Genetics and the causes of ankylosing spondylitis

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Introduction

Genetic studies of ankylosing spondylitis (AS) have over the past decade provided major insights into the etiopathogenesis of the disease that have led to major therapeutic innovations. Some of these new treatments have already entered clinical practice, and others are in trials and undergoing development. It has long been known that susceptibility to and 'severity' of AS are largely genetically determined. Extensive progress has been made identifying susceptibility alleles in the disease, with 113 established loci identified contributing roughly 10% of the heritability of AS, over and above the major effect of HLA-B27, which determines ~20% of the genetic risk. Studies of the genetics of clinical manifestations of AS such as the extent of bony ankylosis or presence of anterior uveitis have been more challenging, though some genes have been found to influence uveitis risk beyond their effects on the risk of AS-itself. This article seeks to present the current state of understanding of the genetic influences in AS, focusing on more recent advances and their contribution to understanding mechanisms of disease.

Major Histocompatibility Complex (MHC) and Ankylosing Spondylitis

Large scale case-control studies of HLA and other MHC genes in AS have demonstrated that the genetic associations at this locus are far more complex than initially thought. Since the discovery of the association of HLA-B27 with AS, there have been many studies

suggesting the presence of additional MHC associated variants [1–5]. With the exception of the association of *HLA-B60* with AS [6,7], until recently none of those have been convincingly replicated.

The MHC is under marked genetic selection pressure and HLA frequencies vary substantially between ethnic groups. The development of methods of HLA-typing using imputation from dense SNP genotyping together with the availability of large reference sets of subjects genotyped at both HLA loci and MHC SNPs has enabled analysis of HLA and MHC associations in large case-control cohorts. Another methodologic advance in recent studies is principal components analysis; population stratification can be identified and controlled for, making the findings robust to differences in allele frequencies due to ethnic variation rather than disease affection status. Two such studies have now been published, one in subjects of European-ancestry [8], and the other in Koreans [9]. Both show convincingly that there are additional HLA-B variants associated with AS, and also other HLA Class I and II variants (Table 1). While these studies do not exclude the presence of other non-HLA MHC genetic associations, they do indicate that it is unlikely that variants of large effect exist within the MHC once the associations of HLA variants are accounted for.

The additional HLA-associations likely contribute to the known association of AS with other diseases. For example, *HLA-B51* which is also a risk variant for AS, is the major risk allele for Behçet's syndrome, a condition that can be complicated by sacroiliitis. *HLA-DRB1*0103* is AS-associated, and is also one of the major risk alleles for Crohn's disease, which frequently co-occurs with AS. *HLA-B7* has a major protective effect on AS, and interestingly is used as a control allele in transgenic rats. In this model, excess copy numbers of *HLA-B27* induce a spondyloarthropathy, whereas rats with similar excess copy numbers of *HLA-B7* remain healthy [10].

Using dense MHC SNP genotyping data, the amino-acid composition of the HLA alleles can be imputed and tested for association with disease, enabling the identification of the key amino-acids involved. This approach has been successfully used to extend the known amino-acids in the rheumatoid arthritis 'shared epitope', which provides a functional explanation for the association of HLA-DRB1 alleles with that disease [11]. In AS, in the populations studied, the identity of the amino-acid at position 97 in HLA-B was shown to determine the direction of association of the major HLA-B alleles with the disease (see Table 2) [8]. This does not mean that this amino acid alone is the sole HLA-B determinant of AS risk; rather it indicates that in the context of the HLA-B alleles involved, this amino acid is a key determinant of disease risk.

Aminopeptidases and Ankylosing Spondylitis

A key recent discovery has been the demonstration that variants of the M1-aminopeptidase gene, *ERAP1*, are associated with AS [12], and interact genetically with both the AS-associated HLA Class I alleles HLA-B27 and HLA-B*4001 [8,13]. Thus *ERAP1* is only associated with AS in HLA-B27 positive cases, or HLA-B27-negative/HLA-B*4001 positive cases. The same *ERAP1* haplotypes interact with HLA-Cw6 in psoriasis [14], and HLA-B51 in Behçet's disease [15]. This locus is also strongly associated with the rare ocular

uveitis birdshot retinopathy, which is strongly associated with HLA-A29, though in this disease the number of subjects studied is too small to determine if the disease association is with *ERAP1*, the neighboring related gene *ERAP2*, or both [16]. The *ERAP2* association with AS is present in both HLA-B27-positive and –negative disease [17] suggesting some subtle difference in its functional mechanism in causing AS. For example ERAP2 may potentially affect on peptide handling by other AS-associated HLA Class I alleles for which ERAP1 peptide cleavage is less influential.

A recent paper has suggested that haplotypes of *ERAP1* variants are more strongly associated with disease and have greater functional effects than individual disease-associated variants [18]. However, the small sample size of this study (19 cases and 17 controls) is too few to distinguish haplotypic from single variant effects, and many of the haplotypic associations reported were not statistically significant [19]. The study also reports the opposite direction of association of the key AS *ERAP1* non-synonymous SNP, rs30187, compared to all other studies, which included more than 1000 times more cases and controls, in multiple ethnic groups [8,20–24]. In the absence of further supportive evidence this study should be considered hypothesis generating.

Extensive studies of the functional mechanism of the associations of ERAP1 and ERAP2 variants and AS are underway. Both proteins are involved in peptide trimming in the endoplasmic reticulum, changing particularly the length but also the amino acid composition of peptides available for HLA Class I presentation [25–2728]. Proposed mechanisms of association include:

- Effects on the peptidome presented by HLA-B27 and thus leading either to presentation of arthritogenic peptides [29] or failure to present disease-protective peptides [30]

- Effects on HLA-B27 free heavy chain expression and KIR interactions, in turn influencing activation of IL-17 producing immune cells [31]

- Effects on HLA-B27 folding and ER accumulation leading to ER stress reactions [32,33].

It is beyond the scope of this article to discuss these hypotheses and studies in detail. However, a common feature of each model is that variants that are disease-protective in AS exhibit reduced peptide cleavage function [34–36]. This, and the fact that apart from with Behçet's disease there is no convincing evidence in either humans or animal models that ERAP deficiency increases disease risk, has led to programs targeting these proteins as therapeutics for AS and related diseases [37].

T-Cells and Ankylosing Spondylitis

How genetic variants predisposing to immune-mediated diseases are tied to altered immune system activity is a question of primary importance. Despite being one of the first proposed mechanisms of disease development in AS, strongly supported by the robust ERAP1-HLA-B27 epistasis identified [36], it has not been conclusively demonstrated that autoreactive T-cells recognize a B27 restricted peptide in AS patients to cause disease. T-cell receptors

(TCRs) develop by the process of random recombination of numerous encoded gene segments to generate highly variable and cell-specific receptor chains. Unique cell surface TCRs on each T-cell have the potential to engage different combinations and conformations of HLA bound peptides (Figure 1). Clonally expanded T-cells that have recognized an antagonistic antigen, and proliferated to hone adaptive immune responses, are a feature of many immune-mediated diseases and contribute to targeted inflammation [38,39]. Little evidence of such occurrence in AS patients has been published since the work of Mamedov *et al.* in 2009 [40], which characterized the T-cell populations of two patients and found stably expanded clones consistently representing between 5 and 50% of the profiled repertoire over a number of years. These T-cells were found to be cytotoxic and proinflammatory in nature, predominantly CD8+/CD27–/CD28– (regarded as terminally differentiated effector or cytotoxic T-cells), and expressed TCRs with homology to a number of previously reported clones in reactive arthritis [41], rheumatoid arthritis [42] and other AS patients [43]. Earlier work demonstrated that a nonamer of the HLA-B*2705 molecule hypervariable region itself can be recognized by cytotoxic T lymphocytes in AS patients [44]. Convergence of TCR sequences used by these nonamer-responsive T-cell populations found in the peripheral blood and synovial fluid of numerous AS patients suggested a mechanism by which self-reactive T-cells contribute to inflammation in a B27-dependent fashion. However other studies including in discordant twin pairs found no sharing of CD4+ or CD8+ peripheral T cell receptor Vβ repertoire[45]. TCR involvement in AS has also been looked at from the point of view of germline variable region genes, but neither linkage nor association of the *TCRA* or *TCRB* loci have been demonstrated with AS [36,46], suggesting that these loci are not important in the familiality of the disease.

The future of immunogenetics studies in immune-mediated diseases with suspected autoreactive T-cell involvement will be in the high resolution profiling of T-cell populations. Although yet to be conducted in an AS cohort, new 'immunosequencing' techniques applied to a diversity of conditions, including juvenile idiopathic arthritis (JIA), has provided insight into the underlying nature of T-cell responses in disease [47]. The JIA study demonstrated a restricted TCRB repertoire in the peripheral blood and synovial fluid regulatory T-cell (Treg) population of patients, with patient sharing of expanded clonotypes lacking in healthy children. Results suggested either appropriate but inefficient control of inflammatory processes by Tregs, that are typically an immensely diverse T-cell population, or perhaps pathogenicity of the expanded clones in disease. Clonal restriction of relevant T-cell populations in AS may very well also be detected with the profiling of hundreds of thousands of T-cell receptors in this fashion, providing support for the arthritogenic peptide model of disease.

Pleiotropy and Ankylosing Spondylitis

AS frequently co-occurs in individuals and families with psoriasis and inflammatory bowel disease, potentially because of shared genetic or environmental risk factors, or both. Utilizing a genotyping chip targeting immunogenetically important loci [48], the extensive role of genetic pleiotropy in these clinical associations has been demonstrated [24]. This 'cross-disease' study showed extensive co-heritability of AS with both ulcerative colitis and Crohn's disease, and with psoriasis albeit to a lesser extent. Utilizing this data, the

investigators identified an additional 17 genome-wide significant AS-associated loci, and 65 loci associated at genome-wide significance with combinations of diseases (pleiotropic loci).

The study further strengthened the evidence of the role of the IL-23 pathway in AS pathogenesis, building on the finding of the association of *IL23R* variants with the disease, which initiated the development of IL-23 pathway inhibitors for the disease [12]. While the exact functional mechanisms underpinning most genetic associations with AS are not yet fully understood, a high proportion of AS-associated genes influence the IL-23 pathway. At some loci multiple disease-associated variants have been identified, often with differential associations with different diseases. For example, Ellinghaus *et al.* identified four independent associations at *IL23R*, and additional variants have been identified as the primary variants associated with other immune-mediated diseases such as Behçet's syndrome, psoriatic arthritis, and Vogt-Koyanagi-Harada syndrome [49]. This suggests that differences in transcriptional regulation such as tissue specificity, or response to particular stimuli, underlie how these genes operate to cause clinically distinct diseases. Functional analysis of such variants will provide important information about the role of particular genes in disease, and inform therapeutic targeting of such genes. An excellent recent example of this has been the functional and immunogenetic dissection of the *RUNX3* locus, known to be associated with AS but also celiac disease, psoriasis[50] and psoriatic arthritis [51], and is a potential therapeutic target for these diseases.

Other novel gene pathways identified in the cross-disease study include:

- DNA methylation: DNA methyltransferase 3a and 3b (*DNMT3A, DNM3TB*) are de novo methyltransferases known to be involved in genomic imprinting and X-chromosome inactivation, to influence haematopoietic stem cell development, and activation of UBE2 ubiquitin ligases, a family of genes also known to be AS-associated. The known functions of these genes raise the hypothesis that they may be involved in the male gender bias in AS, which remains unexplained.

- Gut mucosal immunology: The AS-associated gene *FUT2* encodes a fucosyl transferase that determines the ability to secrete blood group antigens into body fluids. This has a major effect on the gut microbiome providing further evidence that AS is a disease caused by interaction between an abnormal gut microbiome and the host immune system [52].

- JAK (Janus kinase) signaling: JAK2 is the tyrosine kinase that signals from IL-23R, and is the only AS-associated *JAK* [24]. It was therefore predictable that agents such as tofacitinib which primarily target other JAK proteins would be only moderately effective in AS [53], and suggests that more JAK2 specific inhibitors should undergo clinical trials in the disease.

- Toll-like receptor signaling: The association of TLR4 with AS, which drives innate immune reactions, particularly to lipopolysaccharide (a key bacterial cell wall component), provides another non-HLA-B27 dependent pathway involved in causing the disease [24].

These findings demonstrate that further hypothesis-free genetic studies are warranted in AS; although many of the genes identified in recent studies lie in established pathways, new pathways are still being identified through new gene discoveries.

Killer Immunoglobulin-like Receptors (KIR)

Another group of genes with suspected relevance in many immune-mediated diseases are the killer immunoglobulin like receptors (KIRs) encoded within the lymphocyte receptor complex on chromosome 19. KIR disease associations have largely gone undetected by genetic studies because the biological consequences of receptor signaling on immune responses is heavily dependent on a multitude of factors that are highly variable in the population. The KIR locus is immensely polymorphic, encoding variable combinations of 17 different receptors that transduce either excitatory or inhibitory signals to natural killer (NK) and T-cells upon engagement with specific HLA class 1 or HLA-like ligands (Figure 2). Given the large degree of allelic and expression variability that exists at each gene, and that the independent HLA background of an individual governs the compatibility of receptor-ligand engagements, KIR-mediated immune response are uniquely shaped in each individual (reviewed in [54]) and thus difficult to study collectively. KIR associations with AS are varied. One disease model proposes that the ability of KIR3DL2 to recognize abnormal HLA-B27 cell surface homodimers is tied to pathogenicity [55]; others suggest that the balance of inhibitory and excitatory KIR receptors in AS patients is relevant in skewing inflammatory NK cell responses in disease.

Recent molecular studies have demonstrated that the KIR3DL2 receptor is up-regulated on activated CD4+ T-cells. It has been previously demonstrated that CD4+/KIR3DL2+ T-cells are found in increased numbers in AS patients relative to healthy individuals [31,56]; the same research team found them to be expressed in the terminal ileum of early SpA patients [57]. Engagement of KIR3DL2 with HLA-B27 homodimers or free heavy chains has been shown to provide a survival signal to these cell populations and to promote differentiation into Thelper 17 (Th17) cells, which secrete the pro-inflammatory cytokine IL-17 found at increased levels in AS patients[31]. The studies reporting these findings have been small, involved patients with spondyloarthropathies other than AS, and have not been replicated[58]. Only KIR3DL2 and KIR3DL1 usage has been studied in AS to date. A study of *KIR3DL2* genetic variation found no association with AS [59]. Given the potential significance of the studies of KIR3DL2 with AS, there is a clear need for independent replication of this finding, and a more comprehensive survey of KIR usage should be performed.

Genetic studies looking to compare gene dosages of differing *KIR* receptors in AS cases and controls have revealed a number of other genes found more or less frequently in patients, with potential ramifications for the control of NK cell immunity (Table 3). Of relevance are differences in the frequency of genes *KIR3DS1* (increased in AS cohorts), and *KIR3DL1* (decreased in AS cohort)[60–62] given that the latter is an inhibitory receptor known to recognize HLA-Bw4 subgroups including HLA-B27. Highly homologous *KIR3DS1* encodes a lymphocyte activating receptor and has been postulated to also respond to HLA-B27 ligands; KIR3DS1 co-occurrence with HLA-Bw4 is associated with slowed progression to AIDS in HIV individuals, evidence of its immune activating potential [63]. Profiling the

patterns of KIR and HLA inheritance in large disease cohorts is likely to be very informative in characterizing the involvement of KIR-expressing lymphocytes in AS.

Summary

Genetic studies have identified multiple different pathways involved in AS, and as more genes are being identified, more pathways are being uncovered. These findings are helping fill in the mystery about how HLA-B27 is involved in AS, and identifying new potential therapeutic targets. While much more will be learned from further hypothesis free studies in AS genomics, there is the research need now needs to transition increasingly to functional genomics studies to determine the mechanisms underpinning these associations and to turn the genetic discoveries into new treatments, as they have already with regard to IL-23 pathway inhibition in AS.

Acknowledgments

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KEY POINTS

- Ankylosing spondylitis (AS) is a common, highly heritable inflammatory arthritis for which thus far 113 genetic associations have been identified.

- HLA-B27 contributes ~20% of the heritability of AS, and non-MHC loci identified to date contribute another ~10%.

- The HLA associations of AS are complex and multiple non-B27 HLA alleles have been identified as being involved.

- Key pathways identified by AS genetic studies include the IL-23 and M1-aminopeptidase pathways, but multiple other pathways have been identified as increasing numbers of associations have been identified.

- Preliminary evidence suggesting involvement of KIR genes in AS pathogenesis needs replication in other cohorts.

SYNOPSIS

Ankylosing spondylitis (AS) is a common inflammatory arthritis in which genetic factors are the primary determinants of disease risk, and of disease activity and severity. Primarily through linkage disequilibrium mapping substantial progress has been made in identifying genetic pathways involved in the disease, leading to the development of IL-23 pathway inhibitors for AS, and other drug development programs, for example targeting aminopeptidase genes. Multiple other disease pathways have been identified through gene-mapping studies including pathways involving control of DNA methylation, bacterial sensing, and mucosal immunity. Additional pathways will likely be identified as a higher proportion of the genetic risk of AS is determined.
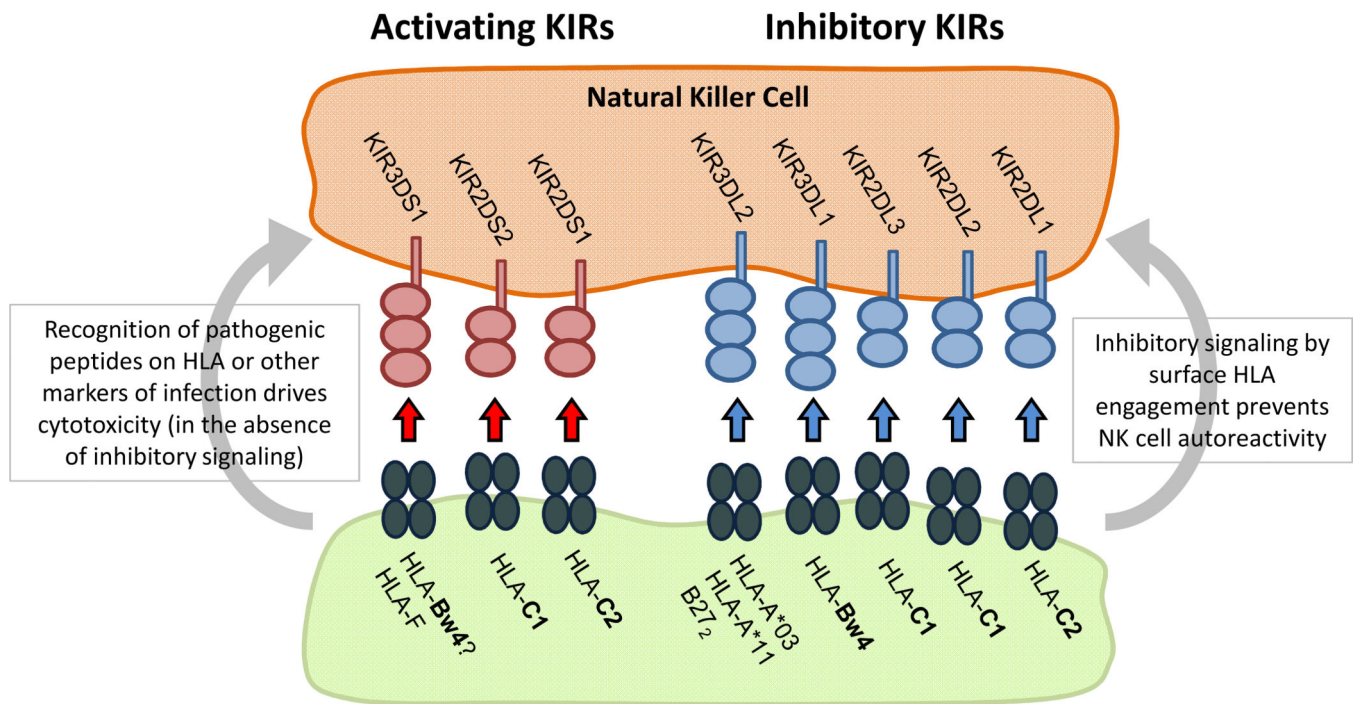
Figure 1:
KIR gene usage and interacting partners, and their effects on NK cell activation or inhibition.
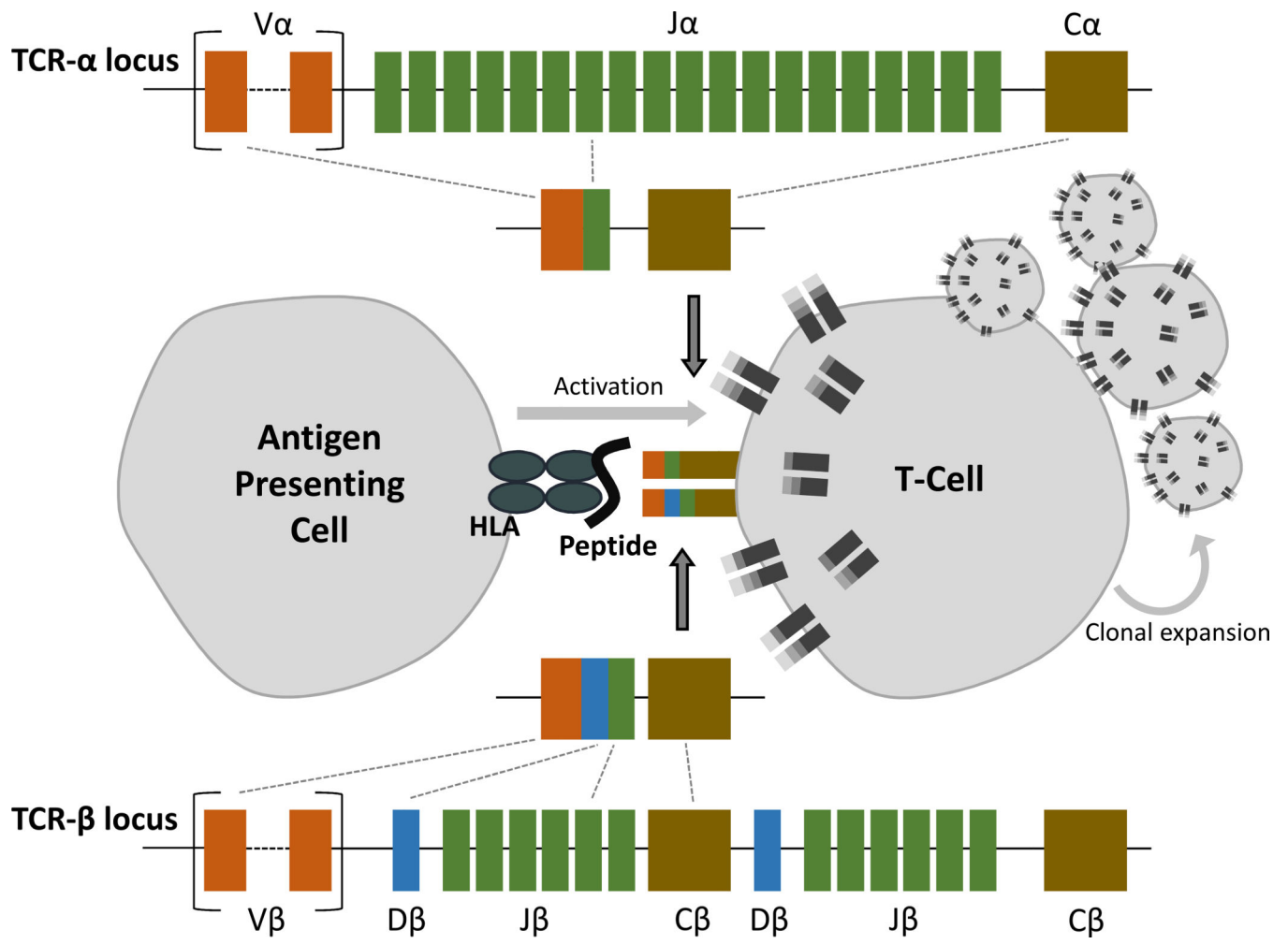
Figure 2:
T-cell receptor gene rearrangement pathway determining T-cell receptor usage by individual T-cells.

Table 1

Association of *HLA-B* alleles with susceptibility to AS in European descent patients. Findings are presented for consecutive conditional analyses, where for rounds 2 and onwards the test conditioned on the previous alleles.

Round	*HLA-B* Allele	Odds Ratio (95% CI)	*P*-value
1	*27:05*	62.41	$< 10^{-321}$
2	*27:02*	43.41	1.07×10^{-122}
3	*07:02*	0.82	5.04×10^{-6}
4	*57:01*	0.75	5.13×10^{-4}
5	*51:01*	1.33	2.14×10^{-3}
6	*47:01*	2.35	2.25×10^{-3}
7	*40:02*	1.59	4.65×10^{-3}
8	*13:02*	1.43	4.29×10^{-3}
9	*40:01*	1.22	4.93×10^{-3}

Table 2

Association analysis of SNPs and amino acids at position 97 of HLA-B.

Amino acid residue	Multivariate OR (95% CI)	*P*-value	Classical *HLA-B* Allele
Asparagine (N)	16.51 (15.43–17.69)	$< 1 \times 10^{-300}$	*27:02, *27:04, *27:05
Threonine (T)	1.12 (1.03–1.21)	4.50×10^{-3}	*13:02, *39:06, *40:06, *51:01, *51:08, *52:01, *55:01, *56:01
Arginine (R)	1.00 (Reference)	1	*15:01, *15:03, *15:10, *15:16, *15:17, *15:18, *18:01, *35:01, *35:02, *35:03, *35:08, *35:12, *37:01, *38:01, *38:02, *39:01, *39:10, *40:01, *41:01, *44:02, *44:03, *44:04, *44:05, *45:01, *47:01, *49:01, *50:01, *53:01, *58:01
Tryptophan (W)	1.00 (0.89–1.12)	0.95	*14:01, *14:02
Serine (S)	0.86 (0.81–0.91)	4.81×10^{-8}	*07:02, *07:05, *08:01, *15:07, *27:07, *40:02, *41:02, *48:01
Valine (V)	0.68 (0.59–0.78)	1.41×10^{-8}	*57:01, *57:03

Table 3

Association studies of *KIR* genes in ankylosing spondylitis.

Study	Sample numbers	Ethnicity	KIR genes typed	Main findings
Lopez-Larrea C, Blanco-Gelaz MA, Torre-Alonso JC, et al. *Arthritis Research & Therapy.* 2006;8(4)	71 AS patients, 105 controls (HLA-B27+ve) 55 AS patients, 75 controls (HLA-B27+ve)	Spanish Portuguese	*KIR3DL1, KIR3DS1*	*KIR3DL1* frequency decreased in patients (Spanish $P < 0.0001$; Azorean $P < 0.003$) *KIR3DS1* frequency increased in patients (Spanish $P < 0.0001$; Azorean $P < 0.003$)
Diaz-Pena R, Blanco-Gelaz MA, Suarez-Alvarez B, et al. *Human Immunology.* 2008;69(7):437–442.	42 AS patients, 30 controls (HLA-B27+ve) 30 AS patients, 16 controls (HLA-B27+ve)	Chinese Thai	*KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5, KIR3DS1, KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL5, KIR3DL1*	*KIR3DS1, KIR2DS5* and *KIR2DL5* frequencies increased in patients (Chinese $P < 0.005$, $P < 0.001$, $p < 0.01$ respectively; Thai $P < 0.05$, $P < 0.05$, $P < 0.05$ respectively) *KIR3DL1/KIR3DL1* frequency decreased in patients (Chinese $P < 0.05$; Thai $P < 0.05$) *KIR3DL1/KIR3DS1* frequency increased in patients (Chinese $P < 0.005$; That $P < 0.05$)
Jiao YL, Ma CY, Wang LC, et al. *Journal of Clinical Immunology.* 2008;28(4):343–349.	119 AS patients, 128 controls	Chinese	*KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1, KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR3DL1, KIR3DL2, KIR3DL3, KIR2DP1, KIR3DP1*	*KIR3DS1* and *KIR2DL5* frequencies increased in patients ($P = 0.016$ and $P = 0.003$ respectively) *HLA-Cw02* frequency increased in patients ($P = 0$) Genotype *HLA-Cw02/KIR2DS1* frequency increased in patients ($P = 0.011$)
Harvey D, Pointon JJ, Sleator C, et al. *Annals of the Rheumatic Diseases.* 2009;68(4):595–598.	200 AS patients, 405 controls (KIR typing) 368 AS patients, 366 controls (KIR3DL2 typing)	Caucasian	*KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1, KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR3DL1, KIR3DL2, KIR3DL3*	No significant difference between KIR gene or *KIR3DL2* allele frequencies between patients and controls.
Jiao YL, Zhang BC, You L, et al. *Journal of Clinical Immunology.* 2010;30(6):840–844.	115 AS patients, 119 controls (HLA-B27+ve)	Chinese	*KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1, KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR3DL1, KIR3DL2, KIR3DL3, KIR2DP1, KIR3DP1*	*KIR2DL1* and *KIR2DL5* frequencies increased in patients ($P = 0.012$ and $P = 0.009$ respectively) *HLA-Cw08* frequency increased in patients ($P = 0.001$)
Zvyagin IV, Mamedov IZ, Britanova OV, et al. *Cellular and Molecular Immunol.* 2010;7(6):471–476.	83 AS patients, 107 controls (HLA-B27+ve)	Russian and Caucasian	*KIR3DL1* (functional), *KIR3DL1*004* (nonfunctional), *KIR3DL1*005* (lowly expressed), *KIR3DL1*007* (lowly expressed) *KIR3DS1*	*KIR3DL1* frequency decreased in patients ($P < 0.01$) *KIR3DS1* frequency increased in patients ($P < 0.01$) *KIR3DL1/KIR3DL1* frequency decreased in patients ($P = 0.005$) *KIR3DL1/KIR3DS1* frequency increased in patients ($P = 0.01$) *KIR3DL1*F* (functional alleles) frequency decreased in patients ($P = 0.005$)

Study	Sample numbers	Ethnicity	KIR genes typed	Main findings
Mahmoudi M, Jamshidi AR, Karami J, et al. *Iranian Journal of Allergy, Asthma and Immunology.* 2016;15(1):27–38	200 AS patients, 200 controls	Iranian	*KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, KIR3DS1, KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4 KIR2DL5, KIR3DL1, KIR3DL2, KIR3DL3 KIR2DP1, KIR3DP1*	*KIR2DL3* frequency increased is patients ($P = 0.005$) *KIR2DL5* frequency decreased in patients ($P = 0.03$) HLA-C2 group and HLA-B27 frequency increased in patients *KIR2DL1$^+$/HLA-CW^{Lys+}, KIR2DL2$^-$/HLA-CW^{asp80-}, KIR2DL3$^+$/HLA-CW^{asp80-}, KIR2DS1$^+$/HLA-CW^{Lys+}, KIR2DS2$^-$/HLA-CW^{asp80-}* frequencies increased in patients ($P = 0.0009$, $P = 0.01$, $P = 0.0008$, $P = 0.009$, $P = 0.002$ respectively) *KIR2DL1$^+$/HLA-CW^{Lys-}, KIR2DL2$^+$/HLA-CW^{asp80+}, KIR2DL3$^-$/HLA-CW^{asp80+}, KIR3DL1$^+$/HLA-Bw4^{Thr+}, KIR2DS1$^-$/HLA-CW^{Lys-}, KIR2DS1$^+$/HLA-CW^{Lys-}, KIR2DS2$^+$/HLA-CW^{asp80+}* frequencies decreased in patients ($P = 0.00008$, $P = 0.01$, $P = 0.002$, $P = 0.07$, $P = 0.02$, $P = 0.006$, $P = 0.004$ respectively)