19	25.9
4	11.701	17.045	13.563	37.32	29.7

Notes: *Development and performance of matrices is described in detail in ref. 48; For other details refer to Methods section of this report.

Table S1B. Peptide sequences of PV- and MMP-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease	MHC	Threshold	Autoantigen	Total predicted binders	Top binders	Score	% optimal
PV-PV	HLA-DQ8 (DQA1*0301/ DQB1*0302)	12.632	desmoglein 3 preproprotein	4	NNRCEMPRS	18.963	36.91
					DAVCSSSPS	15.007	29.21
					WGIEGAHPE	13.2	25.69
					WVKFAKPCR	12.956	25.22
	HLA-DR4 (DRB1*0402)	11.444	desmoglein 3 preproprotein	11	TRYGRPHSG	17.99	40.33
					MRTRHSTGG	17.387	38.98
					IAFKIVSQE	15.149	33.96
					IKFVKNMNR	15.046	33.73
					VKYVMGRND	14.554	32.63
					ITYRISGVG	13.746	30.82
					LLIYDNEGA	12.572	28.19
					IAKITSDYQ	12.438	27.89
					ISRYRVQST	11.643	26.1
					VYFFTSGNE	11.59	25.98
					VRTLTNSLD	11,507	25.8
	HLA-DR4 (DRB1*0404)	12.059	desmoglein 3 preproprotein	5	ILVHGELRI	20.331	53.46
					FPMFRDSQY	16.921	44.49
					KNMNRDSTF	14.837	39.01
					IAFRPASKT	13.182	34.66
					INVREGIAF	12.258	32.23
PV-MMP	HLA-DQ8 (DQA1*0301/ DQB1*0302)	12.632	β4 integrin	**14**	**DERCHLDTT**	**21,785**	**42.4**
					ANRCKKAPV	**20,134**	**39.19**
					KEDHYMLRE	18,019	35.07
					WERPRRPNG	17,709	34.47
					DKPCSGRGE	17,191	33.46
					LRRSQMSPQ	16,831	32.76
					DRRCNTQAE	16,281	31.69
					QYRTQDYPS	14,323	27.88
					DLYILMDFS	14,223	27.68
					NAKAAGSRK	13,872	27.0
					WARLLLAAL	13,739	26.74
					NFKVKMVDE	13,515	26.31
					FHDLKVAPG	13,357	26.0
					LAKHNIIPI	13,172	25.64
		12.632	alpha 1 type XVII collagen (BP180)	**8**	PGRPGIKGE	18,587	36.18
					PAGPAGLPG	17,805	34.66
					NADSGLKAE	16,228	31.59
					DRGPAGPPG	15,018	29.23
					WGPAPAWCP	14,162	27.56
					PKGDRGFPG	13,865	26.99
					DRLQGMAPA	13,846	26.95
					GAKGAMGPA	13.11	25.52
	HLA-DR4 (DRB1*0402)	11.444	β4 integrin	**12**	**ICNGRGHCE**	**20,417**	**45.77**

					ILMDFSNSM	17,384	38.97
					VGFKEDHYM	17,283	38.75
					VRWKVTNNM	15.15	33.97
					VVRWKVTNN	14,177	31.78
					VKYWIQGDS	14,176	31.78
					LAGIMSRND	14,128	31.67
					IITIESQDG	14,068	31.54
					VCYGEGRYE	13,907	31.18
					LRTEVTSKM	13,659	30.62
					VRLLAKHNI	13,509	30.29
					VRRFHVQLS	13.46	30.18
		11.444	alpha 1 type XVII collagen (BP180)	4	ILSYMSSSG	16,967	38.04
					LLTWLLLLG	15,227	34.14
					IRVRLQSAS	14,347	32.17
					IHSYGSSGG	12,689	28.45
	HLA-DR4 (DRB1*0404)	12.059	β4 integrin	**17**	RTGSFHIRR	19,434	51.1
					FRVDGDSPE	18,007	47.35
					KVCAYGAQG	16,458	43.27
					ILMDFSNSM	15,966	41.98
					LVFSTESAF	15,505	40.77
					YTMEGDGAP	15,019	39.49
					RRPNGDIVG	14,632	38.47
					INYSAIHPG	14,598	38.38
					IPVEGELLF	13,889	36.52
					IPIIPDIPI	13,151	34.58
					YVFRVRAQS	13,112	34.48
					FHYEADGAN	13,047	34.3
					YMLRENLMA	13,033	34.27
					PRCERPLQG	13,031	34.26
					LTADQDARG	12,839	33.76
					QRAFHDLKV	12,821	33.71
					RLAFNVVSS	12,808	33.68
		12.059	alpha 1 type XVII collagen (BP180)	**6**	RLLSTDASH	19,689	51.77
					YAGNGGLLG	17,255	45.37
					YLTSPDVRS	14,606	38.4
					RRAHSPAST	13,619	35.81
					KQSLTHGSS	13,497	35.49
					IALAEEVRK	13,157	34.59
MMP-MMP	HLA-DQ7 (DQB1*0301)	31.451	β4 integrin	14	LPPSGKPMG	20.182	44.19
					YRYTVKARN	19.166	41.97
					NYSAIHPGL	19.047	41.7
					APRSAKPAL	18.434	40.36
					YCACCKACL	16.829	36.85
					DVPAGTATL	15.922	34.86
					IRRVLDGGK	14.888	32.6

(*Continued*)

Table S1B. (*Continued*)

Disease	MHC	Threshold	Autoantigen	Total predicted binders	Top binders	Score	% optimal
					FRQQPNAGK	14.639	32.05
					IYQVQLRAL	14.228	31.15
					YSDDVLRSP	13.589	29.75
					RAQSQEGWG	13,035	28.54
					SCVQCQAWG	12,643	27.68
					ADQDARGMV	12,108	26.51
					VIRRVLDGG	11,829	25.9
	HLA-DQ7 (DQB1*0301)	31.451	alpha 1 type XVII collagen (BP180)	14	IRRSILPYG	19.68	43.09
					FDYSELASH	19.381	42.44
					STDASHSRG	16.697	36.56
					ILDANLPSH	16.398	35.9
					AGPAGLPGH	16.234	35.55
					VWSSTLPAG	15.634	34.23
					SLGAGGAFG	13.849	30.32
					IRGPPGPSG	13.625	29.83
					SSQSVSGTY	13.531	29.63
					NTNAYSAGS	13.391	29.32
					YRRAHSPAS	12,928	28.31
					LSSYLHTAG	12,403	27.16
					DIHSYGSSG	12,396	27.14
					APGPAGPAG	11,938	26.14
MMP-PV	HLA-DQ7 (DQB1*0301)	31.451	desmoglein 3 preproprotein	4	ATESGGAAG	17.045	37.32
					LVDYILGTY	15.092	33.05
					ITSDYQATQ	14.73	32.25
					YRLVVSGAD	13.563	29.7

Table S2A. Rankpep analysis of PF- and BP-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease 1	MHC	Performance	Disease 2	Protein	Total sequences	Total predicted binders	Threshold	High binding score	Low binding score	% optimal high	% optimal low
PF	HLA-DR1 (DRB1*0101)	0.75 ± 0.04	PF	desmoglein 1	1041	22	8.009	25.889	8.032	53.66	16.65
	HLA-DR1 (DRB1*0102)	0.72 ± 0.04		desmoglein 1	1041	0	33.00	–	–	–	–
	HLA-DR4 (DRB1*0404)	0.61 ± 0.05		desmoglein 1	1041	5	12.059	18.417	12.178	48.42	32.02
	HLA-DR4 (DRB1*0406)	na		desmoglein 1	1041	0	25.605	–	–	–	–
	HLA-DR1 (DRB1*0101)	0.75 ± 0.04	BP	alpha 1 type XVII collagen (BP180)	1489	**30**	8.009	19.267	8.273	39.93	17.15
	HLA-DR1 (DRB1*0101)	0.75 ± 0.04		dystonin (BP230)	2641	**56**	8.009	21,334	8,064	44.22	16.71
	HLA-DR1 (DRB1*0102)	0.72 ± 0.04		alpha 1 type XVII collagen (BP180)	1489	0	33.00	–	–	–	–
	HLA-DR1 (DRB1*0102)	0.72 ± 0.04		dystonin (BP230)	2641	0	33.00	–	–	–	–
	HLA-DR4 (DRB1*0404)	0.61 ± 0.05		alpha 1 type XVII collagen (BP180)	1489	**6**	12.059	**19.689**	13.157	51.77	34.59
	HLA-DR4 (DRB1*0404)	0.61 ± 0.05		dystonin (BP230)	2641	**11**	12.059	**20,577**	12,103	54.1	31.82
	HLA-DR4 (DRB1*0406)	na		alpha 1 type XVII collagen (BP180)	1489	1	25.605	**36.062**	–	43.23	–
	HLA-DR4 (DRB1*0406)	na		dystonin (BP230)	2641	0	25.605	–	–	–	–
BP	HLA-DQ7 (DQB1*0301)	na	BP	alpha 1 type XVII collagen (BP180)	1489	14	11.701	19.68	11.938	43.09	26.14
	HLA-DQ7 (DQB1*0301)	na		dystonin (BP230)	2641	12	11.701	16,983	12,013	37.19	26.3
	HLA-DQ7 (DQB1*0301)	na	PF	desmoglein 1	1041	6	11.701	18.207	12.73	39.87	27.87

Table S2B. Peptide sequences of PF- and BP-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease	MHC	Threshold	Autoantigen	Total predicted binders	Top binders	Score	% optimal
PF-PF	HLA-DR1 (DRB1*0101)	8.009	desmoglein 1	22 (top ten sequences shown)	YEAMQSLQL	25.889	53.66
					YFCQKAYAY	18.86	39.09
					IKFAAACRE	18.451	38.24
					IRTMNNFLD	16.935	35.1
					VFSMATFAG	16.001	33.16
					VFSMATFAG	16.001	33.16
					YKLKASAIS	14.479	30.01
					ICQEYSGTL	13.778	28.56
					WMAVIFFIS	11.917	24.7
					YCRALNSMG	11.759	24.37
	HLA-DR1 (DRB1*0102)	33	desmoglein 1	0			
	HLA-DR4 (DRB1*0404)	12.059	desmoglein 1	5	RVVSGAGVT	18.417	48.42
					KPLDYEAMQ	13.233	34.79
					MAVIFFISG	12.663	33.29
					TASIGHMRS	12.326	32.41
					GAGSGALSG	12.178	32.02
	HLA-DR4 (DRB1*0406)	25.605	desmoglein 1	0			
PF-BP	HLA-DR1 (DRB1*0101)	8.009	alpha 1 type XVII collagen (BP180)	**30 (top ten sequences shown)**	LTGMPGIRG	19,267	39.93
					YRRAHSPAS	17,904	37.11
					FTASPASIA	16,536	34.27
					YAELSSRIL	15.97	33.1
					MRGLPGAVG	15,095	31.29
					YGAIQGPPG	14.75	30.57
					LQGMAPAAG	14.65	30.36
					IKGEPGAPG	14,646	30.36
					LKAEANGDL	14,629	30.32
					WKWLLGLLL	12,638	26.19
		8.009	dystonin (BP230)	**56 (top ten sequences shown)**	FQAMENRML	21,334	44.22
					YTALVTLMT	18,836	39.04
					IKQMEKDLA	17,855	37.01
					WHKEKADQL	17,566	36.41
					LAFLEAQAA	17,355	35.97
					YRAMVDSQQ	17,008	35.25
					IDKMVALAF	16,637	34.48
					RAAMQTQWS	16,464	34.12
					YIKFAGDSL	16,398	33.99
					YKSTIANLM	15,891	32.94
	HLA-DR1 (DRB1*0102)	33	alpha 1 type XVII collagen (BP180)	0			
		33	dystonin (BP230)	0			
	HLA-DR4 (DRB1*0404)	12.059	alpha 1 type XVII collagen (BP180)	**6**	**RLLSTDASH**	**19,689**	**51.77**
					YAGNGGLLG	17,255	45.37

					YLTSPDVRS	14,606	38.4
					RRAHSPAST	13,619	35.81
					KQSLTHGSS	13,497	35.49
					IALAEEVRK	13,157	34.59
		12.059	dystonin (BP230)	**11**	**KQMEKDLAF**	**20,577**	**54.1**
					MVLFQEESG	17,983	47.28
					FEFFNDAKE	17,551	46.15
					MALRNECSS	15,845	41.66
					IRASNVASI	14,316	37.64
					RQVFHALED	13,846	36.41
					FNINEAIEQ	12,993	34.16
					YDMHTEVTT	12.94	34.02
					VITENDISG	12,309	32.36
					ILAGNALQS	12.14	31.92
					RQIRTPLER	12,103	31.82
	HLA-DR4 (DRB1*0406)	25.605	alpha 1 type XVII collagen (BP180)	**1**	**AAYNADSGL**	**13,157**	**49.23**
			dystonin (BP230)	0			
BP-BP	HLA-DQ7 (DQB1*0301)	11.701	alpha 1 type XVII collagen (BP180)	14	IRRSILPYG	19.68	43.09
					FDYSELASH	19.381	42.44
					STDASHSRG	16.697	36.56
					ILDANLPSH	16.398	35.9
					AGPAGLPGH	16.234	35.55
					VWSSTLPAG	15.634	34.23
					SLGAGGAFG	13.849	30.32
					IRGPPGPSG	13.625	29.83
					SSQSVSGTY	13.531	29.63
					NTNAYSAGS	13.391	29.32
					YRRAHSPAS	12,928	28.31
					LSSYLHTAG	12,403	27.16
					DIHSYGSSG	12,396	27.14
					APGPAGPAG	11,938	26.14
			dystonin (BP230)	12	FESYGHSSH	16,983	37.19
					NFDGDHACS	15.913	34.84
					YRDTYHPLD	14,818	32.45
					LTPSVTPAY	14,805	32.42
					IEPQVHSRL	14,386	31.5
					FAQTLHPSL	14,356	31.43
					ITQSLNSGF	12,997	28.46
					LLQRQKATV	12,573	27.53
					LRHTVTARQ	12,495	27.36
					ADFDFHTGL	12,406	27.16
					ISPTGNEAM	12.06	26.41
					IIDVLIATK	12,013	26.3

(*Continued*)

Table S2B. (*Continued*)

Disease	MHC	Threshold	Autoantigen	Total predicted binders	Top binders	Score	% optimal
BP-PF	HLA-DQ7 (DQB1*0301)	11.701	desmoglein 1	6	YCRALNSMG	18,207	39.87
					SDGAIHSWA	17,857	39.1
					IEGVGSPAG	16,299	35.69
					YALAVRGSD	14,801	32.41
					LTEGVKTSG	12,806	28.04
					ISGGIGSSG	12.73	27.87

Table S3A. Rankpep analysis of PV- and OCP-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease 1	MHC	Performance	Disease 2	Protein	Total sequences	Total predicted binders	Threshold	High binding score	Low binding score	% optimal high	% optimal low
PV	HLA-DQ8 (DQA1*0301/ DQB1*0302)	0.72 ± 0.06	PV	desmoglein 3 preproprotein	991	4	12.635	18.963	12.956	36.91	25.22
	HLA-DR4 (DRB1*0402)	0.72 ± 0.04		desmoglein 3 preproprotein	991	11	11.444	17.99	11.507	40.33	25.8
	HLA-DR4 (DRB1*0404)	0.61 ± 0.05		desmoglein 3 preproprotein	991	5	12.059	20.331	12.258	53.46	32.23
	HLA-DQ8 (DQA1*0301/ DQB1*0302)	0.72 ± 0.06	OCP	alpha 1 type XVII collagen (BP180)	1489	**8**	12.632	18.587	13.11	36.18	25.52
	HLA-DQ8 (DQA1*0301/ DQB1*0302)	0.72 ± 0.06		β4 integrin	1814	**14**	12.632	**21.785**	13.172	42.4	25.64
	HLA-DR4 (DRB1*0402)	0.72 ± 0.04		alpha 1 type XVII collagen (BP180)	1489	4	11.444	16.967	12.689	38.04	28.45
	HLA-DR4 (DRB1*0402)	0.72 ± 0.04		β4 integrin	1814	**16**	11.444	16.62	–	37.04	–
	HLA-DR4 (DRB1*0404)	0.61 ± 0.05		alpha 1 type XVII collagen (BP180)	1489	**6**	12.059	19.698	13.157	51.77	34.59
	HLA-DR4 (DRB1*0404)	0.61 ± 0.05		β4 integrin	1814	**17**	12.059	19.434	12.808	51.1	33.68
OCP	HLA-DQ7 (DQB1*0301)	na	OCP	alpha 1 type XVII collagen (BP180)	1489	14	11.701	19.68	11.938	43.09	26.14
	HLA-DQ7 (DQB1*0301)	na		β4 integrin	1814	14	11.701	20.182	11.829	44.19	25.9
	HLA-DR4 (DRB1*0401)	0.68 ± 0.01		alpha 1 type XVII collagen (BP180)	1489	76	4.853	18.832	4.855	42.71	11.01
	HLA-DR4 (DRB1*0401)	0.68 ± 0.01		β4 integrin	1814	84	4.853	19.344	4.867	43.87	11.04
	HLA-DQ7 (DQB1*0301)	na	PV	desmoglein 3 preproprotein	991	4	11.701	17.045	13.563	37.32	29.7
	HLA-DR4 (DRB1*0401)	0.68 ± 0.01		desmoglein 3 preproprotein	991	43	4.853	**20.296**	4.991	46.03	11.32

Table S3B. Peptide sequences of PV- and OCP-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease	MHC	Threshold	Autoantigen	Total predicted binders	Top binders	Score	% optimal
PV-PV	HLA-DQ8 (DQA1*0301/ DQB1*0302)	12.632	desmoglein 3 preproprotein	4	NNRCEMPRS	18.963	36.91
					DAVCSSSPS	15.007	29.21
					WGIEGAHPE	13.2	25.69
					WVKFAKPCR	12.956	25.22
	HLA-DR4 (DRB1*0402)	11.444	desmoglein 3 preproprotein	11	TRYGRPHSG	17.99	40.33
					MRTRHSTGG	17.387	38.98
					IAFKIVSQE	15.149	33.96
					IKFVKNMNR	15.046	33.73
					VKYVMGRND	14.554	32.63
					ITYRISGVG	13.746	30.82
					LLIYDNEGA	12.572	28.19
					IAKITSDYQ	12.438	27.89
					ISRYRVQST	11.643	26.1
					VYFFTSGNE	11.59	25.98
					VRTLTNSLD	11,507	25.8
	HLA-DR4 (DRB1*0404)	12.059	desmoglein 3 preproprotein	5	ILVHGELRI	20.331	53.46
					FPMFRDSQY	16.921	44.49
					KNMNRDSTF	14.837	39.01
					IAFRPASKT	13.182	34.66
					INVREGIAF	12.258	32.23
PV-OCP	HLA-DQ8 (DQA1*0301/ DQB1*0302)	12.632	alpha 1 type XVII collagen (BP180)	**8**	PGRPGIKGE	18,587	36.18
					PAGPAGLPG	17,805	34.66
					NADSGLKAE	16,228	31.59
					DRGPAGPPG	15,018	29.23
					WGPAPAWCP	14,162	27.56
					PKGDRGFPG	13,865	26.99
					DRLQGMAPA	13,846	26.95
					GAKGAMGPA	13.11	25.52
		12.632	β4 integrin	**14**	**DERCHLDTT**	**21,785**	**42.4**
					ANRCKKAPV	**20,134**	**39.19**
					KEDHYMLRE	18,019	35.07
					WERPRRPNG	17,709	34.47
					DKPCSGRGE	17,191	33.46
					LRRSQMSPQ	16,831	32.76
					DRRCNTQAE	16,281	31.69
					QYRTQDYPS	14,323	27.88
					DLYILMDFS	14,223	27.68
					NAKAAGSRK	13,872	27.0
					WARLLLAAL	13,739	26.74
					NFKVKMVDE	13,515	26.31
					FHDLKVAPG	13,357	26.0
					LAKHNIIPI	13,172	25.64
	HLA-DR4 (DRB1*0402)	11.444	alpha 1 type XVII collagen (BP180)	4	ILSYMSSSG	16,967	38.04
					LLTWLLLLG	15,227	34.14

					IRVRLQSAS	14,347	32.17
					IHSYGSSGG	12,689	28.45
		11.444	β4 integrin	**19**	**ICNGRGHCE**	**20,417**	**45.77**
					ILMDFSNSM	17,384	38.97
					VGFKEDHYM	17,283	38.75
					VRWKVTNNM	15.15	33.97
					VVRWKVTNN	14,177	31.78
					VKYWIQGDS	14,176	31.78
					LAGIMSRND	14,128	31.67
					IITIESQDG	14,068	31.54
					VCYGEGRYE	13,907	31.18
					LRTEVTSKM	13,659	30.62
					VRLLAKHNI	13,509	30.29
					VRRFHVQLS	13.46	30.18
					ISGVHKLQQ	13,011	29.17
					LCNDRGRCS	12,779	28.65
					IVVMESSFQ	11,947	26.78
					LKKMGQNLA	11,872	26.62
					LTLGAQHLE	11,738	26.32
					RRFHVQLSN	11,603	26.01
					LVRLLAKHN	11,572	25.94
	HLA-DR4 (DRB1*0404)	12.059	alpha 1 type XVII collagen (BP180)	6	RLLSTDASH	19,689	51.77
					YAGNGGLLG	17,255	45.37
					YLTSPDVRS	14,606	38.4
					RRAHSPAST	13,619	35.81
					KQSLTHGSS	13,497	35.49
					IALAEEVRK	13,157	34.59
		12.059	β4 integrin	15	RTGSFHIRR	19,434	51.1
					FRVDGDSPE	18,007	47.35
					KVCAYGAQG	16,458	43.27
					ILMDFSNSM	15,966	41.98
					LVFSTESAF	15,505	40.77
					YTMEGDGAP	15,019	39.49
					RRPNGDIVG	14,632	38.47
					INYSAIHPG	14,598	38.38
					IPVEGELLF	13,889	36.52
					IPIIPDIPI	13,151	34.58
					YVFRVRAQS	13,112	34.48
					FHYEADGAN	13,047	34.3
					YMLRENLMA	13,033	34.27
					PRCERPLQG	13,031	34.26
					LTADQDARG	12,839	33.76
					QRAFHDLKV	12,821	33.71
					RLAFNVVSS	12,808	33.68
OCP-OCP	HLA-DQ7 (DQB1*0301)	11.701	alpha 1 type XVII collagen (BP180)	14	IRRSILPYG	19.68	43.09
					FDYSELASH	19.381	42.44

(*Continued*)

Table S3B. (*Continued*).

Disease	MHC	Threshold	Autoantigen	Total predicted binders	Top binders	Score	% optimal
					STDASHSRG	16.697	36.56
					ILDANLPSH	16.398	35.9
					AGPAGLPGH	16.234	35.55
					VWSSTLPAG	15.634	34.23
					SLGAGGAFG	13.849	30.32
					IRGPPGPSG	13.625	29.83
					SSQSVSGTY	13.531	29.63
					NTNAYSAGS	13.391	29.32
					YRRAHSPAS	12,928	28.31
					LSSYLHTAG	12,403	27.16
					DIHSYGSSG	12,396	27.14
					APGPAGPAG	11,938	26.14
			β4 integrin	14	LPPSGKPMG	20.182	44.19
					YRYTVKARN	19.166	41.97
					NYSAIHPGL	19.047	41.7
					APRSAKPAL	18.434	40.36
					YCACCKACL	16.829	36.85
					DVPAGTATL	15.922	34.86
					IRRVLDGGK	14.888	32.6
					FRQQPNAGK	14.639	32.05
					IYQVQLRAL	14.228	31.15
					YSDDVLRSP	13.589	29.75
					RAQSQEGWG	13,035	28.54
					SCVQCQAWG	12,643	27.68
					ADQDARGMV	12,108	26.51
					VIRRVLDGG	11,829	25.9
	HLA-DR4 (DRB1*0401)	4,853	alpha 1 type XVII collagen (BP180)	76 (top ten sequences shown)	YHNNMTTQS	18,832	42.71
					YRRAHSPAS	17.57	39.85
					YAGNGGLLG	16,995	38.55
					YGAIQGPPG	16,763	38.02
					HVWSSTLPA	16,655	37.78
					FRGIVGPPG	15,877	36.01
					YAKTASLGG	14,274	32.37
					WWKWLLGLL	14,086	31.95
					YAAENSDSF	13,332	30.24
					LRGEVGLPG	13,088	29.68
			β4 integrin	84 (top ten sequences shown)	YRTQDGTAQ	19.344	43.87
					YWIQGDSES	19.108	43.34
					FWWLIPLLL	14.965	33.94
					WWLIPLLLL	14.619	33.16
					KVQARTTEG	13.835	31.38
					LHRMTTTSA	13.518	30.66
					YMLRENLMA	13.312	30.19

					GWSGQTCNC	12.611	28.6
					WRPDSTHLL	12.471	28.29
					RRSQMSPQG	12.112	27.47
					KMFQKTRTG	11.537	26.17
OCP-PV	HLA-DQ7 (DQB1*0301)	11.701	desmoglein 3 preproprotein	4	ATESGGAAG	17,045	37.32
					LVDYILGTY	15,092	33.05
					ITSDYQATQ	14.73	32.25
					YRLVVSGAD	13,563	29.7
	HLA-DR4 (DRB1*0401)	4,853	desmoglein 3 preproprotein	43 (top ten sequences shown)	WFEIQTDPR	20,296	46.03
					FVKCQTLSG	15,321	34.75
					LRFQVTDLD	14,638	33.2
					WVKFAKPCR	13,127	29.77
					YFSQKAFAC	13,066	29.63
					YRVQSTPVT	12,754	28.93
					AVWSITTLN	12,398	28.12
					YLVTETYSA	12,309	27.92
					FVKNMNRDS	11.28	25.58
					YSASGSLVQ	10,616	24.08

Table S4A. Rankpep analysis of PV- and BP-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease 1	MHC	Performance	Disease 2	Protein	Total sequences
PV	HLA-DQ8 (DQA1*0301/DQB1*0302)	0.72 ± 0.06	PV	desmoglein 3 preproprotein	991
	HLA-DR4 (DRB1*0402)	0.72 ± 0.04		desmoglein 3 preproprotein	991
	HLA-DR4 (DRB1*0404)	0.61 ± 0.05		desmoglein 3 preproprotein	991
	HLA-DQ8 (DQA1*0301/DQB1*0302)	0.72 ± 0.06	BP	alpha 1 type XVII collagen (BP180)	1489
	HLA-DQ8 (DQA1*0301/DQB1*0302)	0.72 ± 0.06		dystonin (BP230)	2641
	HLA-DR4 (DRB1*0402)	0.72 ± 0.04		alpha 1 type XVII collagen (BP180)	1489
	HLA-DR4 (DRB1*0402)	0.72 ± 0.04		dystonin (BP230)	2641
	HLA-DR4 (DRB1*0404)	0.61 ± 0.05		alpha 1 type XVII collagen (BP180)	1489
	HLA-DR4 (DRB1*0404)	0.61 ± 0.05		dystonin (BP230)	2641
BP	HLA-DQ7 (DQB1*0301)	na	BP	BP180 alpha 1 type XVII collagen	1489
	HLA-DQ7 (DQB1*0301)	na		dystonin (BP230)	2641
	HLA-DQ7 (DQB1*0301)	na	PV	desmoglein 3 preproprotein	991

Total predicted binders	Threshold	High binding score	Low binding score	% optimal high	% optimal low
4	12.635	18.963	12.956	36.91	25.22
11	11.444	17.99	11.507	40.33	25.8
5	12.059	20.331	12.258	53.46	32.23
8	12.632	18.587	13.11	36.18	25.52
12	12.632	**24,137**	13,043	46.98	25.39
4	11.444	16.967	12.689	38.04	28.45
28	11.444	16,557	12,753	37.12	28.59
6	12.059	19.698	13.157	51.77	34.59
11	12.059	**20,577**	12,103	54.1	31.82
14	11.701	19.68	11.938	43.09	26.14
12	11.701	16,983	12,013	37.19	26.3
4	11.701	17.045	13.563	37.32	29.7

Table S4B. Peptide sequences of PV- and BP-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease	MHC	Threshold	Autoantigen	Total predicted binders	Top binders	Score	% optimal
PV-PV	HLA-DQ8 (DQA1*0301/ DQB1*0302)	12.632	desmoglein 3 preproprotein	4	NNRCEMPRS	18.963	36.91
					DAVCSSSPS	15.007	29.21
					WGIEGAHPE	13.2	25.69
					WVKFAKPCR	12.956	25.22
	HLA-DR4 (DRB1*0402)	11.444	desmoglein 3 preproprotein	11	TRYGRPHSG	17.99	40.33
					MRTRHSTGG	17.387	38.98
					IAFKIVSQE	15.149	33.96
					IKFVKNMNR	15.046	33.73
					VKYVMGRND	14.554	32.63
					ITYRISGVG	13.746	30.82
					LLIYDNEGA	12.572	28.19
					IAKITSDYQ	12.438	27.89
					ISRYRVQST	11.643	26.1
					VYFFTSGNE	11.59	25.98
					VRTLTNSLD	11.507	25.8
	HLA-DR4 (DRB1*0404)	12.059	desmoglein 3 preproprotein	5	ILVHGELRI	20.331	53.46
					FPMFRDSQY	16.921	44.49
					KNMNRDSTF	14.837	39.01
					IAFRPASKT	13.182	34.66
					INVREGIAF	12.258	32.23
PV-BP	HLA-DQ8 (DQA1*0301/ DQB1*0302)	12.632	alpha 1 type XVII collagen (BP180)	**8**	PGRPGIKGE	18,587	36.18
					PAGPAGLPG	17,805	34.66
					NADSGLKAE	16,228	31.59
					DRGPAGPPG	15,018	29.23
					WGPAPAWCP	14,162	27.56
					PKGDRGFPG	13,865	26.99
					DRLQGMAPA	13,846	26.95
					GAKGAMGPA	13.11	25.52
		12.632	dystonin (BP230)	**12**	**ITRAHAVAE**	**24,137**	**46.98**
					IKRCKETSE	**20,169**	**39.26**
					PAYTPGFPS	18,052	35.14
					VSWHYLINE	15,507	30.18
					VQRVAKLRD	15,481	30.13
					AYRAAMQTQ	15,471	30.11
					DEIMALRNE	15,444	30.06
					VRGIRVPPE	15,033	29.26
					IVREKEAAE	14,276	27.79
					VLKGVVDPE	13,926	27.11
	HLA-DR4 (DRB1*0402)	11.444	alpha 1 type XVII collagen (BP180)	4	ILSYMSSSG	16,967	38.04
					LLTWLLLLG	15,227	34.14
					IRVRLQSAS	14,347	32.17
					IHSYGSSGG	12,689	28.45

		11.444	dystonin (BP230)	**28 (top ten sequences shown)**	LLNWVDEMQ	16,557	37.12
					IANRVQRDS	16,481	36.95
					LSNLQSRFE	16,202	36.32
					LLRWTQEPQ	15,783	35.38
					VRNIRLRLE	15.26	34.21
					FRKKMEKLM	15,105	33.86
					IIQLKPRNS	14,966	33.55
					LRRKRDNEE	14,692	32.94
					LLNFRNQLE	14,444	32.38
					IARKKDYHA	14,323	32.11
	HLA-DR4 (DRB1*0404)	12.059	alpha 1 type XVII collagen (BP180)	**6**	RLLSTDASH	19,689	51.77
					YAGNGGLLG	17,255	45.37
					YLTSPDVRS	14,606	38.4
					RRAHSPAST	13,619	35.81
					KQSLTHGSS	13,497	35.49
					IALAEEVRK	13,157	34.59
		12.059	dystonin (BP230)	**11**	**KQMEKDLAF**	**20,577**	**54.1**
					MVLFQEESG	17,983	47.28
					FEFFNDAKE	17,551	46.15
					MALRNECSS	15,845	41.66
					IRASNVASI	14,316	37.64
					RQVFHALED	13,846	36.41
					FNINEAIEQ	12,993	34.16
					YDMHTEVTT	12.94	34.02
					VITENDISG	12,309	32.36
					ILAGNALQS	12.14	31.92
BP-BP	HLA-DQ7 (DQB1*0301)	11.701	alpha 1 type XVII collagen (BP180)	14	IRRSILPYG	19.68	43.09
					FDYSELASH	19.381	42.44
					STDASHSRG	16.697	36.56
					ILDANLPSH	16.398	35.9
					AGPAGLPGH	16.234	35.55
					VWSSTLPAG	15.634	34.23
					SLGAGGAFG	13.849	30.32
					IRGPPGPSG	13.625	29.83
					SSQSVSGTY	13.531	29.63
					NTNAYSAGS	13.391	29.32
					YRRAHSPAS	12,928	28.31
					LSSYLHTAG	12,403	27.16
					DIHSYGSSG	12,396	27.14
					APGPAGPAG	11,938	26.14
			dystonin (BP230)	12	FESYGHSSH	16,983	37.19
					NFDGDHACS	15.913	34.84
					YRDTYHPLD	14,818	32.45
					LTPSVTPAY	14,805	32.42
					IEPQVHSRL	14,386	31.5
					FAQTLHPSL	14,356	31.43

(*Continued*)

Table S4B. (*Continued*)

Disease	MHC	Threshold	Autoantigen	Total predicted binders	Top binders	Score	% optimal
					ITQSLNSGF	12,997	28.46
					LLQRQKATV	12,573	27.53
					LRHTVTARQ	12,495	27.36
					ADFDFHTGL	12,406	27.16
					ISPTGNEAM	12.06	26.41
					IIDVLIATK	12,013	26.3
BP-PV	HLA-DQ7 (DQB1*0301)	11.701	desmoglein 3 preproprotein	4	ATESGGAAG	17,045	37.32
					LVDYILGTY	15,092	33.05
					ITSDYQATQ	14.73	32.25
					YRLVVSGAD	13,563	29.7

Table S5A. Rankpep analysis of PV- and MCTD-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease 1	MHC	Performance	Disease 2	Protein	Total sequences	Total predicted binders	Threshold	High binding score	Low binding score	% optimal high	% optimal low
PV	HLA-DQ8 (DQA1*0301/ DQB1*0302)	0.72 ± 0.06	PV	desmoglein 3 preproprotein	991	4	12.635	18.963	12.956	36.91	25.22
	HLA-DR4 (DRB1*0402)	0.72 ± 0.04		desmoglein 3 preproprotein	991	11	11.444	17.99	11.507	40.33	25.8
	HLA-DR4 (DRB1*0404)	0.61 ± 0.05		desmoglein 3 preproprotein	991	5	12.059	20.331	12.258	53.46	32.23
	HLA-DQ8 (DQA1*0301/ DQB1*0302)	0.72 ± 0.06	MCTD	U1-snRNP	429	4	12.635	**20.599**	13.862	40.09	26.98
	HLA-DR4 (DRB1*0402)	0.72 ± 0.04		U1-snRNP	429	3	11.444	**24.837**	11.702	55.68	26.24
	HLA-DR4 (DRB1*0404)	0.61 ± 0.05		U1-snRNP	429	0	12.059	–	–	–	–
MCTD	HLA-DR1 (DRB1*0101)	0.75 ± 0.04	MCTD	U1-snRNP	429	3	8.009	18.186	12.243	37.69	25.38
	9mer-HLA-B07	na		U1-snRNP	429	2	19.18	28.526	27.961	63.48	62.22
	HLA-DR1 (DRB1*0101)	0.75 ± 0.04	PV	desmoglein 3 preproprotein	991	**18**	8.009	**21.359**	8.202	44.27	17.00
	9mer-HLA-B07	na		desmoglein 3 preproprotein	991	0	19.18	–	–	–	–

Table S5B. Peptide sequences of PV- and MCTD-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease	MHC	Threshold	Autoantigen	Total predicted binders	Top binders	Score	% optimal
PV-PV	HLA-DQ8 (DQA1*0301/ DQB1*0302)	12.632	desmoglein 3 preproprotein	4	NNRCEMPRS	18.963	36.91
					DAVCSSSPS	15.007	29.21
					WGIEGAHPE	13.2	25.69
					WVKFAKPCR	12.956	25.22
	HLA-DR4 (DRB1*0402)	11.444	desmoglein 3 preproprotein	11	TRYGRPHSG	17.99	40.33
					MRTRHSTGG	17.387	38.98
					IAFKIVSQE	15.149	33.96
					IKFVKNMNR	15.046	33.73
					VKYVMGRND	14.554	32.63
					ITYRISGVG	13.746	30.82
					LLIYDNEGA	12.572	28.19
					IAKITSDYQ	12.438	27.89
					ISRYRVQST	11.643	26.1
					VYFFTSGNE	11.59	25.98
					VRTLTNSLD	11.507	25.8
	HLA-DR4 (DRB1*0404)	12.059	desmoglein 3 preproprotein	5	ILVHGELRI	20.331	53.46
					FPMFRDSQY	16.921	44.49
					KNMNRDSTF	14.837	39.01
					IAFRPASKT	13.182	34.66
					INVREGIAF	12.258	32.23
PV-MCTD	HLA-DQ8 (DQA1*0301/ DQB1*0302)	12.632	U1-snRNP	4	**IKRIHMVYS**	**20,599**	**40.09**
					RERARRERE	16,863	32.82
					FEDPRDAPP	16,611	32.33
					PSEAGDAPP	13,862	26.98
	HLA-DR4 (DRB1*0402)	11.444	U1-snRNP	3	**LKMWDPHND**	**24,837**	**55.68**
					IEYEHERDM	13,188	29.57
					VKGWRPRRL	11,702	26.24
	HLA-DR4 (DRB1*0404)	12.059	U1-snRNP	0			
MCTD-MCTD	HLA-DR1 (DRB1*0101)	8.009	U1-snRNP	3	YKHADGKKI	18.186	37.69
					YCGIAPYIR	13.599	28.19
					FVARVNYDT	12.243	25.38
	9mer-HLA-B07	19.18	U1-snRNP	2	RPRRLGGGL	28.526	63.48
					APRDPIPYL	27.961	62.22
MCTD-PV	HLA-DR1 (DRB1*0101)	8.009	desmoglein 3 preproprotein	**18**	**YFSQKAFAC**	**21,359**	**44.27**
					YEQLQSVKL	**20,238**	**41.95**
					CRALNAQGL	**18,448**	**38.24**
					YRLVVSGAD	**18,426**	**38.19**
					FKKLAEISL	16,908	35.04
					YDNEGADAT	13,857	28.72
					IAFRPASKT	12,468	25.84
					YQATQKITY	12,421	25.74
					YTGPYTFAL	12.22	25.33
					YLMIDSKTA	11,791	24.44

CCSFIADDL	11,427	23.68				
LLLLLAPLL	11,135	23.08				
WLAVYFFTS	10,792	22.37				
YVMGRNDGG	10,472	21.7				
ISSVPGNLA	9,889	20.5				
ITTLNATSA	9,397	19.48				
LRFQVTDLD	9,214	19.1				
YQAIDEDTN	8,202	17.0				
			9mer-HLA-B07	19.18	desmoglein 3 preproprotein	0

Table S6A. Rankpep analysis of PF- and MCTD-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease 1	MHC	Performance	Disease 2	Protein	Total sequences
PF	HLA-DR1 (DRB1*0101)	0.75 ± 0.04	PF	desmoglein 1	1041
	HLA-DR1 (DRB1*0102)	0.72 ± 0.04		desmoglein 1	1041
	HLA-DR4 (DRB1*0404)	0.61 ± 0.05		desmoglein 1	1041
	HLA-DR4 (DRB1*0406)	na		desmoglein 1	1041
	HLA-DR1 (DRB1*0101)	0.75 ± 0.04	MCTD	U1-snRNP	429
	HLA-DR1 (DRB1*0102)	0.72 ± 0.04		U1-snRNP	429
	HLA-DR4 (DRB1*0404)	0.61 ± 0.05		U1-snRNP	429
	HLA-DR4 (DRB1*0406)	na		U1-snRNP	429
MCTD	HLA-DR1 (DRB1*0101)	0.75 ± 0.04	MCTD	U1-snRNP	429
	9mer-HLA-B07	na		U1-snRNP	429
	HLA-DR1 (DRB1*0101)	0.75 ± 0.04	PF	desmoglein 1	1041
	9mer-HLA-B07	na		desmoglein 1	1041

Total predicted binders	Threshold	High binding score	Low binding score	% optimal high	% optimal low
22	8.009	25.889	8.032	53.66	16.65
0	33	–	–	–	–
5	12.059	18.417	12.178	48.42	32.02
0	25.605	–	–	–	–
3	8.009	18.186	12.243	37.69	25.38
0	33	–	–	–	–
0	12.059	–	–	–	–
1	25.605	**25.891**	–	35.35	–
3	8.009	18.186	12.243	37.69	25.38
2	19.18	28.526	27.961	63.48	62.22
22	8.009	**25.889**	16.001	53.66	33.16
0	19.18	–	–	–	–

Table S6B. Peptide sequences of PF- and MCTD-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease	MHC	Threshold	Autoantigen	Total predicted binders	Top binders	Score	% optimal
PF-PF	HLA-DR1 (DRB1*0101)	8.009	desmoglein 1	22 (top ten sequences shown)	YEAMQSLQL	25.889	53.66
					YFCQKAYAY	18.86	39.09
					IKFAAACRE	18.451	38.24
					IRTMNNFLD	16.935	35.1
					VFSMATFAG	16.001	33.16
					VFSMATFAG	16.001	33.16
					YKLKASAIS	14.479	30.01
					ICQEYSGTL	13.778	28.56
					WMAVIFFIS	11.917	24.7
					YCRALNSMG	11.759	24.37
	HLA-DR1 (DRB1*0102)	33	desmoglein 1	0			
	HLA-DR4 (DRB1*0404)	12.059	desmoglein 1	5	RVVSGAGVT	18.417	48.42
					KPLDYEAMQ	13.233	34.79
					MAVIFFISG	12.663	33.29
					TASIGHMRS	12.326	32.41
					GAGSGALSG	12.178	32.02
	HLA-DR4 (DRB1*0406)	25.605	desmoglein 1	0			
PF-MCTD	HLA-DR1 (DRB1*0101)	8.009	U1-snRNP	3	YKHADGKKI	18,186	37.69
					YCGIAPYIR	13,599	28.19
					FVARVNYDT	12,243	25.38
	HLA-DR1 (DRB1*0102)	33	U1-snRNP	0			
	HLA-DR4 (DRB1*0404)	12.059	U1-snRNP	0			
	HLA-DR4 (DRB1*0406)	25.605	U1-snRNP	**1**	**SRYDERPGP**	**25,891**	**35.35**
MCTD-MCTD	HLA-DR1 (DRB1*0101)	8.009	U1-snRNP	3	YKHADGKKI	18.186	37.69
					YCGIAPYIR	13.599	28.19
					FVARVNYDT	12.243	25.38
	9mer-HLA-B07	19.18	U1-snRNP	2	RPRRLGGGL	28.526	63.48
					APRDPIPYL	27.961	62.22
MCTD-PF	HLA-DR1 (DRB1*0101)	8.009	desmoglein 1	**22 (top ten sequences shown)**	**YEAMQSLQL**	**25,889**	**53.66**
					YFCQKAYAY	**18.86**	**39.09**
					IKFAAACRE	**18,451**	**38.24**
					IRTMNNFLD	16,935	35.1
					VFSMATFAG	16,001	33.16
					YKLKASAIS	14,479	30.01
					ICQEYSGTL	13,778	28.56
					WMAVIFFIS	11,917	24.7
					YCRALNSMG	11,759	24.37
					YVMGNNPAD	11,497	23.83
	9mer-HLA-B07	19.18	desmoglein 1	0			

Table S7A. Rankpep analysis of BP- and MCTD-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease 1	MHC	Performance	Disease 2	Protein	Total sequences	Total predicted binders	Threshold	High binding score	Low binding score	% optimal high	% optimal low
BP	HLA-DQ7 (DQB1*0301)	na	BP	alpha 1 type XVII collagen (BP180)	1489	14	11.701	19.68	11.938	43.09	26.14
	HLA-DQ7 (DQB1*0301)	na		dystonin (BP230)	2641	12	11.701	16,983	12,013	37.19	26.3
	HLA-DQ7 (DQB1*0301)	na	MCTD	UI-snRNP	429	3	11.701	15.071	11.87	33	25.99
MCTD	HLA-DR1 (DRB1*0101)	0.75 ± 0.04	MCTD	U1-snRNP	429	3	8.009	18.186	12.243	37.69	25.38
	9mer-HLA-B07	na		U1-snRNP	429	2	19.18	28.526	27.961	63.48	62.22
	HLA-DR1 (DRB1*0101)	0.75 ± 0.04	BP	alpha 1 type XVII collagen (BP180)	1489	**30**	8.009	**19.267**	8.273	39.93	17.15
	HLA-DR1 (DRB1*0101)	0.75 ± 0.04		dystonin (BP230)	2641	**56**	8.009	**21,334**	8,064	44.22	16.71
	9mer-HLA-B07	na		alpha 1 type XVII collagen (BP180)	1489	**9**	19.18	26.397	20.217	58.74	44.99
	9mer-HLA-B07	na		dystonin (BP230)	2641	1	19.18	23.701	–	52.74	–

Table S7B. Peptide sequences of BP- and MCTD-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease	MHC	Threshold	Autoantigen	Total predicted binders	Top binders	Score	% optimal
BP-BP	HLA-DQ7 (DQB1*0301)	11.701	alpha 1 type XVII collagen (BP180)	14	IRRSILPYG	19.68	43.09
					FDYSELASH	19.381	42.44
					STDASHSRG	16.697	36.56
					ILDANLPSH	16.398	35.9
					AGPAGLPGH	16.234	35.55
					VWSSTLPAG	15.634	34.23
					SLGAGGAFG	13.849	30.32
					IRGPPGPSG	13.625	29.83
					SSQSVSGTY	13.531	29.63
					NTNAYSAGS	13.391	29.32
					YRRAHSPAS	12,928	28.31
					LSSYLHTAG	12,403	27.16
					DIHSYGSSG	12,396	27.14
					APGPAGPAG	11,938	26.14
			dystonin (BP230)	12	FESYGHSSH	16,983	37.19
					NFDGDHACS	15.913	34.84
					YRDTYHPLD	14,818	32.45
					LTPSVTPAY	14,805	32.42
					IEPQVHSRL	14,386	31.5
					FAQTLHPSL	14,356	31.43
					ITQSLNSGF	12,997	28.46
					LLQRQKATV	12,573	27.53
					LRHTVTARQ	12,495	27.36
					ADFDFHTGL	12,406	27.16
					ISPTGNEAM	12.06	26.41
					IIDVLIATK	12,013	26.3
BP-MCTD	HLA-DQ7 (DQB1*0301)	11.701	U1-snRNP	3	SKRSGKPRG	15,071	33.0
					WRPRRLGGG	12.75	27.92
					DRDREHKRG	11.87	25.99
MCTD-MCTD	HLA-DR1 (DRB1*0101)	8.009	U1-snRNP	3	YKHADGKKI	18.186	37.69
					YCGIAPYIR	13.599	28.19
					FVARVNYDT	12.243	25.38
	9mer-HLA-B07	19.18	U1-snRNP	2	RPRRLGGGL	28.526	63.48
					APRDPIPYL	27.961	62.22
MCTD-BP	HLA-DR1 (DRB1*0101)	8.009	alpha 1 type XVII collagen (BP180)	**30 (top ten sequences shown)**	**LTGMPGIRG**	**19,267**	**39.93**
					YRRAHSPAS	17,904	37.11
					FTASPASIA	16,536	34.27
					YAELSSRIL	15.97	33.1
					MRGLPGAVG	15,095	31.29
					YGAIQGPPG	14.75	30.57
					LQGMAPAAG	14.65	30.36
					IKGEPGAPG	14,646	30.36

				LKAEANGDL	14,629	30.32
				WKWLLGLLL	12,638	26.19
	dystonin (BP230)	**56 (top ten sequences shown)**		**FQAMENRML**	**21,334**	**44.22**
				YTALVTLMT	**18,836**	**39.04**
				IKQMEKDLA	17,855	37.01
				WHKEKADQL	17,566	36.41
				LAFLEAQAA	17,355	35.97
				YRAMVDSQQ	17,008	35.25
				IDKMVALAF	16,637	34.48
				RAAMQTQWS	16,464	34.12
				YIKFAGDSL	16,398	33.99
				YKSTIANLM	15,891	32.94
	9mer-HLA-B07	19.18	alpha 1 type XVII collagen (BP180)	**9**	GPPGPVTTI	26,397	58.74
					GPPGPPGAM	23,355	51.97
					GPPGPPGPV	23,008	51.2
					GPPGVSGAL	22,519	50.11
					GPRGPPGPS	22,357	49.75
					GPPGPPGSI	21,477	47.79
					GPRGPPGVS	21,201	47.18
					GPRGPPGPP	20,932	46.58
					GPPGDSRLL	20,217	44.99
			dystonin (BP230)	1	TPRSPLLRW	23,701	52.74

Table S8A. Rankpep analysis of DH- and MCTD-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease 1	MHC	Performance	Disease 2	Protein	Total sequences
DH	HLA-DQ2 (DQA1*0501/DQB1*0201)	0.88 ± 0.06	DH	transglutaminase 3	685
	HLA-DQ8 (DQA1*0301/DQB1*0302)	0.72 ± 0.06		transglutaminase 3	685
	HLA-DQ2 (DQA1*0501/DQB1*0201)	0.88 ± 0.06	MCTD	U1-snRNP	429
	HLA-DQ8 (DQA1*0301/DQB1*0302)	0.72 ± 0.06		U1-snRNP	429
MCTD	HLA-DR1 (DRB1*0101)	0.75 ± 0.04	MCTD	U1-snRNP	429
	9mer-HLA-B07	na		U1-snRNP	429
	HLA-DR1 (DRB1*0101)	0.75 ± 0.04	DH	transglutaminase 3	685
	9mer-HLA-B07	na		transglutaminase 3	685

Total predicted binders	Threshold	High binding score	Low binding score	% optimal high	% optimal low
0	31.451	29.276	16.04	40.74	22.32
3	12.635	15.737	14.644	30.63	28.5
4	31.451	**20.599**	13.862	40.09	26.98
0	12.635	–	–	–	–
3	8.009	18.186	12.243	37.69	25.38
2	19.18	28.526	27.961	63.48	62.22
17	8.009	**19.6**	8.343	40.62	17.29
1	19.18	21.332	–	47.47	–

Table S8B. Peptide sequences of DH- and MCTD-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease	MHC	Threshold	Autoantigen	Total predicted binders	Top binders	Score	% optimal
MCTD	HLA-DR1 (DRB1*0101)	8.009	U1-snRNP	3	YKHADGKKI	18.186	37.69
					YCGIAPYIR	13.599	28.19
					FVARVNYDT	12.243	25.38
	9mer-HLA-B07	19.18	U1-snRNP	2	RPRRLGGGL	28.526	63.48
					APRDPIPYL	27.961	62.22
MCTD-DH	HLA-DR1 (DRB1*0101)	8.009	transglutaminase 3	**17**	**IKAMLSIDV**	**19.6**	**40.62**
					WTIIYNGTL	16,633	34.47
					FILLFNPWL	15,353	31.82
					YVQEDAGII	15,052	31.2
					YDPMGNPLD	14,795	30.67
					WKNSVNSHT	11,181	23.17
					FDILPSRSG	11,107	23.02
					FAEVNADRI	10,445	21.65
					YISTKAVGS	10,043	20.82
					FPAIKAMLS	9,733	20.17
					FAGTLNTAL	9,729	20.16
					WKDSATMSL	9,673	20.05
					LQASNGNTL	9,352	19.38
					CVLMVEGSG	9,057	18.77
					CNKFPAIKA	8,643	17.91
					YGQCWVFAG	8,436	17.49
					FRRDAATDV	8,343	17.29
	9mer-HLA-B07	19.18	transglutaminase 3	1	APIGRYTMA	21,332	47.47
DH-DH	HLA-DQ2 (DQA1*0501/ DQB1*0201)	31.451	transglutaminase 3	0			
	HLA-DQ8 (DQA1*0301/ DQB1*0302)	12.635	transglutaminase 3	3	DFSCNKFPA	15.737	30.63
					ALRSLGIPS	14.828	28.86
					SATMSLDPE	14.644	28.5
DH-MCTD	HLA-DQ2 (DQA1*0501/ DQB1*0201)	31.451	U1-snRNP	0			
	HLA-DQ8 (DQA1*0301/ DQB1*0302)	12.635	U1-snRNP	**4**	**IKRIHMVYS**	**20,599**	**40.09**
					RERARRERE	**16,863**	**32.82**
					FEDPRDAPP	**16,611**	**32.33**
					PSEAGDAPP	13,862	26.98

Table S9A. Rankpep analysis of OCP- and MCTD-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease 1	MHC	Performance	Disease 2	Protein	Total sequences	Total predicted binders	Threshold	High binding score	Low binding score	% optimal high	% optimal low
OCP	HLA-DQ7 (DQB1*0301)	na	OCP	alpha 1 type XVII collagen (BP180)	1489	14	11.701	19.68	11.938	43.09	26.14
	HLA-DQ7 (DQB1*0301)	na		β4 integrin	1814	14	11.701	20.182	11.829	44.19	25.9
	HLA-DR4 (DRB1*0401)	0.68 ± 0.01		alpha 1 type XVII collagen (BP180)	1489	76	4.853	18.832	4.855	42.71	11.01
	HLA-DR4 (DRB1*0401)	0.68 ± 0.01		β4 integrin	1814	84	4.853	19.344	4.867	43.87	11.04
	HLA-DQ7 (DQB1*0301)	na	MCTD	UI-snRNP	429	3	11.701	15.071	11.87	33	25.99
	HLA-DR4 (DRB1*0401)	0.68 ± 0.01		UI-snRNP	429	6	4.853	12.955	6.599	29.38	14.97
MCTD	HLA-DR1 (DRB1*0101)	0.75 ± 0.04	MCTD	U1-snRNP	429	3	8.009	18.186	12.243	37.69	25.38
	9mer-HLA-B07	na		U1-snRNP	429	2	19.18	28.526	27.961	63.48	62.22
	HLA-DR1 (DRB1*0101)	0.75 ± 0.04	OCP	alpha 1 type XVII collagen (BP180)	1489	**30**	8.009	**19.267**	8.273	39.93	17.15
	HLA-DR1 (DRB1*0101)	0.75 ± 0.04		β4 integrin	1814	**29**	8.009	17.695	8.039	36.68	16.66
	9mer-HLA-B07	na		alpha 1 type XVII collagen (BP180)	1489	**9**	19.18	26.397	20.217	58.74	44.99
	9mer-HLA-B07	na		β4 integrin	1814	**5**	19.18	27.886	20.604	62.05	45.85

Table S9B. Peptide sequences of DH- and MCTD-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease	MHC	Threshold	Autoantigen	Total predicted binders	Top binders	Score	% optimal
MCTD-MCTD	HLA-DR1 (DRB1*0101)	8.009	U1-snRNP	3	YKHADGKKI	18.186	37.69
					YCGIAPYIR	13.599	28.19
					FVARVNYDT	12.243	25.38
	9mer-HLA-B07	19.18	U1-snRNP	2	RPRRLGGGL	28.526	63.48
					APRDPIPYL	27.961	62.22
MCTD-OCP	HLA-DR1 (DRB1*0101)	8.009	alpha 1 type XVII collagen (BP180)	**30 (top ten sequences shown)**	**LTGMPGIRG**	**19,267**	**39.93**
					YRRAHSPAS	17,904	37.11
					FTASPASIA	16,536	34.27
					YAELSSRIL	15.97	33.1
					MRGLPGAVG	15,095	31.29
					YGAIQGPPG	14.75	30.57
					LQGMAPAAG	14.65	30.36
					IKGEPGAPG	14,646	30.36
					LKAEANGDL	14,629	30.32
					WKWLLGLLL	12,638	26.19
		8.009	β4 integrin	**29 (top ten sequences shown)**	WKELQVKLL	17,695	36.68
					IRALDSPRG	17,581	36.44
					YSSLVSCRT	16,281	33.75
					YQLLNGGEL	15,387	31.89
					YRQISGVHK	14,592	30.24
					LAALISVSL	13,672	28.34
					FTALSPDSL	13,477	27.93
					WARLLLAAL	12,948	26.84
					YTQYRTQDY	12.79	26.51
					WRPDSTHLL	12,782	26.49
	9mer-HLA-B07	19.18	alpha 1 type XVII collagen (BP180)	**9**	GPPGPVTTI	26,397	58.74
					GPPGPPGAM	23,355	51.97
					GPPGPPGPV	23,008	51.2
					GPPGVSGAL	22,519	50.11
					GPRGPPGPS	22,357	49.75
					GPPGPPGSI	21,477	47.79
					GPRGPPGVS	21,201	47.18
					GPRGPPGPP	20,932	46.58
					GPPGDSRLL	20,217	44.99
		19.18	β4 integrin	**5**	APRSAKPAL	27,886	62.05
					RPRRPNGDI	26,186	58.27
					SPRGLRTEV	24,271	54.01
					APGPNSTVL	21,118	46.99
					RPIGPMKKV	20,604	45.85
OCP-OCP	HLA-DQ7 (DQB1*0301)	11.701	alpha 1 type XVII collagen (BP180)	14	IRRSILPYG	19.68	43.09
					FDYSELASH	19.381	42.44

				STDASHSRG	16.697	36.56
				ILDANLPSH	16.398	35.9
				AGPAGLPGH	16.234	35.55
				VWSSTLPAG	15.634	34.23
				SLGAGGAFG	13.849	30.32
				IRGPPGPSG	13.625	29.83
				SSQSVSGTY	13.531	29.63
				NTNAYSAGS	13.391	29.32
				YRRAHSPAS	12,928	28.31
				LSSYLHTAG	12,403	27.16
				DIHSYGSSG	12,396	27.14
				APGPAGPAG	11,938	26.14
			β4 integrin	LPPSGKPMG	20.182	44.19
				YRYTVKARN	19.166	41.97
				NYSAIHPGL	19.047	41.7
				APRSAKPAL	18.434	40.36
				YCACCKACL	16.829	36.85
				DVPAGTATL	15.922	34.86
				IRRVLDGGK	14.888	32.6
				FRQQPNAGK	14.639	32.05
				IYQVQLRAL	14.228	31.15
				YSDDVLRSP	13.589	29.75
				RAQSQEGWG	13,035	28.54
				SCVQCQAWG	12,643	27.68
				ADQDARGMV	12,108	26.51
				VIRRVLDGG	11,829	25.9
HLA-DR4 (DRB1*0401)	4853		alpha 1 type XVII collagen (BP180)	YHNNMTTQS	18,832	42.71
				YRRAHSPAS	17.57	39.85
				YAGNGGLLG	16,995	38.55
				YGAIQGPPG	16,763	38.02
				HVWSSTLPA	16,655	37.78
				FRGIVGPPG	15,877	36.01
				YAKTASLGG	14,274	32.37
				WWKWLLGLL	14,086	31.95
				YAAENSDSF	13,332	30.24
				LRGEVGLPG	13,088	29.68
			β4 integrin	YRTQDGTAQ	19.344	43.87
				YWIQGDSES	19.108	43.34
				FWWLIPLLL	14.965	33.94
				WWLIPLLLL	14.619	33.16
				KVQARTTEG	13.835	31.38
				LHRMTTTSA	13.518	30.66
				YMLRENLMA	13.312	30.19
				GWSGQTCNC	12.611	28.6
				WRPDSTHLL	12.471	28.29
				RRSQMSPQG	12.112	27.47

(*Continued*)

Table S9B. (*Continued*)

Disease	MHC	Threshold	Autoantigen	Total predicted binders	Top binders	Score	% optimal
OCP-MCTD	HLA-DQ7 (DQB1*0301)	11.701	U1-snRNP	3	KMFQKTRTG	11.537	26.17
					SKRSGKPRG	15,071	33.0
					WRPRRLGGG	12.75	27.92
	HLA-DR4 (DRB1*0401)	4,853	U1-snRNP	6	DRDREHKRG	11.87	25.99
					YIREFEDPR	12,955	29.38
					GYLMEAAPE	9,074	20.58
					GGLGGTRRG	7,653	17.36
					TVKGWRPRR	7,229	16.4
					WDPHNDPNA	6,835	15.5
					YMESEGGDG	6,599	14.97

Table S10A. Rankpep analysis of MMP- and MCTD-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease 1	MHC	Performance	Disease 2	Protein	Total sequences	Total predicted binders	Threshold	High binding score	Low binding score	% optimal high	% optimal low
MMP	HLA-DQ7 (DQB1*0301)	na	MMP	β4 integrin	1814	14	11.701	20.182	11.829	44.19	25.9
	HLA-DQ7 (DQB1*0301)	na	MCTD	U1-snRNP	429	3	11.701	15.071	11.87	33.00	25.99
MCTD	HLA-DR1 (DRB1*0101)	0.75 ± 0.04	MCTD	U1-snRNP	429	3	8.009	18.186	12.243	37.69	25.38
	9mer-HLA-B07	na		U1-snRNP	429	2	19.18	28.526	27.961	63.48	62.22
	HLA-DR1 (DRB1*0101)	0.75 ± 0.04	MMP	β4 integrin	1814	**29**	8.009	17.695	8.039	36.68	16.66
	HLA-DR1 (DRB1*0101)	0.75 ± 0.04		alpha 1 type XVII collagen (BP180)	1489	**30**	8.009	**19,267**	8,273	39.93	17.15
	9mer-HLA-B07	na		β4 integrin	1814	**5**	19.18	27.886	20.604	62.05	45.85
	9mer-HLA-B07	na		alpha 1 type XVII collagen (BP180)	1489	**9**	19.18	26,397	20,217	58.74	44.99

Table S10B. Peptide sequences of MMP- and MCTD-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease	MHC	Threshold	Autoantigen	Total predicted binders	Top binders	Score	% optimal
MCTD-MCTD	HLA-DR1 (DRB1*0101)	8.009	U1-snRNP	3	YKHADGKKI	18.186	37.69
					YCGIAPYIR	13.599	28.19
					FVARVNYDT	12.243	25.38
	9mer-HLA-B07	19.18	U1-snRNP	2	RPRRLGGGL	28.526	63.48
					APRDPIPYL	27.961	62.22
MCTD-MMP	HLA-DR1 (DRB1*0101)	8.009	β4 integrin	**29 (top ten sequences shown)**	WKELQVKLL	17,695	36.68
					IRALDSPRG	17,581	36.44
					YSSLVSCRT	16,281	33.75
					YQLLNGGEL	15,387	31.89
					YRQISGVHK	14,592	30.24
					LAALISVSL	13,672	28.34
					FTALSPDSL	13,477	27.93
					WARLLLAAL	12,948	26.84
					YTQYRTQDY	12.79	26.51
					WRPDSTHLL	12,782	26.49
		8.009	alpha 1 type XVII collagen (BP180)	**30 (top ten sequences shown)**	**LTGMPGIRG**	**19,267**	**39.93**
					YRRAHSPAS	17,904	37.11
					FTASPASIA	16,536	34.27
					YAELSSRIL	15.97	33.1
					MRGLPGAVG	15,095	31.29
					YGAIQGPPG	14.75	30.57
					LQGMAPAAG	14.65	30.36
					IKGEPGAPG	14,646	30.36
					LKAEANGDL	14,629	30.32
					WKWLLGLLL	12,638	26.19
	9mer-HLA-B07	19.18	β4 integrin	**5**	APRSAKPAL	27,886	62.05
					RPRRPNGDI	26,186	58.27
					SPRGLRTEV	24,271	54.01
					APGPNSTVL	21,118	46.99
					RPIGPMKKV	20,604	45.85
		19.18	alpha 1 type XVII collagen (BP180)	**9**	GPPGPVTTI	26,397	58.74
					GPPGPPGAM	23,355	51.97
					GPPGPPGPV	23,008	51.2
					GPPGVSGAL	22,519	50.11
					GPRGPPGPS	22,357	49.75
					GPPGPPGSI	21,477	47.79
					GPRGPPGVS	21,201	47.18
					GPRGPPGPP	20,932	46.58
					GPPGDSRLL	20,217	44.99
MMP-MMP	HLA-DQ7 (DQB1*0301)	31.451	β4 integrin	14	LPPSGKPMG	20.182	44.19
					YRYTVKARN	19.166	41.97
					NYSAIHPGL	19.047	41.7

				APRSAKPAL	18.434	40.36
				YCACCKACL	16.829	36.85
				DVPAGTATL	15.922	34.86
				IRRVLDGGK	14.888	32.6
				FRQQPNAGK	14.639	32.05
				IYQVQLRAL	14.228	31.15
				YSDDVLRSP	13.589	29.75
				RAQSQEGWG	13,035	28.54
				SCVQCQAWG	12,643	27.68
				ADQDARGMV	12,108	26.51
				VIRRVLDGG	11,829	25.9
	HLA-DQ7 (DQB1*0301)	31.451	alpha 1 type XVII collagen (BP180)	IRRSILPYG	19.68	43.09
				FDYSELASH	19.381	42.44
				STDASHSRG	16.697	36.56
				ILDANLPSH	16.398	35.9
				AGPAGLPGH	16.234	35.55
				VWSSTLPAG	15.634	34.23
				SLGAGGAFG	13.849	30.32
				IRGPPGPSG	13.625	29.83
				SSQSVSGTY	13.531	29.63
				NTNAYSAGS	13.391	29.32
				YRRAHSPAS	12,928	28.31
				LSSYLHTAG	12,403	27.16
				DIHSYGSSG	12,396	27.14
				APGPAGPAG	11,938	26.14
MMP-MCTD	HLA-DQ7 (DQB1*0301)	31.451	U1-snRNP	SKRSGKPRG	15,071	33.0
				WRPRRLGGG	12.75	27.92
				DRDREHKRG	11.87	25.99

Table S11A. Rankpep analysis of BP- and DH-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease 1	MHC	Performance	Disease 2	Protein	Total sequences
BP	HLA-DQ7 (DQB1*0301)	na	BP	alpha 1 type XVII collagen (BP180)	1489
	HLA-DQ7 (DQB1*0301)	na		dystonin (BP230)	2641
	HLA-DQ7 (DQB1*0301)	na	DH	transglutaminase 3	685
DH	HLA-DQ2 (DQA1*0501/DQB1*0201)	0.88 ± 0.06	DH	transglutaminase 3	685
	HLA-DQ8 (DQA1*0301/DQB1*0302)	0.72 ± 0.06		transglutaminase 3	685
	HLA-DQ2 (DQA1*0501/DQB1*0201)	0.88 ± 0.06	BP	alpha 1 type XVII collagen (BP180)	1489
	HLA-DQ2 (DQA1*0501/DQB1*0201)	0.88 ± 0.06		dystonin (BP230)	2641
	HLA-DQ8 (DQA1*0301/DQB1*0302)	0.72 ± 0.06		alpha 1 type XVII collagen (BP180)	1489
	HLA-DQ8 (DQA1*0301/DQB1*0302)	0.72 ± 0.06		dystonin (BP230)	2641

Total predicted binders	Threshold	High binding score	Low binding score	% optimal high	% optimal low
14	11.701	19.68	11.938	43.09	26.14
12	11.701	16,983	12,013	37.19	26.3
6	11.701	**20,579**	11.92	45.06	26.1
0	31.451	–	–	–	–
3	12.635	15.737	14.644	30.63	28.5
1	31.451	32.97	–	45.89	–
0	31.451	–	–	–	–
8	12.635	**18.587**	13.11	36.18	25.52
12	12.635	**24,137**	13,043	46.98	25.39

Table S11B. Peptide sequences of BP- and MCTD-derived autoantigens predicted to bind their HLA-associated alleles in a one- and two-disease model.

Disease	MHC	Threshold	Autoantigen	Total predicted binders	Top binders	Score	% optimal
BP-BP	HLA-DQ7 (DQB1*0301)	11.701	alpha 1 type XVII collagen (BP180)	14	IRRSILPYG	19.68	43.09
					FDYSELASH	19.381	42.44
					STDASHSRG	16.697	36.56
					ILDANLPSH	16.398	35.9
					AGPAGLPGH	16.234	35.55
					VWSSTLPAG	15.634	34.23
					SLGAGGAFG	13.849	30.32
					IRGPPGPSG	13.625	29.83
					SSQSVSGTY	13.531	29.63
					NTNAYSAGS	13.391	29.32
					YRRAHSPAS	12,928	28.31
					LSSYLHTAG	12,403	27.16
					DIHSYGSSG	12,396	27.14
					APGPAGPAG	11,938	26.14
			dystonin (BP230)	12	FESYGHSSH	16,983	37.19
					NFDGDHACS	15.913	34.84
					YRDTYHPLD	14,818	32.45
					LTPSVTPAY	14,805	32.42
					IEPQVHSRL	14,386	31.5
					FAQTLHPSL	14,356	31.43
					ITQSLNSGF	12,997	28.46
					LLQRQKATV	12,573	27.53
					LRHTVTARQ	12,495	27.36
					ADFDFHTGL	12,406	27.16
					ISPTGNEAM	12.06	26.41
					IIDVLIATK	12,013	26.3
BP-MCTD	HLA-DQ7 (DQB1*0301)	11.701	transglutaminase 3	6	GSDSVWNFH	20,579	45.06
					DDNGVLAGN	17,095	37.43
					YVGRVLSAM	14,523	31.8
					DPRSWNGSV	13.59	29.76
					AEHPIKISY	13,343	29.22
					ITAVCKVPD	11.92	26.1
DH-DH	HLA-DQ2 (DQA1*0501/ DQB1*0201)	31.451	transglutaminase 3	0			
	HLA-DQ8 (DQA1*0301/ DQB1*0302)	12.635	transglutaminase 3	3	DFSCNKFPA	15.737	30.63
					ALRSLGIPS	14.828	28.86
					SATMSLDPE	14.644	28.5
DH-MCTD	HLA-DQ2 (DQA1*0501/ DQB1*0201)	31.451	alpha 1 type XVII collagen (BP180)	1	RGREGPMGP	32.97	45.89
		31.451	dystonin (BP230)	0			
	HLA-DQ8 (DQA1*0301/ DQB1*0302)	12.635	alpha 1 type XVII collagen (BP180)	**8**	**PGRPGIKGE**	**18,587**	**36.18**
					PAGPAGLPG	**17,805**	**34.66**

			NADSGLKAE	**16,228**	**31.59**
			DRGPAGPPG	15,018	29.23
			WGPAPAWCP	14,162	27.56
			PKGDRGFPG	13,865	26.99
			DRLQGMAPA	13,846	26.95
			GAKGAMGPA	13.11	25.52
12.635	dystonin (BP230)	12	ITRAHAVAE	24,137	46.98
			IKRCKETSE	20,169	39.26
			PAYTPGFPS	18,052	35.14
			VSWHYLINE	15,507	30.18
			VQRVAKLRD	15,481	30.13
			AYRAAMQTQ	15,471	30.11
			DEIMALRNE	15,444	30.06
			VRGIRVPPE	15,033	29.26
			IVREKEAAE	14,276	27.79
			VLKGVVDPE	13,926	27.11