Journal of Human Genetics (2015) 60, 697–702

www.nature.com/jhg

REVIEW

Associations of human leukocyte antigens with autoimmune diseases: challenges in identifying the mechanism

Hiroko Miyadera[1,2] and Katsushi Tokunaga[2]

The mechanism of genetic associations between human leukocyte antigen (*HLA*) and susceptibility to autoimmune disorders has remained elusive for most of the diseases, including rheumatoid arthritis (RA) and type 1 diabetes (T1D), for which both the genetic associations and pathogenic mechanisms have been extensively analyzed. In this review, we summarize what are currently known about the mechanisms of *HLA* associations with RA and T1D, and elucidate the potential mechanistic basis of the *HLA*–autoimmunity associations. In RA, the established association between the shared epitope (SE) and RA risk has been explained, at least in part, by the involvement of SE in the presentation of citrullinated peptides, as confirmed by the structural analysis of DR4-citrullinated peptide complex. Self-peptide(s) that might explain the predispositions of variants at 11β and 13β in *DRB1* to RA risk have not currently been identified. Regarding the mechanism of T1D, pancreatic self-peptides that are presented weakly on the susceptible *HLA* allele products are recognized by self-reactive T cells. Other studies have revealed that DQ proteins encoded by the T1D susceptible *DQ* haplotypes are intrinsically unstable. These findings indicate that the T1D susceptible *DQ* haplotypes might confer risk for T1D by facilitating the formation of unstable HLA–self-peptide complex. The studies of RA and T1D reveal the two distinct mechanistic basis that might operate in the *HLA*–autoimmunity associations. Combination of these mechanisms, together with other functional variations among the *DR* and *DQ* alleles, may generate the complex patterns of *DR*–*DQ* haplotype associations with autoimmunity.
Journal of Human Genetics (2015) **60,** 697–702; doi:10.1038/jhg.2015.100; published online 20 August 2015

INTRODUCTION

Human leukocyte antigen (HLA) class II molecules are heterodimeric transmembrane glycoproteins that present self- and non-self-peptides to the surface of antigen-presenting cells for recognition by T cell receptors. HLA class II consists of three isotypes, including *HLA-DR* (encoded by *HLA-DRA* and *-DRB1*, and *-DRB3*, *4*, and *5* in certain haplotypes), *HLA-DQ* (encoded by *HLA-DQA1* and *-DQB1*) and *HLA-DP* (encoded by *HLA-DPA1* and *-DPB1*). With the exception of *DRA*, each locus has a large number of alleles. For example, *DRB1* has >1 700 alleles, *DQB1* has >700 alleles and *DPB1* has >500 alleles that have been registered to date according to the IMGT/HLA database.[1] Polymorphic variants of *HLA* class II are accumulated mainly in exon 2, which encodes the α1 or β1 domain of each subunit that forms the peptide-binding groove.

Genetic associations between *HLA* and autoimmune diseases were reported in the early 1970s (McDevitt and Bodmer[2] and references therein). Up until now, it has been confirmed that *HLA* have the strongest association signals, compared with any other loci, to a variety of autoimmune and inflammatory disorders. However, the mechanism that might underlie the *HLA*–autoimmunity associations has remained elusive for most of the autoimmune diseases, including rheumatoid arthritis (RA) and type 1 diabetes (T1D), to which the hierarchies of *HLA-DR* and *-DQ* alleles or haplotypes that are associated with susceptibility or protection have been established in Europeans and other ethnicities. The pathogenic mechanisms of RA and T1D have also been extensively analyzed in human and the murine models. In this review, we summarize what are currently known and what are remained elusive about the mechanisms of *HLA* associations with RA and T1D, and elucidate the potential mechanistic basis of the *HLA*–autoimmunity associations.

ASSOCIATIONS BETWEEN *HLA* CLASS II AND RA

RA is a chronic inflammatory disease of the synovial joints. Strong associations of *DRB1*04:01*, **04:04*, **01:01* and **10:01*, which carry a shared epitope (SE)[3] at amino acid positions 70β to 74β (Figure 1) (QKRAA at *DRB1*04:01*, QRRAA at *DRB1*01:01* and **04:04*, and RRRAA at *DRB1*10:01*), with RA risk have been reported and confirmed in numerous studies. In Europeans, the association between *DRB1* and RA is stronger in anti-citrullinated peptide antibody-positive RA than in anti-citrullinated peptide antibody-negative RA.[4,5] Among

[1]Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Chiba, Japan and [2]Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
Correspondence: Dr H Miyadera, Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, 1-7-1 Kohnodai, Ichikawa, Chiba 272-8516, Japan.
E-mail: miyadera-h@umin.net
Received 11 May 2015; revised 22 July 2015; accepted 23 July 2015; published online 20 August 2015

the SE alleles, *DRB1*04:01* and **04:04* confer a stronger predisposition to RA than *DRB1*01:01* and **10:01*.[4,6] *DRB1*04:01* homozygosity and *DRB1*04:01/*04:04* heterozygosity are associated with increased risk for RA.[4] The RA protective *DRB1* alleles include *DRB1*13:01*[6,7] and the alleles that carry Ile67β or Asp70β.[4] In East Asian populations, *DRB1*04:01* and **04:05* confer risk, whereas *DRB1*13:02* and **14:05* confer protection[8–10] (see Furukawa *et al.*[11] in this issue for details of *HLA* association with RA).

The associations between *HLA* and RA have been analyzed mainly for the *DR* loci. However, the strong linkage disequilibrium between *DR* and *DQ* suggests that both *DR* and *DQ* may contribute to predisposition to RA, similar to what has been observed in T1D. In Europeans, RA susceptible *DRB1* alleles are found mainly in *DRB1*04:01-DQA1*03-DQB1*03:01*, *DRB1*04:01-DQA1*03-DQB1*03:02*, *DRB1*04:04-DQA1*03-DQB1*03:02*, *DRB1*01:01-DQA1*01-DQB1*05:01* and *DRB1*10:01-DQA1*01-DQB1*05:01* haplotypes. In East Asian populations, RA susceptible *DR–DQ* haplotypes include *DRB1*04:01-DQA1*03-DQB1*03:01* and *DRB1*04:05-DQA1*03-DQB1*04:01*. *DQA1*03-DQB1*03* and *DQA1*03-DQB1*04:01* may confer susceptibility, and *DQA1*01-DQB1*05* may confer mildly predisposing effect.[12,13]

POTENTIAL MECHANISM OF RA SUSCEPTIBILITY AND PROTECTION

It has been thought that *DRB1* alleles that carry SE confer disease susceptibility through the selective presentation of self-peptides[14] or the mechanism that involves alteration in the peripheral T cell repertoire (see, for example, Auger *et al.*[15] and Roudier[16]). It was later found that DRB1*04:01 protein interacted with citrullinated peptides with higher affinity than with non-citrullinated peptides.[17] A structural study confirmed that DRB1*04:01 and *04:04 proteins presented citrullinated vimentin and aggrecan peptides via interactions between Lys71β or Arg71β and citrulline at the P4-binding pocket.[18] These findings indicate that the SE alleles exert pathogenic effects through the presentation of citrullinated peptides, which are recognized as non-self by T cells. The critical roles for Lys71β and Arg71β in the presentation of citrullinated peptides are consistent with the findings that *DRB1* alleles that carry Glu71β, such as *DRB1*04:02*, **13:01* and **13:02*, are not associated with RA risk. In addition to SE, variants at amino acids 11β and 13β in *DRB1* (Figure 1) also predispose strongly to RA.[6,19] Although these two residues could affect binding preferences for peptides, self-peptide(s) that might explain the predispositions of 11β and 13β to RA have not currently been identified.

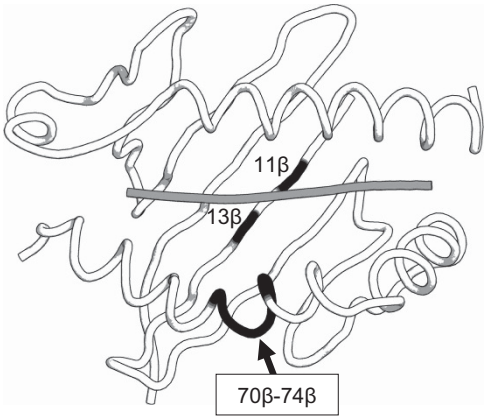

Figure 1 Locations of amino acid residues in *DRB1* product that are associated with rheumatoid arthritis (RA). Locations of 11β, 13β and shared epitope (SE) residues in the structure of DR protein (PDB: 2seb)[75] are shown.

The mechanism of protective associations between *DR–DQ* haplotypes and RA are not well characterized. A recent study revealed that the citrullinated DERAA motif, which was found in the protective *DRB1* allele products, including DRB1*13 protein, and in vinculin, can be presented by the DQ proteins that were encoded on the RA susceptible *DR–DQ* haplotypes.[20] The protective effect of *DRB1*13* against RA was explained by the cross-reactivity of self-reactive T cells to the citrullinated DERAA motif in vinculin and DRB1*13 protein, and the absence of these self-reactive T cells in the *DR4/DR13* heterozygotes.[20]

Self-peptides that are derived from other potential self-antigens, including type II collagen and nuclear ribonucleoprotein A2 can also be presented to the susceptible *DR* or *DQ* allele products and are involved in the pathogenesis of RA.[21–25] The binding affinity of DR4 with class II-associated invariant chain peptide (CLIP) may also affect RA risk.[26] The presentation of misfolded immunoglobulin G heavy chain by DR protein is also shown to contribute to the susceptibility to RA.[27]

To summarize, studies of RA revealed that the association patterns of the most predisposing and the most protective *DRB1* alleles can be explained, at least in part, by variants at 71β that mediate the presentation of citrullinated peptides.[18] The proposed mechanism of protection against RA also involves the allele-specific presentation of self-peptides.[20] Therefore, the selective presentation of self-peptides on the susceptible and protective *HLA* allele products might be one of the key mechanisms of *DR–DQ* haplotype associations with RA (Figure 2a).

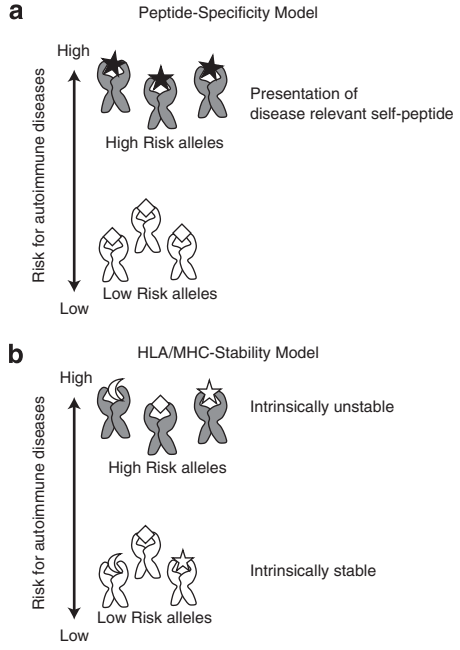

Figure 2 Hypothetical mechanisms for human leukocyte antigen (HLA)–autoimmunity associations.[59] (**a**) *HLA* associations with autoimmune diseases may be explained by the selective presentation of disease-relevant self-peptides by the disease susceptible *HLA* allele products (grey). The disease-relevant peptides (black) and irrelevant peptides (white) are shown. (**b**) The HLA/major histocompatibility complex (MHC) stability model. This model proposes that intrinsically unstable HLA proteins (grey), which form unstable HLA-peptide complex through the presentation of diverse self-peptides, confer a risk for autoimmune diseases.

ASSOCIATIONS BETWEEN *HLA* CLASS II AND T1D

T1D is caused by the autoimmune-mediated destruction of insulin-producing beta cells in the pancreas. *DR* and *DQ* are the strongest susceptibility loci for T1D. In European descendants, the highest risk is conferred by *DR3-DQA1*05-DQB1*02* and *DR4-DQA1*03-DQB1*03:02* haplotypes, and the highest protection is conferred by *DR15-DQA1*01:02-DQB1*06:02.*[28–30] Heterozygosity of *DR3-DQA1*05-DQB1*02/DR4-DQA1*03-DQB1*03:02* confers the strongest genotypic risk for T1D.[28–30] These susceptible haplotypes are partly shared in a variety of other ethnic groups.[29] In the Japanese population, in which these susceptible haplotypes are infrequent, susceptibility to T1D is conferred by *DR9-DQA1* 03-DQB1*03:03* and *DR4-DQA1*03-DQB1*04:01* haplotypes.[31] The strong association between non-Asp57β in *DQB1* and T1D risk is found in Europeans[32,33] but not in the Japanese population, in which the T1D susceptible *DQB1* alleles carry Asp57β. *DR15-DQA1*01:02-DQB1*06:02* haplotype confers protection against T1D in both Europeans and Japanese populations.[29,31]

It has been established that both *DR* and *DQ* loci confer a predisposing and/or protective effect to T1D in a manner independent of or dependent on the allele at the other locus. One of the examples is *DR4-DQA1*03-DQB1*03:02* haplotype, which can be subgrouped into the susceptible haplotypes that contain *DRB1*04:01* or **04:05,* or the neutral-to-protective haplotypes that contain *DRB1*04:03* or **04:06.*[28–31] Another example is the haplotype that contains *DRB1*07*, the association of which can vary from susceptible to protective, depending on *DQA1* and *DQB1* alleles.[28,30,34]

The major autoantigens involved in the pathogenesis of T1D are insulin, glutamic acid decarboxylase, zinc transporter 8 and islet antigen-2.[35–37] The HLA-peptide binding studies revealed that the self-peptides derived from these autoantigens bound promiscuously to the susceptible, neutral and protective *DR* and *DQ* allele products,[38–40] which may reflect the facts that the peptide-binding spectrum of *DR* and *DQ* allele products partially overlaps across alleles.[41,42] It has remained unknown whether the *HLA* associations with T1D can be explained by the selective presentation of certain pancreatic self-peptides on the susceptible *HLA* allele products. Potential contributions of other functional variations among the alleles, such as the promoter activity[43] and the dependency of HLA proteins to invariant chain and HLA-DM,[44–46] to T1D risk have also been reported.

The association between non-Asp57β in *DQB1* and T1D in Europeans, and the presence of non-Asp57β in *I-A*^{g7} of the non-obese diabetogenic (NOD) mice suggested that non-Asp57β had a critical role in the pathogenesis of T1D. Studies of the structure of *DQA1*03-DQB1*03:02* and *I-A*^{g7} products revealed that non-Asp57β in *DQB1* and *I-A*^{g7} facilitated the accommodation of acidic residue at the P9-binding pocket, thereby allowing for the presentation of certain pancreatic self-peptides, such as insulin $B_{9\text{-}23}$, which carried acidic residue at the p9.[47–49] It was later found in both human and the NOD mice that insulin and other pancreatic self-peptides that were presented weakly on the susceptible HLA/major histocompatibility complex allele products were the targets of self-reactive T cells.[50–56] These studies established a notion that the T1D pathogenesis was mediated through the formation of unstable HLA/major histocompatibility complex-peptide complex.[57] The presence of non-Asp57β does not appear to be a prerequisite for the accommodation of self-peptides in a weak binding register. Non-Asp57β is present in the neutral haplotype *DQA1*02-DQB1*02* in Europeans and is absent in the T1D susceptible *DQB1* alleles in the Japanese population. These findings suggest that non-Asp57β in *DQB1* may not be an essential component in the shared mechanism of T1D across ethnicities.

ASSOCIATIONS BETWEEN DQ PROTEIN INSTABILITY AND T1D

One of the additional factors that might contribute to the associations between *DR–DQ* haplotypes and T1D is the protein instability of DQ. It was reported in the 1990s that *DQA1*05-DQB1*02* and *DQA1* 03-DQB1*03:02* haplotypes that predisposed to T1D in Europeans generated SDS unstable DQ protein, whereas the protective haplotype *DQA1*01:02-DQB1*06:02* generated SDS stable DQ protein.[58] However, SDS stability can be affected by both the stability of HLA protein and affinity of the bound peptides. We therefore validated, through a cell-surface HLA expression assay, whether the stability of DQ protein might differ intrinsically among the allele products. We confirmed a steep allelic hierarchy in the stability of *DQ* haplotype products.[59] Consistent with previous study,[58] the T1D susceptible *DQ* haplotypes in Europeans generated intrinsically unstable DQ proteins. Unstable DQ proteins were also generated by *DQA1*05* and *DQB1*03:02* allele products that can be formed in *DQA1*05-DQB1* 02/DQA1*03-DQB1*03:02* heterozygotes. *DQA1*03-DQB1*03:03* and *DQA1*03-DQB1*04:01*, which were associated with T1D risk in the Japanese population, also generated unstable DQ proteins. The protective haplotype *DQA1*01:02-DQB1*06:02* generated highly stable protein (Figure 3).[59] When all of the major *DQ* haplotypes were analyzed, the protein stability of DQ was associated inversely with T1D risk, indicating that the protein instability of DQ might contribute to T1D risk irrespective of ethnicity.[59]

One of the polymorphic variants that regulated the protein stability of DQ was 57β; Asp57β stabilized DQ protein through the interactions with peptide and/or Arg79α. The variants at 47α in *DQA1*, which were located outside of the peptide-binding groove (Figure 4), also regulated the intrinsic stability of DQ protein. Associations between the destabilizing variants at these sites, such as non-Asp57β in *DQB1*

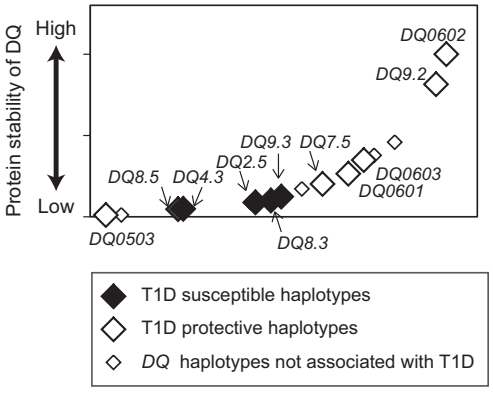

Figure 3 Allelic hierarchy of DQ protein stability. Protein stability of DQ proteins that are encoded by major *DQ* haplotypes in European and Japanese populations are displayed in order of increasing protein stability (*x* axis) with the estimated DQ protein stability plotted on the *y* axis.[59] The type 1 diabetes (T1D) susceptible haplotypes (black, large diamond), T1D protective haplotypes (white, large diamond) and haplotypes not associated with T1D (white, small diamond) are identified in the Swedish[76] and Japanese populations.[31] The following abbreviations are used for each *DQ* haplotype; *DQA1*01:02-DQB1*06:02* (*DQ0602*), *DQA1*02-DQB1* 03:03* (*DQ9.2*), *DQA1*01:03-DQB1*06:03* (*DQ0603*), *DQA1*01:03-DQB1* 06:01* (*DQ0601*), *DQA1*05-DQB1*03:01* (*DQ7.5*), *DQA1*03-DQB1*03:03* (*DQ9.3*), *DQA1*03-DQB1*03:02* (*DQ8.3*), *DQA1*05-DQB1*02* (*DQ2.5*), *DQA1*03-DQB1*04:01* (*DQ4.3*), *DQA1*05/DQB1*03:02* (*trans* combination) (*DQ8.5*) and *DQA1*01-DQB1*05:03* (*DQ0503*).

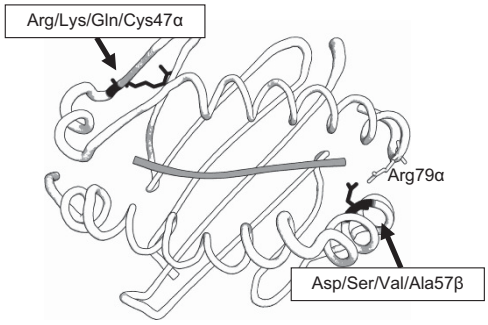

Figure 4 Locations of amino acid residues in *DQA1* and *DQB1* products that are associated with type 1 diabetes (T1D). Locations of 47α and 57β in the structure of DQ protein (PDB: 1uvq)[77] are shown.

and Gln47α in *DQA1*, and T1D risk[59] indicated that non-Asp57β in *DQB1* may confer T1D risk through destabilizing the DQ proteins.

Based on the inverse association between the DQ protein stability and T1D risk, and the strong association signals detected at the protein destabilizing variants at 57β and 47α, we proposed that the intrinsic instability of HLA protein may be one of the important functional components that conferred T1D risk (Figure 2b).[59] This hypothesis is consistent with the established concept of T1D pathogenesis, and indicates that an intrinsically unstable HLA may increase a risk for T1D through facilitating the formation of unstable HLA–self-peptide complex, which may permit the thymic escape of self-reactive T cells.

The mechanisms of protection against T1D of *DQA1*01:02-DQB1*06:02* and *DQA1*02-DQB1*03:03* haplotypes are not known. As the mechanisms of protection, the 'affinity model' and the 'determinant capture model' have been proposed.[60–62]

MECHANISMS OF *DR–DQ* HAPLOTYPE ASSOCIATIONS WITH AUTOIMMUNE DISEASES

Mechanism of *HLA*-associated autoimmune diseases has generally been studied through the identification of self-peptides that are presented selectively to the susceptible *HLA* allele products. Similar to the findings in RA, the association between *DRB1*04:06-DQA1*03-DQB1*03:02* haplotype and insulin autoimmune syndrome[63] has also been explained by the presentation of the disease-relevant self-peptide, the reduced form of insulin, to DRB1*04:06 protein, but not to *DQB1*03:02* and the non-risk allele *DRB1*04:05* products.[64]

Regarding the mechanism of T1D, pancreatic self-peptides that might explain the association of *HLA* have not currently been identified. Studies of multiple sclerosis also documented the promiscuous binding patterns and variable affinity levels in the interactions of self-peptides with the susceptible and non-susceptible *HLA* allele products.[65–67] These findings may indicate a possibility that a variety of low-affinity self-peptides, most of which have not been identified readily in the HLA-peptide binding studies, are implicated in the pathogenesis of certain autoimmune diseases, including T1D.

Genetic association studies of other autoimmune and inflammatory diseases have revealed that the T1D susceptible haplotypes *DR3-DQA1*05-DQB1*02* and *DR4-DQA1*03-DQB1*03:02* in Europeans and *DR9-DQA1*03-DQB1*03:03* and *DR4-DQA1*03-DQB1*04:01* in the Japanese population predispose to multiple autoimmune diseases, including autoimmune polyglandular syndrome type II,[68] celiac disease[69,70] and antineutrophil cytoplasmic antibody-associated vasculitis.[71] The T1D protective haplotype *DR15-DQA1*01:02-DQB1*06:02* confers protection against autoimmune polyglandular syndrome type II and III[68,72] and selective immunoglobulin A deficiency.[73,74] The associations of T1D susceptible/protective *DR–DQ* haplotypes with a variety of autoimmune diseases indicate that the mechanism of autoimmune susceptibility and/or protection may partly be shared among certain autoimmune diseases. As distinct sets of self-peptides are involved in individual diseases, it would not be possible to explain the shared associations by the peptide-binding spectrum of DR and DQ proteins. The HLA/major histocompatibility complex stability model or other molecular mechanism(s), which could operate irrespective of the presented self-peptides, may underlie these associations.

CONCLUDING REMARKS

The studies of RA and T1D suggest that the two distinct mechanistic basis, which might involve allelic variations in the peptide-binding preferences and the protein stability of HLA, might explain the associations of *HLA* with autoimmune diseases. These two mechanisms may constitute a part of the whole mechanism of *HLA*–autoimmunity associations, which can also be affected by allelic variations in the expression levels and the dependency to accessory molecules. In addition to the functional diversity at each locus, their interplay between the loci may also contribute to the complex pattern of *DR–DQ* haplotype associations with autoimmunity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was funded by JSPS KAKENHI grant numbers 22133008 (K Tokunaga) and 22133006 (H Miyadera).

1 Robinson, J., Halliwell, J. A., Hayhurst, J. D., Flicek, P., Parham, P. & Marsh, Steven, G. E. The IPD and IMGT/HLA database: allele variant databases. *Nucleic Acids Res.* **43**, D423–D431 (2015).
2 McDevitt, H. O. & Bodmer, W. F. HL-A, immune-response genes, and disease. *Lancet* **1**, 1269–1275 (1974).
3 Gregersen, P. K., Silver, J. & Winchester, R. J. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum.* **30**, 1205–1213 (1987).
4 Mackie, S. L., Taylor, J. C., Martin, S. G., Consortium, Y., Consortium, U., Wordsworth, P. *et al.* A spectrum of susceptibility to rheumatoid arthritis within HLA-DRB1: stratification by autoantibody status in a large UK population. *Genes Immun.* **13**, 120–128 (2012).
5 Morgan, A. W., Thomson, W., Martin, S. G.Yorkshire Early Arthritis Register, C., Carter, A. M., Consortium, U. K. R. A. G. *et al.* Reevaluation of the interaction between HLA-DRB1 shared epitope alleles, PTPN22, and smoking in determining susceptibility to autoantibody-positive and autoantibody-negative rheumatoid arthritis in a large UK Caucasian population. *Arthritis Rheum.* **60**, 2565–2576 (2009).
6 Raychaudhuri, S., Sandor, C., Stahl, E. A., Freudenberg, J., Lee, H. S., Jia, X. *et al.* Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis. *Nat. Genet.* **44**, 291–296 (2012).
7 van der Woude, D., Lie, B. A., Lundstrom, E., Balsa, A., Feitsma, A. L., Houwing-Duistermaat, J. J. *et al.* Protection against anti-citrullinated protein antibody-positive rheumatoid arthritis is predominantly associated with HLA-DRB1*1301: a meta-analysis of HLA-DRB1 associations with anti-citrullinated protein antibody-positive and anti-citrullinated protein antibody-negative rheumatoid arthritis in four European populations. *Arthritis Rheum.* **62**, 1236–1245 (2010).
8 Jun, K. R., Choi, S. E., Cha, C. H., Oh, H. B., Heo, Y. S., Ahn, H. Y. *et al.* Meta-analysis of the association between HLA-DRB1 allele and rheumatoid arthritis susceptibility in Asian populations. *J. Korean Med. Sci.* **22**, 973–980 (2007).
9 Lee, H. S., Lee, K. W., Song, G. G., Kim, H. A., Kim, S. Y. & Bae, S. C. Increased susceptibility to rheumatoid arthritis in Koreans heterozygous for HLA-DRB1*0405 and *0901. *Arthritis Rheum.* **50**, 3468–3475 (2004).
10 Mitsunaga, S., Suzuki, Y., Kuwana, M., Sato, S., Kaneko, Y., Homma, Y. *et al.* Associations between six classical HLA loci and rheumatoid arthritis: a comprehensive analysis. *Tissue Antigens* **80**, 16–25 (2012).

11 Furukawa, H., Oka, S., Shimada, K., Hashimoto, A. & Tohma, S. Human leukocyte antigen polymorphisms and personalized medicine for rheumatoid arthritis. *J. Hum. Genet.* **60**, 691–696 (2015).
12 Zanelli, E., Breedveld, F. C. & de Vries, R. R. HLA class II association with rheumatoid arthritis: facts and interpretations. *Hum. Immunol.* **61**, 1254–1261 (2000).
13 Seidl, C., Korbitzer, J., Badenhoop, K., Seifried, E., Hoelzer, D., Zanelli, E. *et al.* Protection against severe disease is conferred by DERAA-bearing HLA-DRB1 alleles among HLA-DQ3 and HLA-DQ5 positive rheumatoid arthritis patients. *Hum. Immunol.* **62**, 523–529 (2001).
14 Hammer, J., Gallazzi, F., Bono, E., Karr, R. W., Guenot, J., Valsasnini, P. *et al.* Peptide binding specificity of HLA-DR4 molecules: correlation with rheumatoid arthritis association. *J. Exp. Med.* **181**, 1847–1855 (1995).
15 Auger, I., Toussirot, E. & Roudier, J. Molecular mechanisms involved in the association of HLA-DR4 and rheumatoid arthritis. *Immunol. Res.* **16**, 121–126 (1997).
16 Roudier, J. Association of MHC and rheumatoid arthritis. Association of RA with HLA-DR4: the role of repertoire selection. *Arthritis Res.* **2**, 217–220 (2000).
17 Hill, J. A., Southwood, S., Sette, A., Jevnikar, A. M., Bell, D. A. & Cairns, E. Cutting edge: the conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis-associated HLA-DRB1*0401 MHC class II molecule. *J. Immunol.* **171**, 538–541 (2003).
18 Scally, S. W., Petersen, J., Law, S. C., Dudek, N. L., Nel, H. J., Loh, K. L. *et al.* A molecular basis for the association of the HLA-DRB1 locus, citrullination, and rheumatoid arthritis. *J. Exp. Med.* **210**, 2569–2582 (2013).
19 Okada, Y., Kim, K., Han, B., Pillai, N. E., Ong, R. T., Saw, W. Y. *et al.* Risk for ACPA-positive rheumatoid arthritis is driven by shared HLA amino acid polymorphisms in Asian and European populations. *Hum. Mol. Genet.* **23**, 6916–6926 (2014).
20 van Heemst, J., Jansen, D. T., Polydorides, S., Moustakas, A. K., Bax, M., Feitsma, A. L. *et al.* Crossreactivity to vinculin and microbes provides a molecular basis for HLA-based protection against rheumatoid arthritis. *Nat. Commun.* **6**, 6681 (2015).
21 Ohnishi, Y., Tsutsumi, A., Sakamaki, T. & Sumida, T. T cell epitopes of type II collagen in HLA-DRB1*0101 or DRB1*0405-positive Japanese patients with rheumatoid arthritis. *Int. J. Mol. Med.* **11**, 331–335 (2003).
22 Diab, B. Y., Lambert, N. C., L'Faqihi, F. E., Loubet-Lescoulie, P., de Preval, C. & Coppin, H. Human collagen II peptide 256-271 preferentially binds to HLA-DR molecules associated with susceptibility to rheumatoid arthritis. *Immunogenetics* **49**, 36–44 (1999).
23 Rosloniec, E. F., Whittington, K. B., Zaller, D. M. & Kang, A. H. HLA-DR1 (DRB1*0101) and DR4 (DRB1*0401) use the same anchor residues for binding an immunodominant peptide derived from human type II collagen. *J. Immunol.* **168**, 253–259 (2002).
24 Matsushita, S., Nishi, T., Oiso, M., Yamaoka, K., Yone, K., Kanai, T. *et al.* HLA-DQ-binding peptide motifs. 1. Comparative binding analysis of type II collagen-derived peptides to DR and DQ molecules of rheumatoid arthritis-susceptible and non-susceptible haplotypes. *Int. Immunol.* **8**, 757–764 (1996).
25 Trembleau, S., Hoffmann, M., Meyer, B., Nell, V., Radner, H., Zauner, W. *et al.* Immunodominant T-cell epitopes of hnRNP-A2 associated with disease activity in patients with rheumatoid arthritis. *Eur. J. Immunol.* **40**, 1795–1808 (2010).
26 Patil, N. S., Pashine, A., Belmares, M. P., Liu, W., Kaneshiro, B., Rabinowitz, J. *et al.* Rheumatoid arthritis (RA)-associated HLA-DR alleles form less stable complexes with class II-associated invariant chain peptide than non-RA-associated HLA-DR alleles. *J. Immunol.* **167**, 7157–7168 (2001).
27 Jin, H., Arase, N., Hirayasu, K., Kohyama, M., Suenaga, T., Saito, F. *et al.* Autoantibodies to IgG/HLA class II complexes are associated with rheumatoid arthritis susceptibility. *Proc. Natl Acad. Sci. USA* **111**, 3787–3792 (2014).
28 Koeleman, B. P. C., Lie, B. A., Undlien, D. E., Dudbridge, F., Thorsby, E., de Vries, R. R. P. *et al.* Genotype effects and epistasis in type 1 diabetes and HLA-DQ trans dimer associations with disease. *Genes Immun.* **5**, 381–388 (2004).
29 Thomson, G., Valdes, A. M., Noble, J. A., Kockum, I., Grote, M. N., Najman, J. *et al.* Relative predispositional effects of HLA class II DRB1-DQB1 haplotypes and genotypes on type 1 diabetes: a meta-analysis. *Tissue Antigens* **70**, 110–127 (2007).
30 Erlich, H., Valdes, A. M., Noble, J., Carlson, J. A., Varney, M., Concannon, P. *et al.* HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk: analysis of the type 1 diabetes genetics consortium families. *Diabetes* **57**, 1084–1092 (2008).
31 Kawabata, Y., Ikegami, H., Awata, T., Imagawa, A., Maruyama, T., Kawasaki, E. *et al.* Differential association of HLA with three subtypes of type 1 diabetes: fulminant, slowly progressive and acute-onset. *Diabetologia* **52**, 2513–2521 (2009).
32 Todd, J., Bell, J. & McDevitt, H. HLA-DQβ gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature* **329**, 599–604 (1987).
33 Morel, P. A., Dorman, J. S., Todd, J. A., McDevitt, H. O. & Trucco, M. Aspartic acid at position 57 of the HLA-DQ beta chain protects against type I diabetes: a family study. *Proc. Natl Acad. Sci. USA* **85**, 8111–8115 (1988).
34 Noble, J. A., Johnson, J., Lane, J. A. & Valdes, A. M. HLA class II genotyping of African American type 1 diabetic patients reveals associations unique to African haplotypes. *Diabetes* **62**, 3292–3299 (2013).
35 Baekkeskov, S., Landin, M., Kristensen, J. K., Srikanta, S., Bruining, G. J., Mandrup-Poulsen, T. *et al.* Antibodies to a 64,000 Mr human islet cell antigen precede the clinical onset of insulin-dependent diabetes. *J. Clin. Invest.* **79**, 926–934 (1987).
36 Payton, M. A., Hawkes, C. J. & Christie, M. R. Relationship of the 37,000- and 40,000-M(r) tryptic fragments of islet antigens in insulin-dependent diabetes to the protein tyrosine phosphatase-like molecule IA-2 (ICA512). *J. Clin. Invest.* **96**, 1506–1511 (1995).
37 Wenzlau, J. M., Juhl, K., Yu, L., Moua, O., Sarkar, S. A., Gottlieb, P. *et al.* The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. *Proc. Natl Acad. Sci. USA* **104**, 17040–17045 (2007).
38 Harfouch-Hammoud, E., Walk, T., Otto, H., Jung, G., Bach, J., van Endert, P. *et al.* Identification of peptides from autoantigens GAD65 and IA-2 that bind to HLA class II molecules predisposing to or protecting from type 1 diabetes. *Diabetes* **48**, 1937–1947 (1999).
39 Geluk, A., van Meijgaarden, K. E., Schloot, N. C., Drijfhout, J. W., Ottenhoff, T. H. & Roep, B. O. HLA-DR binding analysis of peptides from islet antigens in IDDM. *Diabetes* **47**, 1594–1601 (1998).
40 Ettinger, R. A. & Kwok, W. W. A peptide binding motif for HLA-DQA1*0102/DQB1*0602, the class II MHC molecule associated with dominant protection in insulin-dependent diabetes mellitus. *J. Immunol.* **160**, 2365–2373 (1998).
41 Sidney, J., del Guercio, M.-F., Southwood, S. & Sette, A. The HLA molecules DQA1*0501/B1*0201 and DQA1*0301/B1*0302 share an extensive overlap in peptide binding specificity. *J. Immunol.* **169**, 5098–5108 (2002).
42 Sidney, J., Steen, A., Moore, C., Ngo, S., Chung, J., Peters, B. *et al.* Divergent motifs but overlapping binding repertoires of six HLA-DQ molecules frequently expressed in the worldwide human population. *J. Immunol.* **185**, 4189–4198 (2010).
43 Britten, A. C., Mijovic, C. H., Barnett, A. H. & Kelly, M. A. Differential expression of HLA-DQ alleles in peripheral blood mononuclear cells: alleles associated with susceptibility to and protection from autoimmune type 1 diabetes. *Int. J. Immunogenet.* **36**, 47–57 (2009).
44 Busch, R., De Riva, A., Hadjinicolaou, A. V., Jiang, W., Hou, T. & Mellins, E. D. On the perils of poor editing: regulation of peptide loading by HLA-DQ and H2-A molecules associated with celiac disease and type 1 diabetes. *Expert Rev. Mol. Med.* **14**, e15 (2012).
45 Busch, R., Rinderknecht, C. H., Roh, S., Lee, A. W., Harding, J. J., Burster, T. *et al.* Achieving stability through editing and chaperoning: regulation of MHC class II peptide binding and expression. *Immunol. Rev.* **207**, 242–260 (2005).
46 Zhou, Z. & Jensen, P. E. Structural characteristics of HLA-DQ that may impact DM editing and susceptibility to Type-1 diabetes. *Front. Immunol.* **4**, 262 (2013).
47 Corper, A. L., Stratmann, T., Apostolopoulos, V., Scott, C. A., Garcia, K. C., Kang, A. S. *et al.* A structural framework for deciphering the link between I-A^{g7} and autoimmune diabetes. *Science* **288**, 505–511 (2000).
48 Latek, R. R., Suri, A., Petzold, S. J., Nelson, C. A., Kanagawa, O., Unanue, E. R. *et al.* Structural basis of peptide binding and presentation by the type I diabetes-associated MHC class II molecule of NOD mice. *Immunity* **12**, 699–710 (2000).
49 Lee, K. H., Wucherpfennig, K. W. & Wiley, D. C. Structure of a human insulin peptide-HLA-DQ8 complex and susceptibility to type 1 diabetes. *Nat. Immunol.* **2**, 501–507 (2001).
50 Levisetti, M. G., Suri, A., Petzold, S. J. & Unanue, E. R. The insulin-specific T cells of nonobese diabetic mice recognize a weak MHC-binding segment in more than one form. *J. Immunol.* **178**, 6051–6057 (2007).
51 Levisetti, M. G., Lewis, D. M., Suri, A. & Unanue, E. R. Weak proinsulin peptide-major histocompatibility complexes are targeted in autoimmune diabetes in mice. *Diabetes* **57**, 1852–1860 (2008).
52 Stadinski, B. D., Delong, T., Reisdorph, N., Reisdorph, R., Powell, R. L., Armstrong, M. *et al.* Chromogranin A is an autoantigen in type 1 diabetes. *Nat. Immunol.* **11**, 225–231 (2010).
53 Stadinski, B. D., Zhang, L., Crawford, F., Marrack, P., Eisenbarth, G. S. & Kappler, J. W. Diabetogenic T cells recognize insulin bound to IA^{g7} in an unexpected, weakly binding register. *Proc. Natl Acad. Sci. USA* **107**, 10978–10983 (2010).
54 Crawford, F., Stadinski, B., Jin, N., Michels, A., Nakayama, M., Pratt, P. *et al.* Specificity and detection of insulin-reactive CD4+ T cells in type 1 diabetes in the nonobese diabetic (NOD) mouse. *Proc. Natl Acad. Sci. USA* **108**, 16729–16734 (2011).
55 Boyton, R. J., Lohmann, T., Londei, M., Kalbacher, H., Halder, T., Frater, A. J. *et al.* Glutamic acid decarboxylase T lymphocyte responses associated with susceptibility or resistance to type I diabetes: analysis in disease discordant human twins, non-obese diabetic mice and HLA-DQ transgenic mice. *Int. Immun.* **10**, 1765–1776 (1998).
56 Yang, J., Chow, I.-T., Sosinowski, T., Torres-Chinn, N., Greenbaum, C. J., James, E. A. *et al.* Autoreactive T cells specific for insulin B:11-23 recognize a low-affinity peptide register in human subjects with autoimmune diabetes. *Proc. Natl Acad. Sci. USA* **111**, 14840–14845 (2014).
57 James, E. A. & Kwok, W. W. Low-affinity major histocompatibility complex-binding peptides in type 1 diabetes. *Diabetes* **57**, 1788–1789 (2008).
58 Ettinger, R. A., Liu, A. W., Nepom, G. T. & Kwok, W. W. Exceptional stability of the HLA-DQA1*0102/DQB1*0602 αβ protein dimer, the class II MHC molecule associated with protection from insulin-dependent diabetes mellitus. *J. Immunol.* **161**, 6439–6445 (1998).
59 Miyadera, H., Ohashi, J., Lernmark, A., Kitamura, T. & Tokunaga, K. Cell-surface MHC density profiling reveals instability of autoimmunity-associated HLA. *J. Clin. Invest.* **125**, 275–291 (2015).
60 Nepom, G. T. A unified hypothesis for the complex genetics of HLA associations with IDDM. *Diabetes* **39**, 1153–1157 (1990).
61 Deng, H., Apple, R., Clare-Salzler, M., Trembleau, S., Mathis, D., Adorini, L. *et al.* Determinant capture as a possible mechanism of protection afforded by major histocompatibility complex class II molecules in autoimmune disease. *J. Exp. Med.* **178**, 1675–1680 (1993).

62 Eerligh, P., van Lummel, M., Zaldumbide, A., Moustakas, A. K., Duinkerken, G., Bondinas, G. *et al.* Functional consequences of HLA-DQ8 homozygosity versus heterozygosity for islet autoimmunity in type 1 diabetes. *Genes Immun.* **12**, 415–427 (2011).
63 Uchigata, Y., Kuwata, S., Tokunaga, K., Eguchi, Y., Takayama-Hasumi, S., Miyamoto, M. *et al.* Strong association of insulin autoimmune syndrome with HLA-DR4. *Lancet* **339**, 393–394 (1992).
64 Ito, Y., Nieda, M., Uchigata, Y., Nishimura, M., Tokunaga, K., Kuwata, S. *et al.* Recognition of human insulin in the context of HLA-DRB1*0406 products by T cells of insulin autoimmune syndrome patients and healthy donors. *J. Immunol.* **151**, 5770–5776 (1993).
65 Wall, M., Southwood, S., Sidney, J., Oseroff, C., del Guericio, M.-F., Lamont, A. G. *et al.* High affinity for class II molecules as a necessary but not sufficient characteristic of encephalitogenic determinants. *Int. Immunol.* **4**, 773–777 (1992).
66 Valli, A., Sette, A., Kappos, L., Oseroff, C., Sidney, J., Miescher, G. *et al.* Binding of myelin basic protein peptides to human histocompatibility leukocyte antigen class II molecules and their recognition by T cells from multiple sclerosis patients. *J. Clin. Invest.* **91**, 616–628 (1993).
67 Muraro, P. A., Vergelli, M., Kalbus, M., Banks, D. E., Nagle, J. W., Tranquill, L. R. *et al.* Immunodominance of a low-affinity major histocompatibility complex-binding myelin basic protein epitope (residues 111-129) in HLA-DR4 (B1*0401) subjects is associated with a restricted T cell receptor repertoire. *J. Clin. Invest.* **100**, 339–349 (1997).
68 Weinstock, C., Matheis, N., Barkia, S., Haager, M. C., Janson, A., Markovic, A. *et al.* Autoimmune polyglandular syndrome type 2 shows the same HLA class II pattern as type 1 diabetes. *Tissue Antigens* **77**, 317–324 (2011).
69 Sollid, L. M., Markussen, G., Ek, J., Gjerde, H., Vartdal, F. & Thorsby, E. Evidence for a primary association of celiac disease to a particular HLA-DQ α/β heterodimer. *J. Exp. Med.* **169**, 345–350 (1989).
70 Spurkland, A., Sollid, L. M., Polanco, I., Vartdal, F. & Thorsby, E. HLA-DR and -DQ genotypes of celiac disease patients serologically typed to be non-DR3 or non-DR5/7. *Hum. Immunol.* **35**, 188–192 (1992).
71 Tsuchiya, N. Genetics of ANCA-associated vasculitis in Japan: a role for HLA-DRB1*09:01 haplotype. *Clin. Exp. Nephrol.* **17**, 628–630 (2013).
72 Hashimoto, K., Maruyama, H., Nishiyama, M., Asaba, K., Ikeda, Y., Takao, T. *et al.* Susceptibility alleles and haplotypes of human leukocyte antigen DRB1, DQA1, and DQB1 in autoimmune polyglandular syndrome type III in Japanese population. *Horm. Res.* **64**, 253–260 (2005).
73 Rioux, J. D., Goyette, P., Vyse, T. J., Hammarstrom, L., Fernando, M. M., Green, T. *et al.* Mapping of multiple susceptibility variants within the MHC region for 7 immune-mediated diseases. *Proc. Natl Acad. Sci. USA* **106**, 18680–18685 (2009).
74 Ferreira, R. C., Pan-Hammarström, Q., Graham, R. R., Fontán, G., Lee, A. T., Ortmann, W. *et al.* High-density SNP mapping of the HLA region identifies multiple independent susceptibility loci associated with selective IgA deficiency. *PLoS Genet.* **8**, e1002476 (2012).
75 Dessen, A., Lawrence, C. M., Cupo, S., Zaller, D. M. & Wiley, D. C. X-ray crystal structure of HLA-DR4 (DRA*0101, DRB1*0401) complexed with a peptide from human collagen II. *Immunity* **7**, 473–481 (1997).
76 Steenkiste, A., Valdes, A. M., Feolo, M., Hoffman, D., Concannon, P., Noble, J. *et al.* 14th International HLA and Immunogenetics Workshop: report on the HLA component of type 1 diabetes. *Tissue Antigens* **69** (Suppl 1), 214–225 (2007).
77 Siebold, C., Hansen, B. E., Wyer, J. R., Harlos, K., Esnouf, R. E., Svejgaard, A. *et al.* Crystal structure of HLA-DQ0602 that protects against type 1 diabetes and confers strong susceptibility to narcolepsy. *Proc. Natl Acad. Sci. USA* **101**, 1999–2004 (2004).