Concise Report

Interleukin-1 promoter region polymorphism role in rheumatoid arthritis: a meta-analysis of IL-1B-511A/G variant reveals association with rheumatoid arthritis

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Objectives. IL-1 has a central role mediating inflammation and joint destruction in RA. Single nucleotide polymorphisms (SNPs) and haplotype structure in the promoter region can modulate IL-1 function. This study examined the effects of four common promoter SNPs in the IL-1 region on susceptibility and clinical characteristics of RA in British Caucasian patients and assessed the risk of RA by meta-analysis of published studies.

Methods. Using PCR-based methods, 756 RA patients and 625 healthy controls (HCs) were genotyped for IL-1A (–889 C/A, rs17561), IL-1B (–511 A/G, rs18944), IL-18 (–1464 C/G, rs1143623) and IL-1B (–3737 G/A, rs4848306) SNPs. Further meta-analysis was performed for IL-1B (–511 A/G) incorporating 3712 RA patients and 2331 HC from six association studies.

Results. The IL-1B (–1464 C/G) G allele was found to be less common in the RA group \( P = 0.01; \) odds ratio (OR) 1.24; 95% CI 1.04, 1.48. There was no association between IL-1 SNPs and the presence of HLA-DRB1 shared epitope, RF or clinical characteristics. Meta-analysis revealed statistically significant association between IL-1B (–511 A/G) and RA (\( P = 0.02; \) pooled OR 1.13; 95% CI 1.02, 1.26).

Conclusions. There may be a protective effect in RA from the IL-1B (–1464 C/G) G variant. No direct association between the polymorphisms studied and clinical severity characteristics were observed. Further meta-analysis revealed IL-1B (–511 A/G) to be associated with increased susceptibility to RA.

Key Words: Rheumatoid arthritis, Meta-analysis, Interleukin-1, Interleukin-1A, Interleukin-1B, Association study, Major histocompatibility complex, Radiographic severity, Genetics, Polymorphism.

Introduction

Cytokines are small regulatory proteins that act as intercellular mediators in the generation and control of immune and inflammatory responses. In RA, two main cytokines, TNF and IL-1, are particularly involved. The most studied of the IL-1 proteins are IL-1α and IL-1β. They function as agonist molecules that activate target cells by binding to IL-1 receptor. The production and activity of IL-1α and IL-1β are regulated by transcriptional and post-translational mechanisms as well as from a natural competitive antagonist. The IL-1 gene cluster (comprising 10 related genes) maps to 2q12. Both IL-1A (MIM 147760) and IL-1B (MIM 147720) may have arisen through gene duplications, and hence encode similar protein products [1]. Strong linkage disequilibrium (LD) has been demonstrated across this gene cluster in Caucasian populations [2].

A genetic linkage study in European RA families provided suggestive evidence of linkage of RA to the IL-1 region [3]. The reported associations of certain IL-1 gene cluster polymorphisms with susceptibility and severity, especially in the more erosive form of RA, have been variable [4–8]. These studies have generally been conducted on relatively small RA patient samples, apart from the one recent exception [8]. In the latter, the authors performed a comprehensive analysis of IL-1A and IL-1B of a large number of North American and Spanish RA populations but identified no major contribution to RA from these genes.

We examined four common single nucleotide polymorphisms (SNPs) and haplotypes in the IL-1A and IL-1B promoter regions and their association with susceptibility and severity with RA in well-characterized British Caucasian patients. We performed meta-analysis of published data on IL-1B-511 to evaluate the effect of this polymorphism on susceptibility to RA among Caucasians.

Patients and methods

Patients and study design

A total of 756 British Caucasian RA patients (542 females, 72%) of Northern European descent attending the Nuffield Orthopaedic Centre in Oxford, UK, were recruited. All satisfied the 1987 ACR criteria for RA and mean age of disease onset was 48 yrs. The controls (\( n = 625 \)) were healthy British Caucasian blood donors from the same geographical area. All subjects gave informed written consent and approval was granted by the Oxford Research Ethics Committee.

Medical records were examined for the RF status of each patient. Patients who were positive at two different time-points were considered seropositive. Where data were not available, RF was measured using nephelometric technique and a titre over 40 IU/ml was considered positive. Overall, 586 patients (78%) deemed to be RF positive.

Severity of RA was determined by the presence of rheumatoid nodules or the requirement of major joint replacement as established from medical records. The association with radiographic outcome was examined in 206 RA patients with minimum disease duration of 3 yrs. Hand radiographs were scored using Rau–Ratingen method [9].

Genotyping

Genomic DNA was extracted from whole blood. Cases and healthy controls (HCs) were genotyped for IL-1A (–889 C/A, rs17561), IL-1B (–511 A/G, rs18944), IL-1B (–1464 C/G, rs1143623) and IL-1B (–3737 G/A, rs4848306) SNPs by PCR-based methods (details available on request). Limited HLA-DRB1 and further DRB1*04 subtyping was performed as published [10]. All samples were genotyped in duplicates, and positive and
negative controls were included. The HLA-DRB1 shared epitope (SE) was defined by the presence of one or two copies of the HLA DRB1*01,0401, *0404, *0405, *0408 and *10 alleles.

Statistical analysis

Case–control association analysis. Statistical analysis for association and Hardy–Weinberg equilibrium were evaluated using \( \chi^2 \)-test. \( P < 0.05 \) was considered statistically significant. Corrections for multiple testing were applied as necessary [11]. For continuous variables, SPSS version 15.0 for Windows (SPSS Inc., IL, USA) was used. Haplotypes estimations and frequencies among HC are 0.67). In the meta-analysis, we compared the variant ‘G’ allele with ancestral A as well as recessive (G/G vs G/A + AA) and dominant (G/G + G/A vs AA) models for the G allele. Genotype-specific risks such as G/G vs A/A and G/A vs AA were also analysed. Statistical analyses were performed using StatsDirect software. Single odd ratios (OR) and 95% CIs for the variant allele in RA patients against HC were calculated from fixed-effects model, as no significant heterogeneity was observed. Forest plots were generated in StatsDirect to display the OR and 95% CI.

Genealogical power calculations for the meta-analysis were done using software program Quanto v1.2.3 (available at http://hydra.usc.edu/gxe) assuming a disease prevalence of 1% and ‘G’ allele frequency of 0.67. Under log-additive model, we calculated the effect of 0.05 with OR of 1.2 (average OR of all combined studies).

Results

Case–control association study

The genotyping results and allele counts for RA patients and HCs are presented in Table 1. IL-1B (−1464 C/G) ‘G’ allele was less common among RA patients (\( P = 0.01 \) OR 1.24; 95% CI 1.04, 1.48). The minor allele frequencies for cases and controls were 24 and 29%, respectively. The association remained significant after applying corrections for false discovery rate.

Haplotype analysis revealed a global difference in haplotype frequencies between the RA and HC (\( P = 0.01 \)). This effect was entirely due to the IL-1B (−1464 C/G) and there was no single over-represented haplotype.

Each of the polymorphisms was investigated in the RA cohort according to RF, SE status and presence of high-risk HLA-DRB1*04 (DRB1*0401, 0404, 0405 or 0408) or a low-risk allele (DRB1*01, 0406, 0407 and *10). No significant difference was observed between the groups (data not shown).

Analysis according to the severity features of RA did not reveal any independent association with the presence of nodules.

Meta-analysis

Search strategy and study selection. Meta-analysis was conducted for IL-1B (−511 A/G) SNPs based on the PubMed database using the terms ‘rheumatoid arthritis’ and ‘interleukin-1’.

The meta-analysis included 3712 RA patients and 2331 controls.

Table 1. Distribution of IL-1A (-889 C/A), IL-1B (-511 A/G), IL-1B (-1464 C/G) and IL-1B (-3737 G/A) alleles and genotypes among RA patients and HC

<table>
<thead>
<tr>
<th>Allele counts (%)</th>
<th>Genotype counts (%)</th>
<th>Genotype counts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2 P-value, OR 95% CI</td>
<td>11 12 22 P-value, OR 95% CI</td>
<td>11 12 + 22 P-value, OR 95% CI</td>
</tr>
<tr>
<td><strong>IL-1A -889</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RA 1031 (70) 447 (30)</td>
<td>535 (48) 321 (43) 63 (9) ( P = 0.91, OR = 1.01 )</td>
<td>355 (48) 384 (52) ( P = 0.80, OR = 1.03 )</td>
</tr>
<tr>
<td>HC 841 (70) 367 (30)</td>
<td>286 (47) 269 (45) 49 (8) ( P = 0.01, OR = 1.19 )</td>
<td>286 (47) 318 (53) ( P = 0.94, 1.28 )</td>
</tr>
<tr>
<td><strong>IL-1B -511</strong></td>
<td></td>
<td></td>
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<tr>
<td>RA 1022 (69) 460 (31)</td>
<td>347 (42) 328 (44) 68 (9) ( P = 0.20, OR = 1.10 )</td>
<td>357 (42) 394 (53) ( P = 0.39, OR = 1.10 )</td>
</tr>
<tr>
<td>HC 796 (66) 404 (34)</td>
<td>267 (45) 262 (44) 71 (11) ( P = 0.01, OR = 1.24 )</td>
<td>267 (45) 333 (55) ( P = 0.88, 1.37 )</td>
</tr>
<tr>
<td><strong>IL-1B -1464</strong></td>
<td></td>
<td></td>
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<tr>
<td>RA 1116 (76) 382 (24)</td>
<td>421 (57) 274 (37) 44 (6) ( P = 0.05 )</td>
<td>421 (57) 318 (43) ( P = 0.03, OR = 1.28 )</td>
</tr>
<tr>
<td>HC 874 (71) 352 (29)</td>
<td>312 (51) 250 (41) 51 (8) ( P = 0.01, OR = 1.24 )</td>
<td>312 (51) 301 (49) ( P = 1.02, 1.59 )</td>
</tr>
<tr>
<td><strong>IL-1B -3737</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RA 778 (54) 676 (46)</td>
<td>211 (29) 356 (49) 160 (22) ( P = 0.26 )</td>
<td>211 (29) 516 (71) ( P = 0.10, OR = 0.82 )</td>
</tr>
<tr>
<td>HC 669 (56) 523 (44)</td>
<td>198 (33) 273 (46) 125 (21) ( P = 0.01, OR = 1.05 )</td>
<td>198 (33) 398 (67) ( P = 0.65, 1.05 )</td>
</tr>
</tbody>
</table>

*Alleles were named according to their frequency in the general population i.e. allele 1 being the frequent or more common allele, allele 2 the rare one.
families with 0 probability of sharing two cluster markers associated with erosive RA, particularly in sib pair analysis presented here is the largest to have investigated the Arthritis Sequential Study cohort. The association of the GG genotype and ‘G’ allele with RA. The affected sib pair RA families, Cox relationship between different analyses, we were unable to demonstrate any significant IL-1B-511 identical by descent. Evidence of less severe disease progression IL-1β-1464G has shown weaker promoter activity IL-1β (−1464 G/C) SNP is the ‘causal’ variant. This polymorphism should be replicated in an independent cohort in order to confirm the result. In our data set, IL-1B (−1464 C/G) is in complete LD with IL-1B (−511 A/G) (D’ > 0.90, r² > 0.80). However, the minor allele frequencies in our cohort for IL-1B (−511 A/G) and IL-1B (−1464 C/G) are dissimilar (0.31 and 0.24 for RA and 0.34 and 0.29 for controls, respectively), indicating that independent genotyping is warranted in order to investigate the IL-1B (−1464 C/G) association. It remains unknown whether the IL-1B (−1464 C/G) SNP is the ‘causal’ variant. This polymorphism has been shown to exhibit allele-specific difference in gene expression as IL-1B-1464G has shown weaker promoter activity than IL-1B-1464C on electromobility gel shift assay [18]. This promoter region harbours a core-binding motif for GATA-binding family proteins and IL-1B (−1464 C/G) could affect protein binding [18]. It is possible that the real ‘protective’ variant is elsewhere and exhibits different LD with IL-1B (−511 A/G) and IL-1B (−1464 C/G). Further studies investigating genetic variations across whole gene regulatory regions and more detailed functional analysis are essential. Although both IL-1α and IL-1β mRNA have been detected in RA synovial fluid, it is IL-1α that has been found to be strongly expressed at the cartilage-pannus junction [19]. In a study of affected sib pair RA families, Cox et al. [4] found four IL-1 gene cluster markers associated with erosive RA, particularly in sib pair families with 0 probability of sharing two HLA-DRB1 alleles identical by descent. Evidence of less severe disease progression after 20 yrs in Swiss RA patients carrying one or two copies of the IL-1B-511 minor allele has also been reported [7]. Using several different analyses, we were unable to demonstrate any significant relationship between IL-1 polymorphisms and radiographic progression. Similar conclusions were reached in the recent study of Johnsen et al. [8] where no association was observed with erosive disease in a large sample of the Brigham Rheumatoid Arthritis Sequential Study cohort. Meta-analysis allows us to maximize the sample size, increase statistical power and provide more robust findings. The meta-analysis presented here is the largest to have investigated the effects of IL-1B (−511) in RA. We demonstrated a significant association of the GG genotype and ‘G’ allele with RA. The power of this meta-analysis was over 90% to detect modest effect (OR 1.2 at a significance level of 0.05). However, the magnitude of the genetic risk associated with this polymorphism is relatively small and further studies on a much larger RA sample are desirable to confirm the true association.

In summary, the role of the genetic factors in RA is complex and the magnitude of the effects is likely to vary greatly. We have demonstrated an association of IL-1B (−511) with RA patients of Caucasian origin by conducting a meta-analysis. The potential protective influence of IL-1B (−1464) found in this study will require further confirmation in other populations.

**Rheumatology key messages**

- There may be a protective effect in RA from the IL-1B (−1464 C/G) polymorphism.
- Meta-analysis of IL-1B (−511 A/G) revealed an association with increased susceptibility to RA.

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**References**