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# Juvenile rheumatoid arthritis and asthma, but not childhood-onset systemic lupus erythematosus are associated with *FCRL3* polymorphisms in Mexicans☆

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ABSTRACT

A regulatory single nucleotide polymorphism located in the 5′ region (−169T/C) of the *Fc receptor-like 3* (*FCRL3_3*) gene has been associated with both susceptibility and protection in immune diseases. This case–control study aimed to evaluate the association between *FCRL3* polymorphisms and juvenile rheumatoid arthritis (JRA), asthma, and childhood-onset systemic lupus erythematosus (SLE) in a Mexican population. We performed PCR-based genotyping to identify four *FCRL3* single nucleotide polymorphisms (*FCRL3_3 to FCRL3_6)* in patients with JRA ($n = 202$), asthma ($n = 239$), or childhood-onset SLE ($n = 377$), and healthy controls ($n = 400$). The case–control analysis showed a male-gender dependent association between the *FCRL3_3C*, *FCRL3_5C*, and *FCRL3_6A* alleles and either JRA (OR = 0.57, $p = 0.003$; OR = 0.55, $p = 0.002$; OR = 0.53, $p = 0.0007$, respectively) or asthma (OR = 0.72, $p = 0.04$; OR = 0.74, $p = 0.05$; OR = 0.70, $p = 0.02$, respectively). As expected, minor alleles of these SNPs with the CGCA haplotype were also significantly associated with JRA (OR = 0.35, $p = 0.00005$) and asthma (OR = 0.61, $p = 0.007$). We found no association between *FCRL3* SNPs or haplotypes and childhood-onset SLE. These results supported the notion that *FCRL3* is involved in the etiology of several immune diseases. Our results also suggested that SNPs located in the *FCRL3* gene were protective against JRA and asthma in male Mexican patients.


## 1. Introduction

Immune disorders involve complex etiologies, but many have a strong genetic component in their pathogenesis (Becker et al., 1998; Phelan et al., 2006; Moser et al., 2009; Lee et al., 2011). Genome-wide linkage studies have identified a group of genetic factors that are shared among many autoimmune and other immune-mediated diseases (Becker et al., 1998; Lee et al., 2011). One of the regions implicated in susceptibility to multiple autoimmune diseases is the cytoband 1q21–23. This region harbors the Fcγ receptor (*FcγR*) II/III and Fc receptor-like family genes (*FCRL1* through *FCRL5*) (Davis et al., 2001). The ligands and function of the FCRLs remain unknown; however, some studies have suggested that FCRL proteins may contribute to the pathogenesis of chronic immune diseases, because they affect B-Cell receptor-mediated signaling, growth, and proliferation (Ehrhardt et al., 2003, 2007; Leu et al., 2005; Kochi et al., 2009). Several association studies have supported the hypothesis that *FCRLs* play an important role in immune-mediated pathologies, either as risk or protection factors (Kochi et al., 2005; WTCCC, 2007; Owen et al., 2007; Martínez, 2007; Matesanz et al., 2008; Chen et al., 2011). Furthermore, meta-analysis studies have shown that the *FCRL3_3C* allele was a significant risk factor for rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) in Asians, but not in Caucasians (Lee et al., 2010; Mao et al., 2010). On the other hand, *FCRL3* SNPs have been reported to have a protective effect against autoimmune thyroid disease (AITD), Addisonĩs disease (AAD) and multiple sclerosis (MS) (WTCCC, 2007; Owen et al., 2007; Martínez, 2007; Matesanz et al., 2008). These findings have suggested that *FCRLs* have differential effects on different populations.

We investigated whether *FCRL3* SNPs were associated with juvenile rheumatoid arthritis (JRA), asthma, and childhood-onset SLE in Mexican pediatric patients.

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## 2. Patients and methods

### 2.1. Study subjects for genetic analysis

This study included 818 patients with JRA ($n = 202$), asthma ($n = 239$), or SLE ($n = 377$). All cases were under 16 years of age and were recruited from five tertiary level Institutions in Mexico City. The diagnosis of asthma was based on criteria published by the American Thoracic Society and the Global Initiative for Asthma. The diagnosis of JRA and SLE were based on criteria published by the American College of Rheumatology. We also included 400 unrelated, healthy control subjects, ethnically matched to the patients, with no history of autoimmune or inflammatory diseases. No previous studies have shown a correlation between the *FCRL3* alleles and age; therefore, the controls were older than 18 years of age. Additionally, allelic frequencies of *FCRL3* polymorphisms were not different between genders; therefore, we used the same control group for all analyses. Patients and controls were descended from parents and grandparents born in Mexico. Ethics and Research Committee approvals were obtained from all participating Institutions, and informed consent was obtained from each individual. Parents provided consent for child participation, and all children assented.

### 2.2. Analysis of genetic polymorphisms

We obtained genomic DNA from peripheral blood leukocytes with a standard salt precipitation technique (QIAgen Systems, Inc., Valencia, CA, USA). Ethnic matching between cases and controls was previously evaluated with a panel of 10 ancestry informative markers (AIMs: rs4884, rs2695, rs17203, rs2862, rs3340, rs722098, rs203096, rs223830, rs1800498, and rs281478) (Jiménez-Morales et al., 2009). These AIMs mainly identify Amerindian and European ancestry in Mexican population. We performed genotyping to reveal *FCRL3_3* (−169T/C or rs7528684), *FCRL3_4* (−110G/A or rs11264799), *FCRL3_5* (+358G/C or rs945635), and *FCRL3_6* (+1381G/A or rs3761959) polymorphisms. The PCR assay used to identify these four SNPs was performed with TaqMan MGB chemistry (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Each PCR reaction contained 10 ng of DNA, 2.5 μl of TaqMan Master Mix, 0.065 μl of 40× assay mixture, and 2.435 μl of distilled, DNase free water in a final volume of 5 μl. The amplification protocol included denaturing at 95 °C for 10 min, followed by 40 cycles of denaturing at 95 °C for 15 s and annealing and extension at 60 °C for 1 min. Genotypes were assigned by detecting allele-specific fluorescence with SDS 2.2.3 software for allelic discrimination (Applied Biosystems, Foster City, CA). The overall genotype call rate was 99.9%, and 30% of randomized samples showed 100% reproducibility in duplicate assays for the four polymorphisms. Genotyping accuracy was confirmed by direct sequencing of PCR products in 50 randomly chosen samples. The PCR products were sequenced directly with a DNA Sequencing Kit and the Big Dye Terminator on an automated ABI PRISM 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA). The primer sequences used to recognize the *FCRL3* were 5′-GAAGACACGAAAGCAATCAAGGAA-3′ and 5′-CATATGGGAAACCCCTTCACTACC-3′. PCR was performed in a 50 μl reaction mixture that contained 60 ng of genomic DNA, 2.5 mM MgCl2, 1.25 units of Amplitaq Gold (Applied Biosystems), 2.5 mM of each primer, 250 mM dNTPs mixed (Takara), and 1X PCR buffer (Applied Biosystems). The amplification protocol included denaturing at 95 °C for 10 min, followed by 35 cycles of denaturing at 94 °C for 30 s, annealing at 58 °C for 30 s, and extending at 72 °C for 35 s; with a final extension at 72 °C for 5 min.

## 3. Statistical analysis

The FINETTI program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) was used to test for Hardy–Weinberg equilibrium (HWE) in genotype distributions for the cases and controls. Association between *FCRL3* SNPs and the three diseases were analyzed with $X^2$ tests on $2 \times 2$ and $2 \times 3$ contingency tables for allele and genotype frequencies, respectively. Statistical analysis was performed with a standard statistical package (Epic Info 2005 V.3.2; Centers of Disease Control and Prevention, Atlanta, GA). Odds ratios (ORs) with 95% confidence intervals (95% CI) were calculated with the same software. Haplotype frequencies and linkage disequilibrium (LD; $r^2$) were calculated with the Haploview software (Barrett et al., 2005). The $p$-value was corrected by applying a permutation test based on 100,000 permutations. The ADMIXMAP program (McKeigue et al., 2000; Hoggart et al., 2003) was used to test the possible effect of population stratification, as described previously (Bonilla et al., 2004; Choudhry et al., 2006), in the two primary parental populations of the Mexican–Mestizo (Amerindian and European).

## 4. Results

The mean (±SD) age at onset of JRA, asthma, and SLE were $8.7 \pm 2.46$, $8.4 \pm 2.8$, and $11.62 \pm 2.46$ years, respectively. The female/male gender distribution was 115/87 (57/43%) for JRA, 92/147 (38/62%) for asthma, and 312/65 (83/17%) for SLE. The control population comprised 200 (50%) females and 200 (50%) males. We used the same control group for all analyses, because no differences between genders were found in the allele frequencies of *FCRL3_3*, *FCRL3_4*, *FCRL3_5*, and *FCRL3_6* SNPs. Genotype distributions of polymorphisms were in HWE for both cases and controls. The AIM distributions were not significantly different between cases and controls.

When the JRA, asthma, and childhood-onset SLE cohorts were compared to controls, only the JRA population exhibited significant differences, and only in the *FCRL3_3* and *FCRL3_6* minor allele frequencies (MAF) ($p = 0.03$ and $p = 0.01$, respectively). After gender stratification, the *FCRL3_5C* allele exhibited a male gender-dependent association with JRA. The *FCRL3_3C*, *FCRL3_5C*, and *FCRL3_6A* allele frequencies were higher in the control group than in the JRA group (OR = 0.57, 95% CI 0.40–0.83, $p = 0.003$; OR = 0.55, 95% CI 0.38–0.80, $p = 0.002$; and OR = 0.53, 95% CI 0.36–0.76, $p = 0.0007$, respectively). Similarly, the same alleles were higher in the control group than in the asthma group (OR = 0.72, 95% CI 0.54–0.98, $p = 0.04$; OR = 0.74, 95% CI 0.55–1.00, $p = 0.05$; and OR = 0.70, 95% CI 0.52–0.95, $p = 0.02$, respectively). Homozygosity for the *FCRL3_3C* minor allele conferred higher protection from JRA (OR = 0.38, 95% CI 0.18–0.78, $p = 0.007$) and asthma (OR = 0.54, 95% CI 0.29–0.99, $p = 0.05$) compared to the other genotypes. However, after applying the Bonferroni correction test, statistical significance persisted only for homozygous *FCRL3_3C* protection against JRA (Table 1).

On the other hand, the haplotype analysis showed that *FcRL3_3*, *FcRL3_5*, and *FcRL3_6* were in high LD, but not *FcRL3_4* (pair-wise $r^2$ values > 0.9) (Fig. 1). We identified a total of four haplotypes with frequencies >0.01 (Table 2). The haplotype CGCA, which carried the three minor alleles, *FcRL3_3C*,*_5C*, and *_6A*, and the major allele, *FcRL3_4G*, showed male gender-dependent protection to JRA and asthma (OR = 0.35, 95% CI 0.20–0.60, $p = 0.00005$; OR = 0.61, 95% CI 0.41–0.89, $p = 0.007$, respectively). This significant association between the CGCA haplotype and protection from both diseases remained after 100,000 permutations.

A comparative analysis between healthy individuals from different populations showed that the frequency of the *FCRL3_3C* allele was higher in Mexicans (49.7%) than in Japanese (37%) and