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Is there a higher genetic load of susceptibility loci in familial ankylosing spondylitis?

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Abstract

Objective—Several genetic risk variants for ankylosing spondylitis (AS) have been identified in genome wide association studies. Our objective was to examine whether familial AS cases have a higher genetic load of these susceptibility variants.

Methods—Overall, 502 AS patients were examined, consisting of 312 who had first-degree relatives (FDR) with AS (familial) and 190 who had no FDR with AS or spondyloarthritis (sporadic). All patients and affected FDRs fulfilled the modified New York Criteria for AS. The patients were recruited from two U.S. cohorts (NASC and PSOAS) and from the United Kingdom- Oxford cohort. The frequencies of AS susceptibility loci in *IL23R, IL1R2, ANTRX2, ERAP1*, two intergenic regions on chromosomes 2p15 and 21q22, and HLA-B27 status as determined by the tag SNP rs4349859 were compared between familial and sporadic cases. Association between SNPs and multiplex status was assessed by logistic regression controlling for sibship size.

Results—HLA-B27 was significantly more prevalent in familial than sporadic cases of AS (p=0.0001, OR: 4.44, CI: (2.06–9.55)). Furthermore, the AS risk allele at chromosome 21q22 intergenic region showed a trend towards higher frequency in the multiplex cases (p=0.08). The frequency of the other AS risk variants did not differ significantly between familial and sporadic cases, either individually or combined.

Conclusions—HLA-B27 is more prevalent in familial than sporadic cases of AS, demonstrating higher familial aggregation of AS in patients with HLA-B27 positivity. The frequency of the recently described non-MHC susceptibility loci is not markedly different between the sporadic and familial cases of AS.

Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disorder primarily affecting the spine, peripheral joints and entheses. Extra-articular features can affect the eye, heart, and gastrointestinal tract. AS can also occur in the setting of other spondyloarthritides (SpA), including psoriatic arthritis, reactive arthritis, and enteropathic arthritis. Genetic factors provide over 90% of the overall susceptibility to AS. AS occurs in up to 75% of monozygotic twins compared to 27% in HLA-B27 positive dizygotic twins, indicating that there is a substantial non-MHC genetic component underlying disease (1). Up to half of the genetic contribution has been attributed to HLA-B27 and possibly other major histocompatibility complex (MHC) genes. Several non-MHC genes, specifically in the endoplasmic reticulum aminopeptidase-1*(ERAP1)*, interleukin 23 receptor (*IL23R*), the interleukin 1 receptor-2 (*IL1R2*), and the anthraxin receptor 2 (*ANTRX2*), as well as two

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intergenic regions at the chromosomes 2 and 21, have been reported as AS susceptibility loci in genome-wide association studies (2;3).

There are several reports of familial aggregation in AS and SpA. The risk of developing AS is up to 21% in HLA-B27 positive first degree relatives (FDRs) of AS cases while this risk decreases to less than 1% in HLA-B27 negative FDRs (4). Furthermore, only 6% of HLA-B27 positive subjects in the general population develop AS (5), underscoring the importance of other genetic loci beyond HLA-B27 in contributing to risk of AS. The frequencies of the above mentioned novel AS susceptibility loci in familial cases in comparison to sporadic AS cases have not been previously reported.

In the current study, we hypothesized that genetic susceptibility variants will be enriched in familial as compared to sporadic cases of AS. Therefore, we examined the genotype frequency of AS susceptibility loci in *IL23R*, *IL1R2, ANTRX2, ERAP*, two intergenic regions at the chromosomes 2 and 21, in addition to a recently described HLA-B27 tag single-nucleotide polymorphism (SNP) in familial and sporadic cases.

Methods

Familial or multiplex AS was defined as more than one family member (parent, sister, brother only - offspring were excluded for this study) with confirmed diagnosis of AS. Singleton or sporadic was defined as only one family member with confirmed AS and no other reported FDRs with AS or SpA. All index cases and FDRs with AS fulfilled the modified New York criteria for AS(6). The investigated cohorts included the North American Spondylitis Consortium (NASC), Prospective Study of Outcomes in Ankylosing Spondylitis (PSOAS), and United Kingdom- Oxford (UK-Oxford) cohorts.

NASC is a collaboration of eight sites in US cities (Houston, TX; Los Angeles, CA; Portland, OR; Cleveland, OH; Minneapolis, MN; Philadelphia, PA; Salt Lake City, UT; and Tampa, FL) as well as the Spondylitis Association of America (SAA), and two sites in Canada (Toronto, ON and Edmonton, AB), examining multiplex families with AS (7). NASC patients included families with 2 or more FDRs meeting the modified 1984 New York criteria for AS and for whom radiographs were available to confirm the diagnosis. The diagnoses were reevaluated by rheumatologists with experience in assessing patients with AS and the radiographs were assessed by study investigators.

The PSOAS study is an ongoing, longitudinal study of AS patients from four sites in the US: Cedars-Sinai Medical center, Los Angeles; the University of Texas at Houston; the University of California, San Francisco; and the National Institute of Health, Bethesda (8). Multiplex and singleton AS cases were enrolled in this study. The presence of sacroiliitis on pelvic radiographs was confirmed by a musculoskeletal radiologist.

The UK-Oxford study is an ongoing cross-sectional genetic study of multicase and sporadic AS cases in which in all cases the diagnosis of AS had been confirmed by a qualified rheumatologist. To further confirm the diagnosis, all cases were interviewed by telephone by study investigators. In patients with atypical medical history or for whom radiographs had not been performed previously, pelvic and lumbosacral spine radiographs were obtained and attending physicians were contacted to confirm the diagnosis(9). In this study, all affected siblings per self-report were assessed according to the above mentioned methodology.

All investigated patients in the current study were of self-reported white European descent. Only multiplex AS cases with confirmed affected FDR (parent and siblings only) and singleton AS cases were investigated. Only one patient with AS per family was included in the analysis.

The following non-MHC susceptibility variants for AS were investigated in the current study: *IL23R*, rs11209026; *IL1R2*, rs2310173; *ANTXR2*, rs4333130; *ERAP1*, rs27434; and two gene desert loci, rs10865331 and rs2242944(2;3). In addition, HLA-B27 was studied using the findings of a recently described HLA-B27 tag SNP, rs4349859(10). The genotype information was obtained from a previously described genome-wide association study conducted in patients enrolled in the NASC, PSOAS and UK-Oxford cohorts(2).

In addition, the clinical features of singleton AS cases in the PSOAS cohort were compared to the multiplex cases in the UK-Oxford cohort. The information on time of disease onset was not available in the NASC cohort. Therefore, patients enrolled in the NASC study were not included in the comparison of clinical features (Table 1).

Statistical Analysis

Association between SNPs and multiplex status was assessed by logistic regression assuming an additive effect of alleles on log odds of being from a multiplex family. Individuals from multiplex families were coded as cases and individuals from simplex families were coded as controls. Best guess genotypes at rs4349859 were imputed using the software MACH(11) as described in Evans et al. (10). Quality control metrics indicated that this SNP could be imputed with a high degree of certainty (RSQR > 0.98). Imputed genotypes at rs4349859 were only available for 292 multiplex and 177 sporadic cases of AS. In order to minimize the effect of possibly misclassifying multiplex cases from smaller families as sporadic cases, we examined the effect of adding sibship size as a covariate in the logistic regression model.

In order to ensure that our results were robust to the effect of population stratification, we also examined the effect of including Eigenstrat derived principal components from a previous genome-wide association study of AS in our analysis (2) and restricting our analysis to US cases only (128 multiplex cases and 176 sporadic cases).

The relationship between the number of affected siblings per family and the number of HLA-B27 alleles in the index case was examined in the UK-Oxford cohort by linear regression.

Results

In the current study, 312 unrelated multiplex and 190 singleton AS patients were investigated. In detail, 160 multiplex AS cases were recruited from the UK-Oxford cohort, on whom the required genotype data were available. These patients had a mean age (SD) of 47.9 (10.2) at enrollment and 60% were male. The mean disease duration at the enrolment was 24.9 (11) years. We also recruited 152 multiplex AS cases from the NASC study. In this group, the mean age at enrollment was 44.4 (10.4) years, 58% of patients were male. Furthermore, 190 singleton cases were recruited from the PSOAS. This patient group had a mean age of 47.1 (16.1) years, was 76% male, and had a mean disease duration of 22.8 (15.7) years at enrollment. Table 1 shows the comparison of clinical features between the singleton and multiplex AS cases. The familial cases had a lower percentage of male patients (p=0.0004). The other clinical features did not differ significantly between the singleton and multiplex cases

Table 2 shows the frequency of susceptibility loci in the multiplex and singleton cases. HLA-B27 was significantly more prevalent in multiplex than in singleton AS cases ($p=8.4\times10^{-8}$, OR: 5.41, CI (2.92–10.03)). The frequency of the other AS susceptibility loci in *IL23R, IL1R2, ANTXR2, ERAP1* genes and the chromosome 2p15 and 21 q22 intergenic regions did not differ significantly between the singleton and multiplex cases. Furthermore,

gender did not modify the significant relationship between HLA-B27 and occurrence of familial AS (p-value for the interaction term between gender and HLA-B27= 0.763).

Similar results were observed after adjustment for sibship size (Table 3). HLA B27 was more prevalent among multiplex cases (p=0.0001). Furthermore, the risk allele in the intergenic region on 21q22 showed a trend towards higher frequency in the multiplex cases (p=0.08, OR: 1.37, CI (0.96–1.95)). The other susceptibility loci did not show a significant association with familial occurrence of AS. As shown in Table 3, these results did not change when the analysis was restricted to patients recruited from the two US cohorts and covariates were added to reflect population substructure.

The number of risk alleles, an individual carries at non-HLA loci, were added together and then divided by the total number of successfully genotyped SNPs at the susceptibility loci to obtain an allelic risk score. This score was regressed against log odds of being from a multiplex family in order to assess the cumulative effect of the AS non-MHC risk alleles on the multiplex status. This allelic score was not associated with risk of being from a multiplex family (p = 0.64).

There was no significant relationship between the number of affected siblings and number of HLA-B27 alleles (p=0.226, OR: 1.2, 95% CI: 0.89–1.62).

Discussion

The current report is the first genetic study to investigate the frequency of recently described susceptibility variants in familial and sporadic AS patients. We report that the relative prevalence of HLA-B27 was higher in familial AS, while the frequency of other risk variants implicated in AS was not significantly increased. This implies higher genetic load of HLA-B27 in familial AS patients.

Significant advances have been made in understanding the genetic basis of AS. The most prominent genetic susceptibility locus remains HLA-B27. Definite non-MHC associations with AS have been demonstrated with polymorphisms in the *ERAP1, IL23R*, *IL1R2*, *ANTXR2* genes in addition to two intergenic regions at chromosome 2p15 and 21q22(2;3). *ERAP1* is the second strongest genetic association after HLA-B27 and the strongest non-MHC susceptibility locus. *IL23R* has also been implicated in AS cases of white European ancestry although not in populations from Eastern Asia. Other genes associated with AS include *IL1R* and *ANTXR2*. Chromosome 2p15 and 21q22 are two intergenic regions or “gene deserts” associated with AS. Though these non-MHC susceptibility loci were confirmed in large independent cohorts(2;3), the effect size of these risk variants is markedly lower than HLA-B27.

The current study compares the genetic data between confirmed familial and sporadic cases of AS. In addition to the above mentioned non-MHC susceptibility loci, the current study investigated an MHC susceptibility locus. Evans *et al* identified a single SNP rs4349859 near the gene MICA which tagged HLA-B27 with sensitivity of 98% and specificity of 99% in AS cases of white European descent. Thus it can be used as an alternative to HLA-B27 genotyping in this ethnicity (10). We report that the HLA-B27 tag SNP was more prevalent in familial AS, while we did not observe a significantly higher prevalence of non-MHC susceptibility loci among familial cases. This implies higher genetic load of HLA-B27 in familial AS patients. While FDR of AS patients who have the HLA-B27 allele are at higher risk of developing AS than their HLA-negative counterparts(4), there are no previous reports of higher frequency of HLA-B27 positivity in familial versus sporadic cases of AS(4). The current study is the first report that demonstrates higher familial aggregation of AS in

patients with HLA-B27 positivity. This finding can be explained by the large contribution of HLA-B27 to susceptibility of AS.

A study of 55 familial and 110 sex and age matched sporadic cases of AS found no difference in disease onset, age at diagnosis or prevalence of peripheral arthritis or uveitis(12). Another study of 160 familial and 160 age and sex matched sporadic cases indicated that familial disease was significantly milder in terms of spinal mobility score, pain score, psychological and social functioning(13). Differences in frequency of severity genes were postulated as an explanation for this finding. However, a selection bias towards milder disease among familial cases is another possible explanation. In the current study, the familial AS cohorts included a higher percentage of female patients. This might be secondary to lower threshold of assessing and diagnosing a female patient with AS if another family member has AS (verification bias). Similar to the above mentioned study (12), the other clinical features did not differ between the comparison groups.

The strengths of the current study are: the relatively large sample size; strict confirmation of diagnosis according to the modified New York criteria(6) in index cases as well as affected FDRs; and the examination of genotype frequencies after correction for sibship size. Although our study is the largest to date comparing familial AS with sporadic AS, it is still underpowered to detect more subtle differences in frequency of non-MHC susceptibility genes between the two investigated groups. Furthermore, we cannot exclude that potential multiplex cases are misclassified as singleton families because of low number of siblings. We tried to account for this possible misclassification by adjusting for sibship size. There are several other potential sources of heritability among familial cases which were not examined in the current study. There might be further relevant SNPs (either common or rare variants) or insertion/deletion polymorphisms not tagged by the investigated SNPs. Moreover, gene-environment interaction might affect familial occurrence of AS.

In summary, HLA tag SNP rs4349859, which tracks closely with HLA-B27, is more prevalent in familial than sporadic cases of AS. This finding demonstrates higher familial aggregation of AS in patients with HLA-B27 positivity. The frequency of the recently described non-MHC susceptibility loci is not markedly different between the sporadic and familial cases of AS although we cannot exclude more subtle differences in frequency of these susceptibility variants between the investigated two groups.

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Reference List

1. Brown MA, Kennedy LG, MacGregor AJ, Darke C, Duncan E, Shatford JL, et al. Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. Arthritis Rheum. 1997; 40(10):1823–8. [PubMed: 9336417]
2. Reveille JD, Sims AM, Danoy P, Evans DM, Leo P, et al. Australo-Anglo-American Spondyloarthritis Consortium. Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. Nat Genet. 2010; 42(2):123–7. [PubMed: 20062062]
3. Burton PR, Clayton DG, Cardon LR, Craddock N, et al. Wellcome Trust Case Control Consortium, Australo-Anglo-American Spondylitis Consortium. Association scan of 14,500 nonsynonymous

SNPs in four diseases identifies autoimmunity variants. Nat Genet. 2007; 39(11):1329–37. [PubMed: 17952073]

4. van der Linden SM, Valkenburg HA, de Jongh BM, Cats A. The risk of developing ankylosing spondylitis in HLA-B27 positive individuals. A comparison of relatives of spondylitis patients with the general population. Arthritis Rheum. 1984; 27(3):241–9. [PubMed: 6608352]
5. Braun J, Bollow M, Remlinger G, Eggens U, Rudwaleit M, Distler A, et al. Prevalence of spondylarthropathies in HLA-B27 positive and negative blood donors. Arthritis Rheum. 1998; 41(1):58–67. [PubMed: 9433870]
6. van der LS, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum. 1984; 27(4):361–8. [PubMed: 6231933]
7. Zhang G, Luo J, Bruckel J, Weisman MA, Schumacher HR, Khan MA, et al. Genetic studies in familial ankylosing spondylitis susceptibility. Arthritis Rheum. 2004; 50(7):2246–54. [PubMed: 15248224]
8. Brionez TF, Assassi S, Reveille JD, Green C, Learch T, Diekman L, et al. Psychological correlates of self-reported disease activity in ankylosing spondylitis. J Rheumatol. 2010; 37(4):829–34. [PubMed: 20156952]
9. Laval SH, Timms A, Edwards S, Bradbury L, Brophy S, Milicic A, et al. Whole-genome screening in ankylosing spondylitis: evidence of non-MHC genetic-susceptibility loci. Am J Hum Genet. 2001; 68(4):918–26. [PubMed: 11231900]
10. Evans DM, Spencer CC, Pointon JJ, Su Z, et al. The Australo-Anglo-American Spondyloarthritis Consortium (, the Wellcome Trust Case Control Consortium. Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. Nat Genet. 2011
11. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. Genet Epidemiol. 2010; 34(8):816–34. [PubMed: 21058334]
12. Paardt M, Dijkmans B, Giltay E, van dH-B I. Dutch patients with familial and sporadic ankylosing spondylitis do not differ in disease phenotype. J Rheumatol. 2002; 29(12):2583–4. [PubMed: 12465156]
13. Calin A, Kennedy LG, Edmunds L, Will R. Familial versus sporadic ankylosing spondylitis. Two different diseases? Arthritis Rheum. 1993; 36(5):676–81. [PubMed: 8489546]

Significance of Innovations

- The first genetic study to compare the frequency of the recently described susceptibility variants between the familial and sporadic AS cases
- There is a higher frequency of HLA-B27 positivity in familial than sporadic cases of AS
- The frequency of the other risk variants implicated in AS was not significantly increased in familial cases, either individually or combined.

Table 1

Comparison of clinical features of multiplex (UK-Oxford cohort) and singleton (PSOAS) AS cases

	Sporadic	Familial	Effect size*	95% C.I.	p-value
Gender %, Male	76.4	60	0.46	0.71, 0.3	0.0004
Age at disease onset, mean, yr	23.7	22.9	−0.84	−2.54, 0.86	0.331
Disease duration at enrollment, mean, yr	24.2	24.9	0.68	−2.03, 3.39	0.62
Uveitis %†	45.2	39.4	0.75	0.49, 1.13	0.16
Psoriasis%†	12	11.3	0.97	0.51, 1.82	0.92

*Reference group: sporadic cases

†Adjusted for disease duration

Table 2

Frequency of the AS susceptibility loci in multiplex and singleton cases

SNP	Candidate Gene	Risk Allele	Odds Ratio (95% C.I.)	P-value
rs11209026	*IL23R*	G	1.23 (0.64, 2.36)	0.54
rs10865331	2p15*	A	0.87 (0.67, 1.12)	0.26
rs2310173	*IL1R2*	T	1.10 (0.86, 1.42)	0.44
rs4333130	*ANTXR2*	T	1.15 (0.87, 1.52)	0.32
rs27434	*ERAP1*	A	0.85 (0.63, 1.13)	0.26
rs2242944	21q22*	G	1.20 (0.91, 1.59)	0.19
rs4349859	HLA-B27 tag SNP	A	5.41 (2.92, 10.03)	$\mathbf{8.4 \times 10^{-8}}$

*These SNPs are located in the intergenic regions

Table 3

Comparison of AS susceptibility loci between multiplex and singleton cases after adjustment for sibship size

SNP	Candidate Gene	Odds ratio (95% C.I.) - overall cohort	P-value overall cohort	Odd ratio (95% C.I.) - US sample	P-value US sample
rs11209026	*IL23R*	1.44 (0.63, 3.27)	0.39	2.33 (0.76, 7.14)	0.14
rs10865331	2p15*	0.85 (0.62, 1.16)	0.31	0.99 (0.69, 1.42)	0.95
rs2310173	*IL1R2*	1.26 (0.9, 1.73)	0.16	1.31(0.91, 1.89)	0.15
rs4333130	*ANTXR2*	1.24 (0.81, 1.64)	0.42	1.15 (0.76,1.72)	0.51
rs27434	*ERAP1*	0.81 (0.55, 1.17)	0.26	0.89 (0.57, 1.38)	0.6
rs2242944	21q22*	1.37 (0.96, 1.95)	0.08	1.45 (0.95, 2.17)	0.08
rs4349859	HLA-B27 tag SNP	4.44 (2.06, 9.55)	0.0001	4.08 (1.7, 9.83)	0.002

*These SNPs are located in intergenic regions