The genetic basis of ankylosing spondylitis: new insights into disease pathogenesis

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Abstract: Ankylosing spondylitis (AS) is a complex disease involving multiple risk factors, both genetic and environmental. AS patients are predominantly young men, and the disease is characterized by inflammation and ankylosis, mainly at the cartilage–bone interface and entheses. *HLA-B27* has been known to be the major AS-susceptibility gene for more than 40 years. Despite advances made in the past few years, progress in the search for non-human leukocyte antigen susceptibility genes has been hampered by the heterogeneity of the disease. Compared to other complex diseases, such as inflammatory bowel disease (IBD), fewer susceptibility loci have been identified in AS. Furthermore, non-major histocompatibility-complex susceptibility loci discovered, such as *ERAP1* and *IL23R*, are likely contributors to joint inflammation. Identification and confirmation of functional variants remains a significant challenge of investigations involving genome-wide association studies (GWAS). It remains unclear why none of the AS-susceptibility genes identified in GWAS appear to be directly involved in the ankylosing process. Numerous reviews have recently been published on the genetics of AS. Therefore, aside from a brief summary of what AS GWAS has successfully achieved thus far, this review will focus on directions that could address unanswered questions raised by GWAS.

Keywords: ankylosing spondylitis, genome-wide association studies, risk loci, ankylosis, joint and gut inflammation, clinical subsets

Introduction

Ankylosing spondylitis

Ankylosing spondylitis (AS) is a subset of spondyloarthritis (SpA), which is characterized by inflammation of the sacroiliac joints, peripheral inflammatory arthropathy, and the absence of rheumatoid factor. Other subsets of SpA include reactive arthritis, psoriatic arthritis, colitic arthropathies (inflammatory bowel disease [IBD]-related SpA), and undifferentiated SpA. With a prevalence of 0.1%–1.4%, AS is an under-recognized form of chronic arthritis.¹ It can lead to significant spinal disease and peripheral arthritis, which can manifest as chronic back pain and a progressive spinal ankylosis. The disease strikes predominantly men between the ages of 20 and 40 years, in their peak productive years, leading to significant loss of work productivity and decreased quality of life.

Diagnosis, progression, and current management of AS

AS is usually diagnosed according to the modified New York criteria,² which include a combination of such clinical features as limited motion of the lumbar spine, persistent lower-back pain, limited chest expansion, and radiographic evidence of sacroiliitis.

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The hallmark of AS is neo-ossification at the site of joint inflammation.³ Although joint inflammation can be detected early in the disease process, eg, in the first year of symptoms, using magnetic resonance-imaging technology, this is not a definitive diagnosis test for AS. The subsequent spinal structural changes, as visualized on radiographs, appear relatively late. This explains in part why it can take 5–10 years to confirm a diagnosis of AS after the initial onset of symptoms.^{4,5}

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the first-line drug treatment for AS patients with symptomatic disease.⁶ Continuous use of NSAIDs appears to slow radiographic progression.^{7,8} If NSAID treatment fails, biologics, particularly tumor necrosis factor (TNF)- α inhibitors, are used in patients with active disease. The TNF blockers adalimumab, etanercept, infliximab, golimumab, and certolizumab have all been proven to be highly effective in controlling inflammation and in improving the quality of life of most AS patients. Whether these TNF blockers could halt progression of structural changes remains a controversial issue. Some studies showed no structural impact,⁹ but a recent study indicated that TNF inhibitors impact structural damage, such as new syndesmophyte appearance, growth of existing syndesmophytes, and development of ankylosis in AS.¹⁰ However, a substantial proportion of AS patients (~25%) do not respond to TNF-inhibitor therapy, and some initial responders developed secondary unresponsiveness.

Joint ankylosis

Current concepts regard new bone formation at the entheses as a pathological response to injury, and that joint inflammation precedes ossification. Early stages of ankylosis involve squaring of the vertebral bodies and formation of syndesmophytes. Total spinal ankylosis and kyphosis are found in the most severe cases. Radiographic changes of the cervical and lumbar spine in AS patients are scored using the modified Stokes AS Spine Score (mSASSS).¹¹ Scoring of sequential radiographs from the same patient over time determines the change in mSASSS per year. Systematic evaluation of ankylosis progression in AS patients is subjective and time-consuming.^{12,13}

There remains some uncertainty whether joint inflammation and ankylosis in AS are linked events or independent processes.^{14–16} For clinicians, this critical issue affects the management strategy for SpA: early intervention with anti-TNF therapy would prevent ankylosis development only if inflammation and ankylosis are sequential and linked processes.

Genetics and AS

Based on family and twin studies, it has been established for a long time that AS has a strong genetic component. The sibling recurrence risk of AS is 9.2% (λ_s is 82) compared to 0.1% in the general population.¹⁷ The heritability of AS is estimated to be >95%. The strong association with human leukocyte antigen (HLA)-B27 was discovered in the early 1970s. Population studies have indicated that 2% of HLA-B27-positive individuals develop AS,¹⁸ implying that other factors – genetic, environmental, or stochastic – contribute importantly to disease development. The most direct evidence that genetic polymorphisms other than HLA-B27 contribute to AS is the difference in concordance rates reported for HLA-B27-positive monozygotic twins (17 of 27 [63%]) and HLA-B27-positive dizygotic twins (four of 15 [27%]).¹⁹ These same statistics indicate that the predisposition to AS is not entirely genetically determined. Nongenetic factors might correspond to an environmental effect, such as a specific microbial infection, as implicated in reactive arthritis, or it might correspond to a stochastic event in development, such as the emergence of specific immune cells.

Recent genome-wide association study findings

A number of updated reviews on AS genetics,^{20–24} including genome-wide association study (GWAS) results, have recently been published, and thus we only briefly summarize the key findings here. Based on the most recent report on AS susceptibility loci detected by ImmunoChip (Illumina, San Diego, CA, USA) genotyping of the largest cohort (more than 10,000 individuals for both cases and controls examined),²⁵ there are at least 25 AS non-major histocompatibility complex (MHC) immune-related risk loci (summarized in Table 1), eleven of which have been identified previously.²⁶ A major achievement of these studies relates to the identification of important biological pathways likely responsible for AS pathogenesis.

Role of the interleukin-23-related pathway

AS-associated genetic variants relating to this pathway include interleukin (IL)-23R, IL-12 β , Tyk2, IL27, and IL-6R. Susceptibility to loci involved in this pathway is shared among many inflammatory diseases, including IBD and psoriasis. It remains to be elucidated how influence on one common pathway leads to the development of different inflammatory diseases. A recent elegant study in *Cell* illustrates the power of using a combination of genetic, clinical, and functional analyses in both mice

Table 1 Summary of ankylosing spondylitis-susceptibility genes identified by genome-wide association studies

<i>RUNX3</i>	Runt-related transcription factor 3
<i>IL23R</i>	Interleukin 23 receptor
<i>IL12Rβ2</i>	Interleukin 12 receptor, β 2
<i>GRP25</i>	G-protein-coupled receptor 25
<i>KIF21B</i>	Kinesin family member 21B
<i>PTGER4</i>	Prostaglandin E receptor 4 (subtype EP ₄)
<i>ERAP1</i>	Endoplasmic reticulum aminopeptidase 1
<i>ERAP2</i>	Endoplasmic reticulum aminopeptidase 2
<i>LNPEP</i>	Leucyl/cystinyl aminopeptidase
<i>IL12B</i>	Interleukin 12B
<i>CARD9</i>	Caspase recruitment-domain family member 9
<i>LTβR</i>	Lymphotoxin β -receptor (TNFR superfamily, member 3)
<i>TNFRSF1A</i>	Tumor-necrosis factor-receptor superfamily member 1A
<i>NPEPPS</i>	Aminopeptidase puromycin-sensitive
<i>TBκBP1</i>	TNFR-associated factor family member-associated nuclear factor- κ B-binding kinase 1-binding protein
<i>TBX21</i>	T-box 21
<i>IL6R</i>	Interleukin 6 receptor
<i>FCGR2A</i>	Fc fragment of immunoglobulin G, low-affinity IIa, receptor (CD32)
<i>UBE2E3</i>	Ubiquitin-conjugating enzyme E2E 3
<i>GPR35</i>	G-protein-coupled receptor 35
<i>NKX2-3</i>	NK2 homeobox 3
<i>ZMIZ1</i>	Zinc finger, MIZ type-containing 1
<i>SH2B3</i>	Src homology 2B adaptor protein 3
<i>GPR65</i>	G-protein-coupled receptor 65
<i>IL27</i>	Interleukin 27
<i>SULT1A1</i>	Sulfotransferase family cytosolic 1A
<i>TYK2</i>	Tyrosine kinase 2
<i>ICOSLG</i>	Inducible T-cell costimulator ligand
<i>EOMES</i>	Eomesodermin
<i>IL7R</i>	Interleukin 7 receptor
<i>BACH2</i>	BTB and CNC homology 1, basic leucine-zipper transcription-factor 2

Abbreviation: CD, classification determinant.

and humans to unravel how a noncoding single-nucleotide polymorphism (SNP) can influence the disease process.²⁷ Intriguingly, this *FOXO3* variant (*rs12212067*), which regulates cytokine production in monocytes, was not associated with susceptibility to the rheumatoid arthritis or Crohn's disease.

It is established that IL-23 drives the differentiation of CD4-positive Th17 cells, which produce IL-17. IL-17 in turn can facilitate the production of other factors (such as IL-6, IL-8, TNF, chemokines, matrix metalloproteinases, and receptor activator of nuclear factor κ B ligand) from a wide range of cell types.²⁸ A French SpA study illustrated that variants at loci in the IL-23/Th17 pathway influence expression levels of genes involved in the differentiation of Th17/Th1 cells, and it is likely that the pathological outcome is dictated by combinatorial assortments of multiple variants.²⁹ A comprehensive discussion on how IL-23/IL-17 pathways

impact on AS pathogenesis is beyond the scope of this review. An excellent review on this aspect has just been published.²⁸

Role of aminopeptidases

In addition to *ERAP1* and *ERAP2*, two other aminopeptidases (*LNPEP* and *NPEPPS*) are associated with AS,²³ reiterating the importance of antigen presentation in AS pathogenesis. Both protective and susceptible *ERAP1* variants associated with AS have been identified. The relative attributable risk of *ERAP1* to AS is about 25%, whereas that of *HLA-B27* is about 50%. These two genes combined provide the two most powerful disease risk factors to AS. Intriguingly, the association of *ERAP1* is restricted to HLA-B27-positive AS patients.²⁵ One recent functional study showed that *ERAP1* variants affect HLA-B27 antigen presentation and stability in vivo.³⁰ Protective variants lead to less *ERAP1* activity, and less efficient trimming of HLA-B27 ligands. Another study supported the notion that AS-associated *ERAP1* variants alter the composition and length of HLA-B27 ligands.³¹ A more in-depth review on the role of *ERAP1* in AS pathogenesis will be discussed in later sections.

ERAP2 is unique to humans, and does not exist in mice. However, a high-frequency variant, when present in homozygosity (about 25% of the population), results in the absence of *ERAP2* protein in these individuals. In *ERAP2*-deficient human B cells, surface MHC-I expression is reduced.³² It remains unclear whether the absence of *ERAP2* might alter/modulate antigen presentation in these individuals, especially patients with such diseases as AS and Crohn's disease in which disease-associated *ERAP2* variants exist. Intriguingly, one *ERAP2* variant (*rs2549782*) confers natural resistance to human immunodeficiency virus-1 infection.³³ Results from the most recent GWAS indicated that *ERAP2* variants are associated with AS in HLA-B27-negative cases.²⁵

Despite substantial sequence homology, similar overall domain organization and structures between *ERAP1* and *ERAP2*, the N-terminal peptide specificities between these two aminopeptidases are quite different, as explained by their crystal structures.³⁴ We showed that an *ERAP1* *ERAP2* haplotype (*rs27044*[G] *rs30187*[T] *rs2549782*[T]) is associated with familial AS.³⁵ A recent study using sequencing haplotypes in 20 individuals showed that this haplotype occurs naturally.³⁶ Amino acid variants coded by *ERAP2* *rs2549782* (N392K) alter both the specificity and activity of *ERAP2*. Amino acid variants coded by *ERAP1* *rs27044* (Q730E) and *rs30187* (K528R) affect peptide-trimming activity. To date,

there has been only one study that assessed the effects of naturally occurring ERAP haplotypes.³⁶ Importantly, results from this study showed that *ERAP* SNPs, when assessed in combination (as a haplotype), showed different effects compared to those assessed singly. Though there are human cells deficient in ERAP2, currently no ERAP1-deficient human cells are available for accurate assessments of the effects of natural ERAP haplotypes, and this poses a limitation on these types of studies. It is expected that different ERAP haplotypes would impact on natural killer cell and cytotoxic T-lymphocyte functions.

Additional AS risk loci contribute to variations in T-cell lineages (*EOMES*, *IL7R*, *RUNX3*, and *ZMIZ1* for CD8⁺ T cells, and *BACH2* and *SH2B3* for CD4⁺ T cells). Variants in G-protein-coupled receptors (*GPR35*, *GPR37*, *GPR65*, and *GPR25*) were also identified, but their involvement with AS pathogenesis is less clear. It has been estimated that all these non-MHC risk loci only account for 4.3% of heritability in AS, while HLA-B27 contribute 20.1%, implying that a majority of risk loci (about 75%) remain undefined.

Another insight emerged from GWAS relates to common risk loci shared among some inflammatory diseases. Most notably is the largest number of loci shared between AS and IBD^{37,38} (12 and 11 AS loci shared with Crohn's disease and ulcerative colitis [UC] respectively). At least 163 risk loci have been identified in IBD and about 28 of them were shared between Crohn's disease and ulcerative colitis. The significant number of risk loci shared between AS and IBD supports the recent concept that gut involvement contributes to disease pathogenesis in a large subset (up to 60%) of AS patients. This issue is further addressed in a later section of this review.

Limitations of GWAS

In general, despite the wealth of new information obtained from GWAS, some unexpected challenges also emerged. Following are examples: 1) The number of risk loci uncovered was not only high (in terms of thousands in some complex traits) but also increased proportionally with the cohort size analyzed.³⁹ There are some indications that variants from different steps of the same biological pathway could contribute similarly to the eventual clinical outcome; 2) The effect size of individual risk loci are usually very modest (odds ratio ~1.05–1.4), and GWAS can only detect common variants with a minor allele frequency of >0.05. For minor causal variants, deep sequencing of the regions of interest is required;^{40,41} 3) Identification of causal variants with direct or indirect functional relevance to disease risk has proved to be difficult. It is challenging to prove that functional consequences of variants (usually assessed

singly) contribute to disease pathogenesis. For complex traits, different combinations of risk factors can lead to similar clinical outcomes, leading to disease heterogeneity. In IBD, of 163 risk loci identified, only one (nucleotide-binding oligomerization domain-containing protein 2) has been shown to correlate with clinical outcomes.⁴² More importantly, the lack of large cohorts with detailed clinical parameters and well-characterized disease outcome renders meaningful analyses of genotypes obtained in GWAS a major challenge; 4) Known AS susceptibility locus (such as the *IL1* gene cluster) can be missed. In the earlier AS genetic studies, positive and negative results were obtained with respect to the association of variants within the *IL1* gene cluster in AS.^{43–47} This locus was not detected in recent AS GWAS. However, a recent French study showed that *IL1A* is associated with AS susceptibility or sacroiliitis in AS.⁴⁸ Likely reasons contributing to the discrepancies among studies include disease heterogeneity, study design, and power limitations. The precise role of IL-1 in AS pathogenesis remains unclear, though it could contribute singly or in combination with other pathways with susceptibility variants (such as IL-23). Despite these inconsistent results from different studies, the latest International Genetics of Ankylosing Spondylitis Consortium GWAS²⁵ concluded that AS is associated with the *IL1R1–IL1R2* locus located on chromosome 2q11. There were two signals, one in each of the *IL1R* genes.

Are there risk loci relating to neo-ossification/ankylosis in AS patients?

In the AS GWAS result published in 2010,²⁶ *ANTXR2* (*CMG2*) was identified as one of the risk loci. Unfortunately, no SNP in this locus was included in the most recent AS GWAS, and thus it is unclear whether this association is replicable. *ANTXR2* is not associated with AS in the Han Chinese,⁴⁹ and the minor allele frequency was too low to analyze this in Koreans. Anthrax toxin-receptor 2 could potentially affect new bone formation, as it is a membrane-bound molecule that can interact with low-density lipoprotein receptor-related protein (LRP)-6.⁵⁰ LRP6 is an important surface receptor in the Wnt/ β -catenin pathway, and thus can affect osteoblastic activity. More work in discerning whether *ANTXR2* plays a role in AS pathogenesis is warranted.

A recent GWAS performed in Han Chinese with AS⁴⁹ detected two risk loci likely with relevance to bone formation (*HAPLN1–EDIL3* at 5q14.3 and *ANO6* at 12q12.1). *HAPLN1* has been shown to be involved with osteophyte formation⁵¹ in Japanese women with spinal osteoarthritis. *EDIL3* has an inhibitory effect on Wnt/ β -catenin signaling.⁵²

ANO6 plays a role in osteoclastogenesis.⁵³ The most recent GWAS on East Asians using the ImmunoChip failed to replicate association of these two loci.²⁵ One explanation is the low frequencies of the variants. This scenario is supported by the absence of association in *IL23R* variants for Han Chinese AS, but recent sequencing of this locus revealed a few rare variants with potential functional relevance.⁵⁴ However, it is also possible that the absence of *ANTRX2*, *HAPLN1-EDIL3*, and *ANO6* association in Han Chinese AS GWAS might be due to other ethnic differences.

There are a few studies implicating the role of IL-23 in excess bone ossification in AS. In a French population, an *IL-23R* variant was associated with radiographic sacroiliitis in AS.⁵⁵ A more indirect finding relates to elevated levels of IL-23 detected in bone marrow cells from AS spinal facet joints obtained in corrective surgery.⁵⁶ A mouse study also illustrated that IL-23 could drive enthesitis via enthesal resident T cells (positive for IL-23R, retinoic acid receptor-related orphan receptor- γ t, and CD3) in an IL-22-dependent manner.⁵⁷

Considering the strength of association of *ERAP1* with AS, we questioned whether genes involved in the antigen-processing pathway could also be a marker of severity in addition to susceptibility. For this study,⁵⁸ a total of 241 Caucasian patients with AS from spondylitis clinics in Toronto and Edmonton were included. Those patients who had at least two full sets of radiographs for mSASSS scoring at a minimum interval of 1.5 years were included in the analysis for genetic predictors of progression. Genotyping was done for a panel of 13 coding region SNPs in the *ERAP1*, *LMP2*, *LMP7*, *TAP1*, and *TAP2* genes. In the univariate analysis for predictors of baseline radiographic severity, the duration of disease was the strongest, followed by male sex and *LMP2* and *ERAP1* variants. In multivariate analysis, the only genetic predictor that remained strongly associated with severity was *LMP2*. This is very interesting, as *LMP2* has previously been reported to be associated with AS and uveitis in AS patients. Due to the association with uveitis, *LMP2* may in fact be a marker of a more aggressive form of AS that could lead to more structural damage. Moreover, the proteasome helps to break down β -catenin, and abnormalities in *LMP2* could lead to excess Wnt/ β -catenin signaling and osteoblastic activity.

A candidate-gene approach to unravel ankylosis-related risk loci in AS; the role of ANKH and TNAP

Inorganic pyrophosphate (PPi) plays an important role in regulating mineralization and bone formation.⁵⁹ The sources of PPi for the cartilage matrix include

intracellular-to-extracellular transport of PPi through the cell membrane in association with a membrane transport system that involves the ANKH (human homolog of progressive ankylosis) protein⁶⁰ and generation of PPi at the cell surface by tissue-nonspecific alkaline phosphatases (TNAPs) or ectonucleotidases.^{61,62}

Using a candidate-gene approach, our linkage- and family-based association analyses⁶³ demonstrated that North American Caucasian AS patients have excess sharing of the *ANKH* gene region, and that AS is significantly associated with a specific *ANKH* haplotype.⁶³ Significantly, we further showed that there are sex differences in the *ANKH* variants associated with AS.⁶⁴ Intriguingly, there is heterogeneity even in multiplex AS families. There were three types of families in our cohort of multiplex AS families: 1) families with affected individuals of both sexes, 2) families with only men affected, and 3) families with only women affected. In the first type of families with affected individuals of both sexes, two *ANKH* SNPs (*rs28006[C]* *rs25957[C]*) were associated with AS only in affected women. This haplotype was transmitted to affected women 79% of the time (15 of 19), but to affected men only 27% of the time (three of eleven). This effect is substantial, as the odds ratio for increased risk approaches 3.0 (0.79/0.27=2.92). In another haplotype (*rs26307[C]* *rs27356[C]*), the frequency of transmission was 70% to affected men (21 of 30) and 43% to affected women (13 of 30). In the subset of families with only men affected, 94% of the time (16 of 17), this haplotype was transmitted to affected men. There were too few informative families with only women affected with this haplotype, and thus we do not have a reliable assessment of the frequency at which this haplotype was transmitted to affected women in this subset for comparison.

Using Family-Based Association Test (Harvard School of Public Health, Boston, MA, USA) analysis in the subset of AS families with affected individuals of both sexes, we also found that a *TNAP* variant (Tyr263His) is significantly associated with AS.⁶⁵ Furthermore, this *TNAP* variant is significantly associated with AS in affected men (dominant model, $P=0.005$). More impressively, the frequency of transmission of this allele was 92.3% (12 of 13) to affected men and only 64.7% (eleven of 17) to affected women. It appears that in this subset of AS families with affected individuals of both sexes, an *ANKH* polymorphism in the intron 1, close to exon 2 region, predisposes the affected women to AS, while a *TNAP* variant (possibly Tyr263His) predisposes the affected men to AS. The *TNAP* Tyr263His is a functional variant,⁶⁶ and is associated with bone mineral density in elderly women.⁶⁷

Osteoporosis is a common occurrence in AS. Investigation on whether this functional TNAP variant (Tyr263His) is associated with osteoporosis in AS patients is warranted.

In our cohort of North American Caucasian multiplex families, both *ANKH* and *TNAP* were associated with AS (summarized in Figure 1). Subsequently, there have been reports of *ANKH* but not *TNAP* association in Japanese⁶⁸ and Han Chinese⁶⁹ cohorts. In an earlier UK study, no *ANKH* association was detected.⁷⁰ A recent presentation at the annual meeting of the American College of Rheumatology reported that *ANKH* variants were associated with disease severity in Korean AS.⁷¹

Gene–gene interactions: the role of ERAP1 and HLA-B27 in AS

The discovery of the association of ERAP with AS opened new opportunities for investigating AS. Gain or loss of ERAP1 function could be used in experimental settings to further decipher the mechanisms by which ERAP1 contributes to the overall pathogenesis of AS. The mechanisms by which ERAP1 and HLA-B27 contribute to AS are under study. The main function of ERAP1 is to trim peptides within the ER into the right length before binding to MHC-I molecules. Abnormal function of ERAP1 can lead to generation of anomalous peptides of incorrect length and sequence, which subsequent to MHC-I binding (eg, HLA-B27) can lead to peptide–MHC-I complex misfolding. These misfolded proteins could either accumulate within the ER, thus contributing to the ER stress, or be transported to the cell surface as a free heavy chain. Both of these mechanisms have been implicated as contributing to AS pathogenesis,

but the degree of their contribution to the overall pathogenesis of AS remains speculative. Loss of ERAP1 function leads to decreased surface expression of MHC-I molecules. Reduced surface expression has been suggested to contribute to the overall pathogenesis of AS. Recent data from our laboratory analyzing immune response to influenza virus have shown a change in the overall flu-peptide repertoire in mice deficient in ERAP function (ERAP1^{−/−}).⁷² Our results confirm the findings of other investigators: ERAP1^{−/−} mice had changes in the presentation of viral epitopes of different types, indicating the importance of ERAP in viral peptide generation and presentation. While these studies implicate the presence of an arthritogenic peptide in AS, the identity of this epitope is unknown. Since loss of ERAP1 function seems to be protective in AS, ERAP1 may be involved in the generation of arthritogenic peptides. However, to date, there has been no direct evidence either confirming or refuting this hypothesis.

Gene–gene interactions: the role of ERAP1 in AS patients following influenza infection

ERAP1 plays an integral role in the immune response following an infection. It can either enhance or diminish the immune response by changing the peptide repertoire presented by antigen-presenting cells.^{73–75} ERAP1, for instance, has been shown to affect the repertoire of peptides generated following influenza infection, one of the most commonly encountered human viruses. However, there are no reports investigating the host response to flu infection in AS patients. Although there are suggestions that an arthritogenic peptide or peptides

Figure 1 Summary of family-based association analyses using multiplex ankylosing spondylitis (AS) families from the North American Spondylitis Consortium (NASC). **Abbreviations:** *ANKH*, human homolog of progressive ankylosis; *TNAP*, tissue-nonspecific alkaline phosphatases.

plays a central role in AS,^{76,77} so far no such peptides have been discovered. Prior flu infection may influence the presentation of such arthritogenic peptides. In addition, AS is a multigenic disease, and as such, studies of AS patients with prior flu infection would be complex to analyze.⁷⁷ Given these limitations, there is a high need for an AS animal model to investigate the role of these aforementioned factors in vivo. An animal model could also be useful to address gene–gene interaction in AS, which is highlighted by the fact that *ERAP1* is associated only with B27-positive AS and not with B27-negative AS.

To overcome this limitation, we have used human HLA transgenic (HLA Tg) mice, which lack both *ERAP1* and endogenous MHC-I molecule expression and which express HLA-B27 (ie, Tg HLA-B27/*ERAP*^{−/−}) to investigate the interaction of *ERAP1* and *HLA-B27* in AS. These two genes are the strongest genetic associations with AS to date,⁷⁸ yet B27/*ERAP*^{−/−} mice manifest no articular abnormalities (unpublished results). It should be noted in this regard that loss-of-function variants of *ERAP1* are protective in AS. On the other hand, since the flu peptides presented by B27 are well known, this animal model can provide important insights into the role of ERAP in generating B27-specific antigenic peptides. Initial studies of flu infection of Tg HLA-B27/*ERAP*^{−/−} mice suggest that *ERAP1* may play a central role in the phenomenon of immunodominance after flu infection.⁷⁹ This animal model could prove valuable in deciphering the interaction of *ERAP1* and *HLA-B27* in an in vivo context.

Animal models with axial ankylosis

Though some AS patients have peripheral arthritis, AS is mainly an axial disease. An informative animal model could unravel the underlying mechanisms that lead to the development of axial inflammation and eventual ankylosis. Because AS is a multifactorial complex disease, it is unlikely that a perfect animal model for AS exists. Since HLA-B27 showed the strongest association with AS, transgenic rats highly expressing HLA-B27 and human β_2 -microglobulin could be a reasonable model. These transgenic rats have spontaneous peripheral and axial inflammation, as well as gut disease.⁸⁰ With elevated β_2 -microglobulin expression, ankylosis developed with concurrent suppression of gut disease and an unfolded protein response.⁸¹

Two mouse models overexpressing TNF α (hTNFtg⁸² and TNF AU-rich elements [Δ ARE]⁸³) develop systemic inflammation, gut disease, and sacroiliitis, but no ankylosis. A recent study⁸⁴ reported that mechanotransduction can lead to enthesitis and neo-ossification at enthesal sites in tail-suspended TNF Δ ARE mice. Enthesitis induction involves a

number of pathways, including signaling via IL-23R-positive enthesal resident cells.

The proteoglycan-induced spondylitis (PGISp) model mimics many features of human AS, including axial inflammation and ankylosis.^{85,86} The spines of the PGISp mice showed decreased levels of Wnt-signaling antagonists (such as Dickkopf-related protein 1 and sclerostin [SOST]).⁸⁷ There is evidence suggesting that enhanced Wnt/ β -catenin signaling contributes to ankylosis in AS patients. Dickkopf-related protein 1, an antagonist of Wnt/ β -catenin signaling, has been reported to be either dysfunctional⁸⁸ or present at lower-than-normal levels in AS patients.⁸⁹ Serum levels of SOST are also lower in AS patients than healthy individuals.⁹⁰ Our recent work on *ank/ank* (progressive ankylosis) mice that have peripheral and spinal ankylosis showed enhanced Wnt/ β -catenin signaling in the joints of these mutant mice.⁹¹

Old male DBA/1 mice develop peripheral arthritis and it has been shown that noggin (NOG) can rescue the enthesopathy.⁹² In contrast, we showed that NOG treatment of *ank/ank* mice led to more severe ankylosis, with concurrent generation of high levels of immunoglobulin (Ig)-G immune complexes (ICs) in which the autoantigens are either NOG (a bone morphogenetic protein-signaling antagonist) or SOST (a Wnt/ β -catenin signaling antagonist).⁹³ These results from the mutant *ank/ank* mice led to our novel finding of similar NOG/SOST IgG ICs in humans, and AS patients have significantly elevated serum levels of these ICs.⁹³ An intriguing possibility is that ICs involving autoantibodies against NOG and SOST at their interacting sites may mimic the inhibitory interaction that naturally occurs between these two proteins. By this mechanism, these autoantibodies would be predicted to play a physiological role in bone homeostasis in normal individuals. However, overabundance of these autoantibodies would lead to reduced levels of functional NOG and SOST, resulting in enhanced bone morphogenetic protein and β -catenin signaling, neo-ossification and eventual spinal ankylosis.

Lessons from GWAS: how might they help in disease management?

Biologics involving inhibition of TNF α have proven to have dramatic efficacy in about 70% of AS patients, but they do not cure the disease. In many patients, challenges remain regarding how best to achieve and maintain remission. As mentioned earlier, treatment targeting IL-1 using anakinra was not very promising.⁹⁴ *IL6R* is one of the AS-susceptibility genes detected in GWAS, yet tocilizumab (anti-human IL-6-receptor monoclonal antibody)⁹⁵ and sarilumab (a fully human monoclonal antibody against IL-6R α)⁹⁶ appear to

have no efficacy in AS patients. Genetic studies implicated the importance of the IL-23 signaling pathway. A recent prospective clinical trial showed promising efficacy and safety in the use of ustekinumab⁹⁷ (anti-IL-12/23p40) to treat AS patients. Promising results were shown in IL-17 blockade.⁹⁸ More studies are warranted to explore novel therapies for AS patients especially for TNF-inhibitor nonresponders.

Future directions

As mentioned earlier, AS is a very heterogeneous complex disease. Different combinations of risk loci, together with other nongenetic factors, would lead to a similar clinical outcome, ie, AS disease. Studies using patient cohorts with bias toward certain subsets would result in controversial and inconsistent results. Genetic analysis of AS subsets would lead to the identification of subset-specific risk loci. The association of *ERAP1* being restricted to HLA-B27-positive AS patients represents a good example.

It has been known for decades that AS patients have extra-articular manifestations. A recent systematic meta-analysis⁹⁹ confirmed that about 25.8% of AS patients have acute anterior uveitis (AAU); 9.3% and 6.8% have concomitant psoriasis and IBD, respectively. Intriguingly, the large overlap of AS patients with AAU is associated with disease duration, implying that the disease process in AS renders the patient susceptible to AAU. In contrast, it was reported that occurrence of IBD or psoriasis in AS was not associated with disease duration. In some such AS patients, IBD and psoriasis might be present prior to the diagnosis of AS. The implication of this is that certain AS subsets might have different etiologic pathways underlying the disease. For example, histopathological findings demonstrated that 50%–60% of AS patients have evidence of gut inflammation,¹⁰⁰ although this is clinically evident in only 5%–10% of AS patients.¹⁰¹ It may be that AS patients with subclinical versus clinical gut inflammation represent distinct subsets of the disease. It is possible that AS patients whose gut and joint inflammation might be triggered by specific microbial exposure and thus leading to distinctive, pathogenic immune responses. In support of this, we recently found that higher-than-normal levels of NOG and SOST IgG immune complexes were detected in sera of AS patients, as well as in patients with both AS and IBD.⁹³ A recent GWAS has already revealed common risk loci between AS and IBD (such as *ERAP1* and *IL23R*).²⁵ It is anticipated that performing GWAS using samples from AS patients with subclinical versus clinical gut inflammation would unravel novel risk loci specific for this AS subset.

In summary, recent GWAS findings provide invaluable clues on pathways key to the development of AS. The logical lines of investigation to follow should focus on the biology of the risk factors, especially in terms of how these risk factors would influence disease pathogenesis and clinical outcome.

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Disclosure

The authors report no conflicts of interest in this work.

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