EXTENDED REPORT

Epistatic interaction between *FCRL3* and *NFκB1* genes in Spanish patients with rheumatoid arthritis

A Martínez, E Sánchez, A Valdivia, G Orozco, M A López-Nevot, D Pascual-Salcedo, A Balsa, B Fernández-Gutiérrez, E G de la Concha, A García-Sánchez, B P C Koeleman, E Urcelay, J Martín

Ann Rheum Dis 2006;**65**:1188–1191. doi: 10.1136/ard.2005.048454

See end of article for authors' affiliations

Correspondence to: A Martínez, Immunology Department, Hospital Clinico San Carlos, C/ Martín Lagos, s/n, 28040 Madrid, Spain; alfmdoncel@terra.es

Accepted 4 February 2006
Published Online First 13 February 2006

Background: A Japanese study has described a strong association between rheumatoid arthritis and several polymorphisms located in the Fc receptor-like 3 (*FCRL3*) gene, a member of a family of genes related to Fc receptors located on chromosome 1q21–23.
Objectives: To evaluate the association between rheumatoid arthritis and *FCLR3* polymorphisms in a large cohort of Caucasian patients with rheumatoid arthritis and healthy controls of Spanish origin. Owing to the described functional link between the *FCRL3* polymorphisms and the transcription factor nuclear factor κB (NFκB), a functional polymorphism located in the *NFκB1* gene was included.
Methods: 734 patients with rheumatoid arthritis from Madrid and Granada, Spain, were included in the study, along with 736 healthy controls. Polymorphisms in the *FCRL3* gene were studied by TaqMan technology. The −94ins/delATTG *NFκB1* promoter polymorphism was analysed by fragment analysis after polymerase chain reaction with labelled primers. Genotypes were compared using 3×2 contingency tables and χ^2 values.
Results: No overall differences were found in any of the *FCRL3* polymorphisms and in the *NFκB1* promoter polymorphism when patients were compared with controls. However, when stratified according to *NFκB1* genotypes, a susceptibility effect of *FCRL3* polymorphisms was observed in patients who were heterozygotes for *NFκB1* ($p_c = 0.003$).
Conclusions: The *FCRL3* polymorphisms associated with rheumatoid arthritis in a Japanese population are not associated per se with rheumatoid arthritis in a Spanish population. A genetic interaction was found between *NFκB1* and *FCRL3* in Spanish patients with rheumatoid arthritis. These findings may provide a general rationale for divergent genetic association results in different populations.

Rheumatoid arthritis is one of the most common autoimmune diseases in Western countries, with an estimated prevalence of 1% in the Spanish population. Recent advances in its genetic basis have been fuelled by an ongoing Japanese genomewide study, which has described several causative polymorphisms in the past few years.[1–3] However, some of these outstanding findings have been difficult to replicate in European populations, as has recently been shown by our group and by others with regard to the gene peptidylarginine deiminase 4 (*PADI4*),[4–6] the haematopoietic isoform of the citrullinating enzyme. *SLC22A4*, an organic cation transporter gene, is another example of lack of association in Caucasian populations of a gene previously shown by the Japanese group[7 8] to be associated with the disease. The causes underlying such discrepancies may vary, but arguably they include both distinct environmental inputs and different genetic structure. The purely genetic reasons for a failure to replicate the association of a polymorphism described as aetiological are many. Firstly, the polymorphism studied may be found in another population in such a low frequency that a significant difference may be difficult to achieve. Secondly, the supposedly aetiological polymorphism may not be really aetiological, but instead it may act as a marker of a nearby causative polymorphism. The associations between different polymorphisms in the same or nearby genes (linkage disequilibrium) may vary widely among populations. Finally, a true aetiological polymorphism may act only on a defined genetic background, and therefore discloses itself only in specific populations. A similar situation has been reported repeatedly in mice, where a defined inactivating mutation may yield different phenotypic outcomes, depending on genetic background.[9]

A recent genetic report from the Yamamoto group[3] has described the association of four single-nucleotide polymorphisms (SNPs) located in the Fc receptor-like 3 (*FCRL3*) gene with an increased susceptibility to rheumatoid arthritis and several other autoimmune diseases, thereby raising the possibility of *FCRL3* being a general autoimmune susceptibility factor. *FCRL3*, also termed *FCRh3*, is one of a family of six related genes located on chromosome 1q21–23, sharing homologies with other classic Fc receptor genes located in the same cluster. These previously known classic genes code for receptors for the constant portion (Fc) of the immunoglobulin molecules. The Fc receptors have relevant roles in diverse

Table 1 Clinical characteristics of patients with rheumatoid arthritis

Mean (SD) age at onset (years)	50 (14)
Female:male ratio	3.2
Erosive disease (%)	81
Shared epitope positivity (%)	60
Rheumatoid factor positivity (%)	75

Abbreviations: *FCRL3*, Fc receptor-like 3; HLA, human leucocyte antigen; NFκB, nuclear factor κB; SNP, single-nucleotide polymorphism

Table 2 Genotypic and allelic distribution of *FCRL3* −169 SNP alleles

	n	AA	AG	GG	Alleles	A	G
Controls from Madrid	489	153 (31)	233 (48)	103 (21)	978	539 (55)	439 (45)
Controls from Granada	229	75 (33)	113 (49)	41 (18)	458	263 (57)	195 (43)
Patients from Madrid	448	117 (26)	229 (51)	102 (23)	896	463 (52)	433 (48)
Patients from Granada	221	61 (28)	122 (55)	38 (17)	442	244 (55)	198 (45)

FCRL3, Fc receptor-like 3; SNP, single-nucleotide polymorphism.
Values in parentheses are percentages.
Overall genotype comparison (Madrid and Granada), p = 0.10.
Minimum detectable allelic RR = 1.24.

aspects of immunobiology.[10] This fact, along with their polymorphic nature, led long ago to their consideration as natural candidates to be susceptibility genes in several autoimmune inflammatory conditions. The region 1q21–23 has been reported to be linked to systemic lupus erythematosus in familial linkage studies with several affected members, but it has not been detected in a recent study on rheumatoid arthritis (interestingly, in the Japanese population) using microsatellites,[11] or in previous genomewide scans in populations of European ancestry. Kochi *et al*[3] examined the region with high-density SNP mapping and found a peak of association not in the classic genes, but on *FCRL3*, a family member expressed on some subsets of B cells. Although they were cautious enough not to discard fully other genes located in the vicinity, the main candidate they propose is clearly *FCRL3* itself. They suggest that the −169C susceptibility allele binds the transcription factor nuclear factor κB (NFκB) more strongly than the non-susceptible −169T allele, thereby enhancing the transcription rate of the *FCRL3* gene.

Although some recent research in the Spanish population has been undertaken to ascertain the role of the classic Fc receptor genes in rheumatoid arthritis,[12] we have tried to replicate directly the Japanese findings, as their functional studies convincingly showed that the polymorphism may be mechanistically relevant to the pathological process. We also looked for possible genetic interactions between the polymorphisms in the *FCRL3* promoter and the functional polymorphism in the promoter of *NFKB1*, coding for the p105 subunit of NFκB. This p105 subunit is proteolytically activated on cell stimulation, yielding the p50 subunit, a component (in combination with c-Rel) of the active transcription factor binding to the −169 *FCRL3* promoter polymorphism.[3]

METHODS

Our study included 734 white Spanish patients with rheumatoid arthritis, consecutively recruited in two hospitals in Madrid (n = 489) and one hospital in Granada (n = 245). The diagnosis was established on the basis of the 1987 American College of Rheumatologists (formerly ARA) criteria.[13] Table 1 shows their main clinical characteristics. Most of these patients have been included in previous studies from our groups. The written consent of the participants was obtained according to the Declaration of Helsinki. The control group included 736 people, 489 from Madrid and 247 from Granada: white Spanish blood donors and healthy laboratory staff and students. Although the blood donors were not specifically asked about the presence of rheumatoid arthritis, the 1% frequency of this disease in the Spanish population precludes this fact from being a major concern. The ethics committees of the participating hospitals (Clinico San Carlos, La Paz, Virgen de las Nieves, Spain) approved this study.

The *FCRL3* polymorphisms were analysed using the C_1741825_10 (*FCRL3* −169, rs7528684, in Madrid only), C_1741826_10 (*FCRL3* −110, rs11264799) and C_2741972_10 (*FCRL3* intron 3, rs1537947, in Granada only) Assays-on-Demand of Applied Biosystems (Foster City, California, USA), according to the conditions recommended by the manufacturer. In all, 94 samples were genotyped for both the −169 SNP and the polymorphism in the intron 3 of the *FCRL3* gene. As described previously (see supplementary table in the Japanese study[3]), the concordance was almost total (>97%; *FCRL3* −169T is equivalent to *FCRL3_6*G, and *FCRL3* −169C is equivalent to *FCRL3_6*A), and it allowed us to consider them as quasi-equivalent markers. They are both referred to as −169SNP hereafter, for simplicity. Alleles were discriminated in an ABI 7900 Sequence Detector (Applied Biosystems). The −94ins/delATTG *NFκB1* promoter polymorphism was analysed as explained previously.[14]

The presence of the shared epitope (defined as DRB1*0101, DRB1*0102, DRB1*0401, DRB1*0404, DRB1*0405, DRB1*0408 or DRB1*1001 alleles) was ascertained as described elsewhere.[15 16] The shared epitope is a common stretch of amino acids present in human leucocyte antigen (HLA)-DRB1 alleles associated with the disease, and it is the strongest rheumatoid arthritis susceptibility factor identified.

Genotype frequencies (3×2 contingency tables), carriage rates (2×2 tables) and allelic frequencies (2×2 tables) were compared with the χ^2 statistics and $p<0.05$ was considered to be significant. The p values were calculated with a standard free software package (Epi Info V.2000, CDC, Atlanta, Georgia, USA). Statistical power (indicated below each table

Table 3 Genotypic and allelic distribution of *FCRL3* −110 SNP

	n	GG	AG	AA	Alleles	G	A
Controls from Madrid	479	235 (49)	196 (41)	48 (10)	958	666 (70)	292 (30)
Controls from Granada	225	115 (51)	89 (40)	21 (9)	450	319 (71)	131 (29)
Patients from Madrid	420	196 (47)	184 (44)	40 (10)	840	576 (69)	264 (31)
Patients from Granada	206	103 (50)	94 (46)	9 (4)	412	300 (73)	112 (27)

FCRL3, Fc receptor-like 3; SNP, single-nucleotide polymorphism.
Values in parentheses are percentages.
Overall genotype comparison (Madrid and Granada), p = 0.23.
Minimum detectable allelic RR = 1.26.

Table 4 Genotypic and allelic distribution of *NFκB1* insertion or deletion in patients with rheumatoid arthritis and in controls

	n	Del/del	Del/ins	Ins/ins	Alleles	Del	Ins
Controls from Madrid	458	74 (16)	214 (47)	168 (37)	916	362 (40)	554 (60)
Controls from Granada	247	35 (14)	107 (43)	105 (43)	494	177 (36)	317 (64)
Patients from Madrid	376	61 (16)	168 (45)	147 (39)	752	290 (39)	462 (61)
Patients from Granada	245	28 (11)	124 (51)	93 (38)	490	180 (37)	310 (63)

del, deletions; ins, insertions; *NFκB1*, nuclear factor κB1; RA, rheumatoid arthritis.
Values in parentheses are percentages.
Overall genotype comparison (Madrid and Granada), p = 0.80.
Minimum detectable allelic RR = 1.25.

as the minimum detectable relative risk (RR) value for p = 0.05 and a statistical power of 80%) was calculated with the online calculator at http://calculators.stat.ucla.edu/powercalc/binomial/case-control/index.php.

RESULTS

SNPs located in the *FCRL3* region (the ones identified as primary sources of the association signal described in the Japanese study[3]) were examined in the Spanish population. The Madrid and Granada populations were very similar in terms of their genotypic distributions. We found no evidence of phenotypic, genotypic or allelic association with rheumatoid arthritis (tables 2 and 3), even when results from both populations were pooled. Shared-epitope stratification did not improve the significance. We could not confirm the association described in the Japanese population.

Table 4 shows the allelic distribution of the −94ins/delATTG *NFKB1* promoter polymorphism found in the promoter region of the *NFKB1* gene. Data from Granada have been published previously[14] and they are included here for completeness, with only minor modifications. No differences were found when patients and controls from each separate location were compared. When controls from Madrid were compared with controls from Granada, we found no differences, showing that the two populations are homogeneous. Similarly, when patients with rheumatoid arthritis from Madrid were compared with those from Granada, we found no differences. This prompted us to pool both populations, to improve statistical power, but again no differences were found between patients with rheumatoid arthritis and controls. No differences were apparent when patients were stratified according to the number of shared epitope alleles carried (data not shown).

As the functional interaction between *NFκB1* and *FCRL3* is proved, we decided to investigate whether any genotype combination showed an altered pattern when patients were compared with controls. Table 5 shows the compound genotypes observed in patients with rheumatoid arthritis and controls. Among patients heterozygotic for the −94ins/delATTG *NFκB1* promoter polymorphism, there is a skewed distribution of the −169 polymorphism (p = 0.001, corrected by three different *NFκB1* genotypes, $p_c = 0.003$). Similarly to the Japanese population, the less common allele was over-represented in patients with rheumatoid arthritis when compared with controls.

DISCUSSION

We report that the association of the promoter polymorphism at position −169 on the *FCRL3* gene depends on the *NFκB1* genotype, thereby suggesting that a genetic and functional link between both genes is relevant in onset of rheumatoid arthritis. This is to our knowledge the first attempt to replicate the *FCRL3* association reported by the Yamamoto group.[3] In contrast with that report, our results show no association of *FCRL3* polymorphisms when considered alone.

The previous Japanese report did not include the polymorphism on the *NFκB1* gene in its analysis. However, their results were highly relevant per se. Our finding of an interaction between both genes seems to reconcile their results with the negative results we obtained when *FCRL3* alone is considered. If the polymorphisms are indeed aetiological this lack of association is otherwise problematic to explain, as the allelic frequencies in our population are by no means low enough to cause problems related to statistical power.

A genetic cooperation between distinct loci is not new in the genetics of rheumatoid arthritis. The relevance of rheumatoid arthritis susceptibility of mutations affecting an intronic *RUNX1* binding site on the *SLC22A4* gene (an organic cation transporter) and of mutations in the *RUNX1* gene itself has been recently described.[1 17] A few years ago, we also described the protection against rheumatoid arthritis afforded by major histocompatibility complex class I chain-related gene A alleles in the presence of the shared epitope at the *HLA-DRB1* locus.[15]

A functional hypothesis explaining our results is not straightforward. The ins/del polymorphism in the *NFκB1* promoter has been related to transcriptional activity of the gene encoding this transcription factor,[18] fundamental in orchestrating immune and inflammatory reactions. But this differential activity is probably not the sole cause behind the results we have presented, because a specific association of *FCRL3* genotypes in *NFκB1* heterozygotes (as opposed to homozygotes or carriers of any allele) is difficult to interpret. There have been a few reports in the literature of the effect of heterozygous genotypes on susceptibility to autoimmune

Table 5 Distribution of patients with rheumatoid arthritis (n = 592) and controls (n = 646) according to the compound *NFκB1* insertion or deletion and *FCRL3* −169SNP genotype

NFκB1	del/del	ins/del	ins/ins
Patients (Madrid and Granada)			
FCRL3			
AA	24 (29)	68 (24)	68 (30)
AG	45 (54)	145 (52)	123 (53)
GG	14 (17)	66 (24)	39 (17)
Controls (Madrid and Granada)			
FCRL3			
A	30 (31)	112 (38)	67 (27)
AG	46 (47)	137 (46)	127 (51)
GG	22 (22)	48 (16)	57 (23)

del, deletions; *FCRL3*, Fc receptor-like 3; ins, insertions; *NFκB1*, nuclear factor κB1; RA, rheumatoid arthritis; SNP, single-nucleotide polymorphism.
Percentages are calculated across columns.
Overall *FCRL3* genotype comparisons for each *NFκB1* genotype, p = 0.54, p = 0.001 and p = 0.28, for del/del, ins/del and ins/ins patients, respectively.

diseases. An *IL4* polymorphism has been described as negatively associated with multiple sclerosis in heterozygosis.[19] The authors suggest that perhaps the association could result from linkage disequilibrium with distant markers. This situation is reminiscent of the one found at the *HLA-DR* locus in coeliac disease. The association of *DR7-DR5* heterozygotes with the disease stems from the fact that *DR7* is strongly linked in our population to *DQB1*02*, and *DR5* is almost invariably linked to *DQA1*05*. These two alleles, located in distinct but nearby genes, are the primary susceptibility factors in coeliac disease, and *DR* is acting only as a linkage disequilibrium marker. A speculative scenario would be that a specific genetic configuration at the *NFκB1* locus confers the ability to become a susceptibility factor to variations found in *FCRL3*. And perhaps what is different between our population and the Japanese population is the distribution of genetic configurations in the *NFκB1* locus.

In summary, our results show complex genetic interactions between different genes to determine the final outcome: susceptibility to rheumatoid arthritis. Further studies are necessary to expand this knowledge and to delineate the genetic susceptibility pathways operating in distinct populations.

ACKNOWLEDGEMENTS

We thank Carmen Martínez Cuervo for her expert technical assistance.

Authors' affiliations

A Martínez, A Valdivia, E G de la Concha, E Urcelay, Immunology Department, Hospital Clinico San Carlos, Madrid, Spain
E Sánchez, G Orozco, J Martín, Lopez-Neyra Biomedicine Institute, CSIC, Granada, Spain
M A López-Nevot, Immunology Department, Hospital Virgen de las Nieves, Granada
D Pascual-Salcedo, Immunology Department, Hospital Universitario La Paz, Madrid
A Balsa, Rheumatology Department, Hospital Universitario La Paz
B Fernández-Gutiérrez, Rheumatology Department, Hospital Clinico San Carlos
A García-Sánchez, Rheumatology Department, Hospital Virgen de las Nieves
B P C Koeleman, Department of Biomedical Genetics, Complex Genetics Section, Utrecht University Medical Centre, Utrecht, The Netherlands

Funding: This work was supported by grant SAF03-3460 from Plan Nacional I+D+I.

Competing interests: AM is an employee with support from the ''Fondo de Investigaciones Sanitarias'' (CP04/00175). EU is a recipient of the ''Ramon y Cajal'' contract from the Spanish Science and Technology Ministry.

REFERENCES

1 **Tokuhiro S**, Yamada R, Chang X, Suzuki A, Kochi Y, Sawada T, *et al.* An intronic SNP in a RUNX1 binding site of SLC22A4, encoding an organic cation transporter, is associated with rheumatoid arthritis. *Nat Genet* 2003;**35**:341–8.
2 **Suzuki A**, Yamada R, Chang X, Tokuhiro S, Sawada T, Suzuki M, *et al.* Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat Genet* 2003;**34**:395–402.
3 **Kochi Y**, Yamada R, Suzuki A, Harley JB, Shirasawa S, Sawada T, *et al.* A functional variant in FCRL3, encoding Fc receptor-like 3, is associated with rheumatoid arthritis and several autoimmunities. *Nat Genet* 2005;**37**:478–85.
4 **Barton A**, Bowes J, Eyre S, Spreckley K, Hinks A, John S, *et al.* A functional haplotype of the PADI4 gene associated with rheumatoid arthritis in a Japanese population is not associated in a United Kingdom population. *Arthritis Rheum* 2004;**50**:1117–21.
5 **Caponi L**, Petit-Teixeira E, Sebbag M, Bongiorni F, Moscato S, Pratesi F, *et al.* A family based study shows no association between rheumatoid arthritis and the PADI4 gene in a white French population. *Ann Rheum Dis* 2005;**64**:587–93.
6 **Martinez A**, Valdivia A, Pascual-Salcedo D, Lamas JR, Fernandez-Arquero M, Balsa A, *et al.* PADI4 polymorphisms are not associated with rheumatoid arthritis in the Spanish population. *Rheumatology* 2005;**44**:263–6.
7 **Newman B**, Wintle RF, van Oene M, Yazdanpanah M, Owen J, Johnson B, *et al.* SLC22A4 polymorphisms implicated in rheumatoid arthritis and Crohn's disease are not associated with rheumatoid arthritis in a Canadian Caucasian population. *Arthritis Rheum* 2005;**52**:425–9.
8 **Barton A**, Eyre S, Bowes J, Ho P, John S, Worthington J. Investigation of the SLC22A4 gene (associated with rheumatoid arthritis in a Japanese population) in a United Kingdom population of rheumatoid arthritis patients. *Arthritis Rheum* 2005;**52**:752–8.
9 **Rieux-Laucat F**, Fischer A, Deist FL. Cell-death signaling and human disease. *Curr Opin Immunol* 2003;**15**:325–31.
10 **Ravetch JV**, Bolland S. IgG Fc receptors. *Annu Rev Immunol* 2001;**19**:275–90.
11 **Tamiya G**, Shinya M, Imanishi T, Ikuta T, Makino S, Okamoto K, *et al.* Whole genome association study of rheumatoid arthritis using 27039 microsatellites. *Hum Mol Genet* 2005;**14**:2305–21.
12 **Nieto A**, Caliz R, Pascual M, Mataran L, Garcia S, Martin J. Involvement of Fc gamma receptor IIIA genotypes in susceptibility to rheumatoid arthritis. *Arthritis Rheum* 2000;**43**:735–9.
13 **Arnett FC**, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;**31**:315–24.
14 **Orozco G**, Sanchez E, Collado MD, Lopez-Nevot MA, Paco L, Garcia A, *et al.* Analysis of the functional NFKB1 promoter polymorphism in rheumatoid arthritis and systemic lupus erythematosus. *Tissue Antigens* 2005;**65**:183–6.
15 **Martinez A**, Fernandez-Arquero M, Balsa A, Rubio A, Alves H, Pascual-Salcedo D, *et al.* Primary association of a MICA allele with protection against rheumatoid arthritis. *Arthritis Rheum* 2001;**44**:1261–5.
16 **Pascual M**, Nieto A, Lopez-Nevot MA, Ramal L, Mataran L, Caballero A, *et al.* Rheumatoid arthritis in southern Spain: toward elucidation of a unifying role of the HLA class II region in disease predisposition. *Arthritis Rheum* 2001;**44**:307–14.
17 **Alarcon-Riquelme ME**. A RUNX trio with a taste for autoimmunity. *Nat Genet* 2003;**35**:299–300.
18 **Karban AS**, Okazaki T, Panhuysen CI, Gallegos T, Potter JJ, Bailey-Wilson JE, *et al.* Functional annotation of a novel NFKB1 promoter polymorphism that increases risk for ulcerative colitis. *Hum Mol Genet* 2004;**13**:35–45.
19 **Kantarci OH**, Schaefer-Klein JL, Hebrink DD, Achenbach SJ, Atkinson EJ, McMurray CT, *et al.* A population-based study of IL4 polymorphisms in multiple sclerosis. *J Neuroimmunol* 2003;**137**:134–9.