Fc-gamma receptor polymorphisms differentially influence susceptibility to systemic lupus erythematosus and lupus nephritis

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Abstract

Objective. To determine relevant Fc-gamma receptor (FcγR) polymorphisms in relation to susceptibility to SLE and LN, and to determine the functional consequences of genetic associations found.

Methods. Using multiplex ligation-dependent probe amplification, copy number regions (CNRs) and relevant known functional single nucleotide polymorphisms of FcγRII and FcγRIII were determined in a LN-enriched cohort of 266 Dutch Caucasian SLE patients and 919 healthy Caucasian controls. Expression of FcγRs on leukocytes was assessed using flow cytometry.

Results. In multivariable analysis, low copy number of CNR1 (including FCGR3B; odds ratio (OR) 2.04; 95% CI: 1.29, 3.23), FCGR2A-131RR (OR 2.00; 95% CI: 1.33, 2.99), and the 2B.4 haplotype of FCGR2B (OR 1.59; 95% CI: 1.13, 2.24), but not FCGR2C open reading frame, were significantly (all P < 0.01) and independently associated with susceptibility to SLE. The 2B.4 haplotype was negatively associated with LN and led to surface expression of FcγRIIb on neutrophils and monocytes.

Conclusion. This study is the first to investigate the most relevant and functional single nucleotide polymorphisms and copy number variations of FcγRII and FcγRIII polymorphisms in one study population, enabling the determination of the individual contribution of each polymorphism in multivariable analysis. Three polymorphisms were shown to be independently associated with susceptibility to SLE. The novel findings of a negative association of the 2B.4 haplotype with LN, and increased expression of FcγRIIb on neutrophils and monocytes as a result of this 2B.4 haplotype warrant future research in the role of these cells and FcγRs in the pathogenesis of SLE and LN.

Key words: Fc-gamma receptor, gene polymorphisms, systemic lupus erythematosus, lupus nephritis, Caucasian, FcγRIIb, FcγRIIC
Introduction

SLE is an autoimmune disease that is characterized by the formation of autoantibodies, deposition of immune complexes and inflammation, and can affect virtually every organ system in the body. Aetiologically, a multifactorial model is presumed, including genetic, hormonal and environmental factors, in which defective clearance and degradation of apoptotic cells is thought to have a pivotal role in developing SLE [1, 2].

The receptors for the Fc portion of IgG [Fc-gamma receptors (FcγRs)] play an important role in the clearance of immune complexes and presentation of the complexed antigen, and provide pro- or anti-inflammatory regulation of immune cell responses [3]. There are three families of FcγRs: of which FcγRI is a high-affinity receptor and FcγRII and FcγRIII are low-affinity receptors [4]. Within these families different subclasses exist. FcγRII Rs trigger activating signalling pathways, except FcγRIIib, which triggers inhibitory signalling pathways. FcγRIIib is a distinct receptor expressed on neutrophils that is not known to associate with signalling molecules. Although it may activate cells in some cases, it may also function as a decoy receptor, helping to clear immune complexes [5, 6]. Failure of FcγRI-mediated clearance of immune complexes and control of inflammatory responses are thought to be predisposing factors for the development of SLE [7].

The relationship between SLE and FcγRII has been extensively studied [8]. The FCGR2/3 locus on chromosome 1q23.3 that encodes the low-affinity FcγRII is subject to both single nucleotide polymorphisms (SNPs) and copy number variation (CNV) (Fig. 1) [9]. Indeed, several of these polymorphisms lead to an altered function of FcγRs [10–13]. Meta-analyses have shown an increased risk for the development of SLE for these polymorphisms, with considerable differences between ethnic groups [14–20]. However, these meta-analyses only studied the effect of a single polymorphism and did not take linkage disequilibrium into account.

Furthermore, studies on the relationship between two specific polymorphisms and susceptibility to clinical manifestations of SLE are very limited or even absent. The first, FCGR2C-open reading frame (ORF), is the result of a SNP in exon 3 on FCGR2C that leads to an ORF, instead of the common stop codon (FCGR2C-Stop) [21, 22]. If an ORF is present, then FcγRIIC is expressed on natural killer cells and is able to induce antibody-dependent cell-mediated cytotoxicity and a rise in intracellular Ca2+ [23, 24]. FCGR2C-ORF has not been previously described in relation to SLE. The other, a SNP in the promoter region of FcγRIIb, also known as 2B4, was found to be more frequently present in SLE patients compared with healthy controls, although the functional consequences of this SNP are not completely clear [25, 26].

Studies on associations between FcγR polymorphisms and specific clinical manifestations of SLE have mainly focused on LN, defined according to ACR criteria (proteinuria and/or cell casts) or biopsy proven. Associations with LN were found for the FCGR3A-158F allele [18, 27, 28], but not for other polymorphisms [14, 19, 20]. Only a limited number of studies investigated the relationship between FcγRI polymorphisms and other clinical SLE manifestations. These studies were performed in patient groups of different ethnic backgrounds and reported various associations without a distinct direction [29–31].

The aim of the present study is to determine associations between several known and one novel (FCGR2C-ORF) genetic polymorphisms and susceptibility to SLE in Caucasian patients. Secondly, to investigate the relationship between specific polymorphisms and renal disease in SLE.

Materials and patients

Subjects

DNA was available from 266 Dutch Caucasian SLE patients and 919 healthy Caucasian controls (199 from the Netherlands, 156 from Australia, 478 from Austria, 86 from the UK) who were randomly selected. SLE patients were recruited from two longitudinal SLE cohorts from VU University Medical Center (VUmc) (n = 98) and University Medical Center Groningen (UMCG) (n = 86), and from the first and second Dutch Lupus Nephritis Study (n = 82) [32]. In both Dutch Lupus Nephritis Studies, patients with a biopsy-proven proliferative LN were included. Both VUmc and UMCG provide primary through tertiary care for SLE patients. Data obtained from these patients include sex and disease duration (in years). Cumulative manifestations according to the updated revised ACR criteria for SLE were available for all SLE patients [33]. These manifestations include malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, serositis, nephrological involvement (i.e. presence of proteinuria >0.5 g/24 h and/or cell casts), neurological involvement (i.e. epilepsy and/or psychosis), haematological involvement (haemolytic anaemia, thrombopenia, leukopenia and lymphopenia), immunological involvement (presence of anti-ds DNA, anti-Sm or aCL and/or presence of lupus anticoagulant) and presence of ANA. Other recorded data were a history of biopsy-proven LN and, if present, the classification of LN according to the WHO classification system [34]. In the case of combined LN classes, the most prominent class in the biopsy was...
The approximate extent of CNRs, which show both duplication and deletion, is indicated by black lines at the bottom. SNPs and haplotypes investigated are indicated with arrows. FCG2A-Q27W: SNP with unknown function; FCG2A-H131R: 131H variant has increased binding affinity to IgG; FCG2A-V158F: 158V variant has increased binding affinity to IgG; FCG2C-ORF/Stop: FCG2C contains variants that can (FCG2C-ORF) or cannot (FCG2C-Stop) be expressed; FCG2B-NA1/NA2/SH: haplotypes determined by six SNPs, respectively encoding the HNA1a/HNA1b/HNA1c antigenic variants of HNA1; FCG2B-promoter 2B.1/2B.4: haplotypes in the promoter of FCG2B that influence expression; FCG2B-I232T: 232T variant has decreased signalling capacity. CNR: copy number variable region; SNP: single nucleotide polymorphism; CNV: copy number variation; HNA: human neutrophil antigen.

recorded. For this study, both definitions of LN were used, namely by clinical presence of proteinuria and/or cell casts (i.e. according to ACR criteria), and/or by histology (i.e. biopsy-proven LN). It should be noted that histological evidence through biopsy is the current gold standard for LN, whereas the ACR criteria for LN are less invasive and use a clinically more feasible method, but are not as reliable as histology since proteinuria and cell casts may have other causes. In this paper, LN according to ACR criteria will be termed nephrological manifestations, whereas histological evidence of LN will be termed biopsy-proven. Written informed consent was obtained from all patients. The VUmc Medical Ethics Committee approved this study.

Multiplex ligation-dependent probe amplification

Multiplex ligation-dependent probe amplification (MLPA) was performed as described extensively by Breunis et al. [23]. In short, MLPA probes were designed specifically for the FCG2A, FCG2B, FCG2C, FCG3A and FCG3B genes on multiple sites in the genes. In this way, CNV regions for FCG2A, FCG3A and FCG3B could be defined (Fig.1). Probes were also included to detect the following SNPs: rs1801274 (FCG2A c.497A > G [p.H131R]), rs9427397 and rs9427398 (combined rs201218629), FCG2A c.184C > T and c.185A > G, p [Q27W], rs1050501 (FCG2B c.695T > C [p.I232T]), rs396991 (FCG3A c.526G > T [p.V158F]) and FCG3B haplotypes (NA1/NA2/SH). The assay also contained a probe specific for the stop codon in exon 3 of the FCG2C gene, rs759550223 c.169T [p.57X] and a non-specific FCG2B/C probe to detect the ORF in exon 3 [p.57Q]. Probes were also included for the splice site mutation at the border of exon 7-intron 7 in FCG2C (rs76277413 c.798 +1 A > G), to distinguish the non-expressed nonclassical FCG2C-ORF variant from the classical FCG2C-ORF that is typically expressed on NK cells, monocytes and neutrophils [22]. Because the nonclassical FCG2C-ORF variant is not expressed [22], it was grouped with FCG2C-Stop in all statistical analyses and expression studies. The assay also contained non-specific probes for the promoter regions of FCG2B and FCG2C, FCG2B/C -386C > G (rs143796418 in FCG2B, rs149754834 in FCG2C and FCG2B/C -120A > T (rs780467580 in FCG2B, rs34701572 in FCG2C)). These promoter polymorphisms make up the promoter haplotypes 2B.1, 2B.2 and 2B.4 as described by Su et al. [35]. Haplotype 2B.1 consists of a G at position -386 and a T at position -120 (nucleotide positions relative to start codon of FCG2B or FCG2C), haplotype 2B.2 consists of -386C and -120T, haplotype 2B.4 consists of -386C and -120A. Exact location of these promoter polymorphisms was determined with a gene-specific long-range PCR for FCG2B and FCG2C as described previously [9]. Since -120A only occurred in the promoter of FCG2B (supplementary Table S1, available at Rheumatology Online), promoter haplotypes were constructed from MLPA data as follows: any C at -386 accompanied by an A at -120 was designated as a 2B.4, and was allocated to FCG2B. For all seven
individuals with 2B.4 haplotypes in FCGR2B studied for expression analysis, this was confirmed with the gene-specific long-range PCR for FCGR2B (supplementary Table S1, available at Rheumatology Online, and data not shown).

Flow cytometry

Whole blood leukocytes were isolated from heparin blood by lysis of red blood cells with an isotonic ammonium chloride buffer. Expression levels of different FcγRs on various types of leukocytes of 136 healthy Dutch Caucasian donors were determined by flow cytometry. The following mAbs were used to detect leukocytes subsets: anti-CD3-Pe-Cy7 clone SK7 (T cells), anti-CD14-Pe-Cy7 clone M5E2 (monocytes), anti-CD19-APC clone HIB19 (B cells) and anti-CD56-APC clone B159 (NK cells), all from BD Pharmingen (San Diego, CA, USA). Neutrophils were selected on the forward scatter (FSC)/side scatter (SSC) pattern. FcγRI expression was measured with anti-FcγRII-FITC clone 3G8 (BD Pharmingen), and anti-FcγRIIb/c clone 2B6, Alexa Fluor 488 labelled (a generous gift from MacroGenics, Rockville, MD, USA). This clone binds FcγRIIb and FcγRIIc equally well [22], but does not bind FcγRIIa [36]. To ensure specific detection of FcγRIIb, only individuals without the FCGR2C-ORF genotype (who cannot express FcγRII) were analysed (n = 105) were included for the analysis of FcγRIIb expression levels. Non-specific binding and background fluorescence were corrected for by subtracting the median fluorescence intensity of relevant isotype controls and UMCG lupus cohorts, nephrological manifestations (i.e. presence of proteinuria > 0.5 g/24 h and/or cell casts) were observed in 71 (38.6%) patients, of which 49 (26.6%) were biopsy proven. All 82 patients from the Dutch Lupus Nephritis Study had a history of nephrological manifestations and biopsy-proven LN.

Susceptibility to SLE

Table 1 shows the frequencies of tested CNVs and SNPs in SLE patients and healthy controls. In all patients and controls, CNV always occurred as a combination of FCGR2C with either FCGR3A or FCGR3B, in three distinct CNV regions (CNRS) as previously defined (Fig. 1) [7]. The CNRs were analysed as such for susceptibility to SLE and this revealed a significant association with low copy number of CNR1 (FCGR2C and FCGR3B) (Table 2). Copy number changes in CNR2 or CNR3 were not significantly associated with susceptibility to SLE. For the SNPs, increased susceptibility to SLE was found for FCGR2A-131R and a trend for the 2B.4 haplotype of FCGR2B (Table 1). FCGR2A-27W, FCGR3A-158V, FCGR2C-ORF, FCGR3B-NA1, FCGR3B-SH and FCGR2B-232I were not associated with susceptibility to SLE (Table 1). In a multivariable regression analysis, FCGR2A-131R, CNR1 and the 2B.4 haplotype were all independently and significantly associated with an increased susceptibility to SLE (Table 2).

Effect of FCGR3B CNV and the -386C/G and −120A/T SNPs in the promoter of FCGR2B on expression levels

Two of the three significantly associated variants conceivably have an impact on the expression levels of FcγRs. In order to test this relationship, the expression levels of different FcγRs on leukocytes were determined in a large group (n = 136) of our Dutch Caucasian healthy controls, using flow cytometry. Hereby, the strong correlation of FCGR3B copy number with expression of FcγRIIb on neutrophils was confirmed (Fig. 2), indicating that CNV
The main findings of our study are an independent and significant association with susceptibility to SLE of FCGR2A-131R, copy number of CNR1 (including FCGR3B) and the 2B.4 haplotype in the promoter of FCGR2B, which encodes the inhibitory Fc\(\gamma\)RIIb. Furthermore, a negative association was observed within the FCGR2C-ORF region. Table 1 summarizes the copy numbers and allele frequencies of Fc\(\gamma\)R II and III genes in healthy controls and SLE patients.

**Discussion**

Focusing on the clinical relevance of these findings, we evaluated the impact of the identified polymorphisms on the expression levels of Fc\(\gamma\)RIIb, a key regulator of immune responses. We demonstrated that the 2B.4 haplotype results in a clear gene-dosage effect. When determining the effect of the promoter haplotype 2B.4 on expression levels of Fc\(\gamma\)RIIb, it is important to consider that monoclonal antibodies that recognize the extracellular domain of Fc\(\gamma\)RIIb will also recognize Fc\(\gamma\)RIIc. This is due to the fact that the extracellular part of these receptors is identical. Individuals with FCGR2C-ORF will express Fc\(\gamma\)RIIc on NK cells, neutrophils and monocytes [22], and possibly B cells [37]. The analysis of expression levels of Fc\(\gamma\)RIIb should therefore be performed in FCGR2C-Stop donors only. Five healthy FCGR2C-Stop donors heterozygous for 2B.4 and two homozygous for 2B.4 were tested in an expression analysis. This revealed that the 2B.4 haplotype causes expression of Fc\(\gamma\)RIIb on neutrophils in the steady state, whereas in individuals with the 2B.1 haplotype, its expression is virtually absent (Fig. 3A). A similar effect was observed in monocytes (Fig. 3B), but on B cells, which express high levels of Fc\(\gamma\)RIIb, no difference between the different promoter haplotypes was observed (Fig. 1D). The 2B.4 haplotype did not cause expression of Fc\(\gamma\)RIIb on T cells or NK cells (Fig. 1C and E).

**Susceptibility to LN**

We then tested within the patient cohort for associations of FCGR2/3 polymorphisms with susceptibility to renal disease in SLE. This revealed that the presence of the FCGR2B promoter haplotype 2B.4 was statistically significant and negatively associated with a clinical history of nephrological manifestations or biopsy-proven LN (Table 3). Only two patients were homozygous for the 2B.4 haplotype and neither had a history of nephrological manifestations or biopsy-proven LN. Other SNPs and CNVs were not significantly associated with renal manifestations or SLE. Out of 131 patients with biopsy-proven LN, 119 (90.8%) had a predominantly class III or IV LN, 9 (6.9%) had class V LN and 2 (1.5%) had class II LN. One patient had a biopsy-proven LN of which the class could not be retrieved by chart review. We did not observe any significant differences between the tested allele polymorphisms and classes III and IV LN compared with the other LN classes (data not shown).

Finally, ORs and CIs of disease manifestations other than renal were calculated for the different polymorphisms (supplementary Table S3, available at Rheumatology Online). In the presence of many clinical manifestations, a relatively small study population and multiple testing, no clear associations were found for the first time, FCGR2C-ORF in SLE was of FCGR2B results in a clear gene-dosage effect. When determining the expression levels of Fc\(\gamma\)RIIb, it is important to consider that monoclonal antibodies that recognize the extracellular domain of Fc\(\gamma\)RIIb will also recognize Fc\(\gamma\)RIIc. This is due to the fact that the extracellular part of these receptors is identical. Individuals with FCGR2C-ORF will express Fc\(\gamma\)RIIc on NK cells, neutrophils and monocytes [22], and possibly B cells [37]. The analysis of expressions levels of Fc\(\gamma\)RIIb should therefore be performed in FCGR2C-Stop donors only. Five healthy FCGR2C-Stop donors heterozygous for 2B.4 and two homozygous for 2B.4 were tested in an expression analysis. This revealed that the 2B.4 haplotype causes expression of Fc\(\gamma\)RIIb on neutrophils in the steady state, whereas in individuals with the 2B.1 haplotype, its expression is virtually absent (Fig. 3A). A similar effect was observed in monocytes (Fig. 3B), but on B cells, which express high levels of Fc\(\gamma\)RIIb, no difference between the different promoter haplotypes was observed (Fig. 1D). The 2B.4 haplotype did not cause expression of Fc\(\gamma\)RIIb on T cells or NK cells (Fig. 1C and E).

**Discussion**

The main findings of our study are an independent and significant association with susceptibility to SLE of FCGR2A-131R, copy number of CNR1 (including FCGR3B) and the 2B.4 haplotype in the promoter of FCGR2B, which encodes the inhibitory Fc\(\gamma\)RIIb. Furthermore, a negative association was observed within the FCGR2C-ORF region. Table 1 summarizes the copy numbers and allele frequencies of Fc\(\gamma\)R II and III genes in healthy controls and SLE patients.

**Table 1** Copy numbers and allele frequencies of Fc\(\gamma\)R II and III genes in 919 healthy controls and 266 SLE patients

<table>
<thead>
<tr>
<th>Variant</th>
<th>Healthy controls, n (%)</th>
<th>SLE patients, n (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
<td></td>
</tr>
<tr>
<td>Copy number region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNR1</td>
<td>1 (0.1) 60 (6.5) 766 (83.4) 84 (9.1) 8 (0.9)</td>
<td>0 (0) 33 (12.4) 205 (77.1) 28 (10.5) 0 (0)</td>
<td>0.0099</td>
</tr>
<tr>
<td>CNR2</td>
<td>0 (0) 11 (1.2) 867 (94.3) 40 (4.4) 1 (0.1)</td>
<td>0 (0) 1 (0.4) 256 (96.2) 9 (3.4) 0 (0)</td>
<td>0.5709</td>
</tr>
<tr>
<td>CNR3</td>
<td>0 (0) 0 (0) 917 (99.8) 2 (0.2) 0 (0)</td>
<td>0 (0) 0 (0) 265 (99.6) 1 (0.4) 0 (0)</td>
<td>0.6497</td>
</tr>
<tr>
<td>Allele frequencies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCGR2A-131R</td>
<td>269 (29.3) 463 (50.4) 187 (20.3) 0 (0) 0 (0)</td>
<td>56 (21.1) 133 (50.0) 76 (28.8) 0 (0) 0 (0)</td>
<td>0.538  0.0034</td>
</tr>
<tr>
<td>FCGR2A-27W</td>
<td>713 (77.6) 194 (21.1) 12 (1.3) 0 (0) 0 (0)</td>
<td>211 (79.3) 51 (19.2) 4 (1.5) 0 (0) 0 (0)</td>
<td>0.111  0.7890</td>
</tr>
<tr>
<td>FCGR3A-158V</td>
<td>385 (41.9) 403 (43.9) 129 (14.0) 2 (0.2) 0 (0)</td>
<td>116 (43.8) 111 (41.7) 39 (14.7) 0 (0) 0 (0)</td>
<td>0.355  0.8886</td>
</tr>
<tr>
<td>FCGR2C-ORF</td>
<td>719 (78.2) 185 (20.1) 14 (1.5) 1 (0.1) 0 (0)</td>
<td>207 (77.8) 53 (19.9) 6 (2.3) 0 (0) 0 (0)</td>
<td>0.122  0.7517</td>
</tr>
<tr>
<td>FCGR2B-NA1</td>
<td>373 (40.6) 428 (46.6) 116 (12.6) 2 (0.2) 0 (0)</td>
<td>97 (36.9) 141 (53.0) 28 (10.3) 0 (0) 0 (0)</td>
<td>0.370  0.2809</td>
</tr>
<tr>
<td>FCGR2B-SH</td>
<td>873 (95.0) 46 (5.0) 0 (0) 0 (0) 0 (0)</td>
<td>254 (95.5) 12 (4.5) 0 (0) 0 (0) 0 (0)</td>
<td>0.023  0.8721</td>
</tr>
<tr>
<td>FCGR2B-232T</td>
<td>720 (78.3) 181 (19.7) 18 (2.0) 0 (0) 0 (0)</td>
<td>216 (81.2) 46 (17.3) 4 (1.5) 0 (0) 0 (0)</td>
<td>0.102  0.6210</td>
</tr>
<tr>
<td>FCGR2B haplotype 2B.4</td>
<td>748 (81.4) 157 (17.1) 14 (1.5) 0 (0) 0 (0)</td>
<td>203 (76.3) 61 (22.9) 2 (0.8) 0 (0) 0 (0)</td>
<td>0.122  0.0809</td>
</tr>
</tbody>
</table>

Text in bold indicates significant values. CNR: copy number region; Fc\(\gamma\)R: Fc gamma receptor; MAF: minor allele frequency.
investigated and an association with increased susceptibility to SLE was not found.

The strength of the present study is that it is the first to investigate the most relevant and functional SNPs and CNVs of FcγRII and FcγRIII polymorphisms in one and the same study population, enabling the determination of the individual contribution of each polymorphism in multivariable analysis. This analysis has not been done before as extensively as in the current cohort study. Previous studies and meta-analyses focused on a single SNP or CNV in relationship to SLE and/or LN susceptibility. Furthermore, detailed data of the SLE patients were available, including the results of the gold standard for LN, which is renal biopsy.

Regarding susceptibility to SLE with FCG2A-131R, two meta-analyses also demonstrated FCG2A-131R to be a susceptible genotype in patients of European descent [16, 17]. In addition, a meta-analysis showed low copy number of FCG3B to be a susceptibility genotype for SLE in a meta-analysis [38]. However, our data now show that CNV of FCG3B occurs solely as a combined deletion of different genes, collectively termed CNR1, and therefore, the interpretation of a low copy number of FCG3B may not be as straightforward as it seems. Deletion of CNR1 in theory has four effects that could contribute to SLE susceptibility, as it contains three genes (FCGR2C, HSPA7, and FCG3B) with CNV, and additionally leads to the ectopic expression of FcγRIIb on NK cells [22]. However, CNV of FCG2C in itself cannot account for susceptibility to SLE, being in most cases a pseudogene (and only the number of copies of the FCG2C-ORF variant is associated with functional differences). Similarly, HSPA7 is a non-expressed pseudogene. On the other hand, individuals with a deletion of CNR1 all had ectopic expression of FcγRIIb on NK cells, confirming our earlier finding in a larger group [22]. Indeed, the ectopic expression of FcγRIIb on NK cells was recently suggested as a potential explanation for the increased susceptibility to SLE [39], although not supported by any experimental evidence. The gene dosage effect of copy number of FCG3B on FcγRIIb expression therefore seems to be the more prominent and logical explanation for the susceptibility to SLE, as it may lead to impaired clearance of immune complexes from the circulation [5, 6]. Finally, the third risk factor identified in our susceptibility study was the promoter haplotype 2B.4, which confirms the findings of two earlier studies [25, 26]. The effects of this promoter haplotype that have been reported thus far are contradictory. One group showed an increase of FcγRIIib on B cells as well.

### Table 2

<table>
<thead>
<tr>
<th>Variant</th>
<th>Contrast</th>
<th>Single model OR (95% CI)</th>
<th>P-value</th>
<th>Multiple model OR (95% CI)</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>CNR1</td>
<td>Overall</td>
<td>0.00 (0.00, ∞)</td>
<td>0.007</td>
<td>0.00 (0.00 - ∞)</td>
<td>0.010</td>
</tr>
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<td></td>
<td>0 vs 2</td>
<td>0.00 (0.00, ∞)</td>
<td>0.992</td>
<td>0.00 (0.00 - ∞)</td>
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<tr>
<td></td>
<td>1 vs 2</td>
<td>2.06 (1.31, 3.24)</td>
<td>0.002</td>
<td>2.04 (1.29, 3.23)</td>
<td>0.002</td>
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<tr>
<td></td>
<td>3 vs 2</td>
<td>1.25 (0.79, 1.97)</td>
<td>0.336</td>
<td>1.15 (0.72, 1.8)</td>
<td>0.565</td>
</tr>
<tr>
<td></td>
<td>4 vs 2</td>
<td>0.00 (0.00, ∞)</td>
<td>0.978</td>
<td>0.00 (0.00, ∞)</td>
<td>0.978</td>
</tr>
<tr>
<td>FCG2A-131H/R</td>
<td>Overall</td>
<td>0.004</td>
<td>0.003</td>
<td>0.004</td>
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<tr>
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<td>HR vs HH</td>
<td>1.38 (0.98, 1.96)</td>
<td>0.067</td>
<td>1.43 (1.00, 2.03)</td>
<td>0.047</td>
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<tr>
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<td>RR vs HH</td>
<td>1.95 (1.32, 2.89)</td>
<td>0.001</td>
<td>2.00 (1.33, 2.99)</td>
<td>0.001</td>
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<td>FCG2B haplotype 2B.4</td>
<td>Overall</td>
<td>0.066</td>
<td>0.026</td>
<td>0.066</td>
<td>0.026</td>
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<td>2B.1/2B.4 vs 2B.1/2B.1</td>
<td>1.44 (1.03, 2.01)</td>
<td>0.033</td>
<td>1.59 (1.13, 2.24)</td>
<td>0.008</td>
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<td>2B.4/2B.4 vs 2B.1/2B.1</td>
<td>0.53 (0.12, 2.34)</td>
<td>0.401</td>
<td>0.69 (0.15, 3.10)</td>
<td>0.629</td>
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</table>

Text in bold indicates significant values. CNR: copy number region; FCG: Fc gamma receptor; OR: odds ratio.

**Fig. 2** Copy number of FCG3B determines expression levels of FcγRIIib on human neutrophils

Individuals were stratified according to their FCG3B copy number; expression levels of FcγRIIib on neutrophils are shown for the different groups. Individuals with 0 copies of FCG3B, n = 2; individuals with 1 copy, n = 10; individuals with 2 copies, n = 109; individuals with 3 copies, n = 13; individuals with 4 copies, n = 2. ns: non-significant; ****P < 0.0001; MFI: median fluorescence intensity.
Fig. 3 Effect of the promoter haplotype 2B.4 in FCGR2B on expression of FcγRIIb on various leukocytes

Individuals were stratified according to their FCGR2B promoter haplotype (haplotype 2B.1 consists of -386G and -120T (nucleotide positions relative to start codon), haplotype 2B.4 consists of -386C and -120A). 2B.1/2B.1: individuals homozygous for the 2B.1 haplotype, n = 98. 2B.1/2B.4: individuals heterozygous for the 2B.1 and 2B.4 haplotype, n = 5. 2B.4/2B.4: individuals homozygous for the 2B.4 haplotype, n = 2. Expression levels are shown for circulating neutrophils (A), monocytes (B), NK cells (C), B cells (D) and T cells (E). In (C), individuals with a deletion of CNR1 are shown as open circles. ns: non-significant; *P < 0.05; ****P < 0.0001; MFI: median fluorescence intensity.
as monocytes and neutrophils when 2B.4 was present [13, 25], whereas another study demonstrated an opposite effect, arguing that the 2B.4 haplotype resulted in a decrease of FcγRIlb expression on B cells [26]. A potential bias that may have resulted in these discordant findings is expression of FcγRIIC, which is highly homologous to FcγRIlb. Because these two receptors are identical in the extracellular domains, monoclonal antibodies detecting surface expression will not be able to distinguish between them. Although FcγRIIC can only be expressed in the subset of individuals (<15%) carrying the FcγRIIC-ORF, this may have led to a substantial bias because the 2B.4 haplotype and the FcγRIIC-ORF often co-occur [23]. For our analysis, we circumvented this problem by selecting only individuals without the FcγRIIC-ORF for an expression analysis in a large group of individuals, now showing conclusive evidence for the fact that only the 2B.4 haplotype leads to de novo expression of FcγRIlb on myeloid cells. We did not find evidence for any effect (be it an increase or a decrease) on the expression of FcγRIlb on circulating B cells by the 2B.4 haplotype.

Another novel finding of the present study is the negative association of the FcγRIIB promoter haplotype 2B.4 with LN (in both clinically and biopsy-proven LN). Whereas the 2B.4 promoter in SLE contributes to susceptibility to SLE, it may also protect against the development of LN. Besides a negative association of the 2B.4 haplotype with LN, we also observed a negative association with specific auto-antibodies (immunological manifestations according to ACR criteria or biopsy proven LN) [40]. Recent studies have shown a clear association between the presence of anti-dsDNA and LN [41]. NETs are able to trap invading microbes and subsequently eliminate them by NETosis. NETs contain DNA and histones, which could be a potential source for autoantigens. Furthermore, degradation of NETs seems to be impaired in SLE and is associated with increased titres of anti-dsDNA and LN [42, 43]. A potential hypothesis for our negative association between 2B.4 haplotype and LN could be that the 2B.4 haplotype results in a decrease of FcγRIlb expression on neutrophils when 2B.4 was present [13, 25], whereas another study demonstrated an opposite effect, arguing that the 2B.4 haplotype resulted in a decrease of FcγRIlb on B cells [26].

### Table 3: Odds ratios of FcγR allele frequencies of 266 SLE patients in relation to LN according to the revised ACR criteria or biopsy proven

<table>
<thead>
<tr>
<th>Clinical variables</th>
<th>FCGR2A 131-R vs H OR (95% CI)</th>
<th>FCGR2A 277W vs Q OR (95% CI)</th>
<th>FCGR3A 158V vs F OR (95% CI)</th>
<th>FCGR3B ORF yes vs no OR (95% CI)</th>
<th>FCGR3B NA1 vs NA2 OR (95% CI)</th>
<th>FCGR2B 2B.4 vs 2B.1 OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephrological manifestations</td>
<td>1.11 (0.79, 1.57)</td>
<td>0.74 (0.43, 1.27)</td>
<td>0.90 (0.63, 1.28)</td>
<td>0.71 (0.40, 1.26)</td>
<td>0.82 (0.57, 1.17)</td>
<td><strong>0.54 (0.31, 0.96)</strong> *</td>
</tr>
<tr>
<td>Biopsy-proven LN</td>
<td>1.20 (0.86, 1.70)</td>
<td>0.73 (0.42, 1.27)</td>
<td>0.92 (0.65, 1.31)</td>
<td>0.88 (0.48, 1.63)</td>
<td>0.81 (0.57, 1.16)</td>
<td><strong>0.51 (0.28, 0.91)</strong> *</td>
</tr>
</tbody>
</table>

*P < 0.05. Text in bold indicates significant values. OR: odds ratio.
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Supplementary data
Supplementary data are available at Rheumatology Online.

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