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Role of Fcγ Receptors IIA, IIIA, and IIIB in Susceptibility to Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To investigate the role of Fcγ receptor (FcγR) genes in susceptibility to rheumatoid arthritis (RA) using family based studies, to examine possible interactions between FcγR genotypes and the shared epitope (SE), and to assess linkage disequilibrium between FcγR loci.

Methods. Association studies were performed in 95 Caucasian, single-case, nuclear Caucasian families with both parents alive using haplotype based haplotype relative risk (HHRR) and transmission disequilibrium test (TDT) statistics. Three FcγR polymorphisms (FcγRIIA-131H/R, FcγRIIIA-158V/F, and FcγRIIIB-NA1/NA2) were genotyped using polymerase chain reaction methods. Linkage analysis was performed using 3 microsatellite markers (D1S498, D1S2844, D1S2762) flanking the FcγR region in an independent set of 90 Caucasian, multiple-case families.

Potential effects of disease heterogeneity, including sex and the presence of rheumatoid factor, SE, and erosive or nodular disease, were taken into account in the analysis. Logistic regression analysis was performed to determine whether FcγR alleles are independent risk factors for the susceptibility to and/or severity of RA. Linkage disequilibrium was calculated using pairwise linkage disequilibrium statistics.

Results. HHRR and TDT analysis showed no evidence of preferential transmission of any FcγR alleles studied, and there were no important associations with any given disease phenotype. Moreover, neither linkage to microsatellite markers close to the FcγR genes on chromosome 1 nor linkage disequilibrium between FcγR loci was present in our population. The distribution of inherited genotypes provided evidence for an interaction between the SE and the FcγRIIIA-158V allele and between the SE and the FcγRIIIA-158V–FcγRIIA-131H 2-locus haplotype since the combined presence of these factors increased the susceptibility to RA (OR 4.13, 95% CI 1.6–10.62 and OR 2.83, 95% CI 1.25–6.38, respectively). However, regression analysis showed that neither the 158V allele nor the 158V-131H haplotype contributed as independent factors to susceptibility or severity of RA.

Conclusion. Isolated FcγR genes do not play a major independent role in susceptibility to RA. To a limited extent, the presence of high-binding alleles at the FcγRIIIA locus or at the FcγRIIIA–FcγRIIA haplotype might predispose to RA in SE positive individuals. (J Rheumatol 2003;30:926–33)

Key Indexing Terms:
RHEUMATOID ARTHRITIS Fc GAMMA RECEPTOR POLYMORPHISMS
ASSOCIATION ANALYSIS LINKAGE ANALYSIS

Rheumatoid arthritis (RA) is a multigenic autoimmune disease characterized by a chronic polyarthritis. It is generally accepted that HLA class II genes are associated to RA, but these explain only 30 to 40% of the genetic component of the disease[1,2]. Other potential susceptibility genes are being investigated using genome-wide scanning and candidate gene strategies[3].

The genes encoding for Fc gamma receptors (FcγR) are obvious candidate genes in RA and other autoimmune diseases. These receptors are expressed on a variety of inflammatory cells and are implicated in the clearance of antibodies and immune complexes, phagocytosis, and in antigen presentation[4-6]. The expression of FcγR is genetically regulated and has strong implications for the development of experimental arthritis[7,8]. Most patients with RA are rheumatoid factor (RF) positive and many have IgG-containing immune complexes and other autoantibodies. It has been proposed that immune complexes within the joint may activate macrophages to induce cartilage damage. The interaction between immune complexes and FcγR might therefore be involved in the pathogenesis of the disease.

The 3 major FcγR subclasses FcγRI (CD64), FcγRII

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(CD32), and FcγRIII (CD16) are encoded by a cluster of genes on the long arm of chromosome 1 (1q21-24)[4-6]. Several genetic polymorphisms in these genes have biological consequences for the function of these receptors[9-11]. Biallelic polymorphisms of the FcγRIIA [histidine (H)/arginine (R) at position 131] and FcγRIIIA [valine (V)/phenylalanine (F) at position 158] have been described. The FcγRIIA-131H and FcγRIIIA-158V isoforms have higher affinity for immune complexes and immunoglobulins than their counterparts[9-12]. FcγRIIIB encompasses the so-called neutrophil antigen system with 2 isoforms, NA1 and NA2, from which the former has a higher functional capacity[10,11].

Two studies suggest that the FcγRIIIA-158 V/F polymorphism may be implicated in susceptibility to RA[13,14], although these results are not concordant and have been challenged by others[15,16]. Studies on the role of FcγRIIA and FcγRIIIB polymorphisms in RA have yielded conflicting results[17-20]. All previous reports had a case-control design and might therefore be hampered by stratification problems[21]. Moreover, most studies have focused on a single polymorphism and did not consider the interactions between alleles and potential linkage disequilibrium between FcγR genes.

We investigated the influence of FcγR genes on RA susceptibility using family based association and linkage studies in 2 independent Caucasian populations collected by the European Consortium on RA Families (ECRAF). Possible interactions between FcγR and the shared epitope (SE) and linkage disequilibrium between FcγR loci were also examined.

MATERIALS AND METHODS

Patients. Association to the FcγRIIA-131H/R, FcγRIIIA-158V/F, and FcγRIIIB-NA1/NA2 polymorphisms was tested in 95 Caucasian, single-case, French families with a proband and 2 healthy parents alive. Linkage to markers located in the vicinity of the FcγR loci was studied in 90 multiple-case Caucasian European families enrolled in the first European genome scan performed by ECRAF [http://www.genethon.fr/projects/genfluo/PR/RES_PR_README][22]. To avoid bias in the linkage study, a single affected sib-pair (ASP) was randomly selected from the multiple-case families. All probands fulfilled the American College of Rheumatology criteria for RA[23]. Patient characteristics are shown in Table 1.

Table 1. Characteristics of the patients included in the association (single-case) and linkage (multiple-case) analysis.

	Single-case Probands, n = 95	Multiple-case Probands, n = 180
Age at onset, median (range), yrs	29.5 (13–50)	55 (27–76)
Disease duration, median (range), yrs	11 (1–33)	15 (1–47)
Female, %	86	79
RF positive, %	71	71
SE positive, %	77	79
Erosive disease, %	87	82
Nodules, %	11	25

Methods. Genomic DNA was extracted from whole blood by standard methods. HLA-DRB1 genotyping was performed with the Inolipa kit (Murex, Chatillon, France) and Dynal kits (Dynal classic SSP, low resolution, DRB1*04 and DRB1*01 kits, Dynal Biotech, Oslo, Norway). HLA-DRB1 alleles encoding the amino acid sequences QRRAA (e.g., *0101, *0102, *0404, *0405, *0408, *1402), QKRAA (*0401, *0409), and RRRAA (*1001) were considered shared epitope (SE) positive.

FcγR typing. Genotyping for FcγRIIA-131H/R, FcγRIIIA-158V/F, and FcγRIIIB-NA1/NA2 was performed using polymerase chain reaction (PCR) methods as described[15,24,25].

Briefly, a hot-start PCR, allele-specific primers (Genset, Paris; Table 2), AmpliTaq Gold polymerase (Perkin-Elmer, Foster City, CA, USA), and an Eppendorf Mastercycler gradient (Eppendorf, Hamburg, Germany) were used in all procedures. Genotyping for FcγRIIA-131H/R was performed using a rapid PCR method using primers for human growth factor (HGH-I and II) as an internal control[24]. For genotyping FcγRIIIB-NA1/NA2, 2 separate allele-specific annealing primer assays (ASPA) were used. Primer sets for a fragment of the transmembrane and 3' untranslated region of both FcγRIIIA and FcγRIIIB and for a fragment on the fourth exon of p22 phos gene were used as controls for the NA1 and NA2 ASPA[25]. PCR products for the FcγRIIA and FcγRIIIB polymorphisms were analyzed by ethidium bromide staining after electrophoresis on a 1% agarose gel (Nusieve/Seakem GTC agarose 3:1, FMC Bioproducts, Rockland, ME, USA).

For typing of FcγRIIIA-158V/F, we used single-strand conformation polymorphism (SSCP) PCR methods as described by Hatta, *et al*[15]. Five microliters of the resulting PCR product were mixed with 5 ml denaturing solution, then incubated at 96°C for 5 min and cooled on ice. Five microliters of this mixture were applied to a 15% polyacrylamide gel with 5% glycerol. After overnight electrophoresis (in 1 mM Tris-borate, 1 mM EDTA, at 4°C, 5 watts), single-strand fragments were visualized with SybeR Green II (Perkin Elmer, Boston, MA, USA).

All PCR protocols had been previously tested on DNA samples with known FcγR polymorphisms. The sequencing of DNA samples was performed at Genethon, Evry, France. Each genotype was independently interpreted by 2 investigators (TR and EP).

Statistical analysis. Allelic frequencies (number of copies of a specific allele divided by the total number of alleles in the group) were calculated. Hardy-Weinberg equilibrium was tested in control genotypes using chi-square tests. Association studies were performed in single-case families using haplotype based haplotype relative risk (HHRR) and transmission disequilibrium testing (TDT)[21,26]. Both tests examine the potential skewing in the transmission of parental alleles to probands. The HHRR applies chi-square statistics to all parental genotypes, whereas TDT assesses the null hypothesis of no association using only heterozygous parental genotypes with the McNemar test.

A sample of 95 single-case, nuclear Caucasian families would provide a power of 63%, 89%, and 72%, respectively, to detect possible associations between RA and the high affinity alleles FcγRII-131H, FcγRIIIA-158V, and FcγRIIIB-NA1 [http://statgen.iop.kcl.ac.uk].

Estimated 2-locus haplotype frequencies were calculated using the HAPLO program[27], and potential linkage disequilibrium (LD) between the FcγRIIA, FcγRIIIA, and FcγRIIIB loci in non-inherited, control haplotypes was calculated using pairwise standard disequilibrium coefficients[28]. The frequencies of the rarer alleles at loci 1 and 2 being p and q, respectively, the LD coefficient (Δ) is calculated as $D^*\sqrt{(p(1-p)\,q(1-q))}$. For double-heterozygous or single-heterozygous genotypes, the haplotypes were determined unambiguously. For double-heterozygous, haplotypes were determined according to the maximum likelihood procedure using the iterative process outlined by Hill[28]. Besides D*, we show the disequilibrium coefficient D', which is the fraction of D_{max} or D_{min} achieved by D*, thus it is less dependent on allelic frequencies. D_{max} and D_{min} are the maximum and minimum possible values of D*, determined as $D_{max} = pP(1-q)$ and $D_{min} = -pq$. To test this hypothesis $\Delta = 0$, a chi-square test is applied with 1 degree of freedom. Additionally, the distributions of 2-locus FcγR haplo-

Table 2. Primers used for the typing of FcγR polymorphisms.

FcγR polymorphism	Primers		Reference
	Upstream	Downstream	
FcγRIIA-131H/R	131H; 5′-ATCCCAGAAATTCTCCCA-3′ 131R; 5′-ATCCCAGAAATTCTCCCG-3′	5′-CAATTTTGCTGCTATGGGC-3′	24
FCγRIIIA-158V/F	5′-TATTTACAGAATGGCAATGG-3′	5′-GTGATGGTGATGTTCACATG-3′	15
FCγRIIIB-NA1/NA2	NA1; 5′-CAGTGGTTTCACAATGTGAA-3′ NA2; 5′-CTCAATGGTACAGCGTGCTT-3′	NA1; 5′-ATGGACTTCTAGCTGCACCG-3′ NA2; 5′-CTGTACTCTCCACTGTCGTT-3′	25

types and LD coefficients were also analyzed excluding double-heterozygous haplotypes.

Linkage analysis was performed in multiple case families using fluorescence based microsatellite markers flanking the FcγR region. The microsatellites analyzed in this study were D1S498, D1S2844, and D1S2762, located respectively at 160.7, 179.2, and 183.3 cM from telomere. Sharing of alleles identical by descent (IBD) between affected sib-pairs was calculated with the program SIBPALNA and compared with the random expectation of IBD = 50%.

Disease heterogeneity was taken into account by stratifying patients according to sex and the presence/absence of rheumatoid factor, shared epitope, and erosive or nodular disease.

Proportions of discrete risk factors were compared using odds ratios and 95% confidence intervals (CI). Between-group comparisons were performed using chi-square tests or Student's t tests as appropriate. Fisher's exact test was used when expected frequencies were lower than 5.

Multiple logistic regression analysis was performed to assess whether the presence of FcγRIIA, FcγRIIIA, and FcγRIIIB alleles was an independent explanatory factor of either the susceptibility for RA or the presence or absence of radiological damage as a marker for disease severity. In both analyses the presence of the shared epitope was used as the dependent variable. Rheumatoid factor was also used as a dependent variable in the analysis for disease severity.

RESULTS

Lack of association between FcγRIIA-131H/R, FcγRIIIA-158V/F, and FcγRIIIB-NA1/NA2 polymorphisms and RA. The allelic frequencies and distribution of genotypes observed in our study (Table 3) were roughly similar to those reported in Caucasians and other ethnic groups[13,15,16,29]. The non-inherited control genotypes were in Hardy-Weinberg equilibrium (p = 0.39, p = 0.95, p = 0.5 for FcγRIIA, FcγRIIIA, and FcγRIIIB, respectively). As shown, the distribution of FcγR alleles and genotypes was similar in the inherited and non-inherited genotypes. Further, both HHRR and TDT analysis showed no preferential transmission of any FcγR alleles to probands (Figure 1). In contrast, and as expected in a cohort with RA, the transmission of SE-encoding alleles to probands was highly skewed (Table 3).

Analysis of disease phenotype showed a trend to preferential transmission of the FcγRIIA-131H allele in patients with non-erosive disease (n = 12, HHRR chi-square = 4.1, p = 0.04), but this difference was not significant after correc-

Table 3. Inherited and non-inherited alleles and genotypes in 95 single-case families.

	Allelic Frequency				Genotype			
	Inherited, n (%)	Non-inherited, n (%)	p†		Inherited*, n (%)	Non-inherited, n (%)	p†	OR (95% CI)
FcγRIIA				FcγRIIA				
131H	107 (56.3)	100 (52.6)	0.47	131HH	31 (32.6)	23 (24.2)	0.36	1.52 (0.83–2.75)
				131HR	45 (47.4)	54 (56.8)		0.68 (0.42–1.11)
131R	83 (43.7)	90 (47.4)		131RR	19 (20)	18 (18.9)		1.07 (0.54–2.13)
FcγRIIIA				FcγRIIIA				
158V	72 (37.9)	71 (37.4)	0.92	158VV	16 (16.8)	14 (14.7)	0.88	1.17 (0.55–2.50)
				158VF	40 (42.1)	43 (45.3)		0.88 (0.53–1.46)
158F	118 (62.1)	119 (62.6)		158FF	39 (41.1)	38 (40)		1.04 (0.62–1.76)
FcγRIIIB				FcγRIIIB				
NA1	48 (25.3)	60 (31.6)	0.17	NA1/NA1	2 (2.1)	7 (7.4)	0.19	0.27 (0.06–1.32)
				NA1/NA2	44 (46.3)	46 (48.4)		0.92 (0.56–1.51)
NA2	142 (74.7)	130 (68.4)		NA2/NA2	49 (51.6)	42 (44.2)		1.34 (0.82–2.21)
SE				SE				
SE+	100 (52.6)	51 (26.7)	< 0.0001	SE+/SE+	27 (28.4)	10 (10.5)	< 0.0001	3.38 (1.57–7.26)
				SE–/SE+	46 (48.4)	31 (32.6)		1.94 (1.14–3.29)
SE–	90 (47.4)	139 (73.2)		SE–/SE–	22 (23.2)	54 (56.8)		0.23 (0.13–0.40)

* Hardy-Weinberg equilibrium for control genotypes (p = 0.39, 0.95, and 0.5 for FcγRIIA, FcγRIIIA, and FcγRIIIB, respectively). † P for inherited versus non-inherited using 2 × 2 (alleles) and 2 × 3 (genotypes) contingency tables.

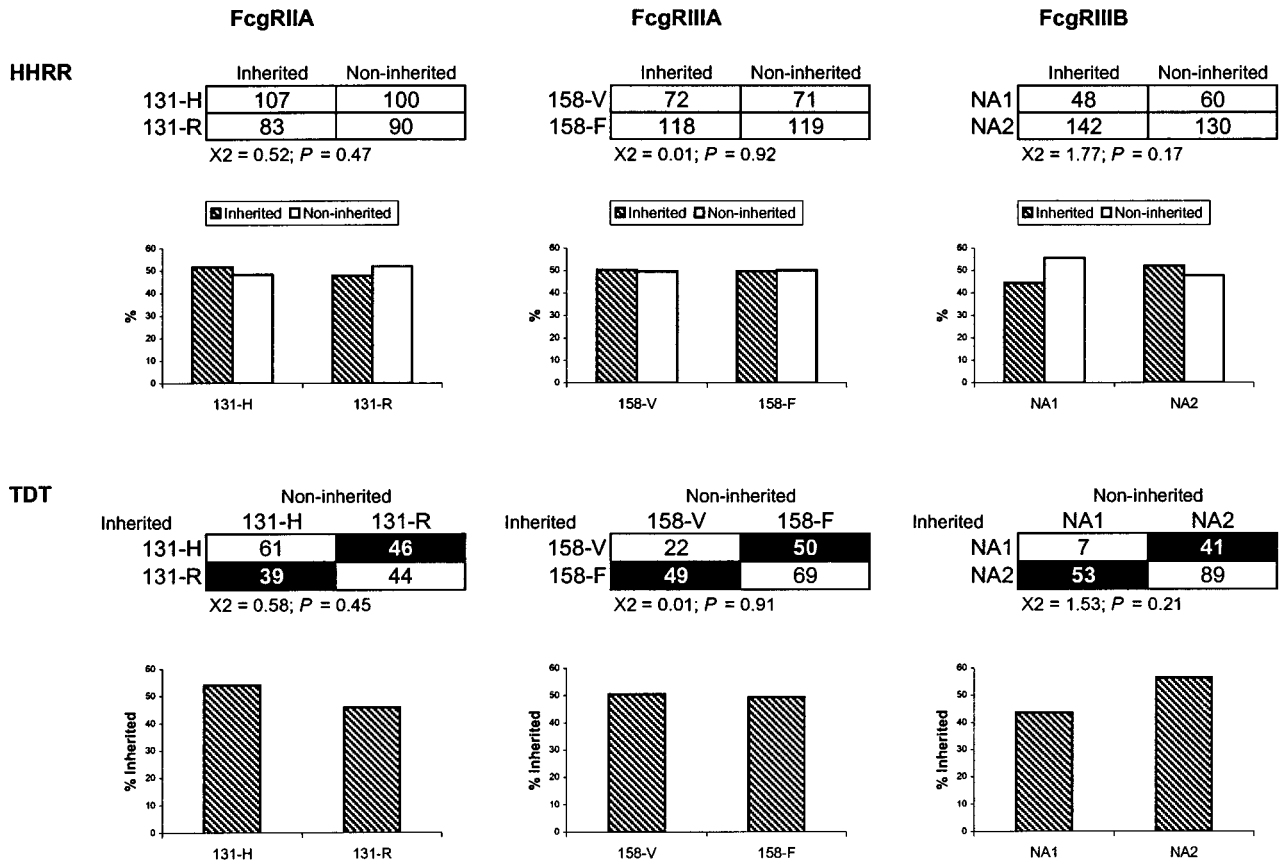

Figure 1. Haplotype based haplotype relative risk (HHRR) analysis and transmission disequilibrium testing (TDT) for the FcγRIIA-131H/R, FcγRIIIA-158V/F, and FcγRIIIB-NA1/NA2 polymorphisms. The HHRR analysis (upper panel) classifies each parent according to which allele is transmitted and which is not, and compares how many times a given parental allele is transmitted versus nontransmitted (chi-square test). The TDT (lower panel) uses only heterozygous parental haplotypes (shaded areas in the 2 × 2 contingency tables). This approach tests the null hypothesis of no association by McNemar test for pairs of transmitted and nontransmitted parental alleles [biallelic TDT chi-square = $(b - c)^2/(b + c)$, where b = transmission of the high-binding allele, c = transmission of the low-binding allele from heterozygous (b/c) parents].

tion for multiple comparisons. With this exception, FcγR genotypes were not associated with other disease characteristics such as sex, RF, and nodular disease (data not shown).

Interaction between FcγR polymorphisms and SE. Analysis of possible interactions between FcγR polymorphisms with the SE was performed by comparing the frequencies of genotypes containing at least one high-binding FcγR allele (FcγRIIA-131H, FcγRIIIA-158V, FcγRIIIB-NA1, respectively) with those who were SE negative and homozygous for the low-binding allele (FcγRIIA-131RR, FcγRIIIA-158FF, and FcγRIIIB-NA2NA2) (Table 4). This analysis yielded evidence for an interaction between the FcγRIIIA-158V allele and the SE. The combined presence of these 2 factors in a given individual increased the risk of RA (OR 4.13, 95% CI 1.6–10.62, p = 0.002) to a greater extent than each factor alone. This was not the case for the FcγRIIIB-NA1 allele, and the combined effect of FcγRIIA-131H and SE did not reach significance (Table 4).

No evidence for linkage disequilibrium at the FcγR locus. The distribution of FcγR 2-locus haplotypes in inherited and non-inherited genotypes is shown in Table 5. This analysis was performed using estimated 2-locus haplotype frequencies calculated with the HAPLO program[27]. Additional analysis was performed considering only genotypes homozygous at one or both loci in order to assign haplotypes unequivocally. A total of 23, 16, and 24 inherited and 22, 20, and 25 control genotypes were double-heterozygous for FcγRIIA-FcγRIIIA, FcγRIIIA-FcγRIIIB, and FcγRIIA-FcγRIIIB, respectively, and were excluded from this latter analysis.

Pairwise standard disequilibrium coefficients (*D* and D'*, which is the fraction of D_{max-} or D_{min-} achieved by D*)[28] of estimated 2-locus haplotypes in controls showed no evidence for linkage disequilibrium between FcγRIIA and FcγRIIIA (*D** = 0.014, D' = 7% of D_{max}; p = 0.56), between FcγRIIIA and FcγIIIB (*D** = 0.027, D' = 23% of D_{max}; p = 0.24), or between FcγRIIA and FcγRIIIB (*D** = 0.026, D' = 17% of D_{max}; p = 0.28). The same was true after exclusion of double-heterozygous haplotypes (data not shown).

Table 4. Interactions between FcγRIIA, FcγRIIIA, and FcγRIIIB alleles and the shared epitope in predisposition to RA.

Allele Status	Shared Epitope	Inherited	Non-inherited	OR (95% CI)*	p*
FcγRIIA-131H					
–	–	6	8	Reference group	
–	+	13	10	1.73 (0.45–6.63)	NS
+	–	16	46	0.46 (0.14–1.54)	NS
+	+	60	31	2.58 (0.82–8.10)	NS
FcγRIIIA-158V					
–	–	9	19	Reference group	
–	+	30	19	3.33 (1.25–8.88)	0.01
+	–	13	35	0.78 (0.28–2.17)	NS
+	+	43	22	4.13 (1.6–10.62)	0.002
FcγRIIIB-NA1					
–	–	13	23	Reference group	
–	+	36	19	3.35 (1.39–8.07)	0.006
+	–	9	31	0.51 (0.19–1.41)	NS
+	+	37	22	2.98 (1.26–7.04)	0.01

* OR and p values were calculated comparing with the reference group consisting of genotypes negative for the shared epitope and homozygous for the low affinity alleles (FcγRIIA-131R, FcγRIIIA-158F, and FcγRIIIB-NA2, respectively). NS: nonsignificant.

Interaction between different FcγR loci: 2-locus haplotypes. Compared to their controls, the distribution of FcγRIIA-FcγRIIIA haplotypes was skewed in RA probands (p = 0.005 in 4 × 2 contigency tables using estimated haplotype frequencies, Table 5). Among inherited genotypes, there was an excess of FcγRIIIA-158V-FcγRIIA-131H and an underrepresentation of FcγRIIIA-158V-FcγRIIA-131R haplotypes. The risk for RA in probands with the FcγRIIIA-158V-FcγRIIA-131H haplotype was increased (OR 1.86, 95% CI 1.15–3.02).

Analysis after exclusion of double-heterozygous parental genotypes showed similar results. Among the latter, out of 49 FcγRIIIA-158V haplotypes, 37 were associated with FcγRIIA-131H alleles and 12 were associated with FcγRIIA-131R. Of 95 FcγRIIIA-158F haplotypes, 47 were associated with FcγRIIA-131H and 48 with FcγRIIA-131R (p < 0.003 using 2 × 2 contingency tables).

Since the FcγRIIIA polymorphism and the SE showed interactions (Table 4), we analyzed the combined effect of the SE and the FcγRIIIA–FcγRIIA 2-locus haplotypes on disease risk. Comparison between inherited and non-inherited genotypes showed that in SE positive probands, the presence of the high-binding FcγRIIIA-158V–FcγRIIA-131H haplotype increased risk almost 3-fold (OR 2.83, 95% CI 1.25–6.38, inherited vs non-inherited). The risk for RA was not increased in SE negative probands carrying the FcγRIIIA-158V–FcγRIIA-131H haplotype (p = 0.59 compared to SE negative probands with other haplotypes).

Multiple logistic regression analysis. As expected, a strong correlation was observed between the presence of the SE and susceptibility to RA (Table 6). However, the additional

Table 5. Frequencies (%) of FcγR 2-locus haplotypes in inherited and non-inherited genotypes.

	Estimated Haplotype Frequencies			
	Inherited	Non-inherited	OR (95% CI)	p*
FcγRIIA–FcγRIIIA				
131H-158V	29.4	18.2	1.86 (1.15–3.02)	0.005
131R-158V	8.5	19.1	0.39 (0.21–0.74)	
131H-158F	35.2	34.4	1.04 (0.68–1.58)	
131R-158F	26.9	28.2	0.94 (0.60–1.47)	
FcγRIIIA–FcγRIIIB				
158V-NA1	4.3	9.1	0.45 (0.19–1.06)	0.2
158V-NA2	33.6	28.3	1.28 (0.83–1.98)	
158F-NA1	20.9	22.5	0.91 (0.56–1.49)	
158F-NA2	41.2	40.2	1.04 (0.69–1.57)	
FcγRIIA–FcγRIIIB				
131H-NA1	15.8	19.2	0.79 (0.47–1.35)	0.4
131H-NA2	40.5	33.4	1.35 (0.89–2.06)	
131R-NA1	9.4	12.4	0.74 (0.38–1.41)	
131R-NA2	34.2	35	0.97 (0.63–1.48)	

* p in 4 × 2 contingency tables.

Table 6. Multiple logistic regression analysis for the extent of contribution of FcγR alleles to RA susceptibility or disease severity.

Dependent Variables	ß	p
RA susceptibility		
SE	1.54	< 0.0001
FcγRIIIA-158	0.23	0.55
FcγRIIIA-158V–FcγRII-131H	0.56	0.88
Disease severity		
SE	1.10	0.09
RF	0.62	0.32
FcγRIIIA-158V	0.38	0.25
FcγRIIIA-158V–FcγRII-131H	–1.49	0.18

Partial regression coefficient (ß) explanatory variables ($\times_1 ... \times_k$). Dependent variable = $ß_0 + ß_1\times_1 + ß_2\times_2 + ... + ß_k\times_k$.

presence of the FcγRIIIA-158V allele or the FcγRIIIA-158V–131H 2-locus haplotype did not further increase this susceptibility. A moderate correlation was found between the presence of SE and disease severity. The addition of the presence of RF, the FcγRIIIA allele, or the FcγRIIIA-158V–131H 2-locus haplotype did not increase the risk for radiological damage in this model.

Linkage study in multiple-case families. Previous findings in the whole genome-wide scan performed by the ECRAF had shown no evidence of linkage to D1S484, a marker located near the FcγR locus at 173 cM to telomere (IBD 51%, $p = 0.4$)[22].

We observed no deviation from the randomly expected 50% identity-by-descent using 3 additional markers located near the FcγRIA locus (D1S498) and the loci for FcγRIIA, FcγRIIIB, and FcγRIIIA (D1S2844 and D1S2762). The same was true after stratification for sex, RF, SE, and erosive or nodular disease.

DISCUSSION

We found no evidence of association or linkage between FcγR genes and susceptibility to RA. Our results do not indicate that individual polymorphisms in the FcγRIIA, FcγRIIIA, or FcγRIIIB genes play an independent role in susceptibility to RA. The association was tested using family based methods in nuclear families. These methods compare inherited genotypes with non-inherited control genotypes for each proband. This ensures genetically well matched controls and avoids spurious differences between probands and nonrelated controls in case-control studies[21,30]. A lack of association between predisposition to RA and FcγRIIA, FcγRIIIB[17,19,20], and FcγRIIIA polymorphisms[16] has been reported by other groups using such case-control approaches.

Despite the lack of association to individual FcγR polymorphisms in the whole RA cohort, our study suggests that interactions between the SE and the high-binding FcγRIIIA-158V allele (Table 4) and between the SE and the FcγRIIIA-158V–FcγRIIA-131H 2-locus haplotype (Table 5) might be involved in susceptibility to RA.

As shown in Table 4, the presence of the FcγRIIIA-158V allele increased the risk for RA in SE positive (OR 4.1, 95% CI 1.6–10.6), but not SE negative individuals (OR 0.8, 95% CI 0.3–1.7). Interestingly, similar interaction was recently reported in a case-control study among UK Caucasians and North-Indians and Pakistanis[13].

Moreover, we also observed an increased risk for RA associated with the high-binding FcγRIIIA-158V–FcγRIIA-131H 2-locus haplotype (Table 5). This association was also observed only in SE positive probands and was less strong than the association observed for the interaction between 158V and SE; this association needs further confirmation. Nevertheless, this phenomenon was not due to linkage disequilibrium, since FcγR haplotypes were randomly distributed in non-inherited controls. As with the FcγRIIIA-158V allele, this association was only present in SE positive probands. Multiple logistic regression analysis showed, nevertheless, that the presence of the FcγRIIIA-158V allele alone or combined with the FcγRIIA-131H allele neither increases the risk for RA nor predicts disease severity. Similar to our findings, no evidence for linkage disequilibrium between FcγRIIA and FcγRIIIA was observed in a recent case-control study in a Hispanic population[31], and only a weak linkage was observed in UK Caucasians[20].

Our findings indicate that genes contributing to disease susceptibility may differ between patient subsets and that disease phenotype needs to be accounted for in association studies. Moreover, the SE is the main factor related to RA both in association and in linkage studies. Its interaction with the FcγR may have hampered the identification of the latter as an independent susceptibility factor for RA in this and previous studies.

It is well known that FcγR clustering, rather than ligand binding, is critical to initiate cell signaling[4,32]. Moreover, since most inflammatory cells express several FcγR isoforms, coaggregation of more than one type of receptor by the same ligand is likely to occur. Expression of high-binding isoforms of one or several stimulatory FcγR, in our case FcγRIIIA-158V and FcγRIIA-131H, may therefore result in increased binding, uptake, and processing of immune complexes, autoantibodies, and opsonized pathogens by antigen-presenting cells[4,32]. In the context of RA, the presentation of arthritogenic peptides by antigen-presenting cells such as dendritic cells and macrophages to T cells is thought to play an essential role in pathogenesis. Interestingly, both dendritic cells and macrophages coexpress FcγRIIIA, FcγRIIA, and HLA class II molecules. Studies have shown that dendritic cell maturation and antigen presentation are mediated by FcγR[33]. Moreover, macrophages are a major effector cell in the perpetuation of the arthritic process[34], and cross-linking of FcγR results in cell activation and release of chemokines[35,36], proinflammatory cytokines[37], and other mediators of cartilage breakdown.

Concerning the findings in previous case-control studies, a modest association of homozygosity for the FcγRIIIA-158V allele with RA, especially in the nodular phenotype, was suggested by Morgan, *et al*[13]. This was not the case in our study or in another large cohort of Caucasian patients with RA[16]. Nevertheless, the prevalence of nodular RA in our nuclear families might have been too low to allow firm conclusions.

Our findings and 2 other reports[13,16] do not corroborate the association of RA to homozygosity for the low-binding FcγRIIIA-158F allele observed in Southern Spain[14]. A straightforward comparison between studies is not easy, since, in contrast to Mid-European Caucasians, the population in Southern Spain is an admixture of European, North

African, Asian, and Jewish[38]. The discrepancies might also be due to phenotypic differences and to confounding due to other inherited susceptibility factors. Thus the mechanism by which a low-binding allele such as FcγRIIIA-158F could determine the predisposition to RA remains unclear.

As in other association studies, our observations do not exclude the possibility that, in the presence of the SE, the observed association with the FcγRIIIA allele or the FcγRIIIA-FcγRIIA haplotype is due to linkage disequilibrium with neighboring genes on chromosome 1. In view of the distance between the loci for FcγRIIIA and FcγRIIA, linkage disequilibrium between these genes might have been detected in a larger cohort, and this could explain the association with the 2-locus haplotype. Finally, the wide confidence intervals in some of our analyses reflect relatively small numbers in some patient subgroups, and require replication in additional family based studies. Whether the observed interactions between SE, FcγRIIIA, and FcγRIIIA-FcγRIIA haplotypes identify subsets of patients with a more severe disease course must be the subject of future studies.

Taken together, our findings do not suggest an independent role for FcγR genes in susceptibility to RA. The combined presence of SE and either the high-binding FcγRIIIA-158V allele or the FcγRIIIA-158V–FcγRIIA-131H haplotype encoding for high-binding at both loci may increase the disease predisposition to some extent. These associations need investigation in other studies.

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