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Ankylosing spondylitis susceptibility loci defined by genome-search meta-analysis

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Abstract In genome scans of ankylosing spondylitis (AS), with the exception of the HLA loci, linkage has not been easy to replicate across studies. We applied the genome-search meta-analysis (GSMA) method to genome scans of AS and spondyloarthropathy (SpA) to assess evidence for linkage across studies. Three AS genome scans and one SpA scan including 430 families with 1,048 affected individuals were used. All four original genome scans mainly analyzed Caucasian families. Seven bins had both *Psumrnk* and *Pord* < 0.05, suggesting these bins most likely contain AS-linked loci; bin 6.2, 6.1, 6.3, 16.3, 19.2, 17.1, and 16.4. The GSMA produced significant genome-wide evidence for linkage on chromosome 6p22.3–6p21.1 (*Psumrnk* = 0.000003), including the HLA locus. In addition to the HLA-B27 locus, strong linkage evidence was found on chromosome 6p25.3–6p22.3 (*Psumrnk* = 0.0013) and 6p21.1–6p15 (*Psumrnk* = 0.043). In the GSMA of four genome scans including one SpA study, the bin 9.4 (9q21.32–9q33.1) was newly found for linkage (*Psumrnk* = 0.043, *Pord* = 0.013). This GSMA added the evidence of the HLA loci as the greatest susceptibility factor to AS and showed evidences of chromosome 6, 16q, 19, 17p, and 9q as non-HLA susceptibility loci.

Keywords Ankylosing spondylitis · Spondyloarthropathy · Genome scan · Linkage · Meta-analysis

Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disorder characterized by inflammation in the spine and sacroiliac joints causing initial bone and joint erosion and subsequent ankylosis (Brown et al. 2002). The peripheral joints and the entheses are frequently involved, and inflammation may involve extra-articular sites such as the uvea, aorta, heart, lungs, and kidneys. AS is the second-most common cause of inflammatory arthritis worldwide, with a prevalence of 0.2–0.9% in white populations (Braun et al. 1998).

Strong genetic factors are implicated in the etiology of the disease. The sibling recurrence risk ratio has been known to be 82 (Brown et al. 2000), and twin studies estimate that disease heritability exceeds 90% (Brown et al. 1997). The human leukocyte antigen (HLA) B27 is the first genetic factor identified in AS and confers the greatest susceptibility to AS (Brewerton et al. 1973). However, there is increasing evidence that non-HLA genes also contribute to AS susceptibility (Breban et al. 2003). Although the prevalence of AS is correlated with the prevalence of the HLA-B27, only 1–5% of B27-positive individuals develop AS (Calin et al. 1983), and HLA-B27 explains less than 50% of the total genetic risk for AS (Brown et al. 1998). The remaining gene loci have yet to be identified. Previous AS genome scans have shown the HLA loci, including HLA-B27, as the strongest linkage region and non-HLA loci as the additional susceptibility regions (Brown et al. 2003; Laval et al. 2001; Miceli-Richard et al. 2004; Zhang et al. 2004). However, with the exception of the HLA loci, linkage has not been easy to replicate across the studies. This is unsurprising because for rare genes, or genes with a weak effect, nonparametric linkage studies are likely to require several hundreds, possibly thousands, of affected sibling pairs to provide sufficient power to detect linkage (Risch and Merikangas 1996). There have been three genome scans of AS and one genome-wide study of spondyloarthropathy (SpA). SpA includes a spectrum of related disorders comprising the prototype AS, psoriatic arthritis, reactive arthritis, arthritis associated with inflammatory bowel disease, and undifferentiated SpA. Until now, no systematic statistical assessment of previous results has yet been carried out in AS and SpA.

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Meta-analysis combines the linkage results from several studies, providing greater statistical power. A genome scan meta-analysis is one step toward defining genomic regions that harbor disease susceptibility loci and can identify regions that may contain disease genes in the pooled studies and identify regions where the genetic effect is too small to be detected in an individual study. Recently, the genome-search meta-analysis (GSMA) method has been proposed as a robust genome scan meta-analysis technique (Levinson et al. 2003). We applied the GSMA method to four genome scans of AS and SpA to assess evidence for linkage across the studies using published data. In addition, in order to confirm the significance provided by the GSMA, we applied Fisher's method to the bins that are most likely to contain linked loci as an alternative method (Guerra et al. 1999).

Materials and methods

Selection of genome scans

Four genome scans were identified though MEDLINE search (Brown et al. 2003; Laval et al. 2001; Miceli-Richard et al. 2004; Zhang et al. 2004). Three genome scans were performed in AS (Brown et al. 2003; Laval et al. 2001; Zhang et al. 2004) and one was studied in SpA (Miceli-Richard et al. 2004). They consisted of three Caucasian and one mixed ethnicity population including 430 families (579 sibling pairs) with 1,048 affected individuals (Table 1). All four original genome scans mainly analyzed Caucasian families. The GSMA method assumes a uniform map in each scan, so we did not consider the second stages of genome scans where candidate regions were more densely mapped or samples were modified. Linkage data such as LOD or NPL scores were obtained from the result tables or linkage graphs in each study for the rank-ordering procedure. LOD scores were used in two genome scans (Brown et al. 2003; Laval et al. 2001), and NPL scores were selected in two genome scans (Miceli-Richard et al. 2004; Zhang et al. 2004). We performed GSMA in three AS genome scans and four genome scans including three AS and one SpA genome scans, respectively.

Genome scan meta-analysis

The GSMA was performed as described (Wise et al. 1999). In brief, the autosomes were divided into 120 30-cM bins

Table 1 Linkage loci in individual genome scans

Chromosomes	Brown et al. (1998)	Laval et al. (2001)	Zhang et al. (2004)	Miceli-Richard et al. (2004)
1	1p36.13–1p32.3 (LOD = 1.70)	1p34.3 (LOD = 0.6)	1q31.1	
	1q23.3 (LOD = 1.0)	1q44 (LOD = 0.7)	(NPL = 1.748)	
	1q44 (LOD = 0.6)			
2	2p21–2p15 (LOD = 1.3)			
	2q14.3 (LOD = 0.7)			
	2q31.1–2q34 (LOD = 1.6)			
3	3p14.2 (LOD = 1.1)	3q28 (LOD = 1.4)	3p14.2–3p13 (NPL = 2.079)	
4			4p15.32 (NPL = 2.114)	
5		5q34 (LOD = 1.4)		5q23.1 (NPL = 2.55)
6	6p22.3–6p12.1 (LOD = 6.9)	6p24.3–6p22.2 (LOD = 4.8)	6p25.1–6p22.3 (NPL = 3.108)	6p22.2 (NPL = 5.29)
	6q14.1 (LOD = 0.6)	6p12.1 (LOD = 0.7)	HLA (NPL = 8.720)	
		6q14.1 (LOD = 2.0)	6p12.1 (NPL = 1.663)	
			6q25.2–6q26 (NPL = 2.457)	
7		7p13 (LOD = 2.6)		
8	8q22.3 (LOD = 0.7)	8q22.3 (LOD = 0.8)		
9	9p24.2 (LOD = 1.6)	9p24.2–9p21.2 (LOD = 1.0)		9q33.2 (NPL = 2.32)
	9p21.2 (LOD = 0.6)	9q22.2 (LOD = 1.2)		
	9q22.2 (LOD = 0.7)	9q33.2 (LOD = 2.1)		
	9q34.2 (LOD = 3.9)			
10	10q23.33–10q26.11 (LOD = 2.4)	10q23.33 (LOD = 1.1)		
11	11p15.5–11p13 (LOD = 1.1)		11q23.1–11q24.3 (NPL = 2.235)	
13				13q14.2 (NPL = 2.55)
15		15q13.3 (LOD = 1.6)		
16	16p12.1 (LOD = 1.3)	16q23.3 (LOD = 1.9)	16q23.1 (NPL = 1.591)	
	16q23.1–16q23.3 (LOD = 2.7)			
17		17p13.3 (LOD = 1.2)	17p13.2–17p12 (NPL = 1.998)	17q24.3 (NPL = 2.44)
19		19p13.12 (LOD = 1.2)	19q12 (NPL = 1.866)	
		19q12–19q13.41 (LOD = 3.58)		
21	21q22.3 (LOD = 1.1)			

Table 2 Characteristics of genome scans included in the meta-analysis. *AS* ankylosing spondylitis, *SpA* spondyloarthropathy

Genome scan (years)	Brown et al. (1998)	Laval et al. (2001)	Zhang et al. (2004)	Miceli-Richard et al. (2004)
Diagnosis	AS	AS	AS	SpA
Ethnicity (%)	Caucasian (100)	Caucasian (100)	Caucasian (95) Mixed (5)	Caucasian (100)
No. of families	99	86	180	65
No. of sibling pairs	130	125	244	80
No. of affected individuals	235	210	423	180
Weighting factor	0.96	0.91	1.29	0.84
Type of analysis program	Nonparametric two, and multipoint	Nonparametric two, and multipoint	Nonparametric two, and multipoint	Nonparametric multipoint
No. of autosomal markers	505	367	400	352
Test statistic output	LOD	LOD	NPL	NPL
Major findings	HLA (LOD = 6.9) 9q34.2 (LOD = 3.9)	HLA (LOD = 4.8) 19q13.31 (LOD = 3.58)	HLA (NPL = 8.720) 11q24.1 (NPL = 2.235)	HLA (NPL = 5.29) 13q14.2 (NPL = 2.55)

defined by Genethon markers (CEPH-Genethon Integrated Map Web site). On the Marchfield map, the average bin width was 29.1 cM. Each marker was placed within one of these bins on the basis of its location on the Genethon or Marshifield map (available at http://www.marshfieldclinic.org/research/genetics). For each study, each bin was assigned a within-study rank (*Rstudy*) based on the maximum linkage score within the bin. Bins were ranked in descending order (120 = most significant result). The summed rank across studies was computed for each bin (*Rsumrnk*). A weighted GSMA was carried out to allow results to reflect the relative contribution of each study. For the weighted analysis, each *Rstudy* value was multiplied by its study's weight $\left(\sqrt{N[\text{affected cases}]}\right)$, divided by the mean of this value over all studies. Two point-wise *P* values were determined, *Psumrnk* and *Pord*, as described and determined by 10,000 permutations of the weighted data set. *Psumrnk* is the probability of observing a bin's summed rank by chance, and *Pord* is the probability of observing the *j*th place bin's summed rank in *j*th place bins in randomly permuted data. The empirical criteria for bins most likely to contain linked loci were both *Psumrnk* and *Pord* < 0.05 and the criterion for genome-wide significance was *Psumrnk* < 0.000417 (0.05 corrected for 120 bins). Therefore, we considered the GSMA results to most likely contain linked loci if both *Psumrnk* and *Pord* were < 0.05 and to have a genome-wide evidence of linkage if the GSMA *Psumrnk* was < 0.000417.

Fisher's method for combining *P* values

As an alternative, we also utilized Fisher's method for combining *P* values (Guerra et al. 1999). Fisher's method is a procedure of combining *P* values for the evaluation of several independent tests of the same null hypothesis. Under the null hypothesis of no-linkage *P* values, p_1, p_2, ..., p_n from *n*, independent studies are uniformly distributed on the interval (0,1) and the combination of *P* values, $-2 \sum_{i=1,n} \ln p_i$, is distributed as a χ^2 random variable with $2n°$ of freedom (df). *P* values are gained by the formula $-\Phi[\text{sign(LOD)}\sqrt{21n(10) \mid \text{LOD} \mid}]$ (Φ is the distribution function for standard normal distribution) or chi-square distribution of NPL score with 1 df. Unfortunately, using the original Fisher's method to some of the genome scan data can introduce a bias in the distribution. This is because most of the model-free linkage methods produce one-tailed LOD scores that truncate all at LOD = 0. To overcome this, we used a *P* value of 0.72 for LOD = 0 (Province et al. 2001).

Results

Each genome scans

Individual genome scans have shown significant linkages in HLA and non-HLA regions. Table 1 shows significant loci in each genome scan. All studies have shown HLA loci as the strongest linkage region. The highest linkage loci outside chromosome 6 were different across four studies. Chromosome 9q34.2, 19q13.31, 11q24.1, and 13q14.2 were the most significant loci outside of chromosome 6 in each study, respectively (Table 2).

Genome-search meta-analyses in AS

In order to increase homogeneity for genetic linkage studies, first of all, we performed the GSMA in AS genome scan studies. Figure 1 shows the summed ranks for each bin giving *Psumrnk*. The summed ranks (vertical axis) are plotted against the bin location by a single point plotted for the summed rank for each bin with chromosome numbers (horizontal axis). A total nine bins lie above 95% confidence level ($P = 0.05$), and five bins are above 99% confidence level ($P = 0.01$). Table 3 summarizes the highest 10% of bins when ordered by summed rank. Out of nine bins (*Psumrnk* < 0.05), seven had both *Psumrnk* and *Pord* < 0.05, suggesting these bins most likely contain AS-linked loci: bins 6.2, 6.1, 6.3, 16.3, 19.2, 17.1, and 16.4.

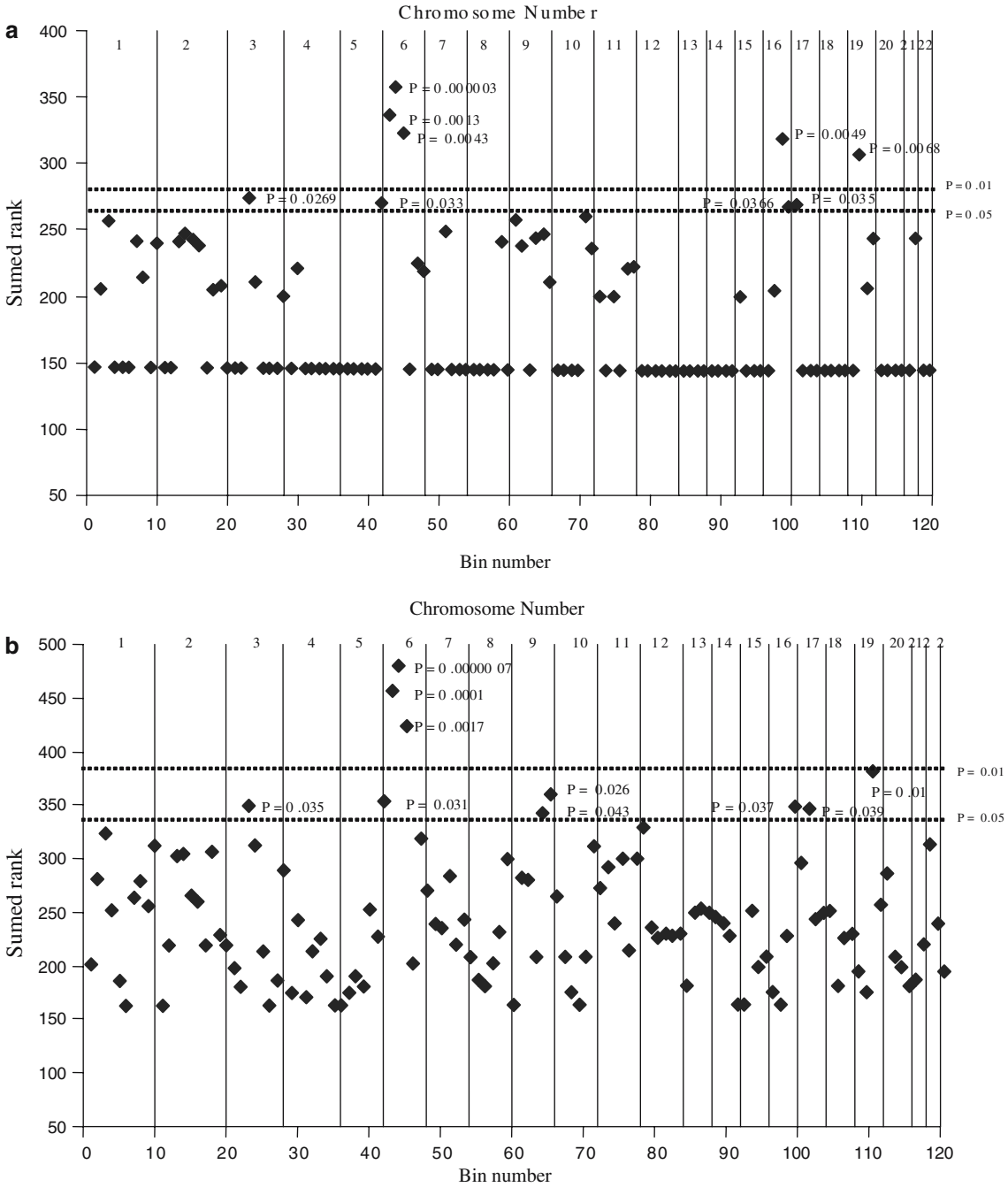

Fig. 1 Genome-search meta-analysis (GSMA) results of genome scans in ankylosing spondylitis (AS) (**a**), and AS and spondyloarthropathy (SpA) (**b**). Individual chromosomes were subdivided into ~30 cM bins (represented by a *dot*), and bins were ranked by the significance after summing weighted data across the studies. Significance levels corresponding to 99% (*Psumrnk* < 0.01) and 95% (*Psumrnk* < 0.05) are shown by the *horizontal lines*

The strongest evidence for linkage based on *Psumrnk* of the AS GSMA occurred on chromosome 6p22.3–6p21.1 (bin 6.2) containing the HLA-B27 region. The AS GSMA produced significant genome-wide evidence for linkage on this chromosome 6p22.3–6p21.1 ($P = 0.000003$). The nominally significant *Psumrnk* of the adjacent bins, 6.1 and 6.3, provided additional evidence for linkage. The significant linkage evidence was found on 6p25.3–6p22.3 (*Psumrnk* = 0.0013) and 6p21.1–6p15 (*Psumrnk* = 0.043) in the adjacent bins. It is likely that the apparent linkage to bins 6.1 and 6.3 merely indicates the linkage of AS to HLA, and the evidence does not indicate a possibility of the existence of other loci near HLA. There may be also other loci on chromosome 6, but it is not supported by the evidence obtained.

Table 3 *Psumrnk* and *Pord* for bins with the highest-summed ranks ($P < 0.1$)

Population	Bin	Cytogenetic location	Weighted analyses				Fisher's method
			Rank	*Psumrnk*	*Pord*	Both *Psumrnk* and *Pord* < 0.05	Combined *P* value
AS	6.2	6p22.3–6p21.1	360.0	0.000003	0.0004	+	2.07E-21
	6.1	6p25.3–6p22.3	337.9	0.001303	0.0094	+	6.60E-07
	6.3	6p21.1–6q15	324.1	0.004336	0.0118	+	0.00019
	16.3	16q12.2–16q23.1	321.4	0.004948	0.0023	+	0.00138
	19.2	19p13.2–19q13.43	310.1	0.006818	0.0007	+	0.00393
	3.3	3p22.2–3p14.1	275.2	0.026916	0.0700		
	5.6	5q34–5q35.3	272.5	0.033005	0.0627		
	17.1	17p13.3–17p12	271.5	0.035052	0.0286	+	0.00967
	16.4	16q23.1–16q24.3	270.8	0.036559	0.0092	+	0.00003
	10.5	10q23.33–10q26.13	263.0	0.056045	0.0730		
	9.1	9p24.3–9p22.3	259.0	0.065902	0.0953		
	1.3	1p36.11–1p32.2	256.9	0.070778	0.0734		
	7.3	7p14.1–7q21.11	250.2	0.085097	0.1473		
	9.5	9q31.1–9q33.3	249.3	0.086249	0.0805		
	2.4	2p16.2–2p12	247.2	0.089584	0.0470		
	9.4	9q21.32–9q33.1	246.5	0.090805	0.0220		
	21.2	21q21.3–21q22.3	246.1	0.092637	0.0099		
	19.4	19q13.33–19q13.43	245.4	0.093602	0.0035		
	2.5	2p12–2q14.2	243.5	0.095504	0.0009		
	8.5	8q22.2–8q24.21	243.1	0.095800	0.0002		
	2.3	2p23.2–2p16.2	242.0	0.098292	0.0001		
	1.7	1q23.3–1q31.1	242.0	0.098292	0.0001		
SpA	6.2	6p22.3–6p21.1	478.3	0.0000008	0.0001	+	1.16E-25
	6.1	6p25.3–6p22.3	455.9	0.0001075	0.0001	+	3.09E-12
	6.3	6p21.1–6q15	422.5	0.0016500	0.0016	+	0.00018
	19.2	19p13.2–19q13.43	379.1	0.0100433	0.0222	+	0.00753
	9.5	9q31.1–9q33.3	357.6	0.0262500	0.1842		
	5.6	5q34–5q35.3	352.5	0.0312733	0.1433		
	3.3	3p22.2–3p14.1	349.1	0.0345433	0.0882		
	16.3	16q12.2–16q23.1	347.0	0.0368633	0.0462	+	0.00430
	17.1	17p13.3–17p12	345.2	0.0385450	0.0191	+	0.01542
	9.4	9q21.32–9q33.1	340.8	0.0431458	0.0132	+	0.00081
	11.6	11q13.4–11q25	328.1	0.0596050	0.0559		
	1.3	1p36.11–1p32.2	323.1	0.0681224	0.0652		
	6.5	6q23.2–6q25.3	316.8	0.0822858	0.1251		
	3.4	3p14.1–3q12.3	311.8	0.0967283	0.2108		
	21.2	21q21.3–21q22.3	311.7	0.0970808	0.1147		
	1.1	1q43–1q44	311.5	0.0977124	0.0610		

The significant linkages on non-HLA loci were found on chromosome 16q12.2–16q23.1 (*Psumrnk* = 0.0049), 19p13.2–19q13.43 (*Psumrnk* = 0.0068), 17p13.3–17p12 (*Psumrnk* = 0.035), and 16q23.1–16q24.3 (*Psumrnk* = 0.036). All had both *Psumrnk* and *Pord* < 0.05.

Genome search meta-analyses in four studies, including three AS and one SpA

We performed GSMA in all four studies, including one SpA genome scan. Ten bins lie above 95% confidence level ($P = 0.05$), and three bins are above 99% confidence level ($P = 0.01$) (Fig. 1). Table 3 summarizes the highest 10% of bins when ordered by summed rank. Out of the ten bins (*Psumrnk* < 0.05), seven had both *Psumrnk* and *Pord* < 0.05, suggesting these bins most likely contain linked loci: bin 6.2, 6.1, 6.3, 19.2, 16.3, 17.1, and 9.4. Bin 9.4 (9q21.32–9q33.1) was newly found for linkage in the GSMA of four genome scans, including one SpA study (*Psumrnk* = 0.043, *Pord* = 0.013). The strongest evidence for linkage based on *Psumrnk* of the GSMA occurs on chromosome 6p22.3–6p21.1 (bin 6.2) containing the HLA-B27 region. This region met a genome-wide significance for linkage ($P = 0.0000008$). The second-highest summed ranks were assigned to bins 6p25.3–6p22.3 (*Psumrnk* = 0.0001) and 6p21.1–6q15 (*Psumrnk* = 0.0016). The next significant linkages were found on chromosomes 19p13.2–19q13.43 (*Psumrnk* = 0.01), 16q12.2–16q23.1 (*Psumrnk* = 0.0368), 17p13.3–17p12 (*Psumrnk* = 0.038), 19p13.2 –19p13.43 (*Psumrnk* = 0.01), and 9q21.32–9q33.1 (*Psumrnk* = 0.043). All had both *Psumrnk* and *Pord* < 0.05.

Fisher's method for combining *P* values

Fisher's method of the combination of probabilities from test of significance is a used tool for the synthesis of linkage evidence across studies. We applied Fisher's combined *P* value method to all of the bins that met an empirical criteria of both *Psumrnk* and *Pord* < 0.05: bins

6.2, 6.1, 6.3, 16.3, 19.2, 17.1, 16.4, and 9.4. Fisher's combined *P* values support the significant GSMA *P* values of the bins for linkage (Table 3).

Discussion

Family and twin studies of AS have demonstrated that predisposition to the disease was not exclusively related to HLA-B27, suggesting that additional susceptibility genes are expected in AS (Jarvinen. 1995). Previous AS and SpA genome-wide studies have shown linkages in HLA and non-HLA regions (Brown et al. 2003; Laval et al. 2001; Miceli-Richard et al. 2004; Zhang et al. 2004). Our meta-analysis provides more evidence confirming that HLA-B27 is a major genetic factor to susceptibility to AS. The highest evidence for linkage was observed in the HLA loci, and the linkage obtained a genome-wide significance ($P = 0.000003$). The nominally significant *Psumrnk* of the adjacent bins, 6.1 and 6.3, provided additional evidence for linkage here. This might be shown because in simulated data, bins adjacent to those containing disease loci often also achieve nominal significance (Cordell et al. 2001), or this finding may suggest the possibility that genes on non-HLA chromosome 6 regions also may play an important role in susceptibility to AS. Beside confirming linkage of the HLA region, the GSMA found evidence for linkage at non-HLA loci such as chromosomes 16q, 19, and 17p. A recent simulation study based on the schizophrenia GSMA indicated that bins with *Psumrnk* and *Pord* < 0.05 had the highest probability of containing a gene (Levinson et al. 2003). Additionally, we also performed the bin-specific Fisher's combining *P* values when both *Psumrnk* and *Pord* are < 0.05 for further assuring the significance. The significance of the bins with both *Psumrnk* and *Pord* < 0.05 by AS GSMA was all supported by Fisher's method.

Previous genome scans have indicated non-HLA susceptibility loci outside chromosome 6, including 9q34.2, 19q13.31, 11q24.1, and 13q14.2. In the GSMA, the linkage evidence on non-HLA loci was shown on 16q12.2–16q23.1, 19p13.2–19q13.43, 17p13.3–17p12, and 16q23.1–16q24.3 across the studies (both *Psumrnk* and *Pord* < 0.05). Linkage studies may have minimal power to detect genes of weak effect, but the combined evidence for linkage at these loci may be increased across studies. In contrast, false-positive results observed in individual studies should decrease in significance. Among non-HLA loci with strong linkage evidence observed in previous genome scans, chromosome 19q was confirmed in the GSMA. Additionally, the GSMA shows that chromosomes 16q, 19, and 17p may harbor non-HLA genes conferring a risk to AS. These regions may be interesting loci for further positional candidate gene studies. However, these data do not exclude the possibility that other chromosomal regions harbor AS susceptibility loci that could not be detected by the present methods or that substantially influence risk in only one or a few populations. GSMA does not also consider X- or Y-chromosome data, so no conclusions can be reached on possible linkage on the chromosomes.

The GSMA of all four genome scans, including one SpA scan, showed similar results with the GSMA data of three AS genome scans except for the linkage on chromosome 9q21.32–9q33.1. The 9q21.32–9q33.1 loci was found only in the GSMA, including the SpA genome scan. This finding suggests that the chromosome 9q region may confer susceptibility to SpA as well as AS.

The linkage loci shown by this meta-analysis may provide a basis for the location of AS susceptibility genes. It is interesting to examine these regions for candidate genes. For example, a possible candidate gene on chromosome 19q13.2 is transforming growth factor beta1 (TGF-β1) gene, which plays a crucial role in inflammatory processes, extracellular matrix synthesis, bone remodeling, and fibrosis and may be important in the biological pathways related to the expression of AS. One study has shown that the TGF-β1 + 1632 polymorphism was associated with a younger age of symptom onset, suggesting that TGF-β1 polymorphisms play a role in AS (Jaakkola et al. 2004).

A review of genome-wide scans of complex diseases has shown that true linkage remains hard to find (Altmuller et al. 2001). A complex disease might have many low- or modest-risk genes involved, with a low probability that the same results would be found in different genome-wide scans. Gene discovery in complex human diseases has been complicated by genetic heterogeneity, genes with small effect, and requirement for large samples (Goring et al. 2001). One potential solution to this issue is to combine data from multiple studies. Meta-analysis is an emerging method in the linkage analysis of complex diseases. The combination of evidence from multiple studies may prove to be critical to the successful localization of genes of small or modest effect in complex diseases. Therefore, the linkage results presented in the GSMA represent an important advance in searching non-HLA loci.

In conclusion, the GSMA added evidence of the HLA loci as the greatest susceptibility factor to AS and shows evidence of chromosomes 6, 16q, 19, 17p, and 9q as non-HLA susceptibility loci. These results provided a basis for future positional candidate gene studies in non-HLA loci.

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