CONCISE REPORT

REPLICATION OF ASSOCIATION OF IL-1 GENE COMPLEX MEMBERS WITH ANKYLOSING SPONDYLITIS IN TAIWANESE CHINESE

Chou, Chung-Tei1*
Timms, Andrew E.2*
Wei, James C.C.3
Tsai, Wen C.4
Wordsworth, B. Paul2
Brown, Matthew A.2

1Veterans General Hospital-Taipei, Taipei, Taiwan.
2Institute of Musculoskeletal Sciences, University of Oxford, Botnar Research Centre, Nuffield Orthopaedic Centre, Oxford, United Kingdom.
3Chung Shan Medical University, Taichung, Taiwan.
4Kaohsiung Medical University Hospital, Kaohsiung, Taiwan.
*These authors contributed equally to this study.

Corresponding author:
Professor Matthew A. Brown,
Institute of Musculoskeletal Sciences, University of Oxford,
Botnar Research Centre,
Nuffield Orthopaedic Centre,
Windmill Road,
Headington, Oxford, OX3 7LD.
United Kingdom.
Email: mbrown@well.ox.ac.uk
ABSTRACT
OBJECTIVE: To test the association of interleukin-1 gene family members with ankylosing spondylitis (AS), previously reported in white Caucasians, in an ethnically remote population.

METHODS: Two hundred Taiwanese Chinese AS patients and two hundred ethnically matched healthy controls were genotyped for 5 SNPs and the IL-1RN.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 IL-1F10.3, IL-1RN.4 and IL-1RN.VNTR was observed with AS (p<0.05). Haplotypes of pairs of these markers and of the markers IL-1RN.6/1 and IL-1RN.6/2 were also significantly associated with AS. The strongest associations observed were with the marker IL-1RN.4, and with the two marker haplotype IL-1RN.4-IL-1RN.VNTR (both p=0.004). Strong linkage disequilibrium was observed between all marker pairs except those involving IL-1B-511 (D’ 0.4-0.9, P<0.01).

CONCLUSION: The IL-1 gene cluster is associated with AS in Taiwanese Chinese. This finding provides strong statistical support that the previously association of this gene cluster with AS is a true positive finding.

KEYWORDS
Ankylosing spondylitis, interleukin-1, association, linkage disequilibrium
INTRODUCTION

Association of members of the IL-1 gene cluster with ankylosing spondylitis (AS) have been reported by several groups studying affected Caucasian families and cases. (1-4) These studies have largely focussed on the genes *IL-1A*, *IL-1B* and *IL-1RN*, apart from one study which examined all 9 members of the IL-1 gene cluster. (4) These genes all have significant homology either with *IL-1A* or *IL-1RN*, and are thought to function primarily as IL-1 agonists or antagonists. Association has been reported by two groups with a VNTR polymorphism in the *IL-1RN* 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 SNPs in the *IL-1RN* 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 AS (5); 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 British Caucasian families and cases, which demonstrated strong association across the IL-1 gene cluster. (4) Logistic regression analysis indicated that the association was particularly with a two-marker SNP haplotype consisting of the IL-1B-511 variant and an SNP in IL-1F10, and that this haplotype carried most, but not all, of the association present in the IL-1 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 IL-1 genetic variants, previously associated with AS in white Caucasian populations, in Taiwanese Chinese.

MATERIALS AND 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 5 SNPs and the IL-1RN.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 2 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 linkage disequilibrium. The results are presented as uncorrected p-values though, as the study is a confirmation study of markers previously associated with AS, and therefore correction would be overly conservative.

Power calculations were performed 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) are HLA-B27 positive. Of the 200 health controls, 84 (42%) are men and 116 (58%) are women, of whom 9/200 are HLA-B27 positive.

The current data was found to be equivalent to 5 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 ≥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 IL-1F10.3, IL-1RN.4, and IL-1RN.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 IL-1B-511. Lower linkage disequilibrium between IL-1B-511 and the neighbouring marker IL-1F10.3 meant that only 50% of haplotypes of this marker pair could be determined with >90% certainty, and therefore these were not analyzed.

For single-markers, associations were observed with IL-1F10.3 (p=0.01), IL-1RN.4 (p=0.004), and the IL-1RN.VNTR (p=0.005).

Significant associations were observed for the two-marker haplotypes IL-1F10.3-IL-1RN.4 (p=0.02), IL-1RN.4 – IL-1RN.VNTR (p=0.004), IL-1RN.VNTR-IL1RN.6/1 (p=0.03) and IL-1RN.6/1-IL-1RN.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: IL-1RN.4-IL1RN.6/1 (p=0.04), IL-1RN.4-IL-1RN.6/2 (p=0.004), IL-1RN.6/1-IL-1RN.6/2 (p=0.05), and IL-1RN.4-IL-1RN.6/1-IL-1RN.6/2 (p=0.004).

DISCUSSION
These findings confirm previous studies documenting association of the IL-1 gene complex and AS, although no association was observed with the IL-1B-511 SNP. The strongest association was observed with the two marker haplotypes IL-1RN.4 – IL-1RN.VNTR and IL-1RN.4-IL-1RN.6/2, the three marker haplotype IL-1RN.4-IL-1RN.6/1-IL-1RN.6/2, and the SNP IL-1RN.4 (all p=0.004). Our findings are very similar to those of Maksymowych and colleagues, who reported substantially stronger haplotypic associations with SNPs of IL-1RN than with individual SNPs(3). Looking at alleles of individual IL-1RN SNPs, Maksymowych and colleagues reported global association of IL-1RN.6/1 and IL-1RN.6/2 alleles with AS (p=0.001 and p=0.04 respectively), and with IL-1RN.4 genotypes (but not alleles) globally (p=0.03), mostly due to under-representation of homozygosity for the minor ‘C’ allele amongst AS cases (p=0.01, odds ratio 0.5). In this study, we also saw under-representation of the ‘C’ allele in AS cases (p=0.004, odds ratio 0.5). We did not
observe association with IL-1RN.6/1 individually, and only a non-significant trend was seen at IL-1RN.6/2 (p=0.08, odds ratio 1.3). Maksymowych and colleagues reported association of specific two- and three- marker haplotypes involving our markers IL-1RN.4, IL-1RN.6/1 and IL-1RN.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 better define the primary association. Furthermore, the linkage disequilibrium between markers, with the exception of IL-1B-511, was quite high, complicating the process of differentiating true association from haplotypic findings. Therefore from this study we cannot determine which is the true associated locus, for which purpose 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 white Caucasians makes it extremely likely that this finding, initially reported in white Caucasians, is a true positive finding, and confirms that IL-1 gene family members are important determinants of susceptibility to AS. The majority of previous studies that have examined the IL-1 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, the manuscript by Kim and colleagues examined 205 AS cases and 200 controls. For the IL-1B+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 IL-1RN.6/2 for an odds ratio of 1.5 is just 37% and the likelihood of a false negative result is high (p>0.1, probability 50%). Jin and colleagues previously reported no linkage or association of the IL-1 gene complex in AS (5), but recently report strong positive association of the IL-1B-511 variant and disease (13). Previous studies of the IL-1RN VNTR have been contradictory with some studies showing no association (4, 11, 12), and some showing positive association of allele 2 with disease (1, 2). We saw significant under-representation of allele 2 of the IL-1RN VNTR (p=0.005) amongst cases. Whether the differences in primary associated genes within the complex are due to statistical reasons (inadequate power to exclude effects or false positive findings), heterogeneity between populations, or gene-gene interactions is uncertain, although the replication here of findings with IL-1F10 and IL-1RN SNPs makes these findings very unlikely to be erroneous. Future studies should focus on the role of these variants in specific ethnic groups, identifying the primary associated variants, determining the utility of the variants in diagnosis (particularly in early disease), studies of the association of the IL-1 gene cluster in other seronegative spondyloarthropathies, and investigation of the mechanism by which these variants increase the risk of the disease.
ACKNOWLEDGEMENTS
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Table 1: Markers genotyped. 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/)

<table>
<thead>
<tr>
<th>RS NUMBER</th>
<th>NAME</th>
<th>GENE</th>
<th>EXON</th>
<th>VARIANT</th>
<th>CHROMOSOME 2 POSITION</th>
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<tr>
<td>rs16944</td>
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<td>IL-1B</td>
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<td>G&gt;A</td>
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<td>rs3811058</td>
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<td>IL-1F10</td>
<td>3</td>
<td>T&gt;C</td>
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<td>rs419598</td>
<td>IL-1RN.4</td>
<td>IL-1RN</td>
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<td>-</td>
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<td>IL-1RN</td>
<td>INT3</td>
<td>-</td>
<td>113983218</td>
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<td>C&gt;G</td>
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Table 2: Allele and Genotype Results. Results for the IL-1RN.VNTR are given for allele 2 compared with other genotypes pooled. Percentages are highlighted in bold.

<table>
<thead>
<tr>
<th>AS PATIENTS</th>
<th>CONTROLS</th>
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<td>GENOTYPE</td>
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<td>IL-1B-511</td>
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<td>IL-1F10.3</td>
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<td>IL-1RN.VNTR</td>
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<tr>
<td>IL-1RN.6/1</td>
<td>31</td>
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<tr>
<td>IL-1RN.6/2</td>
<td>73</td>
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</table>
Table 3: Linkage disequilibrium statistics between IL-1 gene cluster polymorphisms. 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.

<table>
<thead>
<tr>
<th></th>
<th>IL-1B-511</th>
<th>IL-1F10.3</th>
<th>IL-1RN.4</th>
<th>IL-1RN.VNTR</th>
<th>IL-1RN.6/1</th>
<th>IL-1RN.6/2</th>
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<tr>
<td>IL-1B-511</td>
<td>&lt;0.01</td>
<td>0.08</td>
<td>0.90</td>
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<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<tr>
<td>IL-1RN.4</td>
<td>0.07</td>
<td>0.43</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<td></td>
</tr>
<tr>
<td>IL-1RN.VNTR</td>
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<td>0.46</td>
<td>0.97</td>
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<td>&lt;0.01</td>
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<tr>
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<td>0.62</td>
<td>0.74</td>
<td>0.68</td>
<td>0.88</td>
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REFERENCES

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