Genetics of Spondyloarthropathies and Inflammatory Bowel Disease: Searching for Common Susceptibility Factors

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Abstract: Ileocolonoscopic evidence for subclinical gut inflammation is found in a subpopulation of spondyloarthropathy (SpA) patients. The prevalence of microscopic intestinal lesions is even higher and can be classified as either an acute or a chronic type of inflammation. The latter condition is associated with an increased risk of developing overt inflammatory bowel disease (IBD), especially Crohn's disease (CD), over time. Evidence for genetic predisposition in both SpA and IBD is strong, and has resulted in the identification of several linked chromosomal loci and putative candidate genes. The regular co-existence of SpA and IBD within the same family suggests a common genetic component. Interestingly, comparison of genome-wide linkage and association data reveals thirteen disease-associated chromosomal regions that are shared between SpA and IBD. This should convince geneticists to examine genes within these regions as potential susceptibility genes for the development of both SpA and IBD. Significant association of such shared genetic determinants was established for *NOD2* (16q), the major histocompatibility complex I allele *HLA-B27* (6p) and recently also the interleukin 23 receptor (1p). Transgenic animals in which tumor necrosis factor alpha or HLA-B27 is overexpressed suffer both joint and gut abnormalities resembling human SpA/CD pathology, providing additional evidence for a common genetic predisposition for the onset of joint and gut inflammation. In view of a hypothetical pathway leading to intestinal and articular inflammation in SpA and IBD, we review and compare genome-wide linkage and genetic association data obtained for SpA and IBD.

Keywords: Spondyloarthropathy, Crohn's disease, genetic susceptibility, NOD2, HLA-B27, IL23R.

Spondyloarthropathy (SpA) and inflammatory bowel disease (IBD) are chronic inflammatory conditions in which the initiating events are complex. In SpA, the axial skeleton, the sacroiliac joints and, to a lesser degree, peripheral joints become inflamed, whereas in IBD involving Crohn's disease (CD) and ulcerative colitis (UC), the intestine is the primary site of inflammation. The estimated prevalence in Western countries is 0.3-2% for SpA and 0.1-0.3% for IBD. The unity between SpA and IBD is illustrated by a strong clinical as well as molecular overlap. The first symptoms present themselves in early adulthood, usually before the age of 30, but early childhood and late onset disease occur sporadically. Medical treatment is mainly based on diminishing the inflammation and maintaining the remission states. Spondyloarthropathy is a heterogeneous group generally divided into 5 disease categories: ankylosing spondylitis (AS), reactive arthritis (ReA), psoriatic arthritis (PsA), SpA associated with IBD (SpA-IBD) and undifferentiated SpA. In the SpA-IBD group, patients suffer from clinically overt CD or UC. However, the gut is also an important site of inflammation in patients belonging to the other SpA disease categories. In ileocolonoscopic studies of SpA patients, histological signs of gut inflammation were found in more than half of the subjects, while this was not seen in any other inflammatory joint disease [1-6]. Generally, no clinical intestinal manifestations were present. In addition, remission of joint inflammation was always linked with a disappearance of gut inflammation [7]. With histology, two types of inflammation are distinguished: acute inflammation resembling infectious enterocolitis, and chronic inflammation more suggestive of early CD [8]. In a long-term follow-up study of SpA patients, it was shown that a high percentage of patients initially diagnosed with chronic gut inflammation developed clinical CD [7]. The presence of early CD-related immune changes in the gut of patients with SpA and an increase in antigen handling and presentation was shown [9-16]. On the other hand, joint involvement is observed in 10-20% of IBD patients, whereas the presence of radiological sacroiliitis is much more common [17]. In 90% of IBD patients with articular involvement, their symptoms can be classified as SpA [18]. Interestingly, therapeutic intervention in SpA and IBD can have a positive influence on both the articular and the intestinal inflammation [19, 20].

A strong genetic predisposition was shown for CD and SpA, illustrated by familial clustering and a high recurrence risk in relatives of affected individuals [21, 22]. The concordance rate in monozygotic twins is 50-75% for SpA [22] and 20-50% for CD [21], which is significantly higher than in dizygotic twins. Since these numbers do not reach 100%, it is well understood that genes as well as environmental triggers interact to set off the diseases. For a long time, a very strong association with the major histocompatibility complex I allele *HLA-B27* was described for SpA. The genetic component for UC is less than for CD [21, 23]; therefore this review will mainly focus on data obtained for CD.

The vast majority of genetic research in SpA and CD focuses on the individual disease. The presence of shared clinical and pathological features between SpA and IBD, however, encourages the search for common genetic aberrations. To gain insight into common susceptibility for SpA and IBD, we compared genome-wide linkage and association

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data, and focused on chromosomal loci that are linked with both diseases. Secondly, association studies of the most important genes were compared and discussed.

FAMILIAL CLUSTERING OF SPONDYLOARTHROPATHY AND INFLAMMATORY BOWEL DISEASE

In addition to the co-occurrence of SpA and IBD within one patient, these diseases have a high tendency to cluster within families. Several isolated reports of mixed SpA/IBD families exist [24-28], though no systematic estimate within families has been reported to date. In Table **1**, the number of IBD patients among relatives of SpA probands was deducted from other family studies [29-34]. The frequency of IBD in these families ranges from 4 to 10%. In two studies, a disturbed male to female ratio was found [29, 30]. Although no sex difference exists in sporadic or familial IBD [35, 36], linkage to the X chromosome was found twice [37, 38]. Moreover, an increase in maternal transmission was shown in non-Jewish parent-child pairs [39], which was also described in mixed UC/CD families [40]. Whether this sex bias is caused by true genetic association or by imprinting of the X chromosome is not clear.

Familial SpA and IBD is suspected to be genetically more homogenous than sporadic cases. For CD, an earlier age of onset and a higher frequency of exclusively colonic disease was shown in familial cases [41-45]. The familial form of AS is associated with a milder disease phenotype [46], and high heritability of disease severity was shown [47, 48]. To what extent these observations hold true in mixed SpA/IBD families is not known.

Taken together, these numbers indicate a significant increase in the co-occurrence of SpA and IBD within families, and emphasize the importance of family history as a risk factor for the development of SpA and/or IBD. In addition to a genetic component, shared environmental factors such as microbial agents or eating habits in some families cannot be ruled out as specific triggers of the disease [41, 49]. Nevertheless, mixed SpA/IBD families can be extremely useful for the identification of new common risk factors involved in both pathologies.

GENETIC MODELS FOR SPA AND IBD SUSCEPTIBILITY

The question of how many genes are involved in individual genetic susceptibility to SpA and IBD, what degree of penetrance they posses and how they interact remains. Major genes are, by definition, those that lead to the phenotype in most cases, while minor genes are less penetrant and more vulnerable to environmental triggers.

Genome-wide linkage analyses in SpA and CD always result in more than one locus, which suggests an association with more than one chromosomal region and, thus, different genes. Two models that are not mutually exclusive are proposed here, each based on a division between familial and sporadic cases.

The elevated risk of relatives of SpA and CD patients developing disease suggests a model where SpA/CD etiology is reliant on a major gene (Fig. **1**, model 1), e.g. a gene predisposing for "overactive inflammation", with independent major genes acting to develop inflammation at a specific site, the intestine for CD and the axial skeleton for SpA. In this model, different major genes can lead to similar phenotypes, leading to genetic heterogeneity. Especially for the "inflammation" phenotype, every gene within the inflammatory pathway can possibly be involved in genetic susceptibility for overactive or non-self-limiting inflammation. Additional environment-sensitive minor genes independently influence the disease subphenotypes, e.g. colonic or ileal involvement in CD. Disease location in CD is, indeed, highly genetically determined, since this feature is relatively stable within families and is highly concordant in monozygotic twins [50]. Recently, it was suggested that genetic predisposition for familial SpA as a group is due to a shared component, which fits with this model. Indeed, detailed phenotypic studies in SpA multiplex families reported some unexpected findings, most notably the lack of clustering of most manifestations of SpA [30, 31, 51]. In this model, the variation within the SpA concept could be explained as the influence of minor genes acting on the overall SpA phenotype.

On the other hand, most SpA and CD patients are sporadic cases without any diseased relatives. They probably result from a combination of less penetrant minor effects of widespread genetic determinants. A second model states that disease occurs when a specific combination of genes act together (Fig. **1**, model 2). On top, independent minor genes influence the disease phenotype. In this way, overlapping diseases such as CD and SpA can be easily explained. A simple addition of a risk factor in a patient with SpA could lead to intestinal inflammation resembling CD. For example, we showed that common CD-associated variations in the *NOD2* gene are more frequently found in SpA patients with CD-like subclinical chronic gut inflammation [52]. This gene

Table 1. Number of IBD Patients in SpA Multiplex Families

Number of SpA Patients in Multiplex Families	Type of Study	*n* IBD (%)	Male:Female	Ref.
539	Phenotype analysis	25 (4.6)	8:17*	[29]
188	HLA analysis/transmission	8 (4.2)	ND	[34]
445 (AS only)	Whole-genome scan	ND (9)	ND	[32]
393 (AS only)	Whole-genome scan	40 (10.2)	ND	[33]
91	Phenotype segregation	8 (8.8)	ND	[31]
329	Phenotype analysis	17 (5.2)	4:13*	[30]

*Significant difference.
ND: not determined or not cited.

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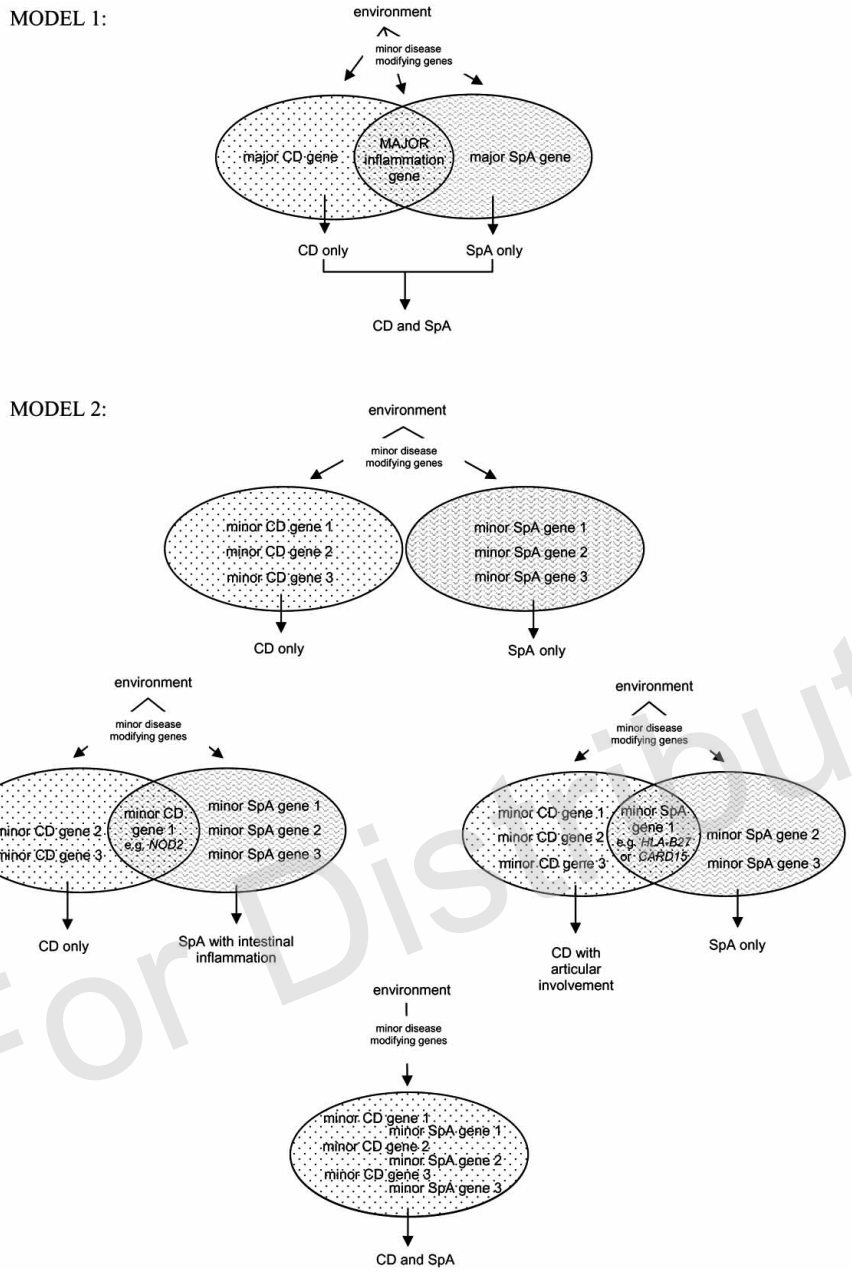

Fig. (1). Two putative models to explain the shared genetic susceptibility for SpA and CD. (**A**) The first model is based on families where both SpA and CD are found. A major gene (Major inflammation gene) is responsible for overactive or persistent inflammation, while independent major genes dictate where the inflammation is typically found, the joints (Major SpA gene) or the intestine (Major CD gene). If the inflammation gene acts together with one of the other two genes, pure CD or SpA is expressed. If, however, the three major genes are present in the patient, he will suffer from both SpA and CD. In addition, independent environment-sensitive minor genes contribute to the overall outcome of the disease in each patient. (**B**) The second model is probably more applicable to sporadic cases. In this graphical representation, the combination of three genes (although this number is purely speculative) is needed to set up either SpA or CD, with environmental factors influencing the disease phenotype. These genes can act independently of each other to produce a specific phenotype in each patient. For example, if a mutation in the *NOD2* gene is present in a patient carrying the combination of the three SpA genes, he will suffer from the chronic gut inflammation phenotype. Similarly, a patient carrying the three genes that set up CD together with the *HLA-B27* allele, he will have articular complaints. In the extreme case where all six genes are present, the patient will have both full-blown SpA and CD at some point in his lifetime, depending on additional environmental factors.

BULLET POINTS

- The frequent co-occurrence of SpA and IBD within a patient and within families suggests shared genetic susceptibility.
- Good candidate genes for such mutual susceptibility are those that are located within loci which have been identified in genome scans of both SpA and IBD. These include *IL23R*, *CTLA4*, *TNF* and *TLR4*.
- The study of animal models that develop typical features of SpA and IBD provide interesting data of how these diseases can be related or how they are initiated.

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is thus a minor gene, which could lead to overt CD in those SpA patients who carry additional minor gene interactions. Similarly, the SpA-associated *HLA-B27* allele is more frequent in IBD patients with associated articular manifestations.

COMMON GENETIC DETERMINANTS OF SPONDYLOARTHROPATHY AND INFLAMMATORY BOWEL DISEASE

The clinical and molecular overlap between SpA and IBD and familial clustering in multiplex families argue for a genetic link between the two diseases. Today, powerful genetic tools can be adopted to find causally important disease-associated genes. Genome-wide linkage and association scans provide insights into the position and number of potentially interesting chromosomal regions linked with the disease. Case-control association studies of mutations/polymorphisms within interesting candidate genes is another approach to find causally important gene aberrations; however, this method is not hypothesis-free and depends on other available information, either positional (because it is located within a known locus) or functional (because of known properties of the encoded protein). In the next section, we compile the known loci and positional candidate gene association studies for SpA and IBD.

Genome-Wide Scans for SpA and IBD

For IBD, 17 genome-wide scans have been performed [37, 53-68]. More than 20 loci were identified with suggestive or significant linkage for CD, UC or IBD in general, and many of them have been independently replicated (*IBD1-9*, 3q, 4q, 6q, 7q, 8q, 10p, 22q, Xp). Four genome-wide scans have been performed for SpA [32, 33, 69, 70], which was recently reviewed by Brown [71]. In addition, a meta-analysis using these four reports was compiled [72], and one study focused on the heritability of age at symptom onset, disease activity, and functional impairment in AS [73]. Three of the four genome-wide scans were performed on isolated AS, which might not be ideal. As discussed earlier, the SpA family is suspected to share susceptibility factors in spite of obvious subclassification.

Interestingly, thirteen chromosomal regions that were identified in one of the genome-wide scans are shared between SpA and IBD (located at 1p, 1q, 2q, 3p, 5q, 6p, 16p, 16q, 19p and 19q, Table **2**). These regions, therefore, are good candidates to look for new genes contributing to com-

Table 2. Summary of Chromosomal Loci Identified in Genome-Wide Scans Shared Between CD and SpA

Shared Locus	Chromosome	SpA		IBD		Candidate Genes
		Locus	Ref.	Locus	Ref.	
1	Chr1	1p32.3	[69]	1p31	[55]	*IL23R*
				1p32	[58]	
				1p33-32.3	[64]	
2		1p36.13	[69]	1p36.13-36.11	[66]	
3		1q23.3	[69]	1q21.3	[64]	
4		1q44	[69]	1q43-q44	[37]	
		1q44	[32]			
5	Chr2	2q31.1-34	[69]	2q32.3	[65]	*CTLA4*
6	Chr3	3p14.2-13	[33]	3p14.2-14.1	[68]	
		3p21	[69]	3p21	[61]	
7	Chr5	5q34	[32]	5q33-35	[59]	*CD14, IL4*
				5q31.1-31.3	[68]	
8	Chr6	6p22.3-12.1	[69]	6p (NOD2 stratified)	[62]	*TNF, TAP2, HLA-B27, SLC22A4*
		6p24.3-22.2	[32]	6p22.2	[54]	
		6p25.1-22.3	[33]	6p22.2-21.2	[68]	
		6p22.2	[70]	6p23-22.3-22.2	[37]	
9		6q25.2-26	[33]	6q25.2-26	[54]	
10	Chr16	16p12.1	[69]	16p13.13	[66]	
				16p11.2-12.1(FM)	[53]	
11		16q23.1-23.3	[69]	16q21-23.1	[63]	*NOD2*
		16q23.3	[32]			
		16q23.1	[33]			
12	Chr19	19p13.12	[32]	19p13.3	[68]	
				19p13.3-13.2	[66]	
13		19q12-13.41	[32]	19q13.31 (NOD2 stratified)	[63]	
		19q12	[33]			

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mon susceptibility to SpA and CD. In the next section, we discuss the most interesting candidate genes within these shared regions.

From Loci to Genes: Association Studies for SpA and IBD

We must bear in mind that, as with all genetic association studies, confirmation in different regionally matched cohorts is crucial. Different findings between association studies are frequently found. This may reflect genuine population differences, or this could mean that the specifically tested variants are not directly involved in etiopathogenesis.

HLA-B27 (6p)

The SpAs are linked by a common genetic risk factor, the *HLA-B27* allele, which is much more prevalent in SpA patients (75-95%) than in other rheumatic diseases or in healthy controls (5-14% in Caucasians). HLA class I molecules are highly polymorphic glycoproteins specialized for antigen-presenting. They are expressed on the surface of nearly all nucleated cells, where they form stable complexes with antigenic peptides and display them for recognition by $CD8^+$ T cells. Currently, more than 30 allelic variants of the *HLA-B27* gene have been identified, and are designated as B*2701 to B*2737 (http://www.anthonynolan.org.uk/HIG/lists/class1list.html). They differ in one or more amino acids in exons 2 and 3 of the gene [74]. HLA-B*2705, which is considered the ancestral subtype, bears the strongest association with SpA. Not all B*27 subtypes are associated with SpA and this seems to be highly dependent on the ethnicity of the study population. Moreover, while most of them are risk factors for SpA, others are reported to be protective for the development of the disease, such as B*2706 and B*2707. The identification of *HLA-B27* subtypes and their association with AS or SpA in general is the subject of much ongoing research. The *HLA-B27* association with SpA is one of the strongest HLA associations found so far. Based on family and twin studies, it was reported that *HLA-B27* contributes to 20 to 50% of the total genetic risk for the disease [75, 76]. Although this number is high, other genes must be involved in SpA etiology. Moreover, only a small fraction of *HLA-B27* positive individuals develop the disease. Which genetic and environmental factors are crucial for eventual development of the disease is not completely understood.

Clinically, SpA pathology in IBD patients is almost identical tot that of idiopathic AS, but there is a difference in *HLA-B27* prevalence. Depending on the study population, 6-33% of IBD patients are positive for *HLA-B27* (Table **3**) [18, 77-86]. However, it is clear that the prevalence is increased in those IBD patients who also suffer from AS. High preva-

Table 3. HLA-B27 Frequency in IBD and IBD-AS Patients

Number of Patients	*HLA-B27* Positive (%)	Number of AS Positive Patients in Study	*HLA-B27* Positive in IBD-AS Patients (%)	Ref.
IBD:				
406	13	15	73	[80]
50 IBP	ND	14	28	[82]
12		12	33	[78]
89	13	4	75	[86]
76	9	8	25	[18]
CD:				
122	12	9	78	[84]
59	10	ND	ND	[18]
51	4	10	20	[77]
100	6	2	50	[79]
78	33	9	100	[85]
102	6	9	33	[81]
213	ND	18	72	[83]
UC:				
58	19	11	36	[77]
17	6	ND	ND	[18]
100	14	5	80	[79]
84	25	7	100	[85]

IBP: IBD patients with inflammatory back pain.
ND: not determined or not cited.

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lences in these patients are found in some studies (73-100%), although others reported rather low prevalences (20-50%) (Table **3**). While each study population was extremely small, they all point out that IBD patients carrying the *HLA-B27* allele are more likely to develop AS than others who do not possess the antigen. The presence of sacroiliitis in the SpA concept or in CD is not associated with *HLA-B27* [81, 84].

NOD2 (16q)

A strong and highly repeated association with *NOD2*, a gene encoding an intracellular receptor for bacterial components, was found for CD. Three single nucleotide polymorphisms (SNPs) were independently associated with the disease [87, 88]. Each SNP carries a 2 to 4-fold increased relative risk if heterozygous, while homozygous or compound heterozygous carriers have a 20-fold increased risk. Despite the strong association with CD (frequency up to 50% in European and North American patients), they are relatively frequent mutations in healthy individuals (~20%). The SNPs in *NOD2* are not associated with susceptibility to UC; however, an epistatic interaction between *NOD2* and the *IBD5* haplotype is suspected [89]. This gene is also involved in the etiology of some other diseases, such as graft *vs* host disease and Blau syndrome [90]. *NOD2* is not a risk factor for SpA [91-96], although one study found an association between one of the SNPs and UC-SpA [93]. Because a locus at 16q has been associated with paternal transmission in PsA [97], the involvement of *NOD2* was more frequently studied in this disease. A positive association with PsA was found once [98], but this was not supported by other studies [99-101]. We also could not show an association of *NOD2* with SpA in general; however, those patients with subclinical chronic gut inflammation contained a significantly higher number of CD-associated SNPs in *NOD2* (38%), which was comparable to the frequency in CD patients from the same institution (49%) [52]. Therefore, we proposed that *NOD2* is a determinant of susceptibility to the development of chronic gut inflammation as such. Since this group of SpA patients is at high risk of developing CD over time [7], it is plausible that the combination of a dysfunctional bacterial receptor with other susceptibility genes or environmental triggers in these patients initiates an abnormally strong immune response toward luminal flora or pathogens.

We described a link between *NOD2* and the presence of radiographic sacroiliitis in CD patients, irrespective of overt AS [81]. Seventy-eight percent of CD patients with sacroiliitis were carriers of at least one SNP in *NOD2*, compared to 48% of those who were wild type for *NOD2*. However, in a second multi-center study, this observation could not be repeated [102]. The reason for this inconsistency is not clear. Poor reproducibility of genetic association studies for complex diseases is not uncommon. However, detecting sacroiliitis on radiographs of the sacroiliac joints is difficult and holds a high inter- and intra- observer variability [103].

An interesting underlying trait in IBD is a compromised intestinal permeability [104]. A "leaky" barrier function can lead to excessive bacterial influx, leading to a continuous triggering of the immune response in the gut mucosa. Whether this process is genetically determined is still controversial [105]. However, a primary role for an increase in intestinal permeability was suggested based on the observation that this abnormality is not only present in CD patients, but also in their healthy relatives [106, 107]. This leaky gut theory was further supported by changes in the unaffected bowel of CD patients [108, 109]. Similarly, an increase in gut permeability was also found in juvenile chronic arthritis [110] and in relatives of patients with AS, irrespective of NSAID intake or disease activity [111-114]. The first genetic evidence of underlying permeability dysfunction in persons carrying a mutated form of *NOD2* comes from the observation that one of these mutations is associated with permeability in familial CD patients and their relatives more than in sporadic CD and their relatives [115, 116]. However, this association was not supported in another study [117], although it is not clear whether familial cases were included in their analysis. Since an increase in gut permeability is not a useful marker for predicting CD development over time, additional genetic components would be of great use to evaluate the risk, especially in relatives of CD patients.

IL23R (1p)

Interestingly, the 1p32 region found in the study of Brown and co-workers has been narrowed down to association with polymorphisms in the receptor for interleukin 23 (IL23R) in CD [55, 58]. Recently, variants in this gene were studied in AS, and a strong association was found [118, 119]. Moreover, IL23 is highly overexpressed in AS gut biopsies as compared to healthy controls, with levels similar to those found in CD biopsies [120]. The role of this cytokine and its receptor will be the subject of further study in SpA-IBD.

TNF (6p)

Tumor necrosis factor alpha (TNF) is a pro-inflammatory cytokine that provides a rapid form of defense against various infections. However, if this cytokine is produced in excess, it can be fatal to the host. This gene was a strong positional and functional candidate gene for CD because TNF levels are increased in the serum, mucosa and stool of these patients, while anti-TNF therapy is very efficacious in CD [121]. The production of TNF is under strong genetic influence [122]. As will be discussed in the next paragraph, the level of TNF production in the TNF transgene mouse model is critical for the development of arthritis and colitis. Therefore, the study of promoter polymorphisms influencing TNF dosage in humans seems highly relevant.

Three SNPs in the promoter of *TNF* (c.-1031C, c.-863A and c.-857T) were associated with susceptibility to CD in a Japanese population [123]. In contrast, none of these SNPs could be associated with IBD in two independent Caucasian populations, while the c.-857C allele was more prevalent in IBD and UC [93]. Interestingly, this variant was also associated with CD when they left out the common *NOD2* allele carriers, meaning that these genes act independently to confer CD susceptibility. Pediatric onset, colonic disease and familial aggregation of CD was associated with the c.-863C>A polymorphism, which is located within a binding site for NFκB in the *TNF* promoter [124]. Moreover, it was demonstrated that exposure of 293T cells to bacterial components stimulates *TNF* gene transcription as a result of NOD2-induced NFκB activation [125]. When this experiment was repeated in cells containing the *NOD2* 1007fs variant, the induction of *TNF* promoter activity was found to be defective. Different combinations of *NOD2* and *TNF* promoter polymorphisms gave rise to distinct TNF transcrip-

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tion levels, which means that *NOD2* and *TNF* promoter polymorphisms interact to exert a functional effect on bacterial induced TNF production. Therefore, this gene-gene interaction may contribute to inter-individual variation in susceptibility to CD.

TNF is in close proximity to the HLA-B locus on chromosome 6. The search for disease-associated alleles within this region is complicated because of high linkage disequilibrium. One report detected an association between -308 and AS [126]. No association was found among four promoter polymorphisms (located at c.-244, c.-376, c.-238 and c.-308) between AS and controls [127, 128]. The CD-associated promoter polymorphisms have not been tested in SpA. However, for PsA, an association was found for the c.-857 SNP [129].

TLR4 (9q)

Lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria, is a major inducer of inflammation, and its signaling is mediated through the cell surface receptor toll-like receptor 4 (TLR4). During intestinal inflammation, TLR4 is up-regulated on epithelial cells, macrophages and dendritic cells, thus providing a first line of defense against enteric Gram-negative bacteria. An association between a polymorphism in the leucine-rich region of TLR4 (c.896A>G, D299G) was reported in a Dutch CD and UC cohort [58]. Allele frequencies of 10.9% were found in CD patients, *vs* 5% in healthy controls. The association was replicated three times [130-132], but could not be reproduced in three other studies [133-135]. This mutation was previously linked with a decreased bronchial responsiveness to LPS [136]. However, no functional defect, such as in cytokine release or LPS recognition, has been attributed to heterozygous carriership of this mutant in CD patients [137, 138].

Because TLR4 is in proximity to a linkage peak in 9q32-33 and the possibility that AS occurs when Gram- negative gut bacteria interact with HLA-B27, this gene was of potential interest to test for association with AS. A small case-control study indeed showed an association [139], but this was not found in two other studies for AS, nor in one for ReA [140-142].

ANIMAL MODELS TO STUDY THE ARTICULAR AND INTESTINAL ASSOCIATION

Two animal models, the TNFΔARE mice and HLA-B27 rats, share some features of both CD and SpA. They thus provide a way to study specific biological mechanisms involved in both pathologies. In these models, TNF and HLA-B27 (together with the human β2-microglobulin) are overexpressed.

The excess production of TNF in SpA and IBD affected tissues and in serum is well known [121, 143]. Moreover, anti-TNF therapy is highly efficient in both diseases. The TNFΔARE mice carry a deletion of AU-rich inhibitory elements of the TNF gene [144]. This results in an increased stability of the TNF mRNA and an increase in constitutive and inducible expression of TNF in circulation and in fibroblasts isolated from the synovium and lung. These mice spontaneously develop transmural ileitis (occasionally proximal colitis) after 8 weeks in the case of heterozygous carriers and show chronic inflammatory polyarthritis at 6-8 weeks. Although the transcription of TNF in the TNFΔARE mice should theoretically be increased in all tissues, it is striking that only extended inflammation is found in the intestine and joints of these mice. Only occasionally do other organs such as lung or liver show some signs of inflammation. This supports the idea that one possible etiopathogenic mechanism in human SpA-IBD is genetically determined overexpression, or lack of repression, of TNF. Therefore, the study of promoter polymorphisms in TNF is very important (see previous section).

The overexpression of the human *HLA-B27* allele in rats represents a very specific model, and provides further evidence for a direct causative role of this allele in human SpA and related intestinal inflammation [145]. These rats develop spontaneous colitis, gastritis, peripheral arthritis and occasionally spondylitis. Importantly, some transgenic mice are healthy, depending on the copy number of *HLA-B27*. Disease only develops if a threshold level of HLA-B27 is reached [146]. This could be one explanation why *HLA-B27* carriers do not all develop SpA. In this view, the regulation of this allele is worth studying. Recently, it was shown that crossing healthy HLA-B27 transgenic rats with those containing healthy human β2-microglobulin transgenes only results in articular problems and not in intestinal inflammation [147]. This indicates that gut inflammation is not absolutely indicated in HLA-B27 rats, and might suggest that this allele could lead to two different phenotypes in human SpA, depending on other factors.

A big advantage of using animal models is that the initiating event, the time course and potential triggers (e.g. bacteria) can be controlled. Interestingly, the HLA-B27 rats don't develop disease when kept in germ free conditions [148]. This directly implies that environmental stimuli are needed to trigger the onset of the disease. Whether this dependence on microflora is also true for the TNFΔARE mice is not known.

CONCLUSIONS

Many complex diseases share overlapping features. Therefore, parallel to the study of genetic susceptibility of the disease in general, subphenotyping in genetic analyses is crucial to understand specific relationships between disorders.

Overlapping chromosomal loci associated with the susceptibility to both SpA and IBD are primary regions of interest in the search for new candidate genes. The association of *NOD2* and *HLA-B27* with the combined presence of SpA and CD in patients provides evidence that these are related polygenic conditions that share some, but not all, susceptibility genes. In addition to a better understanding of the occurrence of specific subphenotypes in SpA and IBD, the identification of common susceptibility genes for SpA and IBD could aid in specifically targeting atypical processes in both diseases. Moreover, once a gene is identified, a whole new area of research can provide new insights into its pathology, as has been nicely illustrated for *NOD2*.

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