Polymorphisms in the interleukin-4 and IL-4 receptor genes modify risk for chronic inflammatory arthropathies in women.


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Polymorphisms in the interleukin-4 and IL-4 receptor genes modify risk for chronic inflammatory arthropathies in women

V. Suppia, M. Rooneyb, K. Vandenbroecka,⁎

a Applied Genomics Research Group, McClay Research Centre, The Queen's University of Belfast, Belfast BT9 7BL, Northern Ireland, UK
b Department of Rheumatology, Musgrave Park Hospital, Belfast, Northern Ireland, UK

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Abstract

Rheumatoid and juvenile idiopathic arthritis (RA, JIA) are chronic inflammatory arthropathies with polygenic autoimmune background. We analysed the IL-4 +33 C/T and IL-4R Q551R single nucleotide polymorphisms (SNPs) in 294 RA, 72 JIA and 165 controls from Northern Ireland. Analysis of the individual phenotypes (RA or JIA) showed that both the IL-4 +33 TT (P = 0.02; OR: 0.25, 95% CI: 0.07–0.87) and the IL-4R Q551R CC genotypes (P = 0.001; OR: 0.19, 95% CI: 0.06–0.56) were exclusively decreased in female RA patients compared to female controls. Similar non-significant trends were observed in female JIA patients (OR: 0.25, 95% CI: 0.03–2.11 and OR: 0.31, 95% CI: 0.07–1.47, respectively). Analysis of the common phenotype (inflammatory arthropathy; i.e. JIA and RA combined) corroborated the unique association of these polymorphisms with female inflammatory arthropathy (P = 0.013 and 0.002, respectively). This is the first demonstration of sex-specific association of the two foremost genes of the IL-4 signalling cascade with chronic inflammatory arthropathies.

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Introduction

Rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA) belong to the same category of diseases known as chronic inflammatory arthropathies. Both of these disorders are believed to display an autoimmune component, with disease manifestations being caused by a chronic destructive, inflammatory response that not only damages the synovial membrane but can also attack extra-articular tissues (Weyand et al., 1998). RA and JIA are distinct disorders even though they share many pathophysiological similarities (Grom et al., 1994). The prevalence of RA in Ireland has been estimated to be 5/1000 (Power et al., 1999).

RA is a collection of adult chronic symmetrical joint inflammation such as Felty’s syndrome, seronegative RA, ‘classical’ RA, progressive and/or relapsing RA and RA with vasculitis, whereas JIA is a collection of childhood inflammatory arthropathies where patients with 4 or less joints affected are classified as having pauciarticular disease while patients with more than 5 affected joints are classified as having polyarticular disease (Arnett et al., 1998).

Like other autoimmune diseases, RA and JIA are influenced by genetic as well as yet-to-be identified environmental factors. The sibling risk ratio for a full sibling of a patient is estimated to be 15 for JIA (Glass and Giannini, 1999) and 8 for RA (Risch, 1987). The best defined genetic link with RA and JIA in many populations involves allelic polymorphisms in the HLA region (Osorio et al., 2004; Eyre et al., 2004; Thompson et al., 2004; Jawaheer et al., 2001). However, association studies have suggested that the HLA region only accounts for a fraction of the genetic component of these diseases.

Naïve T cells can differentiate to become either T helper 1 (Th1) or T helper 2 (Th2) effector cell subtypes. Th1 and Th2 cytokines play an important role in the pathogenesis of many autoimmune diseases (Feldmann et al., 1996). Specific patterns of Th1 or Th2 cytokines have been reported to profoundly influence disease course in RA (van Roon et al., 1997; Miyata et al., 2000) and JIA (Murray et al., 1998). The disease-
limiting role of interleukin-4 (IL-4), in particular, has been documented both for RA and JIA (van Roon et al., 1997; Miyata et al., 2000; Murray et al., 1998). IL-4 is a pleiotropic cytokine that regulates immunoglobulin class switching to IgE and the development of Th2 CD4+ T cells. IL-4 has the ability to inhibit the expression of T helper 1 (Th1) subset such that naive T cells are driven to differentiate towards a Th2 effector profile. T cells that have the ability to produce IL-4 are automatically competent to produce other Th2-specific cytokines such as IL-5, IL-9, IL-10 and IL-13 (O’Gara, 1998; Paul and Seder, 1994). Even though Th2 cytokines may have overlapping biological activities (such as IL-4 and IL-13), these cytokines play unique roles in autoimmune diseases as seen in late asthmatic response (Tomkinson et al., 2001). The human IL-4 gene has been mapped to the q arm (5q23–31) of chromosome 5 and is located within a cluster of other cytokine genes (van Leeuwen et al., 1989; Arai et al., 1989). Both in vitro and in vivo studies suggest that the T allele of the +33 SNP (rs2070874; linked to −590 T allele) is associated with increased IL-4 production (Rosenwasser and Borish, 1997; Kawashima et al., 1998; Noguchi et al., 1998). The IL-4R gene is located at 16p11–12. Substitution of amino acid residues, particularly by the non-synonymous SNP Gln551Arg (rs1801275), has been reported to be functionally important and to impact upon IL-4R signalling (Mirel et al., 2004). This SNP has been found to be significantly associated with various immune-related diseases such as asthma and atopy (Hytönen et al., 2004; Sandford et al., 2000) and connective tissue disease (Youn et al., 2000). IL-4 association studies in JIA patients have yielded mixed results (Cinek et al., 2004; Omoloja et al., 2002; Donn et al., 2001). In a functional study, IL-4 was found to be essential for the suppression of collagen-induced arthritis in mice which is a model for inflammatory arthritis in humans (Myers et al., 2002). In the same study, the authors found that IL-4−/− mice developed severe arthritis despite treatment with intravenous type II collagen. With functional data for both SNPs available, analysis of association may reveal whether genetically programmed IL-4 levels ultimately decrease or increase one’s risk for arthropathy. Both individual (RA and JIA) and common phenotypes (chronic inflammatory arthropathy) were analysed.

Subjects and methods

Subjects

A total of 366 patients (294 with clinically defined RA and 72 with clinically defined JIA (51% with pauciarticular course and 49% with polyarticular course) and 165 healthy controls (79 females and 86 males) were used in this study. All patients were recruited from the Musgrave Park Hospital and Belfast City Hospital in Northern Ireland, and controls were from Northern Irish descent. All patients were assessed by a rheumatologist, and all fulfilled the American College of Rheumatology criteria for RA (Arnett et al., 1998; Petty et al., 1998; Wood, 1978). There were a total of 212 female patients (170 RA and 42 JIA) and 154 male patients (124 RA and 30 JIA) in this study. All the patients were included in the study after giving written informed consent. In the case of the JIA patients, written informed consent was obtained from their legal next-of-kin. This study was approved by the Ethics Committee of Queen’s University Belfast.

Genotyping and statistical analysis

Genotypes of the +33 C/T SNP of the IL-4 gene and the Q551R SNP of the IL-4Rα chain receptor gene were determined as described elsewhere (Suppiah et al., 2005). Briefly, genotypes of the +33 C/T SNP of the IL-4 gene and the Q551R SNP of the IL-4Rα chain receptor gene were determined by Taqman™ assays (ABI, Foster City, USA). The +33 C/T SNP was determined by an Assay-on-Demand (Cat no: C-16176215-10), while the IL-4R α SNP was determined by an Assay-by-Design. The conditions of the PCR and subsequent detection were according to the manufacturer’s instructions. The Taqman™ assays were carried out in the DNA Engine Opticon® 2 system obtained from MJ Research® Inc, Alameda, USA. Chi-squared analysis of genotype, allele and carrier counts was performed for RA and JIA patients and healthy controls, as well as for comparison of RA and JIA patients and total arthritic patients. Data were further stratified in terms of gender for comparison. IL-4−/− IL-4Rα genotype–gene–gene interaction analysis was approached by logistic regression analysis. The SPSS statistical package (SPSS, Chicago, IL, USA) was used for analysis of classical chi-squared and logistic regression analysis. Uncorrected probability values less than or equal to 0.05 were taken to be statistically significant.

Results

+33 C/T SNP in the IL-4 gene

The +33C/T polymorphism in IL-4 was genotyped in the RA and JIA patients and control samples (Table 1). Homozygosity for the +33 C/T*T allele was higher in the controls as compared to the two groups of patients. This difference reached statistical significance in the RA patients (P = 0.011, OR = 0.24, 95% CI = 0.07–0.79; 1.4% TT in the RA patients compared to 5.5% TT in the controls) and total arthritic patients (P = 0.007, OR = 0.24, 95% CI = 0.08–0.73; 1.4% TT in total arthritic patients compared to 5.5% TT in the controls), but was not significant in the JIA patients (P = 0.152, OR = 0.24, 95% CI = 0.03–1.96; 1.4% in the JIA patients compared to 5.5% TT in the controls), though a similar trend was present.

Table 1 shows the distribution of the allele frequency, carriage count and homozygosity of the two groups of patients and control after being stratified for gender. Only female patients and female controls are shown. +33 TT homozygotes were underrepresented in the female RA patients (P = 0.020, OR = 0.25, 95% CI = 0.07–0.87; 2.4% TT in the RA patients compared to 8.9% TT in the controls) and total arthritic patients (P = 0.013, OR = 0.25, 95% CI = 0.08–0.81; 2.4% TT in total arthritic patients compared to 8.9% TT in the controls), but was not significant in the JIA patients (P = 0.172, OR = 0.25, 95% CI = 0.03–2.11; 2.4% TT vs. in the JIA patients compared to 8.9% TT in the controls). The allele, phenotype and genotype frequencies of this polymorphism did not differ significantly between male RA patients and male JIA patients and controls in this population (not shown).

Q551R SNP in the IL-4R gene

The Q551R polymorphism in the IL-4R gene was genotyped in the RA and JIA patients and control samples (Table 1). The allele and phenotype frequencies of this polymorphism did not differ significantly between RA patients and controls. However, homozygosity for the Q551R*C allele was higher in the controls as compared to JIA patients (P = 0.05 for the JIA patients,
OR = 0.25, 95% CI = 0.06–1.11; 2.8% CC in the JIA patients compared to 10.3% CC in the controls) and to total arthritic patients (P = 0.03 for total patients, OR = 0.48, 95% CI = 0.24–0.94; 5.2% CC in total patients compared to 10.3% CC in the controls). A similar non-significant trend was seen in the RA patients (P = 0.076, OR = 0.53, 95% CI = 0.27–1.1; 5.8% CC in the RA patients compared to 10.3% in the controls).

As with the +33 IL-4 SNP, the difference in genotype distribution between patients and controls was attributable to the female patient–control stratum. Female RA patients when compared to female controls (Table 2) showed a significant decrease in homozygosity for the Q551R+C allele as compared to the female controls (P = 0.001, OR = 0.19 95% CI = 0.06–0.56; 2.9% CC in female RA patients compared to 13.9% CC in the female controls), and this was reproduced when female RA and JIA patients were pooled to compare with the female controls (P = 0.002, OR = 0.21 95% CI = 0.08–0.57; 3.3% CC in female arthritic patients compared to 13.9% CC in the female controls). C allele frequencies were decreased in female RA, JIA and combined arthritic patients, compared to controls (P = 0.002, 0.06 and 0.002, respectively). The other groups of patients did not show any significant difference when compared to their respective control groups in allele, phenotype and genotype frequencies of this polymorphism. There was no evidence for IL-4–IL-4R gene–gene interaction in determining susceptibility or disease parameters in the RA or JIA patients in the present study (not shown).

**Discussion**

This study has investigated for the first time the role of two polymorphisms, one in the IL-4 gene known to be linked to high/low production of this Th2 cytokine as well as one in the IL-4R gene also known to affect the receptor activity, in two cohorts of RA and JIA patients. The results of the present study suggest that both SNPs may play a role in a gender-specific manner in RA where female patients differ significantly from their healthy counterparts. Previous studies done on JIA patients examining the genetics of IL-4 seem to show mixed results. One study hinted towards an association with early polyarticular JIA patients (Omoloja et al., 2002) and another with the 1098 T/G polymorphism (Cinek et al., 2004); however, others have found no association at all (Donn et al., 1999). In a study done on RA patients, the authors found a weak association of two polymorphisms in the IL-4 gene, the IL-4 −590C/T allele and IL-4 intron 3 VNTR repeat 1 {which has been shown to be in tight linkage disequilibrium with +33 C/T SNP in an earlier study (Suppiah et al., 2005)} with susceptibility to the disease (Maksymowych et al., 2002). Existing data from in vitro and in vivo studies (Rosenwasser and Borish, 1997; Kawashima et al., 1998; Cantagrel et al., 1999) suggest that the −590 C/T*T allele which is in tight linkage disequilibrium with the +33 C/T*T allele is associated with increased IL-4 expression. In this present study, there is a significant underrepresentation of the +33 C/T*T homozygotes in the female patients.
progesterone promotes the development of Th2 cells which while high levels inhibit specific immune activities whereas pregnancy is associated with a marked increase of estrogen, progesterone and cortisol in the plasma and brings about a surge in Th2 cytokines, in particular, IL-4 while causing a surge in Th2 cytokines, in particular, IL-4.

To our knowledge, no studies have been done to date on the genetics of the IL-4R gene in RA or JIA patients. Data from a functional study (Shirakawa et al., 2000; Hershey et al., 1997) showed that the R551 variant is associated with increased IL-4 receptor signalling and increased total IgE levels. These results indicate that the R551 polymorphism is associated with differentiation of the immune profile into a Th2 type response. The present study supports the hypothesis that changes in IL-4 signalling due to functional polymorphisms in the IL-4 and IL-4 receptor gene may be associated as risk factors in a gender-specific manner with RA. Sexual dimorphism in the immune response and the intimate interaction of sex hormones and their influence on gene regulation promoting differences in men and women clearly warrants further study. An increasing number of reports have pointed to sex-specific association of common gene polymorphisms with autoimmunity. For instance, CD95 was found to be associated with susceptibility to MS in women (Kantarci et al., 2004a), APOE with disease severity of MS in women (Kantarci et al., 2004b), IFNG with susceptibility to MS in men (Goris et al., 2002; Kantarci et al., 2005) and IL26 with susceptibility to RA in women (Vandenbroeck et al., 2003). These data call for a systematic whole-genome scrutiny for gender-specific autoimmune susceptibility factors.

Table 2
Allele frequency, carriage rate and homozygosity of the IL-4 +33 C/T SNP and IL-4R Q551R SNP in female RA and JIA patients and female controls of the Northern Irish population

<table>
<thead>
<tr>
<th>SNP</th>
<th>Female controls (N = 79)</th>
<th>RA counts (N = 170)</th>
<th>P value, odds ratio (95% CI)</th>
<th>JIA counts (N = 42)</th>
<th>P value, odds ratio (95% CI)</th>
<th>Total patients (N = 212)</th>
<th>P value, odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-4 +33 C/T</strong> Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>127 (80.4)a</td>
<td>283 (83.2)</td>
<td>0.437, 0.83 (0.51–1.34)</td>
<td>70 (83.3)</td>
<td>0.574, 0.82 (0.41–1.64)</td>
<td>353 (83.3)</td>
<td>0.417, 0.82 (0.52–1.32)</td>
</tr>
<tr>
<td>T</td>
<td>31 (19.6)</td>
<td>57 (16.8)</td>
<td></td>
<td>14 (16.7)</td>
<td>0.879, 0.96 (0.55–1.67)</td>
<td>71 (16.7)</td>
<td>0.872, 0.96 (0.56–1.64)</td>
</tr>
<tr>
<td><strong>Phenotype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>72 (91.1)</td>
<td>166 (97.6)</td>
<td>0.879, 0.96 (0.55–1.67)</td>
<td>41 (97.6)</td>
<td>0.9, 0.95 (0.44–2.07)</td>
<td>207 (97.6)</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>24 (30.4)</td>
<td>53 (31.2)</td>
<td></td>
<td>13 (31.0)</td>
<td>0.315c</td>
<td>61 (28.8)</td>
<td>0.030c</td>
</tr>
<tr>
<td><strong>Genotype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>55 (69.6)</td>
<td>117 (68.8)</td>
<td>0.020b, 0.25 (0.07–0.87)</td>
<td>29 (69.0)</td>
<td>0.172b, 0.25 (0.03–2.11)</td>
<td>146 (68.9)</td>
<td>0.013b, 0.25 (0.08–0.81)</td>
</tr>
<tr>
<td>CT</td>
<td>17 (21.5)</td>
<td>49 (28.8)</td>
<td>0.044c</td>
<td>12 (28.6)</td>
<td>0.315c</td>
<td>61 (28.8)</td>
<td>0.030c</td>
</tr>
<tr>
<td>TT</td>
<td>7 (8.9)</td>
<td>4 (2.4)</td>
<td></td>
<td>1 (2.4)</td>
<td></td>
<td>5 (2.4)</td>
<td></td>
</tr>
</tbody>
</table>

| **IL-4R Q551R** Allele | | | | | | | |
| C            | 52 (32.9)a              | 69 (20.3)           | 0.002, 0.52 (0.34–0.79)     | 18 (21.4)           | 0.061, 0.56 (0.30–1.03)     | 87 (20.5)                | 0.002, 0.53 (0.35–0.79)     |
| T            | 106 (67.1)              | 271 (79.7)          |                            | 66 (78.6)           | 0.247, 0.66 (0.33–1.33)     | 337 (79.5)               | 0.066, 0.65 (0.41–1.03)     |
| **Phenotype** | | | | | | | |
| C            | 41 (51.9)                | 64 (37.6)           | 0.073, 0.64 (0.40–1.04)     | 16 (38.1)           | 0.121d, 0.31 (0.07–1.47)    | 80 (37.7)                | 0.002d, 0.21 (0.08–0.57)    |
| T            | 68 (86.1)                | 165 (97.1)          |                            | 95 (92.5)           | 0.13a, 0.44 (0.22–1.58)     | 205 (96.7)               | 0.013a, 0.26 (0.08–0.78)    |
| **Genotype** | | | | | | | |
| CC           | 11 (13.9)                | 5 (2.9)             | 0.001b, 0.19 (0.06–0.56)    | 2 (4.8)             | 0.193c                      | 7 (3.3)                  | 0.001c                      |
| CT           | 30 (38.0)                | 59 (34.7)           | 0.002c                      | 14 (33.3)           |                           | 73 (34.4)                |                            |
| TT           | 38 (48.1)                | 106 (62.4)          |                            | 26 (61.9)           |                           | 132 (62.3)               |                            |

a Percentages in parenthesis.

b P value for comparison of TT homozygotes versus CC/CT genotypes.

c P value for comparison of the 3 genotypes.

d P value for comparison of CC homozygotes versus CT/TT genotypes.
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References


