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REVIEW

The genomics and genetics of ankylosing spondylitis

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Abstract: The spondyloarthropathies are a group of arthritides which specifically target the spine and pelvis with ankylosing spondylitis (AS) being the most prevalent and debilitating of these conditions. Unique to AS is the progression to excessive uncontrolled bone formation following an initial inflammatory phase that can result in joint fusion and significant disability. Spondyloarthritis is estimated to affect 1%–2% of the population, twice as many as rheumatoid arthritis and thus constitutes a significant health problem. Currently AS pathogenesis is very poorly understood but recent large-scale genetics and gene expression profiling studies have identified some of the underlying mechanisms and pathways contributing to the disease. Genome-wide association studies have identified a number of candidate genes associated with AS sharing the same pathways which are now being targeted for therapeutic intervention. However, although such approaches can identify genes contributing to the disease process and are very informative as to disease aetiopathogenesis, they cannot profile the actual changes in gene/cell activity at any point in the disease process or possibly more importantly at specific sites. Such information is generated using expression profiling. A number of expression profiling studies have been undertaken in AS, looking at both circulating cells and tissues from affected joints. Although some common genes/pathways have been identified, overall the results to date have been somewhat disappointing due to differences in experimental design and tissue source as well as the low power of the studies. More recent better powered studies have shown some potential in developing gene expression profiling as a diagnostic tool in AS. True future success will rely on larger genetic and genomic studies and the combination of these datasets in eQTL studies requiring significant collaborative efforts. Such larger-scale approaches will also generate sufficient power to target specific disease stages and sites.

Keywords: ankylosing spondylitis, genomewide association studies, gene expression, microarrays, spondylarthritis, inflammatory arthritis

Introduction

The spondyloarthropathies are estimated to affect 1%–2% of the population almost two-fold higher prevalence than rheumatoid arthritis (RA).[1] AS is the prototypic and most prevalent spondyloarthropathy and affects between 0.1%–1.4% of the global population. The condition primarily affects the spine and sacroiliac joints of the pelvis, causing pain and stiffness and eventual fusion. Asymmetric peripheral joint arthritis (~20%) and episodic acute anterior uveitis (~40%) are also common and approximately 10% of AS cases also have either inflammatory bowel disease (IBD) or psoriasis, while approximately 70% have sub-clinical gut inflammation. The disease onset is typically in the second decade of life and thus the disease has a higher impact relative to later onset diseases, such as rheumatoid arthritis, due to its longer disease duration.

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Clinical features

The clinical features of AS can be divided into axial and peripheral components. Axial features include spondylitis, sacroiliitis and skeletal complications. Peripheral features include enthesitis, peripheral arthritis, inflammatory bowel disease (IBD) and anterior uveitis.

Axial features

Axial disease most commonly presents as inflammatory lower back pain and morning stiffness.[2] Morning stiffness lasts from one to several hours and is improved by exercise but not relieved by rest (as in the case of mechanical back pain).[3] Lower back pain results from inflammation of the SI joints and the vertebrae. Sacroiliitis, inflammation of the SI joint, is the most common feature of AS. Sacroiliitis can be detected by conventional x-ray of the pelvis which shows joint space narrowing and eventual sclerosis of the joint (Figure 1).

A distinguishing feature of AS is the site of the initial inflammation. This occurs at the entheses, the sites where tendons or ligaments join bone resulting in enthesitis one of the earliest features of AS (Figure 2). The stooped posture characteristic of AS patients is caused by inflammation of the spine and formation of syndesmophytes, bone growths originating from the initial sites of inflammation. In the spine of AS patients syndesmophytes form in the intervertebral joints and lead to fusion of the vertebrae which sometimes results in a classical ‘bamboo spine’ appearance. Fusion of bone across intervertebral joints ultimately causes flattening of the lumbar spine and limits neck motion and chest expansion. Paradoxically for a disease that induces new bone formation AS patients frequently exhibit reduced bone mineral density leading to osteoporosis driven by the high levels of inflammatory molecules associated with the disease.

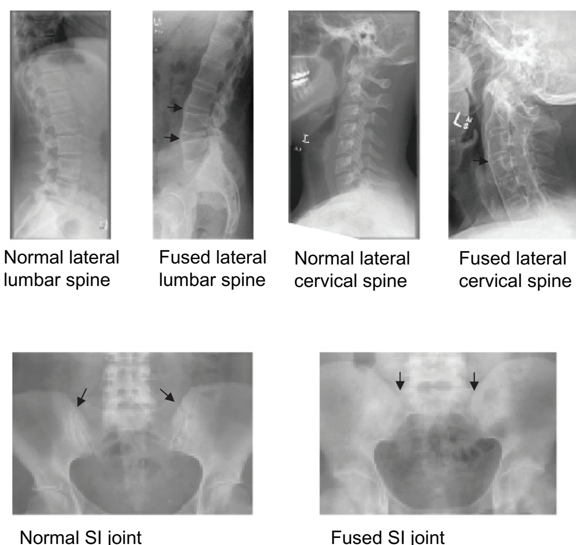

Figure 1 Axial inflammation in AS. In normal lumbar and cervical spine x-rays clear delineation of the intervertebral spaces is evident. However in severely affected fused spinal x-rays extensive bridging across the vertebrae is evident (arrows). In the sacroiliac (SI) joints in normal subjects the joint space is clearly visible (arrows) but in severely affected cases, fusion is evident with blurring of the joint space (arrows). Images courtesy of Linda Bradbury at the University of Queensland Diamantina Institute.

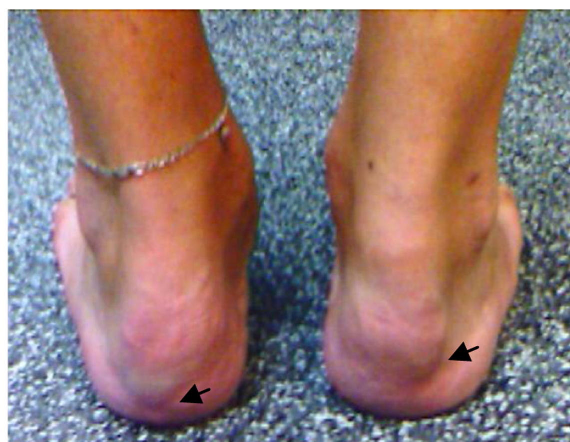

Figure 2 Enthesitis of the Achilles tendon.
Note: Arrows indicate severe inflammation of the Achilles tendon (enthesitis). Image courtesy of Linda Bradbury at the University of Queensland Diamantina Institute.

Peripheral features

Arthritis of peripheral joints including hips, shoulders, knees, wrists, ankles and elbows is common in AS.[4] Radiographically the peripheral arthritis in AS is similar to that observed in rheumatoid arthritis (RA). However, in AS additional ankylosis of wrists, hips, tarsal joints and small joints in the fingers and toes is common. Peripheral joint arthritis is usually asymmetric.[5]

Extra-articular features

IBD

A close relationship exists between gut inflammation and AS. Approximately two-thirds of AS cases display signs of gut inflammation unrelated to clinical gastrointestinal symptoms, and AS cases and their first-degree relatives have increased gut permeability.[6] As will be discussed in more detail later many genetic associations are shared between AS and both Crohn’s disease and ulcerative colitis, including *IL-23R, STAT3, CARD9, IL12B, PTGER4*, and *KIF21B*.[7] These findings suggest common pathogenic processes shared between IBD and AS. Reactive arthritis, an AS- related spondyloarthritis, can result from acute gastrointestinal infections, and these findings suggest that the gut is a major source of antigenic drive in AS.

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However the association between gut and joint disease is not fully understood. Activated T cells expressing members of the β7 family of integrins, which are involved in homing of intestinal lymphocytes, are enriched in the synovium of Spondyloarthropathy patients suggesting an intestinal origin to these cells.[8]

Anterior uveitis

Acute anterior uveitis (AAU) affects approximately 40% of patients with AS and approximately half of all AS patients with AU are B27+.[9] AAU is normally unilateral and characterised by red, sore eyes, increased tear production and photophobia. AAU does not precede axial or peripheral inflammation but is often the first clinical sign presented to doctors by patients who may have lived with lower back pain for several years.

Cardio vascular disease

Recently it has been reported that AS patients are at increased risk for many cardiovascular disorders compared with the general population.[10] The cardiovascular diseases include aortic valvular heart disease, non-aortic valvular heart disease, ischemic heart disease and congestive heart failure. Risk of developing these cardiovascular diseases is greatest in younger AS patients.[10] The reasons for increased risk of cardiovascular events in AS patients are unclear. While it is difficult to determine the contribution of disease to mortality rates a recent study compared mortality among almost 700 AS patients and age, sex and locality-matched controls and found increased mortality in the AS group with circulatory disease the most frequent cause of death among the AS cases.[11]

Aetiology and pathology

There is a strong genetic component in the risk of developing AS. Studies in twins have estimated heritability of AS at >90%.[12] HLA-B27 is established as the major genetic risk factor and the association between HLA-B27 and AS is among the strongest genetic association with any common disease. The genetic aetiology of AS will be discussed in greater detail below. Males are more frequently affected than females at a ratio of 2.5:1. The reasons for this imbalance are unclear.

The initial trigger for AS is currently unknown. With few exceptions, AS has a worldwide distribution, indicating that whatever the trigger it must be ubiquitous. Furthermore, epidemics of AS have not been reported. Studies in the B27 transgenic rat model of AS support the idea of a ubiquitous environmental trigger; B27 transgenic rats exposed to normal commensal bacteria develop disease while rats maintained in germ-free conditions do not.[13] It has been suggested that this ubiquitous environmental factor may be a common microbial pathogen.[14–16] A genetic defect reducing the immune system's ability to clear the pathogen could result in a low-level sub-clinical infection which may result in the autoimmune response seen through the pathogen presenting self-antigens or the pathogen's antigens mimicking self-antigens. Immune cells express various molecules capable of sensing a wide variety of microbial peptides. These include toll-like receptors (TLR), Dectin-1, PAMPs and DAMPs. Defects in recognition of microbial products by immune cells may contribute to an inflammatory cascade, driven by inflammatory cytokines such as IL-23, IL-1 and IL-6. The relative contributions of innate and adaptive immunity to the pathogenesis of AS is still unclear. Understanding the precise role of different immune cell types during different stages of disease and at different sites of disease is important for understanding how to develop novel targeted therapies. Recent studies in our lab have demonstrated that over 25% of γδ T cells in the peripheral blood of AS patients with active disease express IL-23R, secrete large amounts of IL-17 and display a strong Th17 bias.[17] This data suggests an important role for γδ T cells in pathogenesis of AS. Given that γδ T cells bridge the innate-adaptive systems expressing myriad activation receptors such as Dectin-1, Toll-like receptors and NKG2D as well as IL-23R and IL-1, they are attractive candidate cells for further investigation in the pathogenesis of AS.

AS has two phases, an initial inflammatory phase followed by an osteoproliferative/ankylosing phase where the joint fusion is initiated and progresses. The inflammatory phase has similarities with other inflammatory arthrites such as RA with high levels of pro-inflammatory cytokine production and joint damage through osteoclast activity. However, the extent of joint destruction though the cytokine/osteoclast axis is significantly less than in RA with the dominant joint impact stemming from ankylosis occurring as a result of osteoproliferation. The most common site of this excessive bone formation in AS is in the cervical and lumbar vertebrae resulting in squaring of the vertebrae and eventually formation of syndesmophytes from the vertebral corners which can eventually bridge leading to ankylosis. Whether the initial inflammation directly links to the subsequent osteoproliferative stages is subject to significant debate. There is little evidence for a direct link between the two with some evidence that they are temporally distinct.[18,19]

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This is supported by studies of the MRI evidence of inflammation and the subsequent development of syndesmophytes. These show that whilst there is an increased rate of syndesmophytes formation at vertebral corners with preceding MRI evidence of inflammation, most syndesmophytes arise at corners with no prior evidence of inflammation, and most corners with evidence of inflammation do not go on to develop syndesmophytes.[20,21] These studies are consistent with models in which inflammation precedes bone formation but in which the link between inflammation and bone formation is not as direct as, for example, the link between inflammation and erosion in RA.[22,23] There are therefore two key triggers in AS that are currently unknown; the signal for disease initiation, and the switch from inflammation to the damaging osteoproliferation phase. Elucidation of these triggers would be a major advance and identify new therapeutic approaches.

Treatment

The goal of treatment within AS is two-fold: to reduce pain and disability and to prevent progression of disease. Current therapies have provided symptom relief and reduced disability but have shown no efficacy in preventing or even slowing progression of disease. A key issue is the difficulty in diagnosing AS. Currently, firm diagnosis of AS requires confirmation of joint deterioration by X-ray (Modified New York Criteria),[24] which only occurs in quite advanced disease, leading to an average delay between onset of symptoms and diagnosis of 8–11 years. Early diagnosis of AS would allow treatment to be commenced before significant joint damage has occurred possibly increasing the potential to slow disease progress. Trials investigating the effectiveness of this approach are currently underway.

Unlike patients with rheumatoid arthritis, AS patients respond well to physical therapy and long periods of immobility increase pain and immobility. Supervised group physiotherapy shows best symptom relief compared with group exercise or home exercise programs.[25]

Conventional anti-rheumatic drugs such as methotrexate and sulfazalazine are ineffective in treatment axial disease in AS. However, they show some efficacy in management of peripheral and extra-articular features including psoriasis and uveitis.[26,27] Non-steroidal anti-inflammatory drugs (NSAIDS) are the first line of medication for treatment of pain and stiffness in AS. NSAIDS such as naproxen provide some symptom relief and have been shown to significantly reduce inflammatory markers like c-reactive protein in blood of AS patients.[28] However, long term NSAID treatment has the potential for gastrointestinal and cardiovascular toxicity.

The most effective symptom relief is obtained through use of the TNF-antagonist family of medications. Anti-TNF therapy provides substantial symptom relief and has effects on many of the axial, peripheral and extra-articular features of AS.[29–32] However, while anti-TNF therapy reduces inflammation and prevents further joint destruction[33] it neither prevents nor slows bone formation.[34–36] Side effects of long-term anti-TNF therapy include increased susceptibility to tuberculosis infection due to systemic immune suppression.[37]

Since no treatment to date has been shown to have an effect on preventing or slowing the natural course of disease there is a very great need for research to determine what triggers disease and to better understand the inflammatory pathways involved in AS, thereby providing novel therapeutics options. Recent advance in the genetics and genomics of AS have highlighted several important pathways involved in AS. We review here some of those advances and highlight some of the functional consequences of the genetic associations with AS. Further genetic and gene expression studies can generate diagnostic algorithms to aid in early disease detection and improved treatment regimes.

AS genetics

As stated above, AS is a largely genetically controlled disease. Familial aggregation indicating the presence of shared susceptibility factors has been long observed,[38] and studies of disease concordance in twins and families have shown that disease susceptibility is largely controlled by genetic factors.[12,39] Twin studies have shown concordance to be 75% (6/8) in monozygotic twins, 27% (4/15) for dizygotic twins sharing HLA-B27 and 12.5% (4/32) for dizygotic twins overall.[12] AS is highly heritable, with additive genetic factors estimated to account for >90% of susceptibility.[12] Age of onset (40%) and disease severity, as measured by the widely accepted BASDAI (51%) and BASFI (76%) questionnaires, are also highly heritable.[40,41] Below we discuss some of the more established loci contributing to this heritability.

MHC

The most significant AS-associated genetic locus is the major histocompatibility complex (MHC) on chromosome 6p. This genetic association is predominantly due to the association of AS with HLA-B27, however there is evidence for association with other MHC alleles.

HLA-B27

The association of HLA-B27 has been recognised since the early 1970s,[42–44] and is among the strongest genetic

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associations with a common disease. HLA-B27 is carried by approximately 8% of white Europeans, compared with 3% to 5% of Han Chinese,[45] and is rare in African populations (which accounts for the low incidence of AS in Africans).[46] Around 80% to 95% of AS patients are HLA-B27 positive, yet only 1% to 5% of HLA-B27 carriers develop AS,[47–50] therefore screening has not been useful as a sole diagnostic.

There are over 40 different allelic variants of HLA-B27 reported, all of which are ancestrally related to the B*2705 variant.[51] 95% of white British HLA-B27 carriers have this subtype.[51] while Chinese populations mainly have a mixture of B*2704 and B*2705.[45] Of the many HLA-B27 subtypes identified, the majority are rare and their influence on AS has not been investigated.

Although the association between HLA-B27 and AS has been known for many years, the mechanism of disease association is still unclear. The "arthritogenic peptide" theory focuses on the canonical functions of HLA-B27 in antigen presentation to cytotoxic T-lymphocytes.[52–56] The hypothesis suggests that after pathogenic insult, HLA-B27 presents a microbial epitope and elicits a CTL response against it. However, some of the CTLs may cross-react with any self antigens showing molecular mimicry with the microbial epitope. The reaction of CD8+ CTLs with self antigen would result in autoimmune tissue injury and inflammation. Association of *ERAP1*, the gene encoding the protein endoplasmic reticulum aminopeptidase 1 (ERAP1), with AS is only seen in HLA-B27 positive cases. This suggests that in HLA-B27- positive disease aberrant peptide trimming or presentation by ERAP1 and HLA-B27 are involved in AS pathogenesis,[57] and this will be further discussed later.

Non-canonical theories involve properties of HLA-B27 itself. These include the "misfolded protein" hypothesis, in which the slow folding of the HLA-B27 heavy chain causes misfolded proteins that accumulate in the endoplasmic reticulum in the form of covalent homodimers and multimers.[58,59] This accumulation of misfolded proteins causes ER stress, which results in inflammation through the activation of the unfolded protein response (UPR) and the ER-overload response (EOR).[60]

An extension of the misfolding hypothesis is the HLA-B27 surface homodimer hypothesis.[61] Covalently linked HLA-B27 homodimers have been found to be expressed on the cell surface, however they have not been secreted to the cell surface from the ER.[62–64] Signalling through these non-canonical forms of HLA-B27 may play an immunomodulatory role and upset the normal development of HLA class-I responses, with unusual signalling causing excessive pro-inflammatory cytokine release. Additionally, HLA-B27 homodimer expressing APCs have been shown to stimulate the survival, proliferation and IL-17 production of Th17 cells that express the NK-receptor KIR3DL2 in AS patients.[65] This observation provides a link between HLA-B27 and the IL-23 signalling pathway, which will be discussed later.

Other MHC genes

As well as the overwhelming association with HLA-B27, AS also shows association with other genes in the MHC. The MHC class I gene HLA-B60 has been shown to be associated with AS in a UK population.[66] HLA-B60 shows association with both B27-positive and negative AS, and this association has been replicated in a Chinese population.[67]

The MHC class II gene HLA-DRB1 has been found to be associated with AS in B27 matched case control studies[68] and twin studies.[12] A study of non-B27 MHC associations of AS using B27-matched haplotypes in cases and controls has shown that HLA-DRB1 is associated with AS irrespective of whether the haplotype contained HLA-B27.[69] This may contribute to disease by causing aberrant presentation to or selection of CD4+ lymphocytes.

Non-MHC genes

Very little progress was made in the identification of genes associated with AS in the years following the discovery of the HLA-B27 association. A number of linkage studies were performed, identifying association with the interleukin-1 (IL-1) gene complex and *CYP2D6*. There have been several reports of IL-1 complex association with AS,[70–72] including reported association in a Chinese population.[73] The IL-1 gene complex includes genes encoding the pro-inflammatory cytokines IL-1α, IL-1β and their naturally occurring inhibitor IL-1 receptor agonist (IL-1RA). IL-1 is involved in the induction of chemokine and adhesion molecule expression in various cells types, and variation within the IL-1 cluster may contribute to AS pathogenesis through altered inflammation.

Association with AS has also been found for *CYP2D6*, the gene that encodes cytochrome P450 debrisoquine 4-hydroxylase.[48,74] It is possible that CYP2D6 is involved in the metabolism of a ubiquitous environmental factor involved in AS, and that reduced enzymatic activity could lead to increased disease susceptibility, however this association has not been seen in more recent studies.

In recent years there have been a number of studies in which non-MHC genes have been reported as being associated with AS, most of which have been undertaken in populations

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of white-European heritage with confirmed disease according to the Modified New York Criteria. The first study to convincingly identify non-MHC associations with AS was a study of 14,500 non-synonymous SNPs performed by the Wellcome Trust Case Control Consortium (WTCCC) and the Australo-Anglo-American Spondyloarthritis Consortium (TASC), in which associations with *ERAP1* and *IL23R* were identified[75] in a cohort of 1000 AS patients and 1500 healthy controls. The first genome-wide association study (GWAS) in AS was performed by TASC, using Illumina HumHap370 genotyping chips in a cohort of 2500 AS patients and 5000 healthy controls, with replication in a cohort of 900 cases and 1500 controls. This study identified the genes *IL1R2* and *ANTXR2* as being associated with AS, as well as gene deserts at chromosomes 2p15 and 21q22.[76] This was followed by a second GWAS performed by TASC and the Wellcome Trust Case Control Consortium 2 (WTCCC2),using Illumina 660W-Quad and Human 1.2M-Duo chips in a cohort of 3000 AS patients and 4800 controls, with replication in a cohort of 2100 cases and 4500 controls. Association was found with genes including *RUNX3*, *LTBR-TNFRSF1 A* and *IL12B*.[57] In addition to this there have been a number of smaller studies performed in a variety of populations. Table 1 summarizes the genes confirmed to be associated with AS with genome-wide significance from the GWAS performed to date.

Class I presentation associated genes – *ERAP1* and *RUNX3*

ERAP1 was first reported to be associated with AS in the WTCCC/TASC nsSNP study,[75] and this association has been confirmed both the TASC[76] and WTCCC2/TASC GWAS,[57] as well as in a number of different ethnicities including Chinese,[77,78] Portuguese,[79] Korean[80] and Hungarian.[81] *ERAP1* has also been shown to be associated with psoriasis in both white European[82] and Chinese[83] populations.

There are two known roles of ERAP1, both of which may explain the association with AS. ERAP1 is involved in the trimming of peptides within the endoplasmic reticulum to optimal length for MHC class I presentation.[84,85] The strong association with HLA-B27 indicates that AS is primarily an MHC class I mediated disease, but the way B27 increases disease susceptibility is currently unknown. If the association of ERAP1 with AS relates to peptide presentation, this may help explain the association with HLA-B27 (Figure 3). The second function of ERAP1 is the cleavage of cell surface receptors. ERAP1 has been shown to cleave the cell surface receptors for the pro-inflammatory cytokines IL-1 (IL-1R2),[86] IL-6 (IL-6Rα)[87] and TNF (TNFRSF1A),[88] downregulating their signalling. Interestingly, both *IL1R2*[76] and *TNFRSF1A*[57] have been shown to be associated with AS. Genetic variants altering the receptor shedding function of ERAP1 could potentially have pro- or anti-inflammatory effects through this mechanism, however studies in mice have shown no difference in receptor shedding between *Erap1*$^{-/-}$ and C57BL/6 control mice.[57]

The primary AS-associated SNP in *ERAP1* is rs30187, which is an nsSNP encoding the protective p.Lys528Arg amino acid change. There is also an independent secondary association observed with the nsSNPs rs17482078 (p.Arg725Gln) and rs10050860 (p.Asp575 Asn), however these two variants are in complete linkage disequilibrium (LD) and cannot be split genetically.[57] The crystal structure of *ERAP1* has recently been published,[57,89] and has revealed the location of these altered residues around the substrate binding and regulatory sites of ERAP1. Peptide trimming assays performed using recombinant ERAP1 in vitro have shown that the protective rs30187 and rs17482078 variants had ~40% slower rates of trimming than wild-type,[57] suggesting that they are both loss-of-function variants. It has also been shown that while wild-type ERAP1 is able to trim peptides to 8- or 9-mers, the rs30187 variant stops at 11-mers.[90]

Gene-gene interaction between HLA-B27 and *ERAP1* has been observed, with *ERAP1* association only observed with AS in HLA-B27 positive individuals.[57] This restriction in association supports the models in which abnormal peptide trimming or presentation by *ERAP1* and HLA-B27 contribute to HLA-B27 disease. This suggests that inhibition of ERAP1 may be effective in HLA-B27 positive AS.

Table 1 Confirmed candidate genes associated with AS with genomewide significance identified through GWAS studies[1,2]

Chr	SNP rsID	Putative gene of interest	*P* value
1p31	rs11209026	*IL23R*	2.3×10^{-17}
1q32	rs2297909	*KIF21B*	5.2×10^{-12}
1p36	rs11249215	*RUNX3*	9.2×10^{-11}
2q11.2	rs2310173	*IL1R2*	4.8×10^{-7}
2p15	rs10865331	–	6.5×10^{-34}
4q21	rs4389526	*ANTXR2*	9.4×10^{-8}
5q15	rs30187	*ERAP1*	1.8×10^{-27}
5q33	rs6556416	*IL12B*	1.9×10^{-8}
5p13	rs10440635	*PTGER4*	2.6×10^{-7}
6p21	rs4349859	*HLA-B*	$<10^{-200}$
9q34	rs10781500	*CARD9*	1.1×10^{-6}
12p13	rs11616188	*TNFRSF1A*	4.1×10^{-12}
17q21	rs8070463	*TBKBP1*	5.3×10^{-8}
21q22	rs378108	–	2.1×10^{-11}

Abbreviation: GWAS, genome-wide association study.

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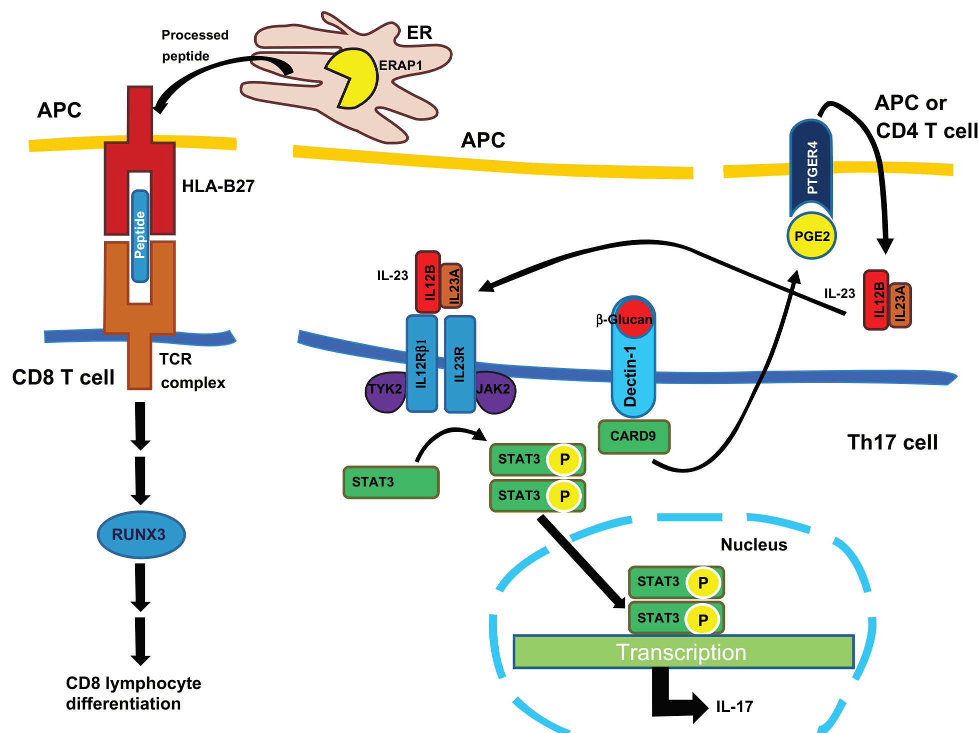

Figure 3 Possible functional roles of AS-associated genes. Differentiation and function of T-cells is driven by antigen presenting cells, with IL-23 production being a key driver of Th17 cell function. β-glucan stimulation of the dectin-1 receptor signals through CARD9, which can drive expression of prostaglandin E2 (PGE2), which stimulates IL-23 production through it's receptor prostaglandin E receptor 4 (subtype EP4) (PTGER4). IL-23 signals through the IL-23 receptor (IL23R), with signal transduction proceeding through JAK2, TYK2 and STAT3. Th17 activity can be further regulated by IL-1β, for which IL-1R2 acts as a high affinity decoy receptor. Another possible contributor to AS susceptibility is antigen presentation in the endoplasmic reticulum (ER), where ERAP1 trims antigens for peptide presentation by MHC class I molecules, such as HLA-B27. Altered antigen processing could alter antigen presentation, which could have an effect on RUNX3 mediated CD8 lymphocyte differentiation.
Abbreviations: APC, antigen processing cells; TCR, T-cell receptor.

RUNX3 is a key gene involved in CD8 lymphocyte differentiation. This gene was identified as being associated with AS in the TASC/WTCCC2 GWAS,[57] and this association supports the hypothesis that HLA-B27 contributes to AS pathogenesis through a mechanism involving peptide presentation to CD8 lymphocytes. It has been shown that AS cases have reduced CD8 lymphocyte counts, as do healthy individuals who carry the AS-associated SNPs.[57] These findings suggest that CD8 lymphocytes are involved in AS pathogenesis, likely in response to presentation of antigenic peptides by HLA-B27 after antigen processing by ERAP1.

IL-23 signalling pathway genes – *IL23R, IL12B, STAT3*

IL23R encodes the receptor for the cytokine interleukin 23 (IL-23), which is a key regulatory cytokine essential for the differentiation of Th17 cells (Figure 3). The association of *IL23R* suggests that Th17 cells are involved in the pathogenesis of AS in white European populations, as well as other *IL23R* associated diseases. *IL23R* has been found to be associated with a number of diseases in White European populations, including Crohn's disease,[91,92] psoriasis,[93] and psoriatic arthritis.[94] These diseases are all closely clinically related members of the seronegative group of autoimmune diseases, and this suggests that *IL23R* may partially explain their coexistence.

The association of *IL23R* was first reported in the 14,500 nsSNP study[75] and has been subsequently confirmed by two GWAS,[57,76] as well as in Portuguese[79] and Spanish[95] populations. Studies in Han Chinese[77,78] and Koreans[96] have shown no association with *IL23R*, and in these studies the primary associated nsSNP in white Europeans, rs11209026, was observed to be non-polymorphic. *IL23R* has also been shown to be not associated with Crohn's disease in a Japanese population, in which rs11209026 was also not polymorphic.[97] However, it is possible that there are rare AS-associated variants of *IL23R* in East Asian populations, and we have next-generation sequencing data that suggests that this is the case in a Han Chinese population (submitted for publication).

The primary AS-associated nsSNP in *IL23R* codes for a Gln381Arg substitution in exon 9, and this variant is protective against disease. There is also an independent secondary association with rs11209032, which lies upstream of *IL23R*, and this variant increases disease susceptibility. There have

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been studies in which the function of the p.Gln381 Arg variant have been investigated.[98,99] In these studies it has been shown that the p.Gln381Arg variant was a loss-of-function change and resulted in decreased levels of IL-23 signalling. This resulted in lower levels of IL-23 mediated IL-17 and IL-22 production, as well as lower levels of circulating Th17 and Tc17 cells. This is consistent with the increased Th17 lymphocyte numbers[65,100,101] and serum IL-17 levels[102,103] observed in AS patients. However, *IL23R* is also expressed on a number of other cell types, including γδ T cells, NK cells and NKT cells,[104–106] and it is not clear which cell type is primarily affected by the disease-associated variant. Our data has shown there are increased levels of IL-23R$^+$ γδ T cells in the peripheral blood of AS patients compared to healthy controls, suggesting that these cells may play a role in disease pathogenesis.[17]

The IL-12p40 subunit shared between IL-12 and IL-23 is encoded by the gene *IL12B*. This gene was previously reported as having suggestive association with AS,[7] and this association was later confirmed at the genome-wide level,[57] further implicating the role of IL-23 signalling in AS pathogenesis. *IL12B* has also been previously reported as being associated with both Crohn's disease and psoriasis.[91,93,107]

STAT3 has been shown to be associated with AS in both white European[7] and Han Chinese[78] populations, as well as being previously shown to be associated with Crohn's disease.[108] STAT3 is a critical transcription factor in the differentiation of Th17 cell populations, and is found directly downstream of IL-23R in the IL-23 signalling cascade (Figure 3).[109,110] Loss of function mutations in STAT3 cause Job syndrome (OMIM 147060), which is characterised by a loss of Th17 cells and is associated with recurrent, severe infections with extracellular bacteria and fungi.[109–111]

Fungal response – *CARD9* and *PTGER4*

CARD9 and *PTGER4* were both identified as being associated with AS in the TASC/WTCCC2 GWAS[57] and have both previously been shown to be associated with Crohn's disease.[112,113] CARD9 mediates signals from the innate immunity receptors dectin-1 and -2, which recognise β-glucan, a component of fungal and some bacterial cell walls. This induces the production of PGE_2, which is the ligand for the prostaglandin E receptor 4, EP4 subtype, which is encoded by *PTGER4*. PTGER4 then induces the production of IL-23 and IL-17, promoting the expansion of Th17 lymphocytes (Figure 3).[114] It has been reported that when SKG mice are treated with β-glucan they develop spondyloarthritis and Crohn's disease,[115] and that this model is characterised by activation of Th17 cells.[116] This model suggests that involvement of the IL-23 signalling pathway in AS pathogenesis may be triggered by ubiquitous pathogens carrying β-glucan. *PTGER4* has also been shown to play an anabolic role in bone remodelling,[117,118] making it a good candidate for a role in the bone formation that characterises AS.

Other associated genes

IL1R2

The IL-1 gene cluster on chromosome 2q has been repeatedly reported as being associated with AS, with association observed with *IL1A*.[119] Neither the WTCCC/TASC nsSNP study nor the TASC or TASC/WTCCC2 GWAS identified association at the IL-1 gene cluster, however the TASC GWAS did identify and confirm association with IL-1 receptor, type II, *IL1R2*.[76] Although the strongest association was observed with *IL1R2*, the association peak is quite broad, and may also include association at *IL1R1*.

IL-1R2 binds IL-1α and IL-1β with high affinity, acting as a decoy receptor after cleavage from myeloid and lymphoid cell membranes, possibly by ERAP1.[86] Further investigation is required to determine the disease mechanism.

ANTXR2

ANTXR2 is the gene encoding the protein capillary morphogenesis protein-2 (CMG2), and was identified as being associated with AS in both the TASC[76] and the TASC/WTCCC2 GWAS.[57] Recessive mutations of *ANTXR2* cause juvenile hyaline fibromatosis (OMIM 228600) and infantile systemic hyalinosis (OMIM 236490), but it has not been previously associated with any complex diseases. It is not clear how this gene functionally contributes to AS pathogenesis.

2p15 and 21q22 gene deserts

The 2p15 and 21q22 intergenic regions or "gene deserts" have been found to be strongly associated with AS in both the TASC[76] and TASC/WTCCC2[57] GWAS. There are no genes within 100 kb of the 2p15 peak associated region. The proteasome assembly chaperone 1 gene *PSMG1* lies nearby the 21q22 locus, however it is separated by a recombination hotspot and it is therefore unlikely that any SNP at 21q22 is in LD with any SNP in *PSMG1.* Possible mechanisms by which these associations contribute to disease include long-range cis-acting regulatory regions controlling expression of nearby genes, or effects on non-coding RNA.

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KIF21B

The gene *KIF21B* encodes for a member of the family of kinesin motor proteins, and has been previously shown to be associated with Crohn's disease. *KIF21B* was first identified as being associated with AS in a study in which genes previously shown to be associated with Crohn's disease were investigated in an AS cohort,[7] and was confirmed in the TASC/WTCCC2 GWAS.[57] Kinesins are used for essential component transport along dendritic and axonal microtubules by neurons. The Kinesin family member *KIF5A* has been associated with rheumatoid arthritis[120] and type-1-diabetes,[121] as well as being close to a locus associated with multiple sclerosis.[122] If the association of *KIF5A* is confirmed as true, this suggests alternate roles for kinesin family members.

TNFRSF1A

The association of *TNFRSF1A* further supports the involvement of TNF pathways in ankylosing spondylitis pathogenesis. In addition to showing association with AS, *TNFRSF1A* has been previously shown to be associated with both Crohn's disease and ulcerative colitis.[123,124] Additionally, a mouse model with constitutive over expression of TNF leads to inflammatory bowel disease, and sacroiliitis resembling AS, in a manner dependent on the expression of TNFR1,[125] supporting a role for *TNFRSF1A* in AS pathogenesis. *TNFRSF1A* has also been shown to be associated with AS in a Han Chinese population,[78] increasing the likelihood of *TNFRSF1A* being a true AS susceptibility gene.

Association has also been observed with *TBKBP1*, which encodes the TNF receptor signalling pathway member TBK binding protein.[57] Suggestive association has also been observed with *TRADD*, the TNF receptor associated death domain protein,[57,75] and further research is needed to elucidate the actual disease mechanism responsible for these associations.

Genomic studies

Expression profiling

Although significant effort has gone into genetic studies of the spondylarthropies, particularly AS including the several large scale GWAS studies described above, large scale gene expression profiling studies have been somewhat less extensive. Other similarly prevalent inflammatory conditions such as RA and Lupus have been the subject of much more extensive study, with over 10 such studies in both diseases in the last two years alone.

Undertaking whole genome expression studies generates powerful datasets that can inform on a number of disease aspects;

1. Identification of genes involved in the disease pathology which can inform on;
    a. The genes directly contributing to disease
    b. Identify pathways that can be targeted therapeutically
    c. Identify environmental factors that contribute to pathology
2. Characterise gene changes through disease progression to identify specific gene involvement at different disease stages
3. Elucidate cell- or tissue-specific genes participating in the disease process
4. Identify biomarkers which can be measured to:
    a. Diagnose early disease
    b. Catalog disease progression
    c. Monitor treatment response.

Expression studies in ankylosing spondylitis

In most large-scale genetic studies germline DNA is used which can be simply extracted from blood or saliva samples. For the majority of these studies in relatively common diseases it is assumed causative mutations are germline making the tissue source less of a consideration. In cases of rarer genetically mosaic conditions such as Melorheostosis, issue of tissue source become extremely important. For expression profiling studies however sample source and handling are always key considerations. The fact that expression profiling "snapshots" the cellular activity in the sample requires consideration to be given to the;

1. Disease and treatment status of the individual
2. Relevance of sample tissue site to disease presentation
3. Handling of tissue sample to minimise potential RNA degradation.

As described earlier there are two aspects to AS pathology, an initial systemic inflammation and the osteoproliferation and joint ankylosis characterising late disease. To monitor these quite different processes, careful consideration has to be given to the suitability of tissue sample. Systemic inflammation can be studied looking at the immune component of the blood, either through RNA extracted from whole blood or the peripheral blood mononuclear cell (PBMC) fraction which are relatively accessible. To study the joint disease processes is considerably more difficult. Biopsies of inflamed or ankylosed joints are rarely performed.

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In cases of severe joint fusion, tissue may be obtained during surgery and usually only warranted in cases of very advanced disease where early key disease-causing changes will not be present.

Disease-site studies

Due to the rarity of informative axial disease-site samples in AS only one expression profiling study in these tissues has been reported. Using sacroiliac joint aspirates from SpA patients, Rihl et al identified sacroiliitis candidate genes and then validated these candidates in knee synovial fluid biopsies from patients with peripheral synovitis.[126] They identified *interleukin-7* (*IL7)* as the best candidate gene but the small size of the study, only 3 spondyloarthropy (SpA) cases and no axial tissue controls, and the small gene array (1200 genes) means these preliminary findings require further confirmation.

Despite axial inflammation being the hallmark of AS, significant peripheral pathology is also found in many patients. Knee synovial biopsies present a more accessible and less invasive tissue candidate for assessment of expression profiles at joint disease sites. To date only two small scale studies have been published using "macroarrays" (600 or 1200 genes). Both of these studies emanated from David Yu's group at UCLA, one comparing osteoarthritis (OA) and SpA knee synovial biopsies[127] and the other comparing SpA and RA synovial fluid mononuclear cells (SFMCs).[128] In the SFMC study, similar patterns of inflammatory cytokines were expressed. One strong candidate gene was identified, the unfolded protein response (UPR) gene, *immunoglobulin heavy chain binding protein* (*BiP*). The UPR has been proposed as a mechanism by which *HLA-B27* might mediate its role in AS.[129] In the synovial biopsy study, SpA and OA samples could be differentiated by their expression profiles, however candidates were not validated.

Our group has recently undertaken an expression profiling study in SpA synovial biopsies using a different approach. Rather than using fresh-frozen biopsies we have utilised Illumina's Whole-Genome DASL® (cDNA-mediated Annealing, Selection, Extension, and Ligation [Illumina Inc, San Diego, CA]) Gene Expression Assay which has been specifically developed for whole-genome expression profiling of degraded RNA samples from archived tissue biopsies. We analyzed the expression profile of 24000 cDNAs in synovial biopsy samples from seronegative spondyloarthropy, AS and osteoarthritis patients and normal control samples (submitted for publication). Several interesting candidate genes were identified. *Matrix metalloproteinase 3* (*MMP3)*, a gene associated with inflammation and bone and cartilage remodelling was strongly upregulated and immunohistochemical confirmation showed MMP3 expression was particularly high in AS biopsies. *MMP3* has previously been identified as a circulating biomarker of AS.[23,130,131] *Dickkopf-3* (*DKK3)*, a Wnt pathway inhibitor, was downregulated. With the Wnt pathway having being recently hypothesised as playing a key role in AS-associated osteoproliferation[132–134] this was particularly interesting. Gene ontology analysis also showed altered immune and inflammation pathways and more interestingly changes in extracellular matrix and osteoblast associated pathways.

Use of peripheral joint samples for expression profiling in AS is not ideal and interpretation of these findings must be undertaken carefully. Unfortunately however axial samples are extremely rare. They are certainly valuable in identifying unique profiles for AS in relation to RA and/or OA samples to eliminate general inflammatory/joint damage genes. Further, many of the early changes in inflammation-mediated cell activity initiating tissue damage would be similar in both peripheral and axial joints.

With the limited number of disease-site studies having been undertaken in AS/SpA, little consensus on key genes have been found. Even using peripheral joint samples, experimental cohorts are small and usually lack sufficient numbers to allow independent validation. To generate sufficient power, consortium approaches should be considered and utilisation of technologies such as DASL to probe archived samples would further increase the numbers of samples available to profile.

Circulating cell studies

As described above, there is also a systemic immune-mediated inflammatory component in AS in addition to the osteoproliferation seen in the axial joints. Thus peripheral blood presents an inviting target tissue to try and elucidate the underlying gene changes that contribute to the early aspects of AS/SpA. The comparative ease with which samples of peripheral blood can be obtained has also meant that expression profiling studies using this RNA source in AS/SpA (as well as many other immune-mediated diseases) have been better powered and more numerous than those described above in joint tissue.

In AS the circulating immune cells may reflect the early changes that contribute both to disease susceptibility and also early stage progression. Most studies to date have utilised the peripheral blood mononuclear cell (PBMC) fraction from

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whole blood which contains the circulating white blood cells including B, T and NK cells, monocytes and granulocytes. Although a widely utilised standard protocol, purification of PBMCs does take several hours and needs to be carried out within 48 hours of blood collection, preferably within a few hours. This is particularly true for transcriptomics where differences in cell handling could cause significant shifts in gene expression patterns. PBMCs can therefore by unsuitable for studies involving multiple centers or tissue sampling from more remote or distant locations. An alternative blood-derived RNA source is to use whole blood. Two kits have been specifically developed for transcriptomic studies in whole blood with the blood collected into an RNA preservative which stabilises the RNA allowing shipping/initial storage at room temperature with minimal degradation (PAXgene from Qiagen [Valencia, CA] or Tempus from Invitrogen/Applied Biosystems [Life Technologies, Grand Island, NY]).

Three whole genome expression profiling studies have been published using whole blood in spondylarthropy all using the PAXgene technology.[135–137] Sharma et al compared healthy controls and spondyloarthropy patients but used a slightly unusual approach. Rather than analysing a main sample group by microarray then confirming candidate genes by qPCR in a second cohort, they split the sample groups into a discovery and a validation set and microarrayed both groups and compared differentially expressed genesets.[137] However, the control and patient groups had significant imbalances in their gender mix, as well as no joint disease scores being ascertained making interpretation difficult. Both Assassi et al and Pimental-Santos et al focussed on AS, using patients with confirmed AS according to the Modified New York Criteria, with Assassi also comparing their datasets with those from systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) patients.[135,136] Both these studies undertook an initial microarray study and confirmed candidate genes by qPCR in a larger 2nd cohort. The Pimentel-Santos study was the largest study with 18 patients and 18 controls in the initial microarray cohort and validated in 156 patients and controls using Taqman Low Density Arrays (TLDAs). Interestingly all three studies identified elements of the innate immune system to be differentially regulated. TLRs 4 and 5,[135,136] NLRP2[137] and CLEC4D[136] were all shown to be differentially expressed. Interestingly TLR4 was also shown to be up regulated at both RNA and protein level in AS PBMCs in a candidate gene study[138] as well as in degenerative intervertebral discs.[139] A role for the innate system in AS has long been postulated due to the proposed link between an as yet unknown pathogen and disease onset.[140] SPARC, a bone matrix protein, was also upregulated in two of the studies[136,137] which suggests dysregulation of the bone matrix which might contribute to the joint ankylosis.

In other immune conditions many studies have been undertaken using PBMCs however this is not the case for SpA/AS. The first SpA expression profiling study used a small (588-gene) array comparing a small control, SpA, RA, and psoriatic arthritis patient cohort (n = 6–7).[141] An arthritis-specific gene signature was identified but only one gene, *myeloid cell nuclear differentiation antigen* (*MNDA*), was able to distinguish SpA from the other arthrites.

Colbert and colleagues adopted a different approach undertaking a large whole genome study (~40000 cDNAs) using purified macrophages derived from normal and AS PBMC samples.[142] They demonstrated a 'reversed' IFNγ signature, with reduced expression of both IFNγ and IFNγ-induced genes.

Gene expression profiling is also a powerful tool to follow response to treatment. Haroon *et al* used whole genome expression arrays to identify a response to anti-TNF treatment.[143] A subset of 1428 genes was differentially expressed in response to treatment and downregulation of 4 inflammation-associated genes, including *LIGHT*, whose downregulation has also been associated with RA suggesting this gene maybe generally associated with inflammatory processes,[144] was confirmed.

Only recently however have large-scale whole genome expression profiling studies on the complete PBMC population been undertaken in AS or SpA by ourselves[145] and David Yu's group at UCLA.[146] Using Illumina's HT-12 whole genome arrays we compared expression profiles of PBMCs from 18 active AS patients (confirmed by Modified New York Criteria) and 18 age- and gender-matched controls identifying a 452-gene signature delineating AS and control samples with 94% accuracy. Downregulation of four immune/inflammation candidate genes, *NR4A2, TNFAIP3, CD69*, and *RORA*, was validated by qPCR. The expression pattern of these four genes generated a diagnostic potential of ~80% for AS which has the potential to be used to distinguish inflammatory from non-inflammatory back pain.

The downregulation of these candidate genes, together with other immune genes, suggests a defect in the capacity of the immune response. In most inflammatory conditions an upregulation of inflammation-associated genes would be expected, such as in RA and systemic lupus erythematosus.[147–151] Our results however are consistent with the macrophage study described above which also detailed a decreased immune response.[142] They also saw a decrease in *TNFAIP3* expression in AS patients.

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Down-regulation or muting of the immune response is compatible with one of the hypotheses proposed for the underlying pathology causing AS, in which a ubiquitous environmental factor acts as a trigger in patients genetically disposed to the disease (such as carrying the *HLA-B27* allele together with other susceptibility alleles from other genes such as *IL23R* and *ERAP1*).[152] A microarray study on whole blood samples from psoriatic arthritis patients, a related spondyloarthropy, also showed a general down-regulation in immune-associated genes suggesting inappropriate immune control.[153]

In addition to comparing AS and control groups, Gu et al also looked at undifferentiated SpA (uSpA) patients.[146] Expression changes were significantly greater in the uSpA patients compared to controls than for the AS samples. Given that uSpA is considered a less severe disease this was somewhat unexpected. Very few genes altered in AS with most of those being up-regulated including *NR4A2* which was downregulated in our study.[145] The Gu et al study was undertaken in a Chinese patient cohort which might explain the different findings to our Caucasian cohort study.

Table 2 summarises the most interesting genes identified through whole genome expression profiling studies in well-powered studies.

As mentioned above, expression profiling can generate powerful diagnostic algorithms. In AS, advanced joint damage is required for firm diagnosis (Modified New York Criteria) to gain subsidized access to the most effective (and expensive) treatments. A need for a reliable early diagnostic in AS is thus very pressing. Both ours[145] and David Yu's[146] studies showed diagnostic power > 80% to predict disease based on the expression profiles.

The results to date have shown a disappointing lack of overlap between the whole blood and PBMC expression studies. Several studies have reported differences in the expression profile of PBMCs vs whole blood.[154–157] Larger cohort studies are required with whole blood profiling the more convenient vehicle to expedite the multicenter approach needed to generate sufficient power.

To date a number of disparate expression profiling studies have been undertaken in AS. Although a number of potentially interesting gene changes have been identified, there is a lack of consensus in the mechanisms highlighted save for an indication of a reduced immune response. A similar problem confronted genetic studies several years ago and this was overcome by large-scale collaborative studies generating large homogenous sample cohorts and enabled strong reproducible candidate gene-associations to be identified. Similar issues need to be addressed for future expression profiling studies in AS. Significant thought must be given to assembling large informative sample cohorts that reflect the stage of disease being investigated. These cohorts need to consist of homogenous case collections targeting specific disease

Table 2 Candidate genes associated with AS identified through genome-wide expression studies

Gene	Samples	Tissue	Platform	Regulation	References
MMP3	AS-SpA vs normal-OA	Synovial biopsy	Illumina DASL	Up	Submitted for publication
DKK3	AS-SpA vs normal-OA	Synovial biopsy	Illumina DASL	Down	Submitted for publication
TLR4	AS vs controls	Whole blood (PAXGene)	Illumina HT12	Up	135,136
TLR5	AS vs controls	Whole blood (PAXGene)	Illumina HT12	Up	135,136
NLRP2	SpA vs controls	Whole blood (PAXGene)	Affymetrix HG-U133 plus 2.0	Down	137
CLEC4D	AS vs controls	Whole blood (PAXGene)	Illumina HT12	Up	136
SPARC	SpA vs controls AS vs controls	Whole blood (PAXGene)	Affymetrix HG-U133 plus 2.0 Illumina HT12	Up	136,137
IFN-regulated genes	AS vs controls	Purified macrophages	Affymetrix HG-U133 plus 2.0	Reverse IFN signature	142
LIGHT	AS patients with and without anti-TNF treatment	Whole blood	Affymetrix HG-U133 plus 2.0	Down	143
NR4A2	AS vs controls	PBMC	Illumina HT12	Down	145
TNFAIP3	AS vs controls	PBMC Purified macrophages	Illumina HT12 Affymetrix HG-U133 plus 2.0	Down	142,145
CD69	AS vs controls	PBMC	Illumina HT12	Down	145
RORA	AS vs controls	PBMC	Illumina HT12	Down	145
NR4A2	AS vs uSpA vs controls	PBMC	Illumina Ref-8	Up	146
RGS1	AS vs uSpA vs controls	PBMC	Illumina Ref-8	Up	146

Abbreviations: PBMC, peripheral blood mononuclear cells; AS, ankylosing spondylitis; SpA, spondyloarthropathy; USpA, undifferentiated spondyloarthropathy; OA, osteoarthritis; IFN, interferon.

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stages, such as early inflammation or late-stage ankylosis, to stand the best chance of being informative. For the development of diagnostic tests, further studies need to be conducted on the suitability of whole blood RNA as a sample source reflective of disease status, as this approach is much more practical for use in clinical practice. Given the scarcity of clinically relevant samples, collaborative approaches would enable such powerful studies. Archived samples should also be utilized using the appropriate technologies developed for such approaches.

With next generation sequencing enabling whole transcriptome studies the future of expression profiling looks to lie in this direction. Costs are rapidly decreasing making large scale studies viable for well-funded labs. RNAseq will allow the full catalog of splice variants, non-coding RNAs and microRNAs to be elucidated and some of these novel species may explain some of the strong GWAS hits in "gene deserts".

Conclusion

Although a number of strong candidate genes have been identified from well-powered GWAS studies there appears to be little correlation with the gene expression profiling studies published to date.

This could be due to;

1. Genetic studies identify for the most part susceptibility genes and expression studies are frequently in patients with established disease. Many alterations in these genes may be finished by the time the expression studies are undertaken
2. Genetic studies may identify genes in which function rather than absolute expression level is altered
3. Genetic disposition to a disease may be the result of very small changes in a number of genes, each of which is below the threshold of detection by expression profiling but cumulatively might generate significant downstream effects which are picked up by the expression studies.

To date no expression quantitative trait loci (eQTL) studies have been published in AS or SpA. There is now a significant body of GWAS and expression profiling data to enable such studies. Such approaches correlating genetic variants with both *cis*- and *trans* expression may further explain to current GWAS/expression profiling disconnect and further reveal the pathways and mechanisms underlying AS.

Disclosure

None of the authors report any conflicts of interest.

References

1. Simmons R, Sharp C, Sims S, et al. High frequency, sustained T cell responses to PARV4 suggest viral persistence in vivo. *The Journal of Infectious Diseases*. May 15, 2011;203(10):1378–1387.
2. Ward MM. Quality of life in patients with ankylosing spondylitis. *Rheum Dis Clin North Am*. Nov 1998;24(4):815–827.
3. Santos H, Brophy S, Calin A. Exercise in ankylosing spondylitis: how much is optimum? *J Rheumatol*. Nov 1998;25(11):2156–2160.
4. Amor B, Santos RS, Nahal R, Listrat V, Dougados M. Predictive factors for the longterm outcome of spondyloarthropathies. *J Rheumatol*. Oct 1994;21(10):1883–1887.
5. Burton PR, Clayton DG, Cardon LR, et al. Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nat Genet*. Nov 2007 print;39(11):1329–1337.
6. Bjarnason I, Helgason KO, Geirsson ÁJ, et al. Subclinical intestinal inflammation and sacroiliac changes in relatives of patients with ankylosing spondylitis. *Gastroenterology*. 2003;125(6):1598–1605.
7. Danoy P, Pryce K, Hadler J, et al. Association of variants at 1q32 and STAT3 with ankylosing spondylitis suggests genetic overlap with Crohn's disease. *PLoS Genetics*. 2010;6(12):e1001195.
8. Plonquet A, Gleizes A, Briot K, et al. Peripheral blood lymphocyte phenotypes in patients with spondyloarthropathy. *Ann Rheum Dis*. April 1, 2004;63(4):467–468.
9. Suhler EB, Martin TM, Rosenbaum JT. HLA-B27-associated uveitis: overview and current perspectives. *Current Opinion in Ophthalmology*. 2003;14(6):378–383.
10. Szabo SM, Levy AR, Rao SR, et al. Increased risk of cardiovascular and cerebrovascular disease in individuals with ankylosing spondylitis: A population-based study. *Arthritis and Rheumatism*. 2011:3294–3304.
11. Bakland G, Gran JT, Nossent JC. Increased mortality in ankylosing spondylitis is related to disease activity. *Ann Rheum Dis*. July 21, 2011.
12. Brown MA, Kennedy LG, MacGregor AJ, et al. Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. *Arthritis Rheum*. Oct 1997;40(10):1823–1828.
13. Taurog JD, Richardson JA, Croft JT, et al. The germfree state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. *The Journal of experimental medicine*. December 1, 1994;180(6):2359–2364.
14. Carter JD, Gerard HC, Espinoza LR, et al. Chlamydiae as etiologic agents in chronic undifferentiated spondylarthritis. *Arthritis and Rheumatism*. May 2009;60(5):1311–1316.
15. Pöllänen R, Sillat T, Pajarinen J, Levón J, Kaivosoja E, Konttinen YT. Microbial antigens mediate HLA-B27 diseases via TLRs. *Journal of Autoimmunity*. Jun 2009;32(3–4):172–177.
16. Rashid T, Ebringer A. Ankylosing spondylitis is linked to Klebsiella – the evidence. *Clinical Rheumatology*. 2007;26(6):858–864.
17. Kenna TJ, Davidson SI, Duan R, et al. γδ T cells are the major IL-23R responsive population in ankylosing spondylitis and may play a critical role in disease pathogenesis. *Arthritis and Rheumatism*. In press. 2011.
18. Lories R, Luyten F, de Vlam K. Progress in spondylarthritis. Mechanisms of new bone formation in spondyloarthritis. *Arthritis Research and Therapy*. 2009;11(2):221.
19. Lories RJU, Derese I, de Bari C, Luyten FP. Evidence for uncoupling of inflammation and joint remodeling in a mouse model of spondylarthritis. *Arthritis and Rheumatism*. 2007;56(2):489–497.
20. Maksymowych WP, Chiowchanwisawakit P, Clare T, Pedersen SJ, Ostergaard M, Lambert RG. Inflammatory lesions of the spine on magnetic resonance imaging predict the development of new syndesmophytes in ankylosing spondylitis: evidence of a relationship between inflammation and new bone formation. *Arthritis Rheum*. Jan 2009;60(1):93–102.
21. Baraliakos X, Listing J, Rudwaleit M, Sieper J, Braun J. The relationship between inflammation and new bone formation in patients with ankylosing spondylitis. *Arthritis Research and Therapy*. 2008;10(5):R104.

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22. Appel H, Rudwaleit M, Sieper J. Relevance of osteoproliferation as an outcome parameter in ankylosing spondylitis. *Nat Clin Pract Rheum*. 2008;4(11):578–579.
23. Maksymowych WP, Landewé R, Conner-Spady B, et al. Serum matrix metalloproteinase 3 is an independent predictor of structural damage progression in patients with ankylosing spondylitis. *Arthritis and Rheumatism*. 2007;56(6):1846–1853.
24. 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.
25. Dagfinrud H, Kvien TK, Hagen KB. Physiotherapy interventions for ankylosing spondylitis. *Cochrane Database Syst Rev*. 2008(1): CD002822.
26. Chen J, Liu C. Sulfasalazine for ankylosing spondylitis. *Cochrane Database Syst Rev*. 2005;2:CD004800.
27. Chen J, Liu C, Lin J. Methotrexate for ankylosing spondylitis. *Cochrane Database Syst Rev*. 2006;4:CD004524.
28. Benhamou M, Gossec L, Dougados M. Clinical relevance of C-reactive protein in ankylosing spondylitis and evaluation of the NSAIDs/coxibs' treatment effect on C-reactive protein. *Rheumatology (Oxford)*. Mar 2010;49(3):536–541.
29. Braun J, Brandt J, Listing J, et al. Treatment of active ankylosing spondylitis with infliximab: a randomised controlled multicentre trial. *Lancet*. April 6, 2002;359(9313):1187–1193.
30. Van den Bosch F, Kruithof E, De Vos M, De Keyser F, Mielants H. Crohn's disease associated with spondyloarthropathy: effect of TNF-alpha blockade with infliximab on articular symptoms. *Lancet*. November 25, 2000;356(9244):1821–1822.
31. Haibel H, Rudwaleit M, Listing J, et al. Efficacy of adalimumab in the treatment of axial spondylarthritis without radiographically defined sacroiliitis: results of a twelve-week randomized, double-blind, placebo-controlled trial followed by an open-label extension up to week fifty-two. *Arthritis and Rheumatism*. Jul 2008;58(7):1981–1991.
32. Dougados M, Combe B, Braun J, et al. A randomised, multicentre, double-blind, placebo-controlled trial of etanercept in adults with refractory heel enthesitis in spondyloarthritis: the HEEL trial. *Ann Rheum Dis*. Aug 2010;69(8):1430–1435.
33. van der Heijde D, Kavanaugh A, Gladman DD, et al. Infliximab inhibits progression of radiographic damage in patients with active psoriatic arthritis through one year of treatment: Results from the induction and maintenance psoriatic arthritis clinical trial 2. *Arthritis and Rheumatism*. Aug 2007;56(8):2698–2707.
34. van der Heijde D, Landewe R, Baraliakos X, et al. Radiographic findings following two years of infliximab therapy in patients with ankylosing spondylitis. *Arthritis Rheum*. Oct 2008;58(10):3063–3070.
35. van der Heijde D, Landewe R, Einstein S, et al. Radiographic progression of ankylosing spondylitis after up to two years of treatment with etanercept. *Arthritis Rheum*. April 25, 2008;58(5):1324–1331.
36. van der Heijde D, Salonen D, Weissman B, et al. Assessment of radiographic progression in the spines of patients with ankylosing spondylitis treated with adalimumab for up to 2 years. *Arthritis Research and Therapy*. 2009;11(4):R127.
37. Dixon WG, Hyrich KL, Watson KD, et al. Drug-specific risk of tuberculosis in patients with rheumatoid arthritis treated with anti-TNF therapy: results from the British Society for Rheumatology Biologics Register (BSRBR). *Ann Rheum Dis*. Mar 2010;69(3):522–528.
38. Jacobs JH, Rose FC. The familial occurrence of ankylosing spondylitis. *British Medical Journal*. November 13, 1954;2(4897):1139–1140.
39. Jarvinen P. Occurrence of ankylosing spondylitis in a nationwide series of twins. *Arthritis Rheum*. Mar 1995;38(3):381–383.
40. Brown MA, Brophy S, Bradbury L, et al. Identification of major loci controlling clinical manifestations of ankylosing spondylitis. *Arthritis Rheum*. Aug 2003;48(8):2234–2239.
41. Brophy S, Hickey S, Menon A, et al. Concordance of disease severity among family members with ankylosing spondylitis? *J Rheumatol*. Sep 2004;31(9):1775–1778.
42. Schlosstein L, Terasaki P, Bluestone R, Pearson C. High association of an HL-A antigen, W27, with ankylosing spondylitis. *N Engl J Med*. April 5, 1973;288(14):704–706.
43. Brewerton DA, Hart FD, Nicholls A, Caffrey M, James DCO, Sturrock RD. Ankylosing Spondylitis and Hl-a 27. *The Lancet*. 1973; 301(7809):904–907.
44. Caffrey MFP, James DCO. Human Lymphocyte Antigen Association in Ankylosing Spondylitis. *Nature*. 1973;242(5393):121.
45. Liu Y, Jiang L, Cai Q, et al. Predominant association of HLA-B*2704 with ankylosing spondylitis in Chinese Han patients. *Tissue Antigens*. Jan 2010;75(1):61–64.
46. Hill AVS, Allsopp CEM, Kwiatkowski D, et al. Common West African HLA antigens are associated with protection from severe malaria. *Nature*. 1991;352(6336):595–600.
47. Brown M, Wordsworth P. Predisposing factors to spondyloarthropathies. *Curr Opin Rheumatol*. Jul 1997;9(4):308–314.
48. Brown MA, Laval SH, Brophy S, Calin A. Recurrence risk modelling of the genetic susceptibility to ankylosing spondylitis. *Ann Rheum Dis*. Nov 2000;59(11):883–886.
49. Gran JT, Husby G, Hordvik M. Prevalence of ankylosing spondylitis in males and females in a young middle-aged population of Tromso, northern Norway. *Ann Rheum Dis*. June 1, 1985;44(6): 359–367.
50. Braun J, Bollow M, Remlinger G, et al. Prevalence of spondylarthropathies in HLA-B27 positive and negative blood donors. *Arthritis and Rheumatism*. 1998;41(1):58–67.
51. Brown M. Human leucocyte antigen-B27 and ankylosing spondylitis. *Internal Medicine Journal*. 2007;37(11):739–740.
52. Benjamin R, Parham P. Guilt by association: HLA-B27 and ankylosing spondylitis. *Immunol Today*. Apr 1990;11(4):137–142.
53. Hermann E, Meyer zum Büschenfelde KH, Fleischer B, Yu DTY. HLA-B27-restricted CD8 T cells derived from synovial fluids of patients with reactive arthritis and ankylosing spondylitis. *The Lancet*. 1993; 342(8872):646–650.
54. Scofield R, Kurien B, Gross T, Warren W, Harley J. HLA-B27 binding of peptide from its own sequence and similar peptides from bacteria: implications for spondyloarthropathies. *Lancet*. June 17, 1995; 345(8964):1542–1544.
55. Frauendorf E, Von Goessel H, May E, Märker-Hermann E. HLA-B27-restricted T cells from patients with ankylosing spondylitis recognize peptides from B*2705 that are similar to bacteria-derived peptides. *Clinical and Experimental Immunology*. 2003;134(2):351–359.
56. Ramos M, Alvarez I, Sesma L, Logean A, Rognan D, Lopez de Castro JA. Molecular Mimicry of an HLA-B27-derived Ligand of Arthritis-linked Subtypes with Chlamydial Proteins. *J Biol Chem*. September 27, 2002;277(40):37573–37581.
57. Evans DM, Spencer CC, Pointon JJ, 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(8):761–767.
58. Mear JP, Schreiber KL, Munz C, et al. Misfolding of HLA-B27 as a Result of Its B Pocket Suggests a Novel Mechanism for Its Role in Susceptibility to Spondyloarthropathies. *J Immunol*. December 15, 1999;163(12):6665–6670.
59. Dangoria NS, DeLay ML, Kingsbury DJ, et al. HLA-B27 misfolding is associated with aberrant intermolecular disulfide bond formation (dimerization) in the endoplasmic reticulum. *J Biol Chem*. June 21, 2002;277(26):23459–23468.
60. Pahl HL, Baeuerle PA. The ER-overload response: activation of NF-[kappa]B. *Trends in Biochemical Sciences*. 1997;22(2):63–67.
61. Bowness P. HLA B27 in health and disease: a double-edged sword? *Rheumatology*. August 1, 2002;41(8):857–868.
62. Allen RL, O'Callaghan CA, McMichael AJ, Bowness P. Cutting edge: HLA-B27 can form a novel {beta}2-microglobulin-free heavy chain homodimer structure. *J Immunol*. May 1, 1999;162(9): 5045–5048.

Advances in Genomics and Genetics downloaded from https://www.dovepress.com/ by 137.108.70.13 on 13-Jan-2020
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63. Kollnberger S, Bird L, Sun M-Y, et al. Cell-surface expression and immune receptor recognition of HLA-B27 homodimers. *Arthritis and Rheumatism*. 2002;46(11):2972–2982.
64. Bird L, Peh C, Kollnberger S, Elliott T, McMichael A, Bowness P. Lymphoblastoid cells express HLA-B27 homodimers both intracellularly and at the cell surface following endosomal recycling. *European Journal of Immunology*. 2003;33(3):748–759.
65. Bowness P, Ridley A, Shaw J, et al. Th17 cells expressing KIR3DL2+ and responsive to HLA-B27 homodimers are increased in ankylosing spondylitis. *The Journal of Immunology*. In press. 2011.
66. 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*. Oct 1996;39(10):1768–1769.
67. Wei JCC, 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*. July 1, 2004 2004;43(7):839–842.
68. Brown MA, Kennedy LG, Darke C, et al. The effect of HLA-DR genes on susceptibility to and severity of ankylosing spondylitis. *Arthritis Rheum*. Mar 1998;41(3):460–465.
69. Sims AM, Barnardo M, Herzberg I, et al. Non-B27 MHC associations of ankylosing spondylitis. *Genes Immun*. 2007;8(2):115–123.
70. Timms AE, Crane AM, Sims AM, et al. The interleukin 1 gene cluster contains a major susceptibility locus for ankylosing spondylitis. *Am J Hum Genet*. Oct 2004;75(4):587–595.
71. van der Paardt M, Crusius JBA, Garcia-Gonzalez MA, et al. Interleukin-1{beta} and interleukin-1 receptor antagonist gene polymorphisms in ankylosing spondylitis. *Rheumatology*. December 1, 2002;41(12):1419–1423.
72. McGarry F, Neilly J, Anderson N, Sturrock R, Field M. A polymorphism within the interleukin 1 receptor antagonist (IL-1Ra) gene is associated with ankylosing spondylitis. *Rheumatology (Oxford)*. Dec 2001;40(12):1359–1364.
73. Chou CT, Timms AE, Wei JC, Tsai WC, Wordsworth BP, Brown MA. Replication of association of IL1 gene complex members with ankylosing spondylitis in Taiwanese Chinese. *Ann Rheum Dis*. Aug 2006;65(8):1106–1109.
74. Beyeler C, Armstrong M, Bird HA, Idle JR, Daly AK. Relationship between genotype for the cytochrome P450 CYP2D6 and susceptibility to ankylosing spondylitis and rheumatoid arthritis. *Ann Rheum Dis*. 1996;55:66–68.
75. WTCCC. Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nat Genet*. 2007;39(11):1329–1337.
76. Reveille JD, Sims AM, Danoy P, et al. Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. *Nat Genet*. Feb 2010;42(2):123–127.
77. Davidson SI, Wu X, Liu Y, et al. Association of ERAP1, but not IL23R, with ankylosing spondylitis in a Han Chinese population. *Arthritis and Rheumatism*. 2009;60(11):3263–3268.
78. Davidson SI, Liu Y, Danoy PA, et al. Association of STAT3 and TNFRSF1A with ankylosing spondylitis in Han Chinese. *Ann Rheum Dis*. Feb 2011;70(2):289–292.
79. Pimentel-Santos F, Ligeiro D, Matos M, et al. Association of IL23R and ERAP1 genes with ankylosing spondylitis in a Portuguese population. *Clin Exp Rheumatol*. Sep–Oct 2009;27(5):800–806.
80. Bang S-Y, Kim T-H, Lee B, et al. Genetic studies of ankylosing spondylitis in Koreans confirm associations with ERAP1 and 2p15 reported in white patients. *The Journal of Rheumatology*. February 1, 2011;38(2):322–324.
81. Pazár B, Sáfrány E, Gergely P, Szántó S, Szekanecz Z, Poór G. Association of ARTS1 gene polymorphisms with ankylosing spondylitis in the Hungarian population: the rs27044 variant is associated with HLA-B*2705 subtype in Hungarian patients with ankylosing spondylitis. *The Journal of Rheumatology*. February 1, 2010;37(2):379–384.
82. GAPC-WTCCC2. A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. *Nat Genet*. 2010;42(11):985–990.
83. Sun L-D, Cheng H, Wang Z-X, et al. Association analyses identify six new psoriasis susceptibility loci in the Chinese population. *Nat Genet*. 2010;42(11):1005–1009.
84. Hammer GE, Gonzalez F, Champsaur M, Cado D, Shastri N. The aminopeptidase ERAAP shapes the peptide repertoire displayed by major histocompatibility complex class I molecules. *Nat Immunol*. 2006;7(1):103–112.
85. Kanaseki T, Blanchard N, Hammer Gianna E, Gonzalez F, Shastri N. ERAAP Synergizes with MHC class I molecules to make the final cut in the antigenic peptide precursors in the endoplasmic reticulum. *Immunity*. 2006;25(5):795–806.
86. Cui X, Rouhani FN, Hawari F, Levine SJ. Shedding of the type II IL-1 decoy receptor requires a multifunctional aminopeptidase, aminopeptidase regulator of TNF receptor type 1 shedding. *J Immunol*. December 15, 2003;171(12):6814–6819.
87. Cui X, Rouhani FN, Hawari F, Levine SJ. An aminopeptidase, ARTS-1, is required for interleukin-6 receptor shedding. *J Biol Chem*. August 1, 2003;278(31):28677–28685.
88. Cui X, Hawari F, Alsaaty S, et al. Identification of ARTS-1 as a novel TNFR1-binding protein that promotes TNFR1 ectodomain shedding. *J Clin Invest*. Aug 2002;110(4):515–526.
89. Nguyen TT, Chang S-C, Evnouchidou I, et al. Structural basis for antigenic peptide precursor processing by the endoplasmic reticulum aminopeptidase ERAP1. *Nat Struct Mol Biol*. 2011;18(5):604–613.
90. Kochan G, Krojer T, Harvey D, et al. Crystal structures of the endoplasmic reticulum aminopeptidase-1 (ERAP1) reveal the molecular basis for N-terminal peptide trimming. *Proceedings of the National Academy of Sciences*. April 20, 2011. In press.
91. Duerr RH, Taylor KD, Brant SR, et al. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science*. December 1, 2006;314(5804):1461–1463.
92. Tremelling M, Cummings F, Fisher SA, et al. IL23R variation determines susceptibility but not disease phenotype in inflammatory bowel disease. *Gastroenterology*. 2007;132(5):1657–1664.
93. Cargill M, Schrodi SJ, Chang M, 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*. Feb 2007;80(2):273–390.
94. Rahman P, Inman RD, Maksymowych WP, Reeve JP, Peddle L, Gladman DD. Association of interleukin 23 receptor variants with psoriatic arthritis. *The Journal of Rheumatology*. January 1, 2009;36(1):137–140.
95. Rueda B, Orozco G, Raya E, et al. The IL23R Arg381Gln non-synonymous polymorphism confers susceptibility to ankylosing spondylitis. *Ann Rheum Dis*. October 1, 2008;67(10):1451–1454.
96. Sung I-H, Kim T-H, Bang S-Y, et al. IL-23R polymorphisms in patients with ankylosing spondylitis in Korea. *The Journal of Rheumatology*. May 2009;36(5):1003–1005.
97. Yamazaki K, Onouchi Y, Takazoe M, Kubo M, Nakamura Y, Hata A. Association analysis of genetic variants in IL23R, ATG16L1 and 5p13.1 loci with Crohn's disease in Japanese patients. *J Hum Genet*. 2007;52(7):575–583.
98. Di Meglio P, Di Cesare A, Laggner U, et al. The IL23R R381Q gene variant protects against immune-mediated diseases by impairing IL-23-induced Th17 effector response in humans. *PLoS ONE*. 2011;6(2):e17160.
99. Sarin R, Wu X, Abraham C. Inflammatory disease protective R381Q IL23 receptor polymorphism results in decreased primary CD4+ and CD8+ human T-cell functional responses. *Proceedings of the National Academy of Sciences*. May 23, 2011. In press.
100. Jandus C, Bioley G, Rivals JP, Dudler J, Speiser D, Romero P. Increased numbers of circulating polyfunctional Th17 memory cells in patients with seronegative spondylarthritides. *Arthritis and Rheumatism*. 2008;8(8):2307–2317.

Advances in Genomics and Genetics downloaded from https://www.dovepress.com/ by 137.108.70.13 on 13-Jan-2020
For personal use only.

101. Shen H, Goodall JC, Hill Gaston JS. Frequency and phenotype of peripheral blood Th17 cells in ankylosing spondylitis and rheumatoid arthritis. *Arthritis and Rheumatism*. 2009;60(6):1647–1656.
102. Wendling D, Cedoz J-P, Racadot E, Dumoulin G. Serum IL-17, BMP-7, and bone turnover markers in patients with ankylosing spondylitis. *Joint Bone Spine*. May 2007;74(3):304–305.
103. Taylan A, Sari I, Kozaci D, et al. Evaluation of the T helper 17 axis in ankylosing spondylitis. *Rheumatology International*. y2011:1–5.
104. Awasthi A, Riol-Blanco L, Jager A, et al. Cutting Edge: IL-23 receptor GFP reporter mice reveal distinct populations of IL-17-producing cells. *J Immunol*. May 15, 2009;182(10):5904–5908.
105. Sutton CE, Lalor SJ, Sweeney CM, Brereton CF, Lavelle EC, Mills KHG. Interleukin-1 and IL-23 induce innate IL-17 production from [gamma][delta] T cells, amplifying Th17 responses and autoimmunity. *Immunity*. 2009;31(2):331–341.
106. Rachitskaya AV, Hansen AM, Horai R, et al. Cutting Edge: NKT cells constitutively express IL-23 receptor and ROR{gamma}t and rapidly produce IL-17 upon receptor ligation in an IL-6-independent fashion. *J Immunol*. April 15, 2008;180(8):5167–5171.
107. Zhang X-J, Huang W, Yang S, et al. Psoriasis genome-wide association study identifies susceptibility variants within LCE gene cluster at 1q21. *Nat Genet*. 2009;41(2):205–210.
108. Barrett JC, Hansoul S, Nicolae DL, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet*. 2008;40(8):955–962.
109. Milner JD, Brenchley JM, Laurence A, et al. Impaired TH17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. *Nature*. April 10, 2008 print;452(7188):773–776.
110. Yang XO, Panopoulos AD, Nurieva R, et al. STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. *J Biol Chem*. March 30, 2007;282(13):9358–9363.
111. Holland SM, DeLeo FR, Elloumi HZ, et al. STAT3 Mutations in the Hyper-IgE Syndrome. *N Engl J Med*. 2007;357(16):1608–1619.
112. Libioulle C, Louis E, Hansoul S, et al. Novel Crohn disease locus identified by genome-wide association maps to a gene desert on 5p13.1 and modulates expression of *PTGER4*. *PLoS Genet*. 2007;3(4):e58.
113. Zhernakova A, Festen EM, Franke L, et al. Genetic analysis of innate immunity in Crohn's disease and ulcerative colitis identifies two susceptibility loci harboring CARD9 and IL18RAP. *The American Journal of Human Genetics*. 2008;82(5):1202–1210.
114. Gagliardi MC, Teloni R, Mariotti S, et al. Endogenous PGE2 promotes the induction of human Th17 responses by fungal β-glucan. *J Leukoc Biol*. November 1, 2010;88(5):947–954.
115. Ruutu M, Yadav B, Thomas G, et al. Fungal beta-glucan triggers spondyloarthropathy and Crohn's disease in SKG mice. *Arthritis and Rheumatism*. 2010;62(S10):1446.
116. Hirota K, Hashimoto M, Yoshitomi H, et al. T cell self-reactivity forms a cytokine milieu for spontaneous development of IL-17+ Th cells that cause autoimmune arthritis. *J Exp Med*. January 22, 2007;204(1):41–47.
117. Yoshida K, Oida H, Kobayashi T, et al. Stimulation of bone formation and prevention of bone loss by prostaglandin E EP4 receptor activation. *Proc Natl Acad Sci U S A*. April 2, 2002;99(7):4580–4585.
118. Tomita M, Li X, Okada Y, et al. Effects of selective prostaglandin EP4 receptor antagonist on osteoclast formation and bone resorption in vitro. *Bone*. 2002;30(1):159–163.
119. Sims AM, Timms AE, Bruges-Armas J, et al. Prospective meta-analysis of IL-1 gene complex polymorphisms confirms associations with ankylosing spondylitis. *Ann Rheum Dis*. September 1, 2008;67(9):1305–1309.
120. Raychaudhuri S, Remmers EF, Lee AT, et al. Common variants at CD40 and other loci confer risk of rheumatoid arthritis. *Nat Genet*. 2008;40(10):1216–1223.
121. Fung EYMG, Smyth DJ, Howson JMM, et al. Analysis of 17 autoimmune disease-associated variants in type 1 diabetes identifies 6q23/TNFAIP3 as a susceptibility locus. *Genes Immun*. 2008;10(2):188–191.
122. ANZgene. Genome-wide association study identifies new multiple sclerosis susceptibility loci on chromosomes 12 and 20. *Nat Genet*. 2009;41(7):824–828.
123. Waschke KA, Villani A-C, Vermeire S, et al. Tumor necrosis factor receptor gene polymorphisms in Crohn's disease: association with clinical phenotypes. *Am J Gastroenterol*. 2005;100(5):1126–1133.
124. Lappalainen M, Halme L, Turunen U, et al. Association of IL23R, TNFRSF1A, and HLA-DRB1*0103 allele variants with inflammatory bowel disease phenotypes in the Finnish population. *Inflammatory Bowel Diseases*. 2008;14(8):1118–1124.
125. Armaka M, Apostolaki M, Jacques P, Kontoyiannis DL, Elewaut D, Kollias G. Mesenchymal cell targeting by TNF as a common pathogenic principle in chronic inflammatory joint and intestinal diseases. *J Exp Med*. February 18, 2008;205(2):331–337.
126. Rihl M, Kellner H, Kellner W, et al. Identification of interleukin-7 as a candidate disease mediator in spondylarthritis. *Arthritis and Rheumatism*. Oct 30 2008;58(11):3430–3435.
127. Rihl M, Baeten D, Seta N, et al. Technical validation of cDNA based microarray as screening technique to identify candidate genes in synovial tissue biopsy specimens from patients with spondyloarthropathy. *Ann Rheum Dis*. May 1, 2004;63(5):498–507.
128. Gu J, Rihl M, Marker-Hermann E, et al. Clues to pathogenesis of spondyloarthropathy derived from synovial fluid mononuclear cell gene expression profiles. *J Rheumatol*. Oct 2002;29(10):2159–2164.
129. Turner MJ, Sowders DP, DeLay ML, et al. HLA-B27 misfolding in transgenic rats is associated with activation of the unfolded protein response. *J Immunol*. August 15, 2005;175(4):2438–2448.
130. Chen C-H, Lin K-C, Yu DTY, et al. Serum matrix metalloproteinases and tissue inhibitors of metalloproteinases in ankylosing spondylitis: MMP-3 is a reproducibly sensitive and specific biomarker of disease activity. *Rheumatology*. April 1, 2006;45(4):414–420.
131. Yang C, Gu J, Rihl M, et al. Serum levels of matrix metalloproteinase 3 and macrophage colony-stimulating factor 1 correlate with disease activity in ankylosing spondylitis. *Arthritis Care and Research*. 2004;51(5):691–699.
132. Daoussis D, Liossis SN, Solomou EE, et al. Evidence that Dkk-1 is dysfunctional in ankylosing spondylitis. *Arthritis and Rheumatism*. December 28, 2009;62(1):150–158.
133. Diarra D, Stolina M, Polzer K, et al. Dickkopf-1 is a master regulator of joint remodeling. *Nat Genet*. Feb 2007 print;13(2):156–163.
134. Uderhardt S, Diarra D, Katzenbeisser J, et al. Blockade of Dickkopf-1 induces fusion of sacroiliac joints. *Ann Rheum Dis*. March 26, 2010;69:592–597.
135. Assassi S, Reveille JD, Arnett FC, et al. Whole-blood gene expression profiling in ankylosing spondylitis shows upregulation of toll-like receptor 4 and 5. *The Journal of Rheumatology*. January 1, 2011;38(1):87–98.
136. Pimentel-Santos F, Ligeiro D, Matos M, et al. Whole blood transcriptional profiling in ankylosing spondylitis identifies novel candidate genes that might contribute to the inflammatory and tissue-destructive disease aspects. *Arthritis Research and Therapy*. 2011;13(2):R57.
137. Sharma S, Choi D, Planck S, et al. Insights in to the pathogenesis of axial spondyloarthropathy based on gene expression profiles. *Arthritis Research and Therapy*. 2009;11(6):R168.
138. Yang ZX, Liang Y, Zhu Y, et al. Increased expression of toll-like receptor 4 in peripheral blood leucocytes and serum levels of some cytokines in patients with ankylosing spondylitis. *Clinical and Experimental Immunology*. 2007;149(1):48–55.
139. Zhang Y-G, Guo X, Sun Z, Jia G, Xu P, Wang S. Gene expression profiles of disc tissues and peripheral blood mononuclear cells from patients with degenerative discs. *Journal of Bone and Mineral Metabolism*. 2010;28(2):209–219.

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140. De Keyser F, Elewaut D, De Vos M, et al. Bowel inflammation and the spondyloarthropathies. *Rheum Dis Clin North Am*. Nov 1998; 24(4):785–813, ix–x.
141. Gu J, Marker-Hermann E, Baeten D, et al. A 588-gene microarray analysis of the peripheral blood mononuclear cells of spondyloarthropathy patients. *Rheumatology*. July 1, 2002;41(7):759–766.
142. Smith JA, Barnes MD, Hong D, Delay ML, Inman RD, Colbert RA. Gene expression analysis of macrophages derived from ankylosing spondylitis patients reveals interferon-gamma dysregulation. *Arthritis Rheum*. May 31, 2008;58(6):1640–1649.
143. Haroon N, Tsui FWL, O'Shea FD, et al. From gene expression to serum proteins: biomarker discovery in ankylosing spondylitis. *Ann Rheum Dis*. January 1, 2010;69(01):297–300.
144. Grcevic D, Jajic Z, Kovacic N, et al. Peripheral blood expression profiles of bone morphogenetic proteins, tumor necrosis factor-superfamily molecules, and transcription factor Runx2 could be used as markers of the form of arthritis, disease activity, and therapeutic responsiveness. *The Journal of Rheumatology*. Feb 2010;37(2): 246–256.
145. Duan R, Leo P, Bradbury L, Brown MA, Thomas G. Gene expression profiling reveals a downregulation in immune-associated genes in patients with AS. *Ann Rheum Dis*. September 1, 2010;69: 1724–1729.
146. Gu J, Wei YL, Wei JC, et al. Identification of RGS1 as a candidate biomarker for undifferentiated spondylarthritis by genome-wide expression profiling and real-time polymerase chain reaction. *Arthritis and Rheumatism*. October 29, 2009;60(11):3269–3279.
147. Batliwalla FM, Baechler EC, Xiao X, et al. Peripheral blood gene expression profiling in rheumatoid arthritis. *Genes Immun*. Aug 2005; 6(5):388–397.
148. Bovin LF, Rieneck K, Workman C, et al. Blood cell gene expression profiling in rheumatoid arthritis. Discriminative genes and effect of rheumatoid factor. *Immunol Lett*. May 15, 2004;93(2–3):217–226.
149. Nikpour M, Dempsey AA, Urowitz MB, Gladman DD, Barnes DA. Association of a gene expression profile from whole blood with disease activity in systemic lupus erythaematosus. *Ann Rheum Dis*. August 1, 2008;67(8):1069–1075.
150. Nzeusseu Toukap A, Galant C, Theate I, et al. Identification of distinct gene expression profiles in the synovium of patients with systemic lupus erythematosus. *Arthritis Rheum*. May 2007;56(5):1579–1588.
151. Baechler EC, Batliwalla FM, Karypis G, et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc Natl Acad Sci U S A*. March 4, 2003;100:2610–2615.
152. Berthelot JM, Glemarec J, Guillot P, Laborie Y, Maugars Y. New pathogenic hypotheses for spondyloarthropathies. *Joint, Bone, Spine*. Mar 2002;69(2):114–122.
153. Batliwalla FM, Li W, Ritchlin CT, et al. Microarray analyses of peripheral blood cells identifies unique gene expression signature in psoriatic arthritis. *Molecular medicine (Cambridge, Mass)*. Jan–Dec 2005;11(1–12):21–29.
154. Alves RDAM, Eijken M, Swagemakers S, et al. Proteomic analysis of human osteoblastic cells: relevant proteins and functional categories for differentiation. *Journal of Proteome Research*. In Press. 2010.
155. Raghavachari N, Xu X, Munson PJ, Gladwin MT. Characterization of whole blood gene expression profiles as a sequel to globin mRNA reduction in patients with sickle cell disease. *PLoS ONE*. 2009;4(8):e6484.
156. Debey S, Schoenbeck U, Hellmich M, et al. Comparison of different isolation techniques prior gene expression profiling of blood derived cells: impact on physiological responses, on overall expression and the role of different cell types. *The Pharmacogenomics Journal*. March 23, 2004 online;4(3):193–207.
157. Min J, Barrett A, Watts T, et al. Variability of gene expression profiles in human blood and lymphoblastoid cell lines. *BMC Genomics*. 2010;11(1):96.

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