CONCISE REPORT

Replication of association of IL1 gene complex members with ankylosing spondylitis in Taiwanese Chinese

C-T Chou*, A E Timms*, J C C Wei, W C Tsai, B P Wordsworth, M A Brown

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Objective: To test the association of interleukin 1 (IL1) gene family members with ankylosing spondylitis (AS), previously reported in Europid subjects, in an ethnically remote population.

Methods: 200 Taiwanese Chinese AS patients and 200 ethnically matched healthy controls were genotyped for five single nucleotide polymorphisms (SNPs) and the IL1RN.VNTR, markers previously associated with AS. Allele, genotype, and haplotype frequencies were compared between cases and controls.

Results: Association of alleles and genotypes of the markers IL1F10.3, IL1RN.4, and IL1RN.VNTR was observed with AS ($p < 0.05$). Haplotypes of pairs of these markers and of the markers IL1RN.6/1 and IL1RN.6/2 were also significantly associated with AS. The strongest associations observed were with the marker IL1RN.4, and with the two-marker haplotype IL1RN.4–IL1RN.VNTR (both $p = 0.004$). Strong linkage disequilibrium was observed between all marker pairs except those involving IL1B-511 (D' 0.4 to 0.9, $p < 0.01$).

Conclusions: The IL1 gene cluster is associated with AS in Taiwanese Chinese. This finding provides strong statistical support that the previously observed association of this gene cluster with AS is a true positive finding.

Association of members of the interleukin 1 (IL1) gene cluster with ankylosing spondylitis (AS) have been reported by several groups studying affected white (Europid) families and cases.^{1–4} These studies have largely focused on the genes *IL1A*, *IL1B*, and *IL1RN*, apart from one study which examined all nine members of the IL1 gene cluster.⁴ These genes all have significant homology with either *IL1A* or *IL1RN*, and are thought to function primarily as IL1 agonists or antagonists. Association has been reported by two groups with a VNTR polymorphism in the *IL1RN* gene, studying respectively Dutch and Scottish AS cases.^{1,2} Using families from the North American Spondyloarthritis Consortium (NASC), Maksymowych and colleagues reported association particularly with haplotypes of single nucleotide polymorphisms (SNPs) in the *IL1RN* gene, suggesting that either combinations of these SNPs were important, or that the true associated polymorphism lay on the associated haplotype but was not one of the individual markers genotyped.³ A linkage study also using the NASC family collection reported no linkage of this region with AS⁵; the inconsistency of these findings is consistent with the known

weakness of linkage as a method of identifying genetic effects in complex genetic diseases such as AS.

The strength and complexity of the association of this locus with AS was demonstrated by studies of white British families and cases, which showed strong association across the IL1 gene cluster.⁴ Logistic regression analysis indicated that the association was particularly with a two-marker SNP haplotype consisting of the IL1B-511 variant and an SNP in IL1F10, and that this haplotype carried most, but not all, of the association present in the IL1 gene cluster. We are currently pursuing further studies in these families to refine the primary genetic associations. Studying ethnically remote cases has great potential not only to assist this process, but also to increase confidence in the role of genetic variants within this cluster in the aetiology of AS. Therefore we sought to test the association of IL1 genetic variants, previously associated with AS in Europid populations, in Taiwanese Chinese.

METHODS

Two hundred unrelated Taiwanese Chinese AS patients (defined by the modified New York criteria) and an equal number of unrelated healthy controls were recruited. All patients were interviewed, examined, and had pelvic radiographs assessed by qualified rheumatologists (CTC, JCCW, WCT). The health controls were blood donors who were also of Taiwanese Chinese descent. HLA-B27 carriage had previously been assessed by flow cytometry.⁶ The study protocol was approved by the local ethics approval board and all participants gave informed consent.

Samples were genotyped by published methods for five SNPs and the IL1RN.VNTR, as outlined in table 1.⁴ Genotype-known control samples were run with each genotyping experiment to confirm accuracy.

Hardy–Weinberg equilibrium was verified by comparison of observed and expected genotype frequencies by contingency table analysis. Marker haplotypes were determined using the program PHASE (version 2.1)⁷; only haplotypes achieving >90% posterior probability were used in further analyses. Contingency table analysis was also used to compare case and control allele, genotype, and two-marker haplotype frequencies. Results were not corrected for multiple comparisons, as this was a confirmation study.

Pairwise linkage disequilibrium was calculated using genotype data from healthy controls, and reported as Lewontin's standardised D' statistic.⁸

To assess the appropriate level of correction for the number of markers assuming this was a discovery study, we applied spectral decomposition analysis.⁹ This method assesses the level of independence of genotypes taking into account

*These authors contributed equally to the study.

Abbreviations: AS, ankylosing spondylitis; SNP, single nucleotide polymorphism

Table 1 Markers genotyped

RS number	Name	Gene	Exon	Variant	Chromosome 2 position
rs16944	IL1B-511	IL1B	Promoter	G>A	113690013
rs3811058	IL1F10.3	IL1F10	3	T>C	113927091
rs419598	IL1RN.4	IL1RN	3	T>C	113982353
–	IL1RN.VNTR	IL1RN	INT3	–	113983218
rs315952	IL1RN.6/1	IL1RN	6	C>T	113985450
rs315951	IL1RN.6/2	IL1RN	6	C>G	113985732

Positions are given from the p-telomere of chromosome 2 in base pairs, according to the human genome map May 2004 release (<http://genome.ucsc.edu/>).

linkage disequilibrium. The results are presented as uncorrected p values, as the study was a confirmation study of markers previously associated with AS and therefore correction would be overly conservative.

Power calculations were done using the Purcell and colleagues "Genetic Power Calculator".¹⁰

RESULTS

The 200 AS cases included 141 men (70.5%) and 59 women (29.5%), of whom 195 (97.5%) were HLA-B27 positive. Of the 200 healthy controls, 84 (42%) were men and 116 (58%) were women, of whom nine were HLA-B27 positive.

The current data were found to be equivalent to five independent markers with no intermarker linkage disequilibrium, and thus a corrected p value of 0.01 is equivalent to $p = 0.05$ uncorrected.

Results of allele and genotype comparisons are given in table 2. All marker genotypes were in Hardy–Weinberg equilibrium, and $\geq 95\%$ were successfully genotyped for any individual SNP. No significant differences were observed in allele or genotype frequencies comparing men and women (data not shown).

Pairwise linkage disequilibrium statistics calculated from the genotype findings in healthy controls are given in table 3. Significant association was observed for IL1F10.3, IL1RN.4, and IL1RN.VNTR in both comparisons of allele and genotype frequencies. Haplotypes could be determined with $>90\%$ certainty for 85% of all possible haplotype pairs involving all markers except IL1B-511. Lower linkage disequilibrium between IL1B-511 and the neighbouring marker IL1F10.3 meant that only 50% of haplotypes of this marker pair could be determined with $>90\%$ certainty, and therefore these were not analysed.

For single markers, associations were observed with IL1F10.3 ($p = 0.01$), IL1RN.4 ($p = 0.004$), and the IL1RN.VNTR ($p = 0.005$).

Significant associations were observed for the two-marker haplotypes IL1F10.3–IL1RN.4 ($p = 0.02$), IL1RN.4–IL1RN.VNTR ($p = 0.004$), IL1RN.VNTR–IL1RN.6/1 ($p = 0.03$), and IL1RN.6/1–IL1RN.6/2 ($p = 0.05$).

SNP haplotypes previously reported to be associated with AS by Maksymowych and colleagues were also globally associated with AS in this study. Specifically the findings were: IL1RN.4–IL1RN.6/1 ($p = 0.04$), IL1RN.4–IL1RN.6/2 ($p = 0.004$), IL1RN.6/1–IL1RN.6/2 ($p = 0.05$), and IL1RN.4–IL1RN.6/1–IL1RN.6/2 ($p = 0.004$).

DISCUSSION

These findings confirm previous studies documenting association of the IL1 gene complex and AS, although no association was observed with the IL1B-511 SNP. The strongest association was observed with the two-marker haplotypes IL1RN.4–IL1RN.VNTR and IL1RN.4–IL1RN.6/2, the three marker haplotype IL1RN.4–IL1RN.6/1–IL1RN.6/2, and the SNP IL1RN.4 (all $p = 0.004$). Our findings are very

similar to those of Maksymowych *et al*, who reported substantially stronger haplotypic associations with SNPs of IL1RN than with individual SNPs.³ Looking at alleles of individual IL1RN SNPs, Maksymowych *et al* reported global association of IL1RN.6/1 and IL1RN.6/2 alleles with AS ($p = 0.001$ and $p = 0.04$, respectively), and with IL1RN.4 genotypes (but not alleles) globally ($p = 0.03$), mostly because of underrepresentation of homozygosity for the minor "C" allele among AS cases ($p = 0.01$, odds ratio = 0.5). In this study, we also saw underrepresentation of the "C" allele in AS cases ($p = 0.004$, odds ratio = 0.5). We did not observe association with IL1RN.6/1 individually, and only a non-significant trend was seen at IL1RN.6/2 ($p = 0.08$, odds ratio = 1.3). Maksymowych *et al* reported association of specific two- and three-marker haplotypes involving our markers IL1RN.4, IL1RN.6/1, and IL1RN.6/2, at significance levels that were generally lower than those reported for individual SNPs. We observed global association with each of these marker combinations.

The sample size studied was too small to employ methods such as logistic regression to define the primary association better. Furthermore, the linkage disequilibrium between markers, with the exception of IL1B-511, was quite high, complicating the process of differentiating true association from haplotypic findings. Therefore we cannot determine from the present study which is the true associated locus; for this, further mapping with a greater marker density in a larger sample size will be required.

Confirmation of the association of this gene cluster with AS in an ethnically remote population to Europids makes it extremely likely that this finding, initially reported in Europids, is a true positive finding, and confirms that IL1 gene family members are important determinants of susceptibility to AS. The majority of previous studies that have examined the IL1 gene complex in AS have also reported associations, though the primary associated variant has varied between studies. Three negative studies have been reported,^{5 11 12} two of which were only sufficiently powered to identify strong positive effects at the locus, and for realistic genetic models were more likely to produce false negative than true positive results. For example, Kim *et al* examined 205 AS cases and 200 controls.¹¹ For the IL1B+3957 variant, for a gene with odds ratio for disease of 1.5, the chance of a true positive finding is very low ($p < 0.05$, 23%), and the study is actually more likely to report a negative finding even if the SNP genotyped was associated with AS ($p > 0.1$, probability 77%). Even for the current study, our power to identify effects at IL1RN.6/2 for an odds ratio of 1.5 was just 37% and the likelihood of a false negative result is high ($p > 0.1$, probability 50%). Jin *et al* previously reported no linkage or association of the IL1 gene complex in AS,⁵ but recently reported strong positive association of the IL1B-511 variant and disease.¹³ Previous studies of the IL1RN.VNTR have been contradictory, with some studies showing no association,^{4 11 12} and some showing positive association of allele 2

Table 3 Linkage disequilibrium statistics between IL1 gene cluster polymorphisms

	IL1B-511	IL1F10.3	IL1RN.4	IL1RN.VNTR	IL1RN.6/1	IL1RN.6/2
IL1B-511		<0.01	0.08	0.90	<0.01	<0.01
IL1F10.3	0.20		<0.01	<0.01	<0.01	<0.01
IL1RN.4	0.07	0.43		<0.01	<0.01	<0.01
IL1RN.VNTR	0.03	0.46	0.97		<0.01	<0.01
IL1RN.6/1	0.26	0.62	0.61	0.56		<0.01
IL1RN.6/2	0.19	0.62	0.74	0.68	0.88	

Linkage disequilibrium is reported using Lewontin's standardised D' measure in bold font the bottom left corner, and the associated p value in the top right corner.

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