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REVIEW

Progress of genome-wide association studies of ankylosing spondylitis

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Ankylosing spondylitis (AS) is an immune-mediated arthritis which primarily affects the spine and sacroiliac joints. Significant progress has been made in discovery of genetic associations with AS by genome-wide association studies (GWAS) over past decade. These findings have uncovered novel pathways involved pathogenesis of the disease and have led to introduction of novel therapeutic treatments for AS. In this Review, we discuss the genetic variations associated with AS identified by GWAS, the major pathways revealed by these AS-associated variations and critical cell types involved in AS development.
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INTRODUCTION

Ankylosing spondylitis (AS) is a common, chronic, highly heritable, immune-mediated arthritis that affects primarily the spine and sacroiliac joints. It can also affect peripheral joints and extra-articular tissues including the eye, gut and skin. AS is the prototypic form of spondyloarthritis (SpA), a group of rheumatic disorders which share clinical, genetic and radiographic features, and which includes psoriatic arthritis (PsA), reactive arthritis (ReA), arthritis of inflammatory bowel disease (IBD), juvenile-onset spondyloarthritis and undifferentiated Spondyloarthritis (USpA). The diagnosis of AS is determined by clinical and radiographic features as defined by the modified New York (mNYAS) classification criteria.[1] This review focuses on mNYAS criteria based studies because multiple lines of evidence demonstrate that classification criteria recently designed to increase sensitivity in early disease[2] lack adequate specificity[3,4] and lead to marked increases in genetic heterogeneity.[5]

The prevalence of AS varies in different countries, being highly correlated with the frequency of the class I major histocompatibility complex (MHC) allele human leucocyte antigen *(HLA)-B*27*.[6] The prevalence of AS in European-descent populations is about 0.55%,[7] 0.2–0.54% in Han Chinese populations[8,9] and 0.074% in South Africa.[10] AS affects men more often than women and the ratio of men with AS to women is about 2–3:1,[11–13] while the ratio in Europe is 3.8:1 and in Asia is 2.3:1.[10] In addition, men are generally more likely to have a younger onset age and more often develop extra-articular features such as IBD and psoriasis.

GENETIC EPIDEMIOLOGY OF ANKYLOSING SPONDYLITIS

AS has been known to run strongly within families, for a long time. The risk ratio of the sibling or first-degree relative of an AS patient is >52 compared with the general population (non-related individuals) risk.[14–16] It was unclear whether the co-familiality was due to shared environment or genetic factors until the association of *HLA-B*27* allele with AS was discovered in the early 1970s.[17–19] The recurrence risk drops rapidly with increasing distance of relationship to the proband (monozygotic (MZ) twins 63%, first-degree relatives 8.2%, second-degree relatives 1.0% and third-degree relatives 0.7% in Europeans;[14] first-degree relatives 3.84%, 2nd degree relatives 0.87% and 3rd degree relatives 0.315% in Han Chinese).[20] The evidence suggests that AS is a polygenic rather than monogenic disease since the frequency of monogenic disease reduces about half with each increase in distance of relationship to the proband, while frequency in polygenetic disease reduces approximate the square root with each increase in distance of relationship to the proband. Also, the concordance rate in dizygotic twins (DZ, 12.5%), or even in *HLA-B*27* positive DZ twins (24–27%), is much lower than in MZ twins (63%), implying the presence of non-*HLA*B27* factors, either environmental factors or other non-*HLA-B*27* genes influencing disease susceptibility.[14,21]

The estimated heritability of AS by twin studies is $>90\%$.[21] The variants associated with AS from that study explain 27.82% of AS heritability, with the greatest contribution coming from the MHC (20.44%) and with 7.38% coming from non-MHC loci.[22,23] Disease activity (BASDAI, 51%),[24] functional impairment (BASFI, 68%),[24] radiographic change (62%),[25] and age of symptom onset,[26] all additionally show significant heritability in AS.

The co-existence of AS and IBD has been known for a long time.[27] Clinically diagnosed IBD presents in 5–10% of the AS patients, and 40–60% of AS patients have developed subclinical inflammation in gut and bowel.[28] Moreover, the risk ratios of IBD were 3.0 and 2.1 in first- and second-degree relatives of patients with AS compared with unrelated individuals, respectively.[15] These findings suggest that these two diseases may have similar aetiology, and multiple genes shared by

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these two diseases have been found.[22,29] Studying the heritability captured by the Immunochip SNP microarray, strong co-heritability was observed between AS and Crohn's disease (40% including and 39% excluding the MHC), ulcerative colitis (33 and 31%) and to a lesser but nonetheless significant extent with psoriasis (27 and 20%) and primary sclerosing cholangitis (23 and 20%).[22]

*HLA-B*27*

The association of AS with *HLA-B*27* was discovered in the early 1970s[17,18] and it is one of the strongest genetic associations with any common human disease. The prevalence of *HLA-B*27* varies in different ethnic groups and populations. The population prevalence of *HLA-B*27* is approximately 8% in British,[30] 4% in black Africans,[31] and 3.6–5.7% of Han Chinese.[8] In general, the population prevalence of AS parallels the frequency of *HLA-B*27* except in West Africans.[32] 80-95% of AS patients of European ancestry are *HLA-B*27* positive.[33] Despite the strong association between *HLA-B*27* and AS, only 2–5% of *HLA-B*27* positive individuals develop AS, suggesting that other factors such as other loci, environmental or stochastic factors also contribute considerably to AS development.[6,34] *HLA-B27* homozygosity moderately increases risk of AS compared with *HLA-B27* heterozygosity.[23,35,36]

The introduction of high-throughput *HLA* sequencing has revealed that *HLA-B*27* is remarkably polymorphic. To date, at least 271 *HLA-B*27* subtypes (*B*27:01-B27:164*) have been reported[37] and the number keeps increasing rapidly. Most are too rare to determine whether they are AS-associated or not, but two subtypes have been shown to have neutral associations with AS, *HLA-B*27:06* (a common subtype in south-east Asia),[38] and *HLA-B*27:09* (a rare subtype found primarily on Sardinia).[39] *HLA-B*27:05* is present in nearly all populations[40] and it is suggested to be the possible ancestral allele. *HLA-B*27:05* and *HLA-B*27:02* are the main subtypes associated with AS in Caucasians, in Asians the main associated subtypes are *HLA-B*27:04* and *HLA-B*27:07* and in Mediterranean populations is *HLA-B*27:02*.[41] *HLA-B*27* positive patients tend to develop AS earlier than *HLA-B*27*-negative patients.[35,42]

NON-*HLA-B*27* MHC ASSOCIATIONS

There are clearly other HLA alleles, and potentially MHC genes, associated with AS. *HLA-B*60* was the first non- *HLA-B*27* alleles identified to be associated with AS in *HLA-B*27* positive patients,[43] and was later confirmed in *HLA-B*27* positive UK cases.[44] In addition to *HLA-B*60, HLA-B*61* was identified to be associated with *HLA-B**27-negative Taiwan Chinese AS patients.[45] Association of *HLA-B*39* with *HLA-B*27* negative AS patients in Japanese was observed[46] in a small cohort, but was not replicated in European-descent cases.[44]

Though the associations of these *HLA-B* alleles with AS were identified, the complexity of MHC region makes it is extremely difficult to further study the associations between MHC loci and diseases until new technology and large study cohort are available. Immunochip is an Illumina Infinium SNP microarray with 195 806 SNPs and 718 small insertion–deletions on the array. The chip has dense coverage of the MHC and killer immunoglobulin-like receptor (*KIR*) loci.[47] Dissection of the associations of MHC with diseases has been greatly improved with the help of Immunochip. Other than these *HLA-B* alleles, *HLA-B*51:01*, *HLA-B*47:01, HLA-B*40:02, HLA-B*13:02* and *HLA-B*40:01* were identified to be associated with increasing risk of AS whilst *HLA-B*07:02* and *HLA-B*57:01* were found to be associated with reduced risk of AS in a study cohort with 9,069 AS cases and 13,578 population controls of European descent using Immunochip.[37]

Furthermore, non-*HLA*B* alleles, *HLA-A* (*HLA-A*02:01*), *HLA-DPB1* and *HLA-DRB1* were associated with AS after controlling for the associated *HLA-B* haplotypes in this study.[37] HLA-B51 is also a known risk factor for the spondyloarthritis-related disease, Behcet's disease, suggesting some shared pathogenic mechanisms with AS. *HLA-B*7* has been shown to be highly protective against AS in Chinese (odds ratio after control for *HLA-B*27* = 0.15).[48] Another study in the Korean population using Immunochip reported that *HLA-C*15:02* allele was associated with AS in addition to *HLA-B*27*.[49]

NON-MHC GENETIC ASSOCIATIONS

Enabled by the development of high-throughput chip-based genotyping and computational genetics from early 2000s, genetic studies were able 10 years to ago to move to a new era, from family linkage study to genome-wide association studies (GWAS). In this review we consider not only association studies using the GWAS chip but also on customized chips, such as the Illumina Immunochip.[47] Table 1 lists the published key AS GWAS/association studies since 2007. The first AS GWAS was conducted by the Wellcome Trust Case Control Consortium (WTCCC) and Australo-Anglo-American Spondylitis Consortium (TASC) in 2007, analysing 14 500 non-synonymous SNPs in 1,000 AS patients and 1500 healthy controls. This study made the first definitive identification of non-MHC susceptibility loci in AS, these being SNPs in endoplasmic reticulum aminopeptidase 1 (*ERAP1*) and interleukin 23 receptor (*IL23R*).[50]

A subsequent and comprehensive GWAS on a larger cohort of 2,053 AS patients and 5,140 controls of European ancestry was performed by TASC in 2010.[33] This study replicated the previous

Table 1 Major genome-wide association studies in AS

Study	*Year*	*Discovery cohort (case/control)*	*Coverage/variant types*	*Ethnicity*	*No. of non-MHC variants reaching genome-wide significance*
Burton *et al.* (WTCCC & TASC)[46]	2007	1000/1500	Non-synonymous variants only	European	2
Reveille *et al.* (TASC)[30]	2010	2053/5140	GWAS	European	4
Evans *et al.* (TASC)[47]	2011	3023/8779	GWAS	European	8
Lin *et al.*[49]	2011	1965/4301	GWAS	Han Chinese	3
Cortes *et al.* (IGAS)[20]	2013	10,619/15,145	Illumina Immunochip	European, East Asian and Latin American	24
Robinson *et al.*[48]	2016	5040/21,133	Illumina Exomechip	European	2
Ellinghaus *et al.*[19]	2016	8726/34,213	Illumina Immunochip	European and East Asian	113

Abbreviation: MHC, major histocompatibility complex.

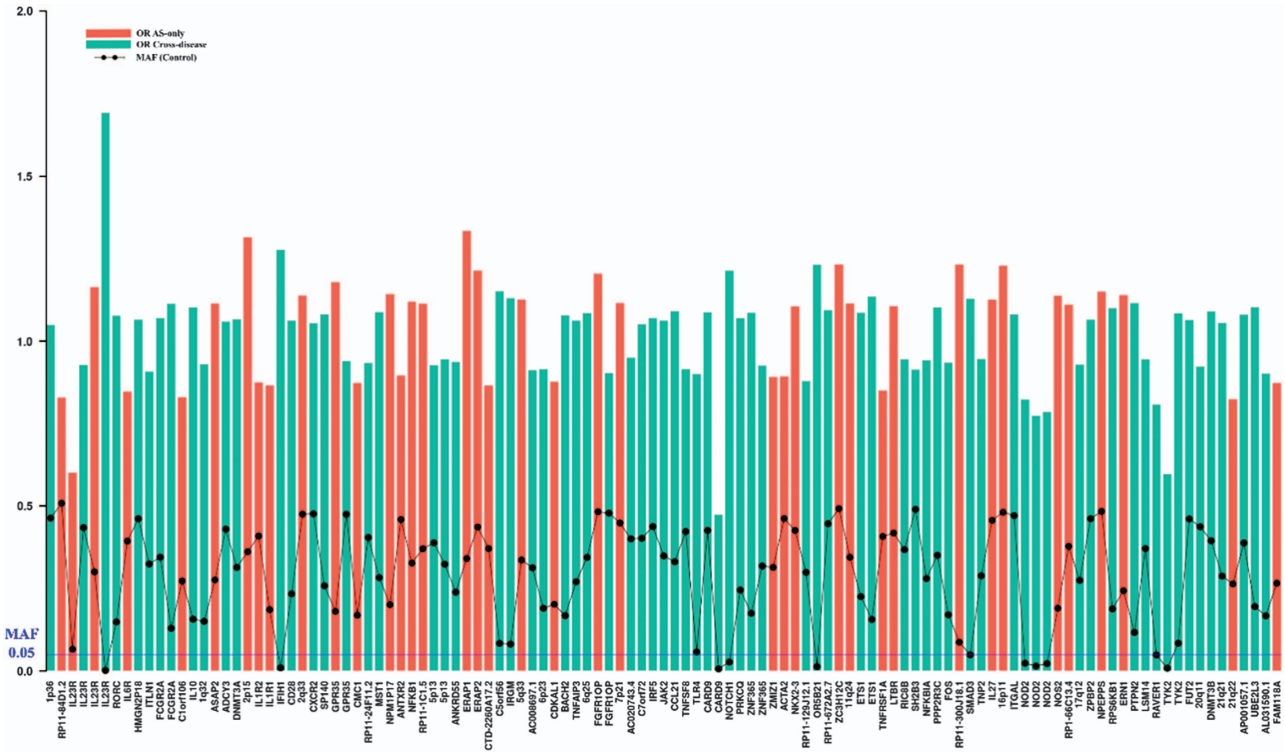

Figure 1 Odds ratios and minor allele frequencies in controls of 113 non-MHC AS-associated variants identified in Ellinghaus *et al.*[22] Each bar and dot represents a GWS variant. The variants are labelled by nearby genes. The red and green bars respectively represent odds ratio of AS-associated variants from AS-only case–control analyses, and analyses leveraging pleiotropy. Black dot represents minor allele frequency in healthy controls. The blue line indicates 5% minor allele frequency.

findings of *ERAP1* and *IL23R*. Moreover, additional loci, including Anthrax Toxin Receptor 2 (*ANTXR2*), Interleukin 1 Receptor Type 2 (*IL1R2*) and two intergenic regions on *2p15* and *21q22* were also found to be associated with AS at genome-wide significance. The association with intergenic regions suggests that they may harbour non-coding functional elements or be involved in regulating the expression of other genes. Further studies are needed to investigate their roles in AS pathogenesis.

In 2011, TASC and WCCC2 published the largest AS GWAS to date, involving a cohort of 3,023 AS patients and 9,141 health controls of European descent.[51] This study identified several new associations, with its larger cohort. For the first time, *RUNX3, LTBR-TNFRSF1A* and *IL12B* were reported to be associated with AS at genome-wide significance level. It also replicated the *HLA-B*27*, *ERAP1, IL23R, 2p15, 21q22* and *KIF21B* findings.

In 2013, International Genetics of Ankylosing Spondylitis Consortium (IGAS) conducted a case–control AS association study on Immunochip which has dense coverage in immune loci and MHC region. This cohort contains 10 619 AS cases and 15 145 controls in populations of European, East Asian and Latin American ancestry.[23] IGAS identified 13 new associations of AS with replication of 11 previous findings other than *HLA-B*27*. These new associations are with SNPs nearby or within *IL6R, FCGR2A, UBE2E3, GPR35, BACH2, ZMIZ1, NKX2-3, SH2B3, GPR65, SULT1A1, NOS2, TYK2* and *ICOSLG*.

In 2016 an AS association study using the Illumina Exomechip was conducted in a cohort of 5,040 AS patients and 21 133 healthy controls of European descent.[52] Technically, this is not a genome-wide study but an exome-wide study. Exomechip has dense coverage of low-frequency and rare variants in addition to common coding variants. Due to the limited power of detecting rare variants and incomplete coverage of rare variants, this study only identified two novel loci (*USP8* and *CDKAL1*) associated with AS at genome-wide significance and confirmed 11 known AS associations.[52]

More recently, the largest AS case–control association study on Immunochip identified 113 AS-associated genome-wide significant variants by combining case cohorts from five related diseases (Crohn's disease, psoriasis, primary sclerosing cholangitis and ulcerative colitis).[22] This study has 8,726 AS cases and 34 213 health controls, and another 43 536 cases in other four diseases on Immunochip. 39 out of these variants are GWS significant in AS-only cohort, while additional 74 non-MHC GWS variants were identified in pleiotropy analysis with other four diseases then confirmed as being independently AS-associated. The larger sample size of this study increased its power to detect loci in disease-specific analyse, and to an even greater extent in analyses of pleiotropic genetic effects. It identified 17 new loci in AS missed in the original Immunochip study simply through the use of a much larger number of controls. The new AS-associated loci are *ITLN1, CTLA4, CMC1, NPM1P17, NFKB1, CDKAL1, FGFR10P, 6p22, 7p21, ACTA2, 11q24, PPP2R3C, CORO1A, 16p11, ERN1, PTPN2* and *FAM118A* (for which suggestive association had already been identified in the AS Exomechip study). Odds ratios and minor allele frequencies in controls of 113 non-MHC variants identified in this study are illustrated in Figure 1. Given its large size, this study was sufficiently powered to identify several rare variants, including four variants with MAF $< 1\%$ and seven with MAF 1–5%. Notably, all the rare variants were identified by pleiotropic analysis with multiple diseases.

Thus far, most AS GWAS or case–control association studies have been performed in white European cohorts. The only GWAS yet

carried out in the Han Chinese population, reported in 2011, identified two novel genome-wide significant non-MHC loci (*2p15*, *5q14.3* and *12q12*), and replicated the known *HLA-B*27* and *2p15* loci.[53] The two novel non-MHC loci were not replicated in the IGAS Immunochip study (either in European descent or a large Chinese case–control analysis), nor the cross-disease study mentioned above, and are thus likely to be false positives.

There is clearly great need for a large GWAS to be performed in Han Chinese population to leverage the difference in genetic makeup of east-Asians to identify further genes and pinpoint AS-genetic associations. For example, it has been shown that the *IL23R* SNPs associated with AS in European descent populations are not associated with AS in Han Chinese.[54] Subsequent studies have shown that this is likely because the key European-associated variant, rs11209026, is not polymorphic in east Asians. Rather, a different variant (rs76418789, G149R) is AS-associated in east Asians.[55] This variant shows weak association in European descent populations too but that effect is difficult to see because of the strength of association of other variants at this locus, notably rs11209026 (R381Q).

There is significant gender bias in AS and women are generally more resistant to getting AS than men. In addition, differences in recurrent risk between the offspring of affected mothers and fathers have been observed. These findings imply loci of X-chromosome may contribute to disease susceptibility. Despite multiple AS GWAS having been performed on autosomes, there are only two AS association studies on X chromosome, each in small cohorts. Thus far, no association or linkage of the X-chromosome with AS have been found.[56,57] Therefore, the sex bias in AS hasn't been explained by X-chromosome–encoded genetic effects, although no formal high-density SNP analysis of this chromosome has yet been reported.

Identified AS susceptibility loci are involved in numerous immunomodulatory pathways affecting innate and adaptive immunity, such as antigen presentation and binding, TNF-α/NF-κB activation and signalling, IL-23R signalling, lymphocyte development and activation, IL-1 cluster genes, G-protein coupled receptor and IL-17/IL-22 mediated immunity. Though there are over 100 AS-associated loci that have been identified, the underlying mechanism by which they are involved remain unclear for most of these loci. There are extensive ongoing studies try to pinpoint the causative variants of AS, understand their roles in AS, and develop new therapeutic treatment for AS. Among these pathways, MHC class 1 presentation and IL-23 pathway are major pathways involved in AS development and also the best studied.

AMINOPEPTIDASE IN AS

ERAP1 is one of the three aminopeptidases (other two are *ERAP2* and *NPEPPS*) have been shown to be associated with AS. Association of *ERAP1* with AS was firstly identified by WTCCC in European Caucasian population in 2007.[50] It was a big leap in AS research since it is one of the two first AS-associated non-MHC genes. This association has been widely replicated different ethnics groups, including (not exclusively) Han Chinese,[53,54] Korean,[58] white European ancestry[59,60] and Iranian populations,[61] suggesting common *ERAP1* variants are involved in AS rather than diverse rare variants. A recent study investigated haplotypes generated from five common *ERAP1* SNPs—rs2287987 (M349V), rs30187 (K528R), rs10050860 (D575N), rs17482078 (R725Q) and rs27044 (Q730E), and further confirmed that the association of *ERAP1* with AS is primarily contributed by individual common *ERAP1* genotypes, rather than by haplotypes.[62]

ERAP1 operates in AS by acting as a molecular ruler, trimming peptides to optimal length (10-16 residues) for presentation by HLA class 1 molecules.[63] The main *ERAP1* variant rs30187 only shows association with AS when *HLA-B*27* or *HLA-B*40* is present.[37] Interaction between *ERAP1* and *HLA-B*27* in AS indicates that peptide trimming and presentation contribute to disease susceptibility. Multiple variants in *ERAP1* have been found to influence the risk of AS. The non-synonymous SNP rs30187 (K528R) is directly associated with AS.[51] An independent association is observed with rs10050860 (D575N), but this is in a very strong LD with a range of other SNPs and it is unclear if itself is directly disease associated.[51] According to ERAP1 enzyme structure studies, ERAP1 variants that are associated with AS are commonly located in the active site of enzyme, on the inner surface of the C-terminal cavity and junctions.[64] The SNP rs30187 is located at the junction domain and it could indirectly affect specificity or enzymatic activity by altering the conformational change between open and closed forms.[65] Indeed, the protective alleles of rs30187 and rs17482078 (R725Q) are reported to be associated with 40% reduction in enzyme activities of peptide trimming *in vitro* using recombinant ERAP1 protein, whereas rs10050860 showed no effect.[51]

These studies show that disease-associated coding variants in *ERAP1* are gain-of-function. The further molecular and immunological mechanisms by which these variants influence AS risk are the subject of active research and have been reviewed elsewhere. It is also clear that *ERAP1* expression is strongly affected by genetic variation at the locus, something first reported by Myriad Genetics[66] and subsequently confirmed by others.[67,68] Thus it is likely that the genetic variants influencing AS risk at this locus do so by a combination of effects on *ERAP1* expression and function.

Apart from *ERAP1*, polymorphisms of *ERAP2* are also found to be associated with AS in multiple studies.[50,69] Particularly, the protective allele (G) of rs2248374 affects the exon 10 splicing site and produces a truncated mRNA. This truncated mRNA is degraded leading to a complete absence of ERAP2. Unlike *ERAP1*, *ERAP2* plays a role in both HLA-B27 positive and negative AS.[70] Though further studies are needed to understand precise mechanism of how *ERAP1* and *ERAP2* affect AS status, this finding together with the protective effects of rs30187 implies that inhibiting these proteins may be potential treatment for AS.

NPEPPS encodes puromycin-sensitive aminopeptidase or alanine aminopeptidase, which is expressed in the cytosol. NPEPPS knock-down has been shown to influence cell surface HLA Class I expression, either increasing or decreasing cell surface expression depending on the HLA Class I antigen involved, and indirectly, through studies of HLA Class I complex stability, to influence the peptide pool available for presentation.[71]

IL-23 PATHWAY

IL23R was first reported to have association with AS in 2007 and this finding revealed the involvement of IL-23 pathway in AS pathogenesis.[50] It encodes the receptor for a proinflammatory cytokine IL-23 which plays important roles in activation of various proinflammatory cells, including T_h17 T cells, gamma-delta T cells, NK cells, mast cells, Paneth cells and others.

Multiple variants involved with IL-23 pathway genes, such as *TYK2, JAK2, IL12B, IL6R, IL27, PTGER4* and *CARD9* have also been found to be associated with AS.[23,72,73] TYK2 is a member of Janus kinases (JAKs) protein families and it plays important roles in transducing a range of signals, including IL-23, IL-10, IL-6, IFN-α and -β and IL-12 signals.[74] *JAK2* encodes the component of JAK-STAT signalling pathway, which is the downstream pathway of IL-23R. *IL12B* encodes

IL-12p40, one of the subunits of IL-23 heterodimer. CARD9 mediates the downstream signalling of Dectin-1 which occurs in response particularly to fungal stimulation, leading to expression of pro-inflammatory cytokines.[75,76] As the receptor for IL-6, IL-6R is involved in the IL-6 signalling pathway which affects T helper (T_h)17 cell differentiation amongst other things. Activation of Prostaglandin E2 receptor 4 (EP4), the protein product of *PTGER4* promotes development of T_h17 cells by decreasing IL-12p70 and increases IL-23 expression level.[77] IL-27 is in the same IL-12 family with IL-23. It has widespread immunological effects, including promotion of T_h1 differentiation, and inhibition of T_h2 cell and T_h17 differentiation.[78] Its expression is increased in response to LPS interacting with TLR4, which is itself AS-associated, and by prostaglandin E2 (reviewed in Wang *et al.*).[79]

Multiple genes in this pathway show pleiotropic effect in different diseases. For example, *IL23R* is known to be associated with AS-related diseases including IBD[80] and psoriasis.[81] Variants in *TYK2* are associated with AS, IBD, psoriasis, type 1 diabetes, multiple sclerosis and rheumatoid arthritis.[82] Multiple genes related with *IL23R* pathway are shared between IBD and AS, including *IL23R, IL12B, TYK2, JAK2, IL27* and *CARD9,* highlighting that the two diseases probably share similar aetiology through IL-23R pathway.[29]

IL-23 was reported to have higher expression level in the terminal ileum of AS patients and CD patients[83] and mouse model studies have revealed that overexpression of IL-23 alone can cause spondyloarthritis.[53,84] Sherlock *et al* identified a population of IL-23-responsive cells from entheseal expressed IL-17 and IL-22 that developed in response to the IL-23 treatment.[84] Clinical paw swelling scores were reduced by inhibiting IL-17 or IL-22, especially by inhibiting both. Over-expression of IL-22 caused disease but overexpression of IL-17 did not. Other animal models of AS, both rat and mouse, have also been shown to be significantly affected by IL-23 pathway targeted interventions.[85] Clinical trials of therapeutic treatments targeting IL-23 pathway shows promising results, with secukinumab being widely used now in treatment of AS, and multiple other agents in development and trial. It will be very interesting to see in humans the differential effect of targeting IL-23, IL-22 and IL-17, which is hard to predict from genetic studies alone.

CRITICAL CELL TYPES INVOLVED IN AS

Similar to other complex trait associated polymorphisms, the vast majority of AS GWS variants lie within intergenic regions and therefore raise a great challenge to unravel the mechanisms through which they operate. For instance, AS-associated loci *2p15* and *21q22* are located in intergenic regions which makes it is difficult to pinpoint the associated genes, let alone to elucidate the underlying biological function and involved pathways. Nevertheless, recent studies have indicated that disease-associated variants often contribute through effects on gene regulatory regions in specific cell types or tissues rather than directly modifying the protein function, such as regulating gene expression level by expression quantitative trait loci (eQTL) effect or by altering the accessibility of chromatin.[72,86] Though the DNA sequence is the same among all the cells and tissues, the regulatory effect of a variant could be different between cell types and tissues. In addition, with the rapidly increasing availability of epigenetic and gene expression data in hundreds of cell types or tissue types, disease-associated variants can be further investigated in the cell types which they primarily operate.

Various cell types have been suggested to be involved in AS, including $CD4^+$, $CD8^+$ and regulatory T cells,[87] T_h17 cell,[88] natural killer (NK) cells,[89] gamma-delta cells,[90] innate lymphoid cells (particularly ILC3 subset),[91] B cells,[92] mast cells[93] and intestinal Paneth cells.[83] These studies indirectly reveal cell types which AS-associated variants predominantly function. However, understandably they have mainly focused on circulating immune cells and tissues that are easily accessed.

A role for NK and CD8 T-cells has been suggested both by evidence that these KIR3DL2 bearing cells of these types interact with HLA-B27 dimers leading to IL-17 production.[89] Further, AS is associated with *TBX21* which encodes T-bet, a transcription factor with effects on differentiation and function of multiple immunological cell types. NK and CD8 T-cells expressing T-bet are increased in AS cases, risk variants of *TBX21* lead to increased expression in AS cases, and *TBX21* knockout mouse are resistant to the development of spondyloarthritis and IBD in the Skg mouse model.[94]

To identify the key cell types involved in the mechanism by which AS-associated genes influence its aetiopathogenesis, we recently integrated AS-genetic and cell-type specific data of epigenomes, transcriptomes and proteomes in a wide range of cell types.[95] This analysis suggested that AS-associated variants primarily operate through effects in $CD8^+$ T cells, $CD4^+$ T cells, NK cells, regulatory T cells, monocytes, as well as gastrointestinal cells. These findings are consistent with a previous study which performed a similar epigenetic analysis with predicted causal AS SNPs.[96,97] In addition, AS-associated variants were shown to be enriched in regulatory elements, especially in enhancer regions, in related immune cells. Moreover, the AS upregulated genes show different enrichment bias in both gene and protein expression compared with AS downregulated genes. The AS upregulated genes are significantly enriched in monocytes, whereas AS downregulated genes showed enrichment in $CD8^+$ T cell and NK cells in mRNA and protein expression.

The strong co-familiality between AS and IBD, the shared susceptibility loci, especially IL-23 pathway genes, suggest that gut microbial is involved in AS development. Differences in microbial signature in the terminal ileum of AS patients compared with healthy individuals have been demonstrated.[98] Our finding that the genes specifically enriched in gut cells are also enriched in 'response to bacterium' and other similar Gene Ontologies further supports that AS status is influenced by the interaction among the host genetics, the intestinal microbiome and the immune response. Over-expression of the anti-microbial peptides alpha-defensin, phospholipase A2 and lysozyme by Paneth cells in human AS cases provides further support for the involvement of these cells and the gut mucosal immune system in AS pathogenesis.[99] Bone cell types showed relative weak enrichment signals, consistent with the hypothesis that bone changes in AS are secondary to inflammatory processes rather than driven by bone-specific genetic effects. However, data on more AS-relevant tissues and cell types, such as enthesial and synovial cell populations, innate lymphoid cells and Paneth cells, is needed to identify more AS-relevant cell types and to further investigate the specific role of AS-associated variants in these cell types.

CHALLENGES

The two main remaining challenges in immunogenetic research in AS are to identify the variants responsible for the large proportion of heritability of the disease that remains unexplained, and determining the functional mechanisms underpinning those genetic associations. The sample size of GWAS that have been performed to date are relatively modest in comparison with the far larger studies that have been funded in other major immune-mediated diseases that have similar or lower disease prevalence, such as type 1 diabetes, IBD, and multiple sclerosis.[100] Further large scale GWAS are clearly indicated.

In addition to discovering more genetic variants associations with AS, another challenge is fine mapping the casual variants through the associated signals. About 70% of the AS-associated SNPs are located in non-coding regions, making them difficult to be interpreted via traditional protein coding theory. Furthermore, these variants may function in specific cell types or tissues. Therefore, integrating cell-type specific multi-omics data, such as transcriptomic, epigenetic and proteomics, is necessary.

Besides genetic factors, only limited studies of epigenetic factors have been reported in AS.[95,101] Larger more systematic studies controlling for the profound effects of age, cigarette smoking and tissue type are required to properly investigate the role of epigenetic factors and AS, and also how genetic factors operate through epigenetic mechanisms to influence disease.

Whilst the main goal of genetic studies in AS is clearly to inform research ultimately leading to therapeutic or preventative approaches for the disease, given the high heritability of AS and its clinical manifestations, it is possible that genetic risk prediction may prove valuable in the disease. Genetic risk scores have been shown to have high discriminatory capacity between AS and healthy controls.[5] Further development of these approaches using whole of GWAS data may prove even more informative given their ability to capture a higher proportion of disease heritability than analyses restricted to genome-wide significant loci.[102] This may prove useful in identifying people either at high risk of disease to enable preventative approaches, or to distinguish that set of patients with suggestive early disease who truly have axial spondyloarthritis rather than non-inflammatory causes of back pain.[103] Given the evidence that delay in implementing effective therapy is linked with worse outcome in AS,[104] reducing the current substantial diagnostic delay in the disease would be a valuable contribution.

CONCLUSIONS

GWAS is a hypothesis-free approach for testing the associations between hundreds of thousands of variants and phenotype. To date, at least 113 non-MHC variants have been identified as well as *HLA-B*27* and these findings provide a wealth of new genetic information for AS, critical clues on biological pathways of development of AS and contributed to the development of new treatments. As mentioned above, at least several treatments targeting genes in IL-23R pathway, which was first uncovered in WTCCC GWAS, have been approved in AS or under clinical trials. In addition to genetic studies, functional studies of genetic variants in AS-relevant cell types will be necessary for understanding AS pathogenesis and developing novel treatments.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheum* 1984; **27**: 361–368.
2 Rudwaleit M, Landewe R, van der Heijde D, Listing J, Brandt J, Braun J *et al.* The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part I): classification of paper patients by expert opinion including uncertainty appraisal. *Ann Rheum Dis* 2009; **68**: 770–776.
3 Akkoc N, Khan MA. Looking into the new ASAS classification criteria for axial spondyloarthritis through the other side of the glass. *Curr Rheumatol Rep* 2015; **17**: 515.
4 Robinson PC, Wordsworth BP, Reveille JD, Brown MA. Axial spondyloarthritis: a new disease entity, not necessarily early ankylosing spondylitis. *Ann Rheum Dis* 2013; **72**: 162–164.
5 Thomas GP, Willner D, Robinson PC, Cortes A, Duan R, Rudwaleit M *et al.* Genetic diagnostic profiling in axial spondyloarthritis: a real world study. *Clin Exp Rheumatol* 2017; **35**: 229–233.
6 Braun J, Bollow M, Remlinger G, Eggens U, Rudwaleit M, Distler A *et al.* Prevalence of spondylarthropathies in HLA-B27 positive and negative blood donors. *Arthritis Rheum* 1998; **41**: 58–67.
7 Akkoc N, Khan MA. Overestimation of the prevalence of ankylosing spondylitis in the Berlin study: comment on the article by Braun *et al. Arthritis Rheum* 2005; **52**: 4048–4049 author reply 4049–4050.
8 Zeng QY, Chen R, Darmawan J, Xiao ZY, Chen SB, Wigley R *et al.* Rheumatic diseases in China. *Arthritis Res Ther* 2008; **10**: R17.
9 Ng SC, Liao Z, Yu DT, Chan ES, Zhao L, Gu J. Epidemiology of spondyloarthritis in the People's Republic of China: review of the literature and commentary. *Semin Arthritis Rheum* 2007; **37**: 39–47.
10 Dean LE, Jones GT, MacDonald AG, Downham C, Sturrock RD, Macfarlane GJ. Global prevalence of ankylosing spondylitis. *Rheumatology (Oxford)* 2014; **53**: 650–657.
11 Lee W, Reveille JD, Davis JC Jr., Learch TJ, Ward MM, Weisman MH. Are there gender differences in severity of ankylosing spondylitis? Results from the PSOAS cohort. *Ann Rheum Dis* 2007; **66**: 633–638.
12 Boonen A, van der Heijde D, Landewe R, Guillemin F, Spoorenberg A, Schouten H *et al.* Costs of ankylosing spondylitis in three European countries: the patient's perspective. *Ann Rheum Dis* 2003; **62**: 741–747.
13 Masi AT, Wilkins WR. Does male:female sex ratio in ankylosing spondylitis change with age? *J Rheumatol* 1996; **23**: 947–948.
14 Brown MA, Laval SH, Brophy S, Calin A. Recurrence risk modelling of the genetic susceptibility to ankylosing spondylitis. *Ann Rheum Dis* 2000; **59**: 883–886.
15 Thjodleifsson B, Geirsson AJ, Bjornsson S, Bjarnason I. A common genetic background for inflammatory bowel disease and ankylosing spondylitis: a genealogic study in Iceland. *Arthritis Rheum* 2007; **56**: 2633–2639.
16 Geirsson AJ, Kristjansson K, Gudbjornsson B. A strong familiality of ankylosing spondylitis through several generations. *Ann Rheum Dis* 2010; **69**: 1346–1348.
17 Caffrey MF, James DC. Human lymphocyte antigen association in ankylosing spondylitis. *Nature* 1973; **242**: 121.
18 Brewerton DA, Hart FD, Nicholls A, Caffrey M, James DC, Sturrock RD. Ankylosing spondylitis and HL-A 27. *Lancet* 1973; **1**: 904–907.
19 Schlosstein L, Terasaki PI, Bluestone R, Pearson CM. High association of an HL-A antigen, W27, with ankylosing spondylitis. *N Engl J Med* 1973; **288**: 704–706.
20 ZM L The Genetic Risk and Genome-wide Linkage and Association Study. 2010.
21 Brown MA, Kennedy LG, MacGregor AJ, Darke C, Duncan E, Shatford JL *et al.* Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. *Arthritis Rheum* 1997; **40**: 1823–1828.
22 Ellinghaus D, Jostins L, Spain SL, Cortes A, Bethune J, Han B *et al.* Analysis of five chronic inflammatory diseases identifies 27 new associations and highlights disease-specific patterns at shared loci. *Nat Genet* 2016; **48**: 510–518.
23 International Genetics of Ankylosing Spondylitis C, Cortes A, Hadler J, Pointon JP, Robinson PC, Karaderi T *et al.* Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci. *Nat Genet* 2013; **45**: 730–738.
24 Hamersma J, Cardon LR, Bradbury L, Brophy S, van der Horst-Bruinsma I, Calin A *et al.* Is disease severity in ankylosing spondylitis genetically determined? *Arthritis Rheum* 2001; **44**: 1396–1400.
25 Brophy S, Hickey S, Menon A, Taylor G, Bradbury L, Hamersma J *et al.* Concordance of disease severity among family members with ankylosing spondylitis? *J Rheumatol* 2004; **31**: 1775–1778.
26 Brown MA, Brophy S, Bradbury L, Hamersma J, Timms A, Laval S *et al.* Identification of major loci controlling clinical manifestations of ankylosing spondylitis. *Arthritis Rheum* 2003; **48**: 2234–2239.
27 Mielants H, Veys EM, Goemaere S, Goethals K, Cuvelier C, De Vos M. Gut inflammation in the spondyloarthropathies: clinical, radiologic, biologic and genetic features in relation to the type of histology. A prospective study. *J Rheumatol* 1991; **18**: 1542–1551.
28 Crane AM, Bradbury L, van Heel DA, McGovern DP, Brophy S, Rubin L *et al.* Role of NOD2 variants in spondylarthritis. *Arthritis Rheum* 2002; **46**: 1629–1633.
29 Parkes M, Cortes A, van Heel DA, Brown MA. Genetic insights into common pathways and complex relationships among immune-mediated diseases. *Nat Rev Genet* 2013; **14**: 661–673.
30 Brown MA, Pile KD, Kennedy LG, Calin A, Darke C, Bell J *et al.* HLA class I associations of ankylosing spondylitis in the white population in the United Kingdom. *Ann Rheum Dis* 1996; **55**: 268–270.
31 Baum J, Ziff M. The rarity of ankylosing spondylitis in the black race. *Arthritis Rheum* 1971; **14**: 12–18.
32 Brown MA, Jepson A, Young A, Whittle HC, Greenwood BM, Wordsworth BP. Ankylosing spondylitis in West Africans–evidence for a non-HLA-B27 protective effect. *Ann Rheum Dis* 1997; **56**: 68–70.
33 Australo-Anglo-American Spondyloarthritis C, Reveille JD, Sims AM, Danoy P, Evans DM, Leo P *et al.* Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. *Nat Genet* 2010; **42**: 123–127.

34 Reveille JD, Arnett FC. Spondyloarthritis: update on pathogenesis and management. *Am J Med* 2005; **118**: 592–603.
35 Jaakkola E, Herzberg I, Laiho K, Barnardo MC, Pointon JJ, Kauppi M *et al.* Finnish HLA studies confirm the increased risk conferred by HLA-B27 homozygosity in ankylosing spondylitis. *Ann Rheum Dis* 2006; **65**: 775–780.
36 Khan MA, Kushner I, Braun WE, Zachary AA, Steinberg AG. HLA–B27 homozygosity in ankylosing spondylitis: relationship to risk and severity. *Tissue Antigens* 1978; **11**: 434–438.
37 Cortes A, Pulit SL, Leo PJ, Pointon JJ, Robinson PC, Weisman MH *et al.* Major histocompatibility complex associations of ankylosing spondylitis are complex and involve further epistasis with ERAP1. *Nat Commun* 2015; **6**: 7146.
38 Nasution AR, Mardjuadi A, Kunmartini S, Suryadhana NG, Setyohadi B, Sudarsono D *et al.* HLA-B27 subtypes positively and negatively associated with spondyloarthropathy. *J Rheumatol* 1997; **24**: 1111–1114.
39 D'Amato M, Fiorillo MT, Carcassi C, Mathieu A, Zuccarelli A, Bitti PP *et al.* Relevance of residue 116 of HLA-B27 in determining susceptibility to ankylosing spondylitis. *Eur J Immunol* 1995; **25**: 3199–3201.
40 Sheehan NJ. HLA-B27: what's new? *Rheumatology (Oxford)* 2010; **49**: 621–631.
41 Taurog JD. The mystery of HLA-B27: if it isn't one thing, it's another. *Arthritis Rheum* 2007; **56**: 2478–2481.
42 Feldtkeller E, Khan MA, van der Heijde D, van der Linden S, Braun J. Age at disease onset and diagnosis delay in HLA-B27 negative vs. positive patients with ankylosing spondylitis. *Rheumatol Int* 2003; **23**: 61–66.
43 Robinson WP, van der Linden SM, Khan MA, Rentsch HU, Cats A, Russell A *et al.* HLA-Bw60 increases susceptibility to ankylosing spondylitis in HLA-B27+ patients. *Arthritis Rheum* 1989; **32**: 1135–1141.
44 Brown M, Bunce M, Calin A, Darke C, Wordsworth P. HLA-B associations of HLA-B27 negative ankylosing spondylitis: comment on the article by Yamaguchi *et al. Arthritis Rheum* 1996; **39**: 1768–1769.
45 Wei JC, Tsai WC, Lin HS, Tsai CY, Chou CT. HLA-B60 and B61 are strongly associated with ankylosing spondylitis in HLA-B27-negative Taiwan Chinese patients. *Rheumatology (Oxford)* 2004; **43**: 839–842.
46 Yamaguchi A, Tsuchiya N, Mitsui H, Shiota M, Ogawa A, Tokunaga K *et al.* Association of HLA-B39 with HLA-B27-negative ankylosing spondylitis and pauciarticular juvenile rheumatoid arthritis in Japanese patients. Evidence for a role of the peptide-anchoring B pocket. *Arthritis Rheum* 1995; **38**: 1672–1677.
47 Cortes A, Brown MA. Promise and pitfalls of the Immunochip. *Arthritis Res Ther* 2011; **13**: 101.
48 Yi L, Wang J, Guo X, Espitia MG, Chen E, Assassi S *et al.* Profiling of HLA-B alleles for association studies with ankylosing spondylitis in the Chinese population. *Open Rheumatol J* 2013; **7**: 51–54.
49 Kim K, Bang SY, Lee S, Lee HS, Shim SC, Kang YM *et al.* An HLA-C amino-acid variant in addition to HLA-B*27 confers risk for ankylosing spondylitis in the Korean population. *Arthritis Res Ther* 2015; **17**: 342.
50 Wellcome Trust Case Control C Australo-Anglo-American Spondylitis C, Burton PR, Clayton DG, Cardon LR, Craddock N *et al.* Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nat Genet* 2007; **39**: 1329–1337.
51 Evans DM, Spencer CC, Pointon JJ, Su Z, Harvey D, Kochan G *et al.* Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. *Nat Genet* 2011; **43**: 761–767.
52 Robinson PC, Leo PJ, Pointon JJ, Harris J, Cremin K, Bradbury LA *et al.* Exome-wide study of ankylosing spondylitis demonstrates additional shared genetic background with inflammatory bowel disease. *npj Genomic Med* 2016; **1**: 16008.
53 Adamopoulos IE, Tessmer M, Chao CC, Adda S, Gorman D, Petro M *et al.* IL-23 is critical for induction of arthritis, osteoclast formation, and maintenance of bone mass. *J Immunol* 2011; **187**: 951–959.
54 Davidson SI, Wu X, Liu Y, Wei M, Danoy PA, Thomas G *et al.* Association of ERAP1, but not IL23R, with ankylosing spondylitis in a Han Chinese population. *Arthritis Rheum* 2009; **60**: 3263–3268.
55 Davidson SI, Jiang L, Cortes A, Wu X, Glazov EA, Donskoi M *et al.* Brief report: high-throughput sequencing of IL23R reveals a low-frequency, nonsynonymous single-nucleotide polymorphism that is associated with ankylosing spondylitis in a Han Chinese population. *Arthritis Rheum* 2013; **65**: 1747–1752.
56 Hoyle E, Laval SH, Calin A, Wordsworth BP, Brown MA. The X-chromosome and susceptibility to ankylosing spondylitis. *Arthritis Rheum* 2000; **43**: 1353–1355.
57 Zhang G, Luo J, Bruckel J, Weisman MA, Schumacher HR, Khan MA *et al.* Genetic studies in familial ankylosing spondylitis susceptibility. *Arthritis Rheum* 2004; **50**: 2246–2254.
58 Bang SY, Kim TH, Lee B, Kwon E, Choi SH, Lee KS *et al.* Genetic studies of ankylosing spondylitis in Koreans confirm associations with ERAP1 and 2p15 reported in white patients. *J Rheumatol* 2011; **38**: 322–324.
59 Maksymowych WP, Inman RD, Gladman DD, Reeve JP, Pope A, Rahman P. Association of a specific ERAP1/ARTS1 haplotype with disease susceptibility in ankylosing spondylitis. *Arthritis Rheum* 2009; **60**: 1317–1323.
60 Pimentel-Santos FM, Ligeiro D, Matos M, Mourao AF, Sousa E, Pinto P *et al.* Association of IL23R and ERAP1 genes with ankylosing spondylitis in a Portuguese population. *Clin Exp Rheumatol* 2009; **27**: 800–806.
61 Mahmoudi M, Jamshidi AR, Amirzargar AA, Farhadi E, Nourijelyani K, Fallahi S *et al.* Association between endoplasmic reticulum aminopeptidase-1 (ERAP-1) and susceptibility to ankylosing spondylitis in Iran. *Iran J Allergy Asthma Immunol* 2012; **11**: 294–300.
62 Roberts AR, Appleton LH, Cortes A, Vecellio M, Lau J, Watts L *et al.* ERAP1 association with ankylosing spondylitis is attributable to common genotypes rather than rare haplotype combinations. *Proc Natl Acad Sci USA* 2017; **114**: 558–561.
63 Chang SC, Momburg F, Bhutani N, Goldberg AL. The ER aminopeptidase, ERAP1, trims precursors to lengths of MHC class I peptides by a 'molecular ruler' mechanism. *Proc Natl Acad Sci USA* 2005; **102**: 17107–17112.
64 Nguyen TT, Chang SC, Evnouchidou I, York IA, Zikos C, Rock KL *et al.* Structural basis for antigenic peptide precursor processing by the endoplasmic reticulum aminopeptidase ERAP1. *Nat Struct Mol Biol* 2011; **18**: 604–U118.
65 Kochan G, Krojer T, Harvey D, Fischer R, Chen L, Vollmar M *et al.* Crystal structures of the endoplasmic reticulum aminopeptidase-1 (ERAP1) reveal the molecular basis for N-terminal peptide trimming. *Proc Natl Acad Sci USA* 2011; **108**: 7745–7750.
66 Inc MG. *Gene Variants and Use Thereof*, In: USPTO (ed.) 2007.
67 Harvey D, Pointon JJ, Evans DM, Karaderi T, Farrar C, Appleton LH *et al.* Investigating the genetic association between ERAP1 and ankylosing spondylitis. *Hum Mol Genet* 2009; **18**: 4204–4212.
68 Costantino F, Talpin A, Evnouchidou I, Kadi A, Leboime A, Said-Nahal R *et al.* ERAP1 gene expression is influenced by nonsynonymous polymorphisms associated with predisposition to spondyloarthritis. *Arthritis Rheumatol* 2015; **67**: 1525–1534.
69 Tsui FW, Haroon N, Reveille JD, Rahman P, Chiu B, Tsui HW *et al.* Association of an ERAP1 ERAP2 haplotype with familial ankylosing spondylitis. *Ann Rheum Dis* 2010; **69**: 733–736.
70 Robinson PC, Costello ME, Leo P, Bradbury LA, Hollis K, Cortes A *et al.* ERAP2 is associated with ankylosing spondylitis in HLA-B27-positive and HLA-B27-negative patients. *Ann Rheum Dis* 2015; **74**: 1627–1629.
71 Kim E, Kwak H, Ahn K. Cytosolic aminopeptidases influence MHC class I-mediated antigen presentation in an allele-dependent manner. *J Immunol* 2009; **183**: 7379–7387.
72 Edwards SL, Beesley J, French JD, Dunning AM. Beyond GWASs: illuminating the dark road from association to function. *Am J Hum Genet* 2013; **93**: 779–797.
73 Davidson SI, Liu Y, Danoy PA, Wu X, Thomas GP, Jiang L *et al.* Association of STAT3 and TNFRSF1A with ankylosing spondylitis in Han Chinese. *Ann Rheum Dis* 2011; **70**: 289–292.
74 Dendrou CA, Cortes A, Shipman L, Evans HG, Attfield KE, Jostins L *et al.* Resolving TYK2 locus genotype-to-phenotype differences in autoimmunity. *Sci Transl Med* 2016; **8**: 363ra149.
75 LeibundGut-Landmann S, Gross O, Robinson MJ, Osorio F, Slack EC, Tsoni SV *et al.* Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nat Immunol* 2007; **8**: 630–638.
76 Gross O, Gewies A, Finger K, Schafer M, Sparwasser T, Peschel C *et al.* Card9 controls a non-TLR signalling pathway for innate anti-fungal immunity. *Nature* 2006; **442**: 651–656.
77 Boniface K, Bak-Jensen KS, Li Y, Blumenschein WM, McGeachy MJ, McClanahan TK *et al.* Prostaglandin E2 regulates Th17 cell differentiation and function through cyclic AMP and EP2/EP4 receptor signaling. *J Exp Med* 2009; **206**: 535–548.
78 Yoshimura T, Takeda A, Hamano S, Miyazaki Y, Kinjyo I, Ishibashi T *et al.* Two-sided roles of IL-27: induction of Th1 differentiation on naive CD4+ T cells versus suppression of proinflammatory cytokine production including IL-23-induced IL-17 on activated CD4+ T cells partially through STAT3-dependent mechanism. *J Immunol* 2006; **177**: 5377–5385.
79 Wang Q, Liu J. Regulation and Immune Function of IL-27. *Adv Exp Med Biol* 2016; **941**: 191–211.
80 Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ *et al.* A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 2006; **314**: 1461–1463.
81 Cargill M, Schrodi SJ, Chang M, Garcia VE, Brandon R, Callis KP *et al.* A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. *Am J Hum Genet* 2007; **80**: 273–290.
82 Diogo D, Bastarache L, Liao KP, Graham RR, Fulton RS, Greenberg JD *et al.* TYK2 protein-coding variants protect against rheumatoid arthritis and autoimmunity, with no evidence of major pleiotropic effects on non-autoimmune complex traits. *PLoS ONE* 2015; **10**: e0122271.
83 Ciccia F, Bombardieri M, Principato A, Giardina A, Tripodo C, Porcasi R *et al.* Overexpression of interleukin-23, but not interleukin-17, as an immunologic signature of subclinical intestinal inflammation in ankylosing spondylitis. *Arthritis Rheum* 2009; **60**: 955–965.
84 Sherlock JP, Joyce-Shaikh B, Turner SP, Chao CC, Sathe M, Grein J *et al.* IL-23 induces spondyloarthropathy by acting on ROR-gammat+ CD3+CD4-CD8- entheseal resident T cells. *Nat Med* 2012; **18**: 1069–1076.
85 Benham H, Rehaume LM, Hasnain SZ, Velasco J, Baillet AC, Ruutu M *et al.* Interleukin-23 mediates the intestinal response to microbial beta-1,3-glucan and the development of spondyloarthritis pathology in SKG mice. *Arthritis Rheumatol* 2014; **66**: 1755–1767.
86 Franke A, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T *et al.* Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nat Genet* 2010; **42**: 1118–1125.
87 Szalay B, Meszaros G, Cseh A, Acs L, Deak M, Kovacs L *et al.* Adaptive immunity in ankylosing spondylitis: phenotype and functional alterations of T-cells before and during infliximab therapy. *Clin Dev Immunol* 2012; **2012**: 808724.
88 Shen H, Goodall JC, Hill Gaston JS. Frequency and phenotype of peripheral blood Th17 cells in ankylosing spondylitis and rheumatoid arthritis. *Arthritis Rheum* 2009; **60**: 1647–1656.

89 Chan AT, Kollnberger SD, Wedderburn LR, Bowness P. Expansion and enhanced survival of natural killer cells expressing the killer immunoglobulin-like receptor KIR3DL2 in spondylarthritis. *Arthritis Rheum* 2005; **52**: 3586–3595.
90 Kenna TJ, Davidson SI, Duan R, Bradbury LA, McFarlane J, Smith M *et al.* Enrichment of circulating interleukin-17-secreting interleukin-23 receptor-positive gamma/delta T cells in patients with active ankylosing spondylitis. *Arthritis Rheum* 2012; **64**: 1420–1429.
91 Ciccia F, Guggino G, Rizzo A, Saieva L, Peralta S, Giardina A *et al.* Type 3 innate lymphoid cells producing IL-17 and IL-22 are expanded in the gut, in the peripheral blood, synovial fluid and bone marrow of patients with ankylosing spondylitis. *Ann Rheum Dis* 2015; **74**: 1739–1747.
92 Niu XY, Zhang HY, Liu YJ, Zhao D, Shan YX, Jiang YF. Peripheral B-cell activation and exhaustion markers in patients with ankylosing spondylitis. *Life Sci* 2013; **93**: 687–692.
93 Noordenbos T, Yeremenko N, Gofita I, van de Sande M, Tak PP, Canete JD *et al.* Interleukin-17-positive mast cells contribute to synovial inflammation in spondylarthritis. *Arthritis Rheum* 2012; **64**: 99–109.
94 Lau MC, Keith P, Costello ME, Bradbury LA, Hollis KA, Thomas R *et al.* Genetic association of ankylosing spondylitis with TBX21 influences T-bet and pro-inflammatory cytokine expression in humans and SKG mice as a model of spondyloarthritis. *Ann Rheum Dis* 2017; **76**: 261–269.
95 Hao J, Liu Y, Xu J, Wang W, Wen Y, He A *et al.* Genome-wide DNA methylation profile analysis identifies differentially methylated loci associated with ankylosis spondylitis. *Arthritis Res Ther* 2017; **19**: 177.
96 Farh KK, Marson A, Zhu J, Kleinewietfeld M, Housley WJ, Beik S *et al.* Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature* 2015; **518**: 337–343.
97 Uddin M, Codner D, Hasan SM, Scherer SW, O'Rielly DD, Rahman P. Integrated genomics identifies convergence of ankylosing spondylitis with global immune mediated disease pathways. *Sci Rep* 2015; **5**: 10314.
98 Costello ME, Ciccia F, Willner D, Warrington N, Robinson PC, Gardiner B *et al.* Brief Report: Intestinal Dysbiosis in Ankylosing Spondylitis. *Arthritis Rheumatol* 2015; **67**: 686–691.
99 Ciccia F, Bombardieri M, Rizzo A, Principato A, Giardina AR, Raiata F *et al.* Over-expression of paneth cell-derived anti-microbial peptides in the gut of patients with ankylosing spondylitis and subclinical intestinal inflammation. *Rheumatology (Oxford)* 2010; **49**: 2076–2083.
100 Visscher PM, Wray NR, Zhang Q, Sklar P, McCarthy MI, Brown MA *et al.* 10 Years of GWAS Discovery: Biology, Function, and Translation. *Am J Hum Genet* 2017; **101**: 5–22.
101 Lai NS, Chou JL, Chen GCW, Liu SQ, Lu MC, Chan MWY. Association between cytokines and methylation of SOCS-1 in serum of patients with ankylosing spondylitis. *Mol Biol Rep* 2014; **41**: 3773–3780.
102 Speed D, Balding DJ. MultiBLUP: improved SNP-based prediction for complex traits. *Genome Res* 2014; **24**: 1550–1557.
103 van der Linden S, Akkoc N, Brown MA, Robinson PC, Khan MA. The ASAS criteria for axial spondyloarthritis: strengths, weaknesses, and proposals for a way forward. *Curr Rheumatol Rep* 2015; **17**: 62.
104 Haroon N, Inman RD, Learch TJ, Weisman MH, Lee M, Rahbar MH *et al.* The impact of tumor necrosis factor alpha inhibitors on radiographic progression in ankylosing spondylitis. *Arthritis Rheum* 2013; **65**: 2645–2654.

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