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## Apoptosis and autoantibodies in systemic lupus erythematosus

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**Fcγ receptor polymorphisms in systemic lupus erythematosus: association with disease and *in vivo* clearance of immune complexes**

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### Summary

Fc receptors for IgG (FcγR) play a prominent role in the clearance of immune complexes in systemic lupus erythematosus (SLE). Polymorphisms of FcγR have been proposed as genetic factors that influence susceptibility to SLE. We analysed three functional FcγR polymorphisms in a strictly Caucasian population of SLE patients, and determined the influence of these polymorphisms on the clearance of immune complexes *in vivo*. Genomic DNA was isolated from 230 Caucasian patients with SLE and 154 controls. Amplification of FcγR-genomic regions in allotype-specific polymerase chain reactions was used to distinguish the genotypes. In addition, we analysed the FcγR genotypes of 13 patients with SLE who participated in a study determining the half-life of IgG-coated erythrocytes in the blood.

We found a strong trend toward skewing of FcγRIIa, with an enrichment of the homozygous FcγRIIa-R/R131 genotype in patients compared with controls. We did not find a correlation between this genotype and the development of lupus nephritis. However, we established that the half-life of IgG-coated erythrocytes in the blood was prolonged in patients expressing the FcγRIIa-R/R131 genotype. The homozygous FcγRIIIa-F/F158 genotype was found more frequently in patients with arthritis and/or serositis. In Caucasian populations, the R/H polymorphism of FcγRIIa is a minor determinant in susceptibility to SLE, whereas the V/F polymorphism of FcγRIIIa is associated with a set of disease manifestations. Notably, the R/H polymorphism of FcγRIIa affects the clearance of immune complexes *in vivo*, which may influence the course of a disease such as SLE.

### Introduction

Systemic lupus erythematosus (SLE) represents the prototype of an immune complex-mediated autoimmune disease. Impaired handling and subsequent tissue deposition of immune complexes are believed to play an important role in the pathogenesis of the disease. Clearance of immune complexes mainly depends on the mononuclear phagocyte system (MPS), in which erythrocytes transport complexes bound via complement receptor 1 to mononuclear phagocytes located in liver and spleen. Abnormalities in complement-mediated clearance have been described in SLE, as well as abnormalities in receptors for the Fc fragment of immunoglobulin G (FcγR) on mononuclear phagocytes [1].

Three distinct classes of FcγR are recognised, which vary in ligand-binding affinity and specificity, as well as in cell distribution [2]. Liver and spleen phagocytes, important constituents of the MPS, express members of all three classes of leukocyte FcγR: FcγRIa, FcγRIIa, and FcγRIIIa. In contrast, neutrophils constitutively express FcγRIIa and FcγRIIIb, and can be induced to express FcγRIa. Many studies have recently identified polymorphisms of FcγR as genetic factors influencing susceptibility to SLE and other

autoimmune diseases. Fc $\gamma$ RIIa, Fc $\gamma$ RIIIa as well as Fc $\gamma$ RIIIb display functional polymorphisms, including an arginine (R) or histidine (H) at amino acid position 131 for Fc $\gamma$ RIIa, a valine (V) or phenylalanine (F) at amino acid position 158 for Fc $\gamma$ RIIIa, and a 4-amino acid variation termed neutrophil antigen (NA) polymorphism (NA1 and NA2) for Fc $\gamma$ RIIIb [3,4]. Indeed, these polymorphisms have a profound influence on human IgG binding; homozygosity for R/R of Fc $\gamma$ RIIa, F/F of Fc $\gamma$ RIIIa, and NA2/NA2 of Fc $\gamma$ RIIIb lessens the ability to interact with specific IgG subclasses [4-9]. Thus, the effect of these polymorphisms on the handling of immune complexes in vivo may have implications for a disease like SLE, although this has not yet been investigated.

In association studies, evidence in support of a role for the Fc $\gamma$ RIIa polymorphism in susceptibility to SLE has been controversial [10-20], whereas evidence for the Fc $\gamma$ RIIIa polymorphism has been less contradictory [6,16,18,20]. No skewing of the Fc $\gamma$ RIIIb polymorphism in patients with SLE has been reported thus far [16]. The discrepancies between studies are striking, in particular for the Fc $\gamma$ RIIa-R/H131 polymorphism. Inconsistencies may arise from variation in population sizes, as became apparent in studies by Song et al [13] and Salmon et al [18]. In addition, variation in the clinical criteria used, specifically with regard to the classification of nephritis, may affect the results. A third influence may be ethnic differences between tested populations [21]. Although most studies are confined to populations of a single ethnic background, some studies investigated populations of mixed ethnic backgrounds [6,14]. Differential distribution of Fc $\gamma$ R polymorphisms among various ethnic populations has been described extensively, for Fc $\gamma$ RIIa in particular [14-15,19,21].

In the present study, we determined the influence of all three functional Fc $\gamma$ R polymorphisms on the susceptibility to SLE in a strictly Caucasian population. We studied 230 Caucasian patients with SLE from 2 large medical centers, and 154 regionally matched controls. We performed our study in a stable, mostly nonmigrating, population, in contrast to several studies conducted previously. To evaluate associations between Fc $\gamma$ R polymorphisms and specific clinical parameters of the disease, the well-defined and uniformly acknowledged SLE criteria of the American College of Rheumatology (ACR) were used, as well as the World Health Organization (WHO) classification criteria for nephritis based on the histologic findings on kidney biopsy. Furthermore, we determined the influence of different Fc $\gamma$ R genotypes on the clearance of immune complexes in 13 patients with SLE, who had participated in a previous study determining the half-life of injected IgG-coated erythrocytes [22].

## Patients and Methods

### *Study population*

Power analysis revealed that 216 patients and 144 controls were required to demonstrate a 15% increase in frequency of the homozygous Fc $\gamma$ RIIa-R/R131 genotype in SLE patients

compared with controls (power of 85%,  $\alpha = 0.05$ ). Subsequently, 230 consecutive, unrelated Caucasian patients with SLE (24 males, 206 females) who were followed up prospectively at the University Hospitals of Groningen and Utrecht in The Netherlands, were included in the study. A total of 154 healthy, unrelated Caucasian blood donors were used for comparison. All patients fulfilled at least 4 of the ACR 1982 revised criteria for the classification of SLE [23]. The median age of the patients was 37 years (range 21-78), with a median disease duration of 9 years (range 1-40). Patient characteristics are shown in table 1.

**Table 1:**

*Clinical characteristics of patients with systemic lupus erythematosus (SLE) in the Fc $\gamma$  receptor (Fc $\gamma$ R) polymorphism association study and the immune complex (IC) clearance study*

Characteristic	Fc $\gamma$ R association study (n=230)	IC clearance study (n=13)
<i>Male / female</i>	24 / 206	4 / 9
<i>Age (years)</i>		
Median (range)	37 (21-78)	38 (24-59)
<i>Disease duration (years)</i>		
Median (range)	9 (1-40)	
<i>ACR criteria (%)</i>		
Malar rash	111 (48)	8 (62)
Discoid rash	49 (21)	3 (23)
Photosensitivity	113 (49)	10 (77)
Oral ulcers	36 (16)	4 (31)
Arthritis	179 (78)	11 (85)
Serositis	76 (33)	2 (15)
Renal involvement	108 (47)	6 (46)
CNS involvement	20 (9)	2 (15)
Haematologic abnormalities	163 (71)	7 (54)
Immunologic abnormalities	209 (91)	12 (92)
Anti-nuclear antibodies	226 (98)	12 (92)

#### *Fc $\gamma$ R genotyping*

Fc $\gamma$ RIIa, Fc $\gamma$ RIIIa and Fc $\gamma$ RIIIb genotyping was performed on genomic DNA of patients and controls using polymerase chain reaction (PCR)-based genotyping methods. The Fc $\gamma$ RIIa-131R/H genotyping assay was performed as described previously [17]. In brief, 2 Fc $\gamma$ RIIa-specific primers were used to amplify a 1,000-bp fragment from the Fc $\gamma$ RIIa gene, containing the polymorphic site. Subsequently, the amplified fragment served as a template to amplify a 278-bp fragment in an allele-specific primer reaction. The genotyping methods for Fc $\gamma$ RIIIa and Fc $\gamma$ RIIIb consisted of allele-specific primer

reactions only, and were performed according to methods described earlier with minor modifications [6,25].

In each experiment, sequence-verified genomic DNA of all 3 genotypes was included and served as controls. Amplified products were analysed by gel electrophoresis (1.5% agarose), stained with ethidium bromide, and visualised under ultraviolet light.

### *Clearance studies*

Clearance studies were performed as described previously [22]. In short, erythrocytes were coated with anti-rhesus antiserum (mainly IgG1 and IgG3, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) and labeled with 500  $\mu$ Ci  $^{99m}$ Tc. Labeling efficiency was ~90%. After washing in saline, cells were injected intravenously in 60 seconds. The injected dose was determined by measuring the syringe before and after injection in a common dose calibrator. Blood samples were taken at 0, 3, 8, 13, 18, and 23 min, and the half-life of IgG-coated erythrocytes in the blood was calculated by regression analyses.

### *Statistical analyses*

Phenotype and genotype frequencies in patients and controls were compared with 3 x 2 and 2 x 2 contingency tables (chi-square test with continuity correction or Fisher's exact test, as appropriate). The 95% confidence intervals (95% CI) of odds ratios (OR) were calculated with the approximation of Woolf. Differences in age at diagnosis between groups were tested by Mann-Whitney U test. Univariate associations between Fc $\gamma$ R genotypes and half-life of IgG-coated erythrocytes in the blood were compared with Spearman's rank correlation test. Two-sided *P* values less than 0.05 were considered significant. Corrections for multiple comparisons were not made due to unknown associations between clinical manifestations.

## **Results**

### *Distribution of genotypes*

In this study, 230 Caucasian patients with SLE (24 males and 206 females), as well as 154 Caucasian controls, were included for Fc $\gamma$ R genotype analysis. The median age of the patients was 37 years (range 21-78), with a median disease duration of 9 years (range 1-40). The median age at diagnosis for women was slightly lower than the median age at diagnosis for men (26 years (range 8-73) versus 32 years (range 14-68), *p* not significant [NS]).

Fc $\gamma$ RIIa, Fc $\gamma$ RIIIa, and Fc $\gamma$ RIIIb genotypes were determined for all patients and controls, using amplification of Fc $\gamma$ R-genomic regions in allotype-specific PCRs. No significant skewing of Fc $\gamma$ RIIa, Fc $\gamma$ RIIIa, or Fc $\gamma$ RIIIb polymorphisms could be observed in this SLE cohort (table 2).

**Table 2:***Distribution of FcγR genotypes in Caucasian patients with SLE and Caucasian controls (%)*

	FcγRIIa			FcγRIIIa			FcγRIIIb		
	R/R 131	R/H 131	H/H 131	V/V 158	V/F 158	F/F 158	NA1/1	NA1/2	NA2/2
<i>Controls</i> (n=154)	32 (21)	80 (52)	42 (27)	15 (10)	73 (47)	66 (43)	27 (17)	66 (43)	61 (40)
<i>SLE</i> (n=230)	68 (30)	108 (47)	54 (23)	30 (13)	108 (47)	92 (40)	42 (18)	101 (44)	87 (38)

*Values are the number (%) of patients. Fcγ = Fcγ receptor; SLE = systemic lupus erythematosus*

However, there was a strong trend toward skewing of the homozygous FcγRIIa-R/R131 genotype, with an enrichment of this genotype in patients with SLE compared with healthy controls (68 of 230 versus 32 of 154, respectively; OR 1.60, 95% CI 0.99-2.59,  $p = 0.071$ ) (table 2). In addition, the median age at diagnosis for patients with the FcγRIIIa-F/F158 genotype was slightly lower than that of patients with other genotypes (25 years (range 8-70) versus 28 years (range 12-73),  $p = 0.082$ ). No differences in sex distribution were observed between the different FcγR genotypes.

#### *Clinical presentation*

Among the SLE patients, 108 patients (47.0%) developed symptoms of nephritis during the course of the disease. The median age at diagnosis in the group of patients with nephritis was slightly lower than in the group without nephritis (25 years (range 8-68) versus 29 years (range 10-73),  $p = \text{NS}$ ). No differences in sex distribution were observed between these 2 groups. When the distribution of FcγR genotypes between patients with nephritis and healthy controls was analysed, no significant skewing was observed for FcγRIIa, FcγRIIIa, or FcγRIIIb (table 3). Remarkably, significant skewing toward the FcγRIIIb-NA1 allele could be detected in patients with nephritis compared with patients without nephritis (frequency of 0.46 versus 0.35, respectively; OR 1.61, 95% CI 1.11-2.35,  $p = 0.016$ ) (table 3).

A renal biopsy was performed in 81 of the 108 patients who presented with nephritis, and the findings were characterised according to WHO classification criteria for nephritis. Within this classification, WHO classes III and IV are of particular interest for this study, since these forms of nephritis are associated with proliferative inflammation, high titers of antinuclear antibodies, as well as complement consumption [26]. However, no significant skewing was observed for FcγRIIa, FcγRIIIa, or FcγRIIIb within this patient group. In contrast, in patients who presented with WHO class II nephritis, marked skewing toward the FcγRIIIb-NA1 allele could be detected. This skewing did not reach statistical significance (frequency of 0.54 versus 0.35 in nonrenal SLE patients; OR 2.18, 95% CI 0.97-4.93,  $p = 0.085$ ) (table 3). Analysis of patients with WHO class V and class VI was not performed due to the small numbers of patients in these groups.

**Table 3:**

*Distribution of Fc $\gamma$ R gene frequencies in controls, SLE patients without nephritis, and SLE patients with nephritis, classified according to World Health Organization (WHO) criteria*

	Fc $\gamma$ RIIa		Fc $\gamma$ RIIIa		Fc $\gamma$ RIIIb	
	R131	H131	V158	F158	NA1	NA2
<i>Controls</i> (n=154)	0.47	0.53	0.33	0.67	0.39	0.61
<i>Non-renal SLE</i> (n=122)	0.54	0.46	0.37	0.63	0.35	0.65
<i>Renal SLE</i> (n=108)	0.52	0.48	0.36	0.64	0.46	0.54
<i>WHO III + IV</i> (n=59)	0.53	0.47	0.36	0.64	0.43	0.57
<i>WHO II</i> (n=13)	0.38	0.62	0.42	0.58	0.54	0.46

*Values are the frequencies of each allele*

With regard to the other ACR criteria, no differences in the distribution of Fc $\gamma$ R genotypes were observed in terms of skin manifestations (malar rash, discoid rash, photosensitivity, oral ulcers), neurologic disorders, or immunologic abnormalities, including the presence of antinuclear antibodies (table 4). However, in patients who presented with symptoms of inflammation associated with an acute-phase response [27, 28], that is, arthritis and/or serositis, significant skewing toward the homozygous Fc $\gamma$ RIIIa-F/F158 genotype could be observed in comparison with patients without these inflammatory manifestations (82 of 190 versus 10 of 40, respectively; OR 2.28, 95% CI 1.05-4.93,  $p = 0.035$ ) (table 4). For arthritis, the Fc $\gamma$ RIIa polymorphism could play an important role additionally, since 19.6% of these patients expressed the combined homozygous Fc $\gamma$ RIIIa-F/F158 and Fc $\gamma$ RIIa-R/R131 genotype, compared with 5.9% of patients without arthritis (35 of 179 versus 3 of 51, respectively; OR 3.89, 95% CI 1.14-13.22,  $p = 0.019$ ). Finally, there was a strong trend toward enrichment of the homozygous Fc $\gamma$ RIIIa-F/F158 genotype in patients with autoantibody-associated cell destruction, that is, haematologic cytopenias, compared with patients without these conditions (72 of 163 versus 20 of 67, respectively; OR 1.86, 95% CI 1.01-3.42,  $p = 0.062$ ) (table 4).

#### *Clearance studies*

The half-life of IgG-coated erythrocytes in the blood, as a measure of Fc receptor function, was determined at the University Hospital of Groningen in a previous study [22]. Thirteen patients who participated in this study were included in our study for Fc $\gamma$ R genotype analysis. One of these 13 patient was deficient for Fc $\gamma$ RIIIb.

When patients with the homozygous Fc $\gamma$ RIIa-R/R131 genotype were compared with patients expressing other Fc $\gamma$ RIIa genotypes, a significant increase in the half-life of IgG-coated erythrocytes in the blood was observed (Spearman's  $r = 0.61$ ,  $p = 0.027$ ) (figure 1a). No differences in half-life of IgG-coated erythrocytes were observed for either Fc $\gamma$ RIIIa or Fc $\gamma$ RIIIb genotypes (figures 1b and 1c). Notably, 2 patients with Fc $\gamma$ RIIa-H/H131, 2 patients with Fc $\gamma$ RIIIa-V/V158 as well as 2 patients with Fc $\gamma$ RIIIb-NA1/NA1 had accelerated clearance rates (figure 1), but these are different patients in each graph.



**Table 4:**

*Distribution of FcγR genotypes in patients with SLE classified according to American College of Rheumatology (ACR) criteria*

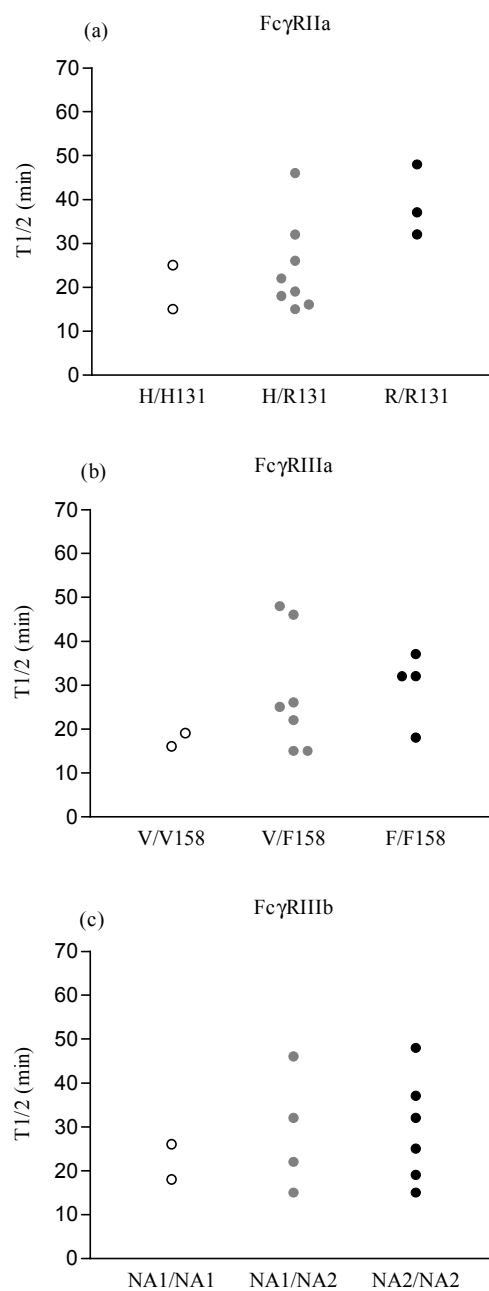
ACR criterion	FcγRIIa			FcγRIIb			FcγRIIIb			
	R/R	H/H + R/H	OR	F/F	V/V + V/F	OR	NA2/2	NA1/1 +1/2	OR	
<i>Malar rash</i>	+	32	79	0.93	48	63	1.30	40	71	0.86
	-	36	83		44	75		47	72	
<i>Discoid rash</i>	+	12	37	0.72	17	32	0.75	23	26	1.62
	-	56	125		75	106		64	117	
<i>Photo-sensitivity</i>	+	30	83	0.75	45	68	0.99	41	72	0.88
	-	38	79		47	70		46	71	
<i>Oral ulcers</i>	+	7	29	0.53	13	23	0.82	13	23	0.92
	-	61	133		79	115		74	120	
<i>Arthritis, serositis</i>	+	58	132	1.32	82	108	2.28*	71	119	0.90
	-	10	30		10	30		16	24	
<i>Renal involvement</i>	+	31	77	0.92	44	64	1.06	30	78	0.44 <sup>†</sup>
	-	37	85		48	74		57	65	
<i>Neurologic symptoms</i>	+	3	17	0.39	10	10	1.56	6	14	0.68
	-	65	145		82	128		81	129	
<i>Haematologic symptoms</i>	+	48	115	0.98	72	91	1.86 <sup>‡</sup>	63	100	1.13
	-	20	47		20	47		24	43	
<i>Immunologic symptoms</i>	+	61	148	0.82	86	123	1.75	79	130	0.99
	-	7	14		6	15		8	13	
<i>Anti-nuclear antibodies</i>	+	66	160	0.41	91	135	2.02	84	142	0.20
	-	2	2		1	3		3	1	

OR = Odds Ratio; \*95% CI 1.05-4.93,  $P = 0.035$ ; <sup>†</sup>95% CI 0.25-0.76,  $P = 0.004$ ; <sup>‡</sup>95% CI 1.01-3.42,  $P = 0.062$ .

## Discussion

In the present study, we determined the influence of three functionally relevant FcγR polymorphisms on susceptibility to SLE and the development of clinical disease manifestations in a strictly Caucasian population. In addition, we determined the influence of different genotypes on the clearance of immune complexes *in vivo* in a pilot study. Analysis of the FcγRIIa-R-H131, FcγRIIIa-V-F158 and FcγRIIIb-NA1-NA2 polymorphisms demonstrated that none of these genotypes constitutes a genetic risk factor for the development of SLE. However, we did find a strong trend toward skewing of FcγRIIa, with an enrichment of the homozygous FcγRIIa-R/R131 genotype in patients with SLE compared to healthy controls.

Remarkably, this genotype was also associated with decreased clearance of IgG-coated erythrocytes in the blood. The results of our study are in accordance with most association studies on the Fc $\gamma$ RIIa-R/H131 polymorphism and susceptibility to SLE, performed in at least five different ethnic backgrounds [11, 14-20]. In contrast, some studies found an apparent correlation between the homozygous Fc $\gamma$ RIIa-R/R131 genotype and the occurrence of SLE [10, 12-13]. Notably, a recent study in a Caucasian German population did not demonstrate an association of the homozygous Fc $\gamma$ RIIa-R/R131 genotype and susceptibility to SLE, but showed that this genotype was significantly correlated with higher frequencies of various clinical and serological parameters, including a younger age at disease onset [17]. We could not reproduce these associations in our study, despite our finding of a trend toward enrichment of the homozygous Fc $\gamma$ RIIa-R/R131 genotype in our patient population in general. Therefore, in agreement with that previous study [17], we conclude that in Caucasian populations, the R/H polymorphism of Fc $\gamma$ RIIa does not represent a strong genetic risk factor for SLE, but constitutes a relatively minor determinant in susceptibility to the disease. We speculate that this polymorphism may influence the course of the disease by influencing the clearance of immune complexes, since we found an apparent correlation between these parameters in our in vivo clearance studies.



**Figure 1:** Half life (T1/2) of IgG-coated erythrocytes in the blood of patients with systemic lupus erythematosus expressing different Fc $\gamma$  receptor (Fc $\gamma$ R) genotypes. Half life was prolonged in patients expressing the Fc $\gamma$ RIIa-R/R131 genotype (a), demonstrating an effect of this genotype on the clearance of immune complexes in vivo. No differences were observed for either Fc $\gamma$ RIIIa (b) or Fc $\gamma$ RIIIb (c) genotypes.

Our results do, however, challenge 2 of 3 studies on the Fc $\gamma$ RIIIa-V-F158 polymorphism and susceptibility to SLE. In a population of mixed ethnic background, as well as in a Caucasian population, apparent skewing was observed toward the homozygous Fc $\gamma$ RIIIa-F/F158 genotype [6, 16]. We could not reproduce these results in our population, which is consistent with the finding of a recent study in an African American population [20]. Finally, we did not find an association between the Fc $\gamma$ RIIIb-NA1/NA2 polymorphism and susceptibility to SLE, as one other study previously described [16].

We also assessed the influence of Fc $\gamma$ R polymorphisms on the development of clinical manifestations. Of particular interest in our analysis was glomerulonephritis, since this complication is associated with the deposition of immune complexes and autoantibodies at the glomerular basement membrane [29]. In our patient population, we could not demonstrate a correlation between the occurrence of nephritis and the R/H polymorphism of Fc $\gamma$ RIIa, or the V/F polymorphism of Fc $\gamma$ RIIIa. This finding is in agreement with most studies on Fc $\gamma$ RIIa [14-20], but in contrast to 3 studies on Fc $\gamma$ RIIIa [6,16,18]. It should be noted, however, that the latter studies were performed in 3 different ethnic backgrounds. In a previous study conducted at our hospitals [11], glomerulonephritis was found to be significantly associated with the Fc $\gamma$ RIIa-R/R131 genotype compared with healthy controls. Although there is some overlap between the cohorts of patients and controls that participated in the 2 studies, we could not reproduce this association in the present study. The observed difference may be attributable to differences in the criteria used to define renal involvement, as well to the different sample sizes between these studies. Notably, no significant association was found upon comparison of patients with and without nephritis in either study.

Remarkably, we did find significant skewing toward the Fc $\gamma$ RIIIb-NA1 allele in patients with nephritis compared with patients without nephritis. This skewing was not apparent in patients who presented with WHO class III or IV nephritis, but was notable in patients with WHO class II nephritis. Because we would argue that the low-binding NA2 allele of Fc $\gamma$ RIIIb is associated with reduced clearance of immune complexes, this finding is rather unexpected. A previous study in a Caucasian population did not demonstrate a correlation between nephritis and Fc $\gamma$ RIIIb alleles [16].

In patients who presented with symptoms of inflammation associated with an acute-phase response [27,28], that is, arthritis or serositis, we observed significant skewing toward the homozygous Fc $\gamma$ RIIIa-F/F158 genotype. These manifestations are often associated with raised C-reactive protein (CRP) levels, as opposed to cerebral lupus or renal involvement [27,28]. Recently, CRP has been demonstrated to interact with Fc $\gamma$ R on phagocytic cells [30], and this interaction might be polymorphism-dependent [31]. Indeed, this finding may have important implications for the inflammatory potential of immune complexes in patients who are homozygous for certain Fc $\gamma$ R alleles. For the development of arthritis, the Fc $\gamma$ RIIa polymorphism could play an important role additionally, since 1 in 5 patients with this disease complication were shown to express the combined Fc $\gamma$ RIIIa-F/F158 and Fc $\gamma$ RIIa-R/R131 genotype. This combined Fc $\gamma$ R genotype has also been shown to

influence the relapse rate in patients with other autoimmune diseases [32], and a critical role for at least 2 Fc $\gamma$ R has been demonstrated in models of collagen-induced arthritis using FcR-deficient mice [33].

A strong trend toward enrichment of the homozygous Fc $\gamma$ RIIIa-F/F158 genotype was also observed in patients with haematologic abnormalities, that is, hemolytic anemia, leukopenia, lymphopenia, and thrombocytopenia. The prognosis of SLE is negatively influenced by the occurrence of autoimmune cytopenias, as has been clearly demonstrated for anemia and thrombocytopenia [34-36]. Indeed, the V-F polymorphism of Fc $\gamma$ RIIIa might influence the clearance of autoantibody-opsonized blood cells, leading to subsequent Fc receptor-mediated destruction of these cells. Notably, a critical role for Fc $\gamma$ R has been demonstrated in models of experimental hemolytic anemia and thrombocytopenia in FcR  $\gamma$ -chain-deficient mice that lack Fc $\gamma$ RI and Fc $\gamma$ RIII expression [37].

Finally, SLE patients with the homozygous Fc $\gamma$ RIIIa-F/F158 genotype presented with disease at a slightly younger age than did patients with other Fc $\gamma$ R genotypes, although this difference did not reach statistical significance. In view of these findings, we conclude that in our Caucasian population, the Fc $\gamma$ RIIIa polymorphism constitutes a factor that influences the clinical disease manifestations and the course of SLE, without representing a genetic risk factor for susceptibility to SLE. However, since corrections for multiple testing were not made due to the explorative nature of this study, and because there may be unknown associations between clinical manifestations, the observed associations deserve to be interpreted with caution.

Genetic linkage studies have recently provided evidence for an SLE-associated locus on the long arm of chromosome 1, either including [38] or excluding [39] the Fc $\gamma$ R-encoding gene cluster (mapped to 1q23-24). Despite the fact that the genes for Fc $\gamma$ RIIa, Fc $\gamma$ RIIIa and Fc $\gamma$ RIIIb are clustered in close proximity on chromosome 1, there is no evidence for non-random distribution of Fc $\gamma$ R genotypes within healthy control populations [40]. Accordingly, we did not find evidence for a skewed distribution of combinations of Fc $\gamma$ R genotypes between SLE patients and healthy controls in our analyses.

In conclusion, the present data support the notion that in Caucasian populations, the allelic variant of Fc $\gamma$ RIIa is a relatively minor determinant in susceptibility to SLE, whereas the allelic variants of Fc $\gamma$ RIIIa may influence the development of clinical disease manifestations. Notably, the R/H polymorphism of Fc $\gamma$ RIIa affects the clearance of immune complexes in vivo, which may indeed profoundly influence the course of a disease like SLE.

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