Regulation of MHC class II gene expression, genetic variation and disease

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Abstract

Major histocompatibility complex (MHC) class II molecules are central to adaptive immune responses and maintenance of self-tolerance. Since the early 1970s the MHC class II region at chromosome 6p21 has been shown to be associated with a remarkable number of autoimmune, inflammatory and infectious diseases. Given that a full explanation for most MHC class II disease associations has not been reached through analysis of structural variation alone, in this review we explore the role of genetic variation in modulating gene expression. We describe the intricate architecture of the MHC class II regulatory system, indicating how its unique characteristics may relate to observed associations with disease. There is evidence that haplotype-specific variation involving proximal promoter sequences can alter the level of gene expression, potentially modifying the emergence and expression of key phenotypic traits. Although much emphasis has been placed on *cis*-regulatory elements, we also explore the role of more distant enhancer elements together with the evidence of dynamic inter- and intra-chromosomal interactions and epigenetic processes. The role of genetic variation in such mechanisms may hold profound implications for susceptibility to common disease.

Keywords

MHC; HLA; transcription; gene regulation; polymorphism; autoimmune

Introduction

Major histocompatibility complex (MHC) class II molecules are cell-surface glycoproteins that play a central role in the immune system by presenting peptides to the antigen receptor of CD4+ T cells.[1] Antigen presentation is not only crucial for the regulation of protective immune responses against invading pathogens, but is also necessary for the maintenance of self-tolerance. Indeed, MHC class II expression directs positive and negative selection processes that shape the specificity of the T-cell-receptor repertoire of the CD4+ T-cell population during its development in the thymus.[2]

It is therefore perhaps not surprising to find that the human MHC class II gene region holds the largest number, and some of the longest recognised, associations with autoimmune diseases of any similar-sized region across the genome (Table 1). Early associations based on serological typing were established for multiple sclerosis[3,4], type I diabetes[5,6] and

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coeliac disease[7],[8] which were subsequently resolved to specific *HLA-DR/DQ* haplotypes[9],[10] while recent genome-wide association studies using common SNP markers have served to underline the remarkable contribution of this region in susceptibility to autoimmune disease[11] which dwarfs any other genetic effect. Although the functional basis for the observed class II associations in autoimmune disease remain incompletely understood, one long held view suggests a breakdown in immunological tolerance to self-antigens through presentation of peptides to auto reactive T cells.[12]

From a genetics standpoint, it has been difficult to clearly determine the disease-causing variant(s) for most MHC class II associated diseases owing to extended linkage disequilibrium and the great sequence diversity exhibited by genes in this region.[13]-[15] However, it is becoming increasingly clear that structural variation alone cannot fully account for disease associations in the MHC class II region and there is increasing interest in defining genetic variants which may modulate gene expression.[16]-[18] Support for this comes from recent genome-wide analyses of gene expression as a quantitative trait which have highlighted the impact of *cis*-acting genetic variation on expression of class II genes such as *HLA-DQA1, HLA-DRB1, HLA-DPA1* and *HLA-DQB1*.[18] The observed variation in class II gene expression makes it possible that association of MHC class II polymorphism with disease may relate to the level of gene expression as much or in addition to the restriction of response to antigen.

MHC class II genes and their expression

The human MHC class II cluster includes three classical class II genes (*HLA-DP, -DQ, -DR*) and two 'non-classical' class II genes (*HLA-DM* and *-DO*) (Figure 1).[19] Some genes are duplicated, one copy of each being functional in the case of *DP* and *DQ*. Depending on the individual, 19 genes may be found in the 0.9Mb of sequence spanned by the class II region including 8 pseudogenes. The antigen presenting molecules comprising α and β chains encoded by the classical class II genes exist as dimers on the cell surface.[20] On the other hand, non-classical class II genes are not expressed on the cell surface, but form heterotetrameric complexes and enable peptide exchange and loading onto classical class II molecules.[21] The class II region includes other functionally clustered genes involved in antigen processing including *TAP1* and *TAP2* (encoding the transporter associated with antigen processing protein), *PSMB8* and *PSMB9* (involved in ubiquitin tagged protein degradation) and, in the extended class II region, *TAPBP*, encoding the TAP binding protein.

Expression of class II genes is tightly regulated, consistent with their critical role in immunity during development and after birth. Their expression is cell-type specific and mainly restricted to thymic epithelial cells (TECs) and bone marrow derived antigen presenting cells (APCs) which include B cells, macrophages and dendritic cells.[22],[23] Levels of class II expression also vary according to the developmental stage of APCs. For example, differentiation of B cells into plasma cells as well as maturation of dendritic cells is characterised by the repression of MHC class II gene expression. Moreover, activated T cells are known to express MHC class II molecules[24] and recent studies provide evidence for a functionally distinct subset of T regulatory cells as defined by HLA-DR expression.[25] Other cell types such as fibroblasts, epithelial and endothelial cells do not express MHC class II molecules unless they are exposed to specific stimuli, notably interferon-gamma (IFN-γ). Levels of class II gene expression also vary in APCs dependent on particular stimuli. MHC class II gene expression may become markedly down regulated in particular physiological or disease states, for example during severe or prolonged infection[26]-[28] or B cell lymphoma[29] where it is associated with poor outcome.

Regulation of MHC class II gene expression

Expression of MHC class II molecules is regulated primarily at the level of transcription by a complex process involving highly conserved sequences located in the proximal promoter regions upstream of all classical and non-classical MHC class II genes which recruit specific binding factors, generating the MHC class II enhanceosome.[23],[30]-[33] At the DNA level this regulatory unit comprises four sequences (S, X, X2 and Y boxes) (Figure 2), the 'SXY module', which is found at all MHC class II genes and highly conserved across vertebrates.

The main trans-acting factors that interact with the SXY module were identified by elegant studies delineating genes involved in loss of MHC class II expression in specific B cell lines and patients with Bare Lymphocyte Syndrome (BLS) (OMIM #209920).[31],[34] BLS is a severe hereditary immunodeficiency disease in which there is defective synthesis of class II molecules. Mutations were found in particular groups of BLS patients involving specific genes encoding the proteins binding the SXY module sequences; these included Regulatory Factor X5 (RFX5)[35], RFX-associated protein (RFXAP)[36] and RFX-associated ankyrin-containing protein (RFXANK) (also known as RFXB).[37] Most significant however was the discovery of the remarkable class II transactivator (CIITA), found to be defective in a specific group of BLS patients and cloned using a cell line showing defective class II expression.[38]

The cytoplasmic association of RFX5 and RFXAP results in a conformational change in RFXAP such that on nuclear translocation it shows a high affinity interaction with RFXANK.[39] Together RFX5, RFXAP and RFXANK form the trimeric RFX complex which binds to the X box of the SXY module[36] while the X2 and Y boxes are bound by cyclic AMP responsive element binding protein (CREB),[40] and nuclear transcription factor Y (NF-Y)[41] respectively (Figure 2). This complex set of protein-DNA and protein-protein interactions generates the MHC enhanceosome which is crucial for protecting class II promoters against DNA methylation[42] and recruitment of CIITA. CIITA acts as an inducible coactivator (dubbed the 'master controller') to regulate transcription of MHC class II gene expression at several different levels including chromatin modifications, transcriptional initiation and elongation, in a cell-type-specific manner (reviewed in [23],[43]). Although the function of the S box is modified by an unidentified factor, CIITA recruitment is highly dependent on the integrity of the S-box and the precise spacing between S and X boxes.[44]

Expression of the *CIITA* gene (also known as *MHC2TA*) is inducible and found to be tightly linked to coordinated MHC class II expression. The regulation of *CIITA* is highly complex involving four different promoters in a cell and stimulus specific manner.[45] The *CIITA* gene is located at chromosome 16p13, flanking a region strongly implicated in type 1 diabetes in genome-wide association studies.[46],[47] A number of autoimmune diseases including rheumatoid arthritis and multiple sclerosis were reported to be associated with a specific promoter SNP of *CIITA*[48] suggesting a general reduction in class II expression may be a risk factor in autoimmune diseases. However the initial disease associations were modest and subsequent studies have been conflicting.[49]-[53]

SXY like sequences and MHC class II gene regulation

Although the concept of CIITA regulated transcription of MHC class II genes is well established there is evidence of additional layers of complexity in the control of MHC class II gene expression. The proximal promoter SXY module alone is not sufficient to direct the normal pattern of MHC class II gene expression. For example, *H2-Ea* (*H2-Ea* is a mouse gene encoding histocompatibility 2, class II antigen E alpha) in B-cells of transgenic mice require both the proximal promoter sequences and a distal region located at a considerable distance away from the gene.[54],[55] Deletion of this distal locus control region results in a

profound reduction of *H2-Ea* expression in B cells while thymic expression remains at a normal level indicating a role for *cis*-variation in tissue specific gene expression.[56]

Remarkably, sequence analysis of the distal locus control region revealed an inverted copy of the SXY module ~1.4 kb upstream of the transcriptional start site.[54] A similar SXY motif was found ~2.3 kb upstream of the orthologous human *HLA-DRA* gene[57] and shown to bind RFX and CIITA leading to induction of long-range histone acetylation, recruitment of RNA polymerase II and production of low levels of intergenic transcripts.[58] Several other distal SXY modules acting as transcriptional enhancers have been identified in the MHC class II region.[59] These distal SXY modules add another tier of complexity to class II gene regulation through long distance control of expression. The notion that they have a tissue specific role is intriguing and it will be of interest to investigate if genetic variation in these distal elements can contribute to disease via cell-type specific MHC class II expression defects.

These findings were further extended using a bioinformatics approach to identify X box-like (XL) sequences not associated with proximal promoters in the class II region. Several XL sequences identified between *HLA-DRA* and *HLA-DRB3* were found to be functionally important based on their ability to bind RFX and CIITA *in vivo*, and drive expression of a reporter gene.[60] Furthermore, Gomez and colleagues identified a structural interaction between an upstream XL-sequence and the *HLA-DRA* promoter using a chromatin-looping assay.[60] Strikingly, it appears that certain XL sequences can contribute to a global increase in chromatin accessibility of the class II region and interact with proximal promoter regulatory sequences through DNA looping. This illustrates the involvement of these XL sequences in a complex three dimensional system of gene expression and we will return to this topic in a later section.

MHC haplotypes, sequence variation and class II regulation

The MHC class II region is extraordinarily polymorphic. There are for example more than 400 different *HLA-DRB1* alleles, many of which are relatively recent in origin and human specific.[61] Analysis of *DRB* loci in this region has defined five major haplogroups in which variable numbers of functional genes and pseudogenes are present (Figure 3).[62,63] The high level of nucleotide diversity in the class II region is likely to reflect balancing selection acting not only on the epitope binding sites[64] but also on the promoter regions.[65,66]

Selection involving such non-coding DNA sequences important to gene regulation is supported by the evidence of haplotype-specific variation in MHC class II gene expression. A number of studies carried out in the early 1990s have highlighted the presence of sequence variation in the proximal promoter regions of MHC class II genes, in particular involving the conserved elements important to regulation such as the S, X, X2 and Y box motifs described previously.

Such work was notable for the *HLA-DRB* genes where sequence variation involving conserved proximal promoter elements[67-70] was associated allelic differences in recruitment of specific binding factors.[71-73] Polymorphism altered gene expression in resting cells[71-74] but interestingly following cytokine activation, variation in the Y rather than the X1 box was significant.[75] For *HLA-DRB1*, low and high expressing alleles were defined based on reporter gene analysis using specific promoter constructs[72,73,76-78] and assays of transcript abundance in immortalised B cell lines or B cells from peripheral blood,[77,79,80] although analysis of nascent and steady state mRNA using the latter did also indicate a role for post-transcriptional mechanisms in some of the differences observed.[81] There was however consistency seen between the *in vitro* reporter assays and assayed transcript levels *in vivo* with higher transcript abundance in resting peripheral B cells with the *DR52* haplogroup

(*HLA-DRB1*03*, *11*, *13* and *14*) as compared to the *DR53* haplogroup (*HLA-DRB1*04*, *01* and *09*).[79],[80]

The evidence from *H-2* genes in the mouse supports haplotype-specific differential gene expression with striking evidence of specific promoter variants, for example in the X2 box, resulting in allele-specific expression. Remarkably, this was specific to particular cell types, in macrophages for example altering the subsequent T cell cytokine response.[82],[83] In humans, quantitative differences in levels of *DRB1* and *DRB3* expression are also highly significant in determining the CD4+ T cell response.[84],[85]

An analysis illustrating the occurrence of sequence diversity in conserved SXY module motifs for the *HLA-DRB1* promoter is shown in Figure 4. We analysed sequence conservation for different haplotypes and confirmed the presence of haplotype-specific variation in the consensus regulatory sequences which may modulate promoter strength and allele-specific differences in transcriptional activity. Our comparative analysis of these regulatory sequences indicates that the lower expressing *DR53* haplogroup may be an evolutionary distinct branch and harbours a higher degree of variation compared to the other haplogroups.

Allele specific differences in expression have also been defined for other MHC class II genes notably *HLA-DRA* and *HLA-DQB* with variants defined involving proximal promoter elements X, X1 and Y which modulate transcription. As well as altering DNA binding sites for specific regulatory factors such as RFX[86], sequence differences affecting the spacing of particular motifs were also seen to be highly significant, for example a TG insertion/deletion polymorphism (rs5875386) between the S and X1 boxes in the *HLA-DRBQ1* promoter. This was associated with differences in reporter gene activity and allele-specific expression at the transcript level, *HLA-DQB1*0301* (TG absent) for example being expressed at significantly higher levels than *0302* (TG present).[87] Analysis of relative allelic expression in cells heterozygous for different class II alleles has been a powerful complimentary approach, helping to resolve differentially expressed alleles such as *HLA-DQB1*0301*.[88]-[91] Such studies also highlighted evidence of stimulus and cell type specificity in the effects of sequence variation involving proximal promoter elements, notably the X box.

Further comprehensive studies are needed using the wealth of resequencing data now available and techniques such as chromatin immunoprecipitation to resolve specific functional regulatory variants *in vivo* based detailed mechanistic studies of haplotype-specific protein-DNA interactions and allele-specific expression. The evidence already available however of differences in transcript abundance among class II alleles, and the role of variation in key regulatory motifs, provides important evidence for the presence of functional regulatory variants. This complements more recent data from genome-wide expression quantitative trait mapping which highlighted *cis*-acting markers involving MHC class II genes such as *HLA-DRB1* and confirmed that expression differences were heritable.[18]

Can differences in promoter strength explain class II associations with disease?

An important question that needs to be addressed is whether allele specific sequence variation affecting expression can provide a mechanism for class II associated diseases. A large number of self-antigens are expressed at highly variable levels in the thymic epithelium.[92] Given the quantitative nature of the thymic selection processes,[93] a general reduction in the expression of the disease associated class II alleles in the thymus could

result in loss of central tolerance where thymocytes with excessive reactivity to self-antigens escape elimination.

However, the association of particular autoimmune diseases with several different MHC class II alleles makes it unlikely that the observed hierarchy of class II promoter strengths alone can account for class II associations with autoimmunity. For example, a predominant role of *HLA-DRB1*03*, *04*, *13* and *15* alleles in susceptibility to multiple sclerosis has been observed in various populations.[94]-[98] These alleles have promoters of different apparent strengths, indicating several haplotypes can contribute to the same autoimmune disease. If expression is important in disease aetiology, it is more likely an additional layer of complexity enables tissue and/or timing specific MHC class II gene regulation in autoimmune diseases resulting in variable levels of class II molecules.

Support for such a model comes from analysis of a variable number tandem repeat (VNTR) polymorphism found upstream of the *INS* insulin gene, shorter (class I) alleles of which are associated with increased susceptibility to type 1 diabetes.[99],[100] Intriguingly, there was evidence of tissue specific differences in differential allelic expression between the short and long alleles of this VNTR with the disease-associated class I alleles showing increased expression in pancreatic islet cells using a number of different approaches but reduced expression in the thymic epithelium.[101]-[104] Similarly, *cis*-regulatory elements in the S, X and Y boxes or flanking regions may interact with tissue and/or timing specific regulatory factors to enable local control of class II expression resulting in tissue type dominance. There is evidence to support the concept of tissue type dominance in relation to regulatory variation from studies of the *HLA-DQB1* promoter region, where the relative expression levels associated with *0301* and *0302* haplotypes was reversed in different cell types.[86] This reinforces the view that future analysis aiming to define functionally important MHC class II alleles will need to carefully consider the context specificity within which particular variants may be acting including cell type, stage of differentiation and stimulus.

Global effects on MHC class II gene expression acting in a non allele-specific manner may also play an important role in disease susceptibility to infectious diseases. Many viruses reduce MHC class II expression to avoid protective immune responses. For example, *Chlamydia trachomatis* has been found to inhibit IFN-γ induced CIITA expression and therefore MHC class II gene expression, by causing degradation of the transcription factor Upstream Stimulatory Factor 1 (USF-1).[105] Moreover, the lack of MHC class II gene expression among patients with BLS is associated with reduced positive selection of CD4+ T cells in the thymus and inability of mature CD4+ cells to respond to antigens in the periphery.[31] As a result, patients suffer from severe and recurrent bacterial and viral infections. Infectious diseases also provide many examples of specific associations with particular MHC class II haplotypes such as *HLA-DR2* in tuberculosis and leprosy[106] where the functional basis for observed allelic risk remain unclear, but environmental influences on MHC class II gene expression may provide some insight.

Environmental factors in MHC class II gene regulation

Several possible mechanisms can contribute to determining temporal and/or tissue specific differences in MHC class II expression. These include epigenetic mechanisms, cell-type specific enhancers or regulatory factors as well as the environment. To illustrate this, we consider here vitamin D and its relationship to MHC class II gene expression and disease. Vitamin D has long been known to exhibit immunomodulatory actions and early studies provided some evidence for an effect of vitamin D on MHC class II gene expression, although no specific mechanism was characterized.[107]

Ultraviolet radiation dependent metabolism provides the major source of vitamin D in humans but at high latitudes solar radiation is too low to produce adequate levels of vitamin D, particularly in the winter.[108],[109] As a result, vitamin D deficiency is associated with numerous autoimmune and infectious diseases. For instance, the world-wide distribution of reduced UVB radiation mirrors that of MS prevalence[110],[111] and MS patients have significantly low circulating vitamin D levels during their adolescence compared to healthy controls.[112] Similarly, associations between vitamin D deficiency and TB susceptibility were described over 20 years ago[113] and there is a good correlation between active disease and low vitamin D levels in TB.[114]

Vitamin D acts in the body through binding to its receptor (VDR). Once this ligand-receptor complex is internalized, it forms a heterodimer with the retinoid X receptor and acts as a transcription factor, binding to vitamin D response elements (VDREs) in the promoter region of several nuclear genes.[115] Variation in vitamin D levels due to latitude and season enable differences in expression of vitamin D responsive genes to be manifested and provide an elegant candidate for triggering autoimmunity. This is supported by the finding that the vitamin D receptor is expressed in the thymus[116] and peripheral mononuclear cells.[117]

We have recently shown how genetic variation defines a functional vitamin D response element (VDRE) in the proximal promoter of *HLA-DRB1* (Figure 5).[118] Strikingly, this VDRE showed haplotype-specific differences, being highly conserved in the major MS associated haplotype *HLA-DRB1*1501* in Northern European populations, but disrupted to varying degrees in non-disease associated haplotypes. Functional assays showed that this VDRE can influence gene expression, conferring vitamin D sensitivity to the *HLA-DRB1*1501* haplotype. It is important to note that the extent of sequence diversity at this site remains to be defined and other haplotypes relevant to MHC class II associated diseases may harbour conserved VDREs.

The role of this interaction in disease aetiology remains to be discovered but it is plausible that lack of vitamin D in early childhood can affect allele-specific expression in the thymus resulting in loss of central tolerance and perhaps increasing the risk of autoimmunity in later life. At this point this is speculative and further work is needed. However it is intriguing that TB and leprosy patients have genetic associations with the VDR itself and/or are deficient in vitamin D,[119],[120] this can result in reduced expression of the disease associated *HLA-DR2* alleles (which include *HLA-DRB1*1501*) perhaps providing a mechanism as to how protective immune responses are bypassed.

Other factors such as estradiol have been shown to down regulate MHC class II gene expression.[117],[121] This may be of particular interest to autoimmunity given that a lower age at puberty is a known risk factor for women[122] and the observed sexual dimorphism in many autoimmune diseases.[123]

The insulator factor CTCF and MHC class II gene regulation

Recent studies of transcriptional regulation have highlighted how gene expression is regulated in a complex three-dimensional system in which spatial organisation of chromatin plays an important role.[124],[125] This raises the question of which factors control nuclear organisation and long-range chromosomal interactions. One leading candidate is CCCTC-binding factor (CTCF), a highly conserved and ubiquitously expressed 11 zinc finger protein.[126] CTCF was initially identified as a transcriptional repressor but subsequently shown to also activate transcription and have a much more fundamental and global role as a transcriptional insulator, acting as a position dependent enhancer blocking protein[127] and binding to boundary elements to prevent spreading of hereterochromatin.[128] Moreover, CTCF has been shown to play a critical role in mediating inter- and intra-chromosomal

interactions through chromosomal looping with diverse roles in transcriptional regulation dependent for example on specific interacting proteins.[126] This includes the regulation of allele specific expression patterns at imprinted gene loci such as *H19/IGF2* relating to methylation status.[129-132]

A recent study elegantly provides evidence for a novel CTCF-dependent MHC class II mechanism of gene regulation, where the expression of *HLA-DRB1* and *HLA-DQA* is dependent on an intergenic X-box like sequence dubbed XL9 located 29 kb centromeric to *HLA-DRB1.* Similar to XL sequences discussed earlier, XL9 displays a histone modification profile that is associated with accessible chromatin involving histone acetylation extending several kilobases from the site.[133] The region of peak acetylation was found to be bound by CTCF and functioned as an enhancer blocking element. Majumder and colleagues more recently provided evidence for long-range chromatin loops between the promoters of *HLA-DRB1* and *HLA-DQA* and XL9.[134] Strikingly, these interactions were dependent on the activity of CTCF, CIITA and RFX, either constitutively or after induction with IFNγ in non-expressing cells. Indeed, CTCF siRNA knock down was found to reduce the long-range interactions and decrease expression of *HLA-DRB1* and *HLA-DQA.*[134]

These findings suggest a novel regulatory mechanism dependent on CTCF, whereby both *HLA-DRB1* and *HLA-DQA* loci can interact simultaneously with XL9. This replaces the outdated linear model of gene regulation with a three dimensional model in which long range intrachromosomal associations orchestrate MHC class II regulation. In general, the *HLA-DR* and *HLA-DQ* genes are thought to be co-regulated, however they can be discordantly regulated for example in certain non-Hodgkin's lymphomas.[135] It is possible that aberrant regulation of the XL9 element may allow these differences to be manifested.

It will be intriguing to investigate whether genetic variation exists in this CTCF binding site. Such variation could give rise to decreased or dysregulated expression of *HLA-DRB1* and *HLA-DQA1* and perhaps contribute to MHC class II associations with disease. Moreover, recent genome-wide analyses of CTCF binding in human cells have identified several other CTCF binding sites in the class II region[136-138] suggesting that multiple CTCF sites may serve to coordinate the expression of nearby class II genes and be candidates for the site of action of regulatory variants.

Missing pieces in the regulation of MHC class II gene expression

In the past decade, we have acquired a remarkably detailed understanding of how MHC class II genes are regulated. As discussed in this review, studies to date have focused on *cis*-regulatory elements in the context of DNA sequence variation. It is becoming increasingly clear however that processes such as epigenetic modifications and noncoding RNAs are also central players in gene regulation.[139] Given that a full explanation for most MHC class II associations with disease has not been reached, it seems rational to explore the potential contribution of such mechanisms to MHC class II gene expression.

Epigenetics refers to processes such as DNA methylation and histone modifications that act as local chemical marks on the DNA to regulate gene activity and, in the stricter definition of the term, result in heritable effects independently of any changes in the DNA sequence.[140,141] Combinations of these epigenetic changes modulate the accessibility of regulatory DNA sequences to transcription factors and may contribute to the cell-type specific expression of class II genes. Furthermore, the recognition that methylation marks are prone to disease-related alterations makes them prime targets in epigenetic analysis and expression studies.[142] Interest in this field has gained great momentum with the recent realisation that epigenetics may underpin gene-environment interactions and that the resulting expression changes could be a major way of adapting to external influences.[143,144] Given the pivotal

role that the MHC class II region plays in autoimmune disease, modulation of epigenetic regulation of class II gene expression by environmental factors is likely to be an important mechanism in disease pathogenesis. So far reports investigating the methylation profiles across the classical MHC including the class II region demonstrate tissue specific and bimodally distributed patterns of methylation.[145],[146] However, to date a complete analysis of methylation profiles in the MHC class II accounting for haplotypic variation has not been carried out.

Epigenetic regulation involving covalent modifications such as histone acetylation and methylation play a critical role in activation of MHC class II genes while gene silencing through for example histone deacetylation are also fundamentally important as seen elsewhere in the genome.[147] There are however unique features to MHC class II genes relating to their particular mode of regulation, notably the role of CIITA in recruitment of histone acetyltransferases (HATs).[148] CIITA can associate with multiple HATs, including CBP/p300 as well as other HATs such as steroid receptor coactivator (SRC1).[149] Intriguingly, this provides a mechanism by which hormones such as estrogens and glucocorticoids could regulate MHC class II genes. As well as the key role of CIITA in histone acetylation,[150] CIITA is also critical to recruitment of ATP-dependent chromatin remodelling complexes; there is also evidence for a role for CIITA and RFX in recruitment of histone deacetylases (HDACs) (reviewed in[147]).

Regulation of CIITA gene expression is itself controlled by epigenetic mechanisms including DNA methylation and histone modifications.[147] These can in turn be modulated by environmental factors such as infectious pathogens. Indeed, *Mycobacterial* infection may inhibit the host immune response through such epigenetic mechanisms, *Mycobacterium tuberculosis* for example inhibiting IFNγ induced CIITA expression through effects on promoter histone acetylation and so reducing MHC class II expression.[151],[152]

Alternative splicing, the process whereby multiple mRNAs may be generated from individual genes, adds another dimension to the regulation of gene expression and considerably expands the versatility of the transcriptome to result in significant proteomic diversity.[153],[154] It is possible genetic variation in the class II region affects splicing efficiency and alters either the total output of a gene or the ratio of alternatively spliced variants. Indeed, haplotype-specific splicing patterns of the *HLA-DQB1* gene have been observed and mapped to specific single nucleotide substitutions within intron 3. These variants were located in a regulatory sequence controlling splicing, specifically a branch point sequence, that altered the binding affinity of splicing factor 1 and led to exclusion of exon 4 for *HLA-DQB1*0302* and **0401*.[155] Similarly, several other class II loci including *HLA-DRB1* and *HLA-DQA1* are known to exhibit haplotypic variation at splice sites, which may affect expression at a post-transcriptional level.[156] A growing number of reports highlight the role of DNA sequence variation in modulating alternative splicing which can contribute to disease susceptibility.[157]

The MHC class II region has already provided one of the clearest examples of a SNP modulating alternative splicing resulting in common disease through the work of Valentonyte and colleagues on genetic determinants of sarcoidosis, a chronic granulomatous condition.[158] A genome-wide linkage and association analysis resolved variants at *BTNL2* (butyrophilin-like 2, a member of the immunoglobulin family) in disease susceptibility and more specifically a G to A nucleotide substitution (rs2076530) within exon 5 which created an alternative splice site, and led to a frameshift and premature stop. The truncated protein associated with the A allele loses its membrane localisation ability which is thought to affect the ability of BTNL2 to regulate T cell activation.[159] Further characterization of the MHC

class II transcriptome and splicing code in the light of sequence diversity may therefore shed light on the molecular basis for class II associations with disease.

Furthermore, it has recently come to light that a family of small non-coding RNAs known as microRNAs (miRNAs) can negatively regulate gene expression by suppressing translation or degrading mRNA transcripts.[160] These miRNAs have important and diversified functions including cell differentiation[161], tumorigenesis[162] and viral defence.[163] As a result, the possibility that class II gene expression could be regulated by miRNAs, and more specifically, differentially regulated based on underlying sequence variation, needs to be examined. An elegant example of how such a mechanism may be involved in gene regulation comes from the MHC class I gene *HLA-G* and variants implicated in susceptibility to asthma. A SNP in the 3′ UTR of *HLA-G* influences the targeting of three miRNAs and therefore affects *HLA-G* protein expression in an allele-specific manner.[164]

Conclusion

The emerging evidence that regulation of gene expression is much more complex than previously thought has significant implications for disease associated MHC class II genes. Identification of regulatory genetic variants involving this region requires further detailed investigation taking into account current insights into coordinate regulation involving proximal and distal elements, intergenic regions and chromosomal looping, epigenetic mechanisms and environmental factors determining cell, differentiation state and stimulus specific gene expression. Although the focus in this review has been on how class II gene expression can be regulated at the transcriptional level, it is known that post-transcriptional modifications affecting the cytoplasmic tail of class II molecules may alter their export to the cell surface.[165] Clearly, we still have much more to learn about how gene expression is regulated in the class II region. Technological advances should continue to improve our ability to characterise class II expression including for example at single cell resolution *in vivo*, allowing analysis in disease-specific cell types and hopefully unlocking some of the mysteries that have long surrounded MHC class II genetic associations with autoimmune and infectious diseases.

Acknowledgments

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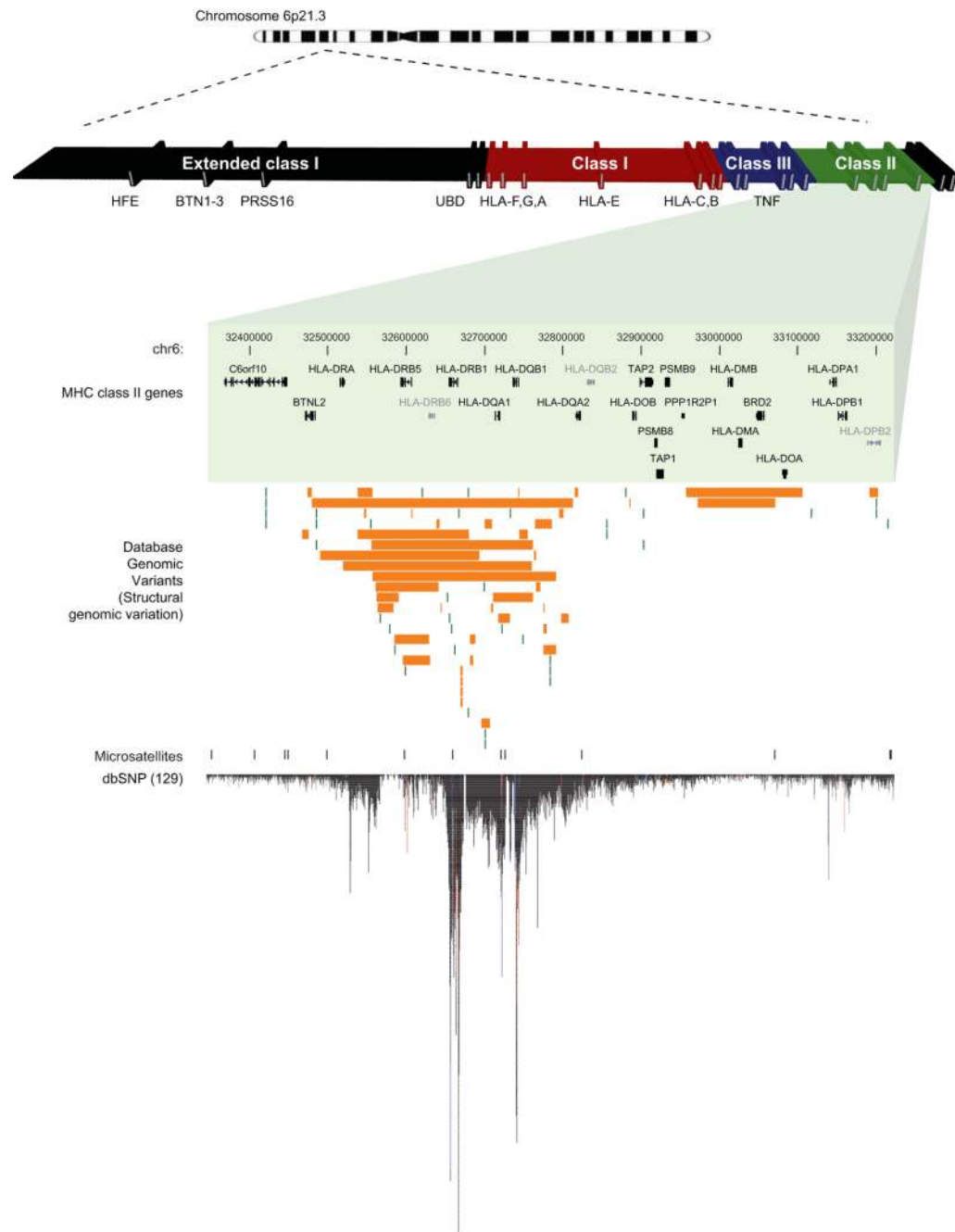
Figure 1. Genes and genetic diversity in the MHC class II region
The classical MHC class I, class III and class II regions are shown at chromosome 6p21
together with a higher resolution plot showing the location of genes within the MHC class II
region chr6:32,250,000-33,300,000 (hg18 build 36). Structural genomic variation from the
Database of Genomic Variants[166] are shown (copy number variants in orange, insertions/
deletions dark green) together with location of microsatellites and sequence level variation
in terms of single nucleotide polymorphisms and small insertions and deletions (indels)
(dbSNP build 129).[167] Images adapted from UCSC Genome Browser.[168]
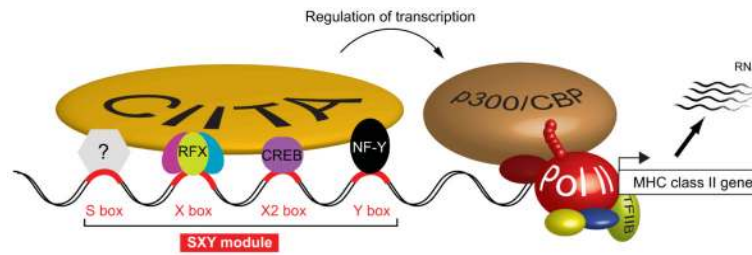
Figure 2. Regulation of MHC class II transcription
Schematic representation of transcriptional regulation for an MHC class II gene. The MHC class II enhanceosome is shown resulting from recruitment of different binding factors including RFX, CREB and NF-Y to the S, X, X2 and Y box sequences located in the proximal promoter region. This recruits the master regulator CIITA which directs transcription.

Figure 3. Major human HLA-DR haplogroups

Five haplogroups are shown denoted *DR1, DR8, DR51, DR52* and *DR53*. These differ by the presence of an additional functional *DRB* gene (*DRB3, DRB4* or *DRB5*) (shown in black) and a varying number of *DRB* pseudogenes (*DRB2, DRB6, DRB7, DRB8* or *DRB9*) (shown in grey). The *DRB1* allelic lineages can be resolved to five families which relate to the five main haplogroups: *HLA-DRB1*01* and *10* (*DR1*), *08* (*DR8*), *15* and *16* (*DR51*), *03*, *11*, *13* and *14* (*DR52*), and *4*, *7* and *9* (*DR53*). Gene transcript structure derived from Ensembl but note figure schematic rather than drawn to scale with respect to location.
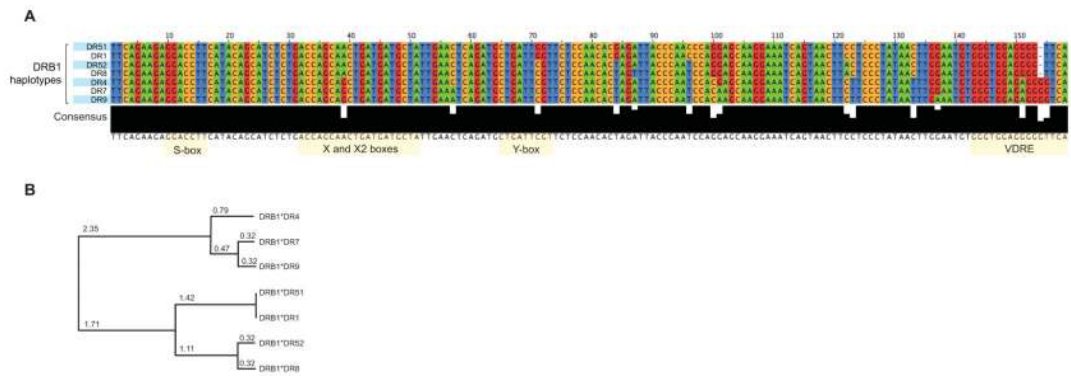
Figure 4. Sequence conservation and regulatory elements in proximal promoter region of *HLA-DRB1*

(**A**) Evidence of haplotype specific sequence variation involving regulatory sequences in *HLA-DRB1* involving both X and Y boxes. DR4, DR7 and DR9 (the *DR53* haplogroup) contain the highest variability. Such variants may explain the observed allele specific differential expression at this locus. (**B**) Dendrogram showing the relationship between the regulatory regions obtained from a pairwise similarity score. In general, regulatory sequence clusters correspond to the different ancestral haplotype groups. The proximal promoter sequence of the DR8 haplotype shows high degrees of similarity to that of the DR52 haplotype. Another cluster of homologous sequences consists of DR4, DR7 and DR9 (the *DR53* haplogroup) and seems to have evolved independently.
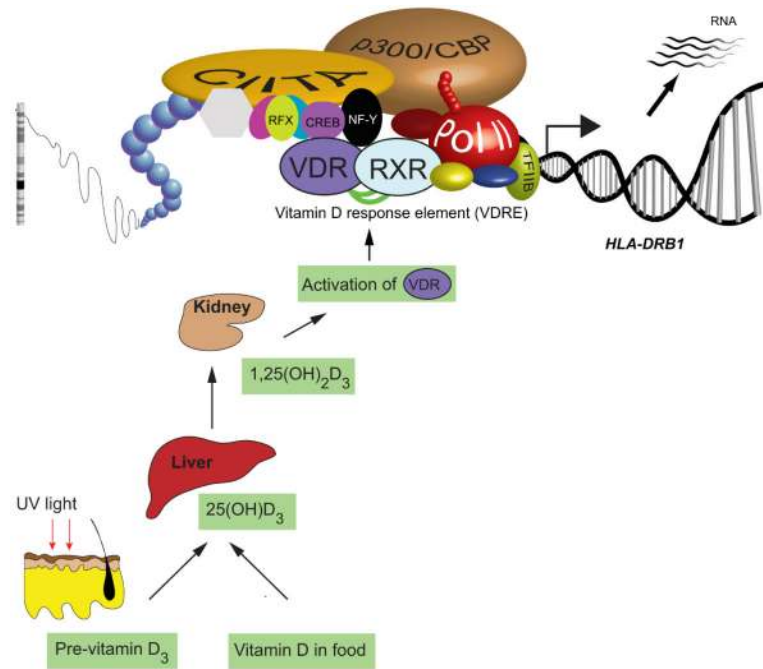
Figure 5. A conserved VDRE confers responsiveness to vitamin D to *HLA-DRB1*
Schematic illustrating the role of a proximal VDRE at the *HLA-DRB1* gene which confers vitamin D responsiveness through binding of VDR/RXR.

Table 1

Summary of major MHC class II associations with autoimmune, inflammatory and infectious diseases

Immune and autoimmune			
Disease	**Class II association**	**Comments**	**Reference**
Celiac disease	*HLA-DQA*0501-DQB1*0201*		169
Crohn's disease	*HLA-DRB1*0103, *04, *0701* and *HLA-DRB3*0301*	The most consistently reproducible finding is *HLA-DRB1*0701*	170_173
Ulcerative colitis	*HLA-DRB1*0103* and *1502*	*HLA-DRB1*0103* is the most reproducible association	170,174,175
Graves disease	*HLA-DRB1*03-DQB1*02-DQA1*0501*	Logistic-regression analysis shows that the association could be explained by either DRB1 or DQA1	176,177
Hashimoto's thyroiditis	*HLA-DRB1*04-DQB1*03-DQA1*03*		178
Multiple sclerosis	*HLA-DRB1*1501– DQB1*0602 HLA-DRB1*0301-DQB1*0201, HLA-DRB1*0405-DQB1*0301*	The most consistently reproducible finding is *HLA-DRB1*1501* in Northern Europeans. Epistasis among *HLA-DRB1, HLA-DQA1*, and *HLA-DQB1* loci has been shown to influence MS risk.	94_98,179
Narcolepsy	*HLA-DQB1*0602*	*HLA-DQB1*0601* associated with protection	180_182
Rheumatoid arthritis	*HLA-DRB1* shared epitope susceptibility alleles *0101, *0401, and *0404* in Europeans and *0405 and *0901* in Asians.	*HLA-DRB1*04* alleles confer high risk and the rest exhibit a more moderate risk	175,183
SLE	*HLA-DR3* and *HLA-DR2* haplotypes		184,185
Type 1 diabetes	Europeans: *DRB1*0401– DQA1*0301– DQB1*0302* and *DRB1*0301– DQA1*0501– DQB1*0201* Asians: *DRB1*0405–DQB1*0401* and *DRB1*0901–DQB1*0303*	Heterozygosity for both European haplotypes confers the greatest risk for T1D	10,186_189
Infectious disease			
Hepatitis B	*HLA-DR7 HLA-DPA1*0103-DPB1*0402* and *HLA-DPA1*0103-DPB1*0401*	*HLA*DP* is associated with chronic hepatitis B in Asians	190,191
Leprosy	*HLA-DR2 HLA-DRB1*10*		192,193
Tuberculosis	*HLA-DR2*		194,195