Association analysis of functional variants of the FcgRIIa and FcgRIIIa genes with type 1 diabetes, celiac disease and rheumatoid arthritis

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FcgRIIa and FcgRIIIa are potent modulators of the immune system which bind (auto)antibodies and activate immune cells. The FcgRIIa*A519G and FcgRIIIa*A559C functional variants have been associated with several immune-related diseases. We studied FcgRIIa*A519G and FcgRIIIa*A559C SNPs in type 1 diabetes (T1D), celiac disease (CD) and rheumatoid arthritis (RA) patients and controls and included a meta-analysis of all recent studies of FcgRIIIa*A559C and RA. Our cohorts comprised 350 T1D, 519 CD, 639 RA patients and 1359 controls, who were genotyped for FcgRIIa*A519G and FcgRIIIa*A559C variants. Regression and expectation maximization (EM) algorithm-based haplotype analyses were used for the data analysis. We found significant differences in genotype frequencies of FcgRIIa between controls and patients with T1D (P = 0.04), CD (P = 0.000005) and RA (P = 0.04). The FcgRIIa*519GG genotype showed an increased risk for both T1D [odds ratio (OR) = 1.51; 95% confidence interval (95% CI) 1.08–2.12; P = 0.015] and CD (OR = 1.81; 95% CI 1.35–2.37; P = 0.000004), but not for RA. There was no difference in the frequency of FcgRIIa*A559C genotypes or allelotype frequencies between controls with T1D, CD and RA. We found that FcgRIIa and FcgRIIIa haplotype frequencies differed significantly between controls and patients with T1D (P = 0.05) and with CD (P = 0.00038) but not with RA. Our meta-analysis showed a significant 1.37(95% CI 1.14–1.66)-fold increased risk of RA for the FcgRIIIa*A559CC (158VV) genotype (P = 0.001). This is the first report that the FcgRIIa*519GG genotype predisposes to T1D and CD. We confirmed that the FcgRIIIa*A559CC genotype is associated with RA. If replicated, our findings would suggest FcgRIIa*519G as a common risk factor for auto-immune diseases. This may have clinical implications with regard to efficacy or safety of antibody-based immuno-modulator therapies.

INTRODUCTION

Fc receptors I, II and III (FcgRI, FcgRII and FcgRIII) have evolved as crucial immune response-modulating molecules that participate in reactivity to environmental antigens (1–3). Eight genes clustered on chromosome 1q21–q24 encode three classes of FcgRs that are expressed at the cell surface, namely the high-affinity receptor FcgRI (CD64), which binds monomeric IgG, and FcgRII (CD32) and FcgRIII (CD16), which bind to multivalent IgG. FcgRII and FcgRIII have different subclasses. FcgRIIa and FcgRIIIa associate with the common FcRgamma-chain containing a

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stimulatory ITAM motif that is also present in the intracellular tail of FcγRIIa, whereas FcγRIIb contains an inhibitory ITIM motif in the cytoplasmic domain. FcγRIIa and FcγRIIa stimulatory receptors are expressed by many leukocytes, including monocytes, dendritic cells, macrophages, natural killer cells, platelets and endothelial cells, and a subpopulation of T-cells, whereas FcγRIIb is expressed by B-lymphocytes, macrophages and dendritic cells (FcγRb2) (4). Upon binding of antibodies or autoantibodies, FcγRIIa and FcγRIIa activate immune cell functions, including phagocytosis, and the release of inflammatory mediators, whereas FcγRIIb nullifies cell activation (3,5). Thus, FcγRs are part of an important regulatory system in intercepting and digesting (auto)antibodies, which modulates antibody-mediated cellular cytotoxicity (1,3,6,7).

FcγR isoforms were therefore linked to the pathogenic consequences triggered by autoantibodies or immune complexes in autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (1,3,7).

FcγRIIa can have either histidine (H131) or arginine (R131) at amino acid position 131 located in the IgG-binding site, which is encoded as FcγRIIIa at amino acid position 131 located in the IgG-binding site, and systemic lupus erythematosus (SLE) (1,3,7).

In autoimmune diseases such as rheumatoid arthritis (RA) system in intercepting and digesting (auto)antibodies, which

T-cells, whereas FcγRIIb nullifies cell acti-

158 (32.61), 1.81 (1.35–2.37)5

117 (18.72), 0.86 (0.65–1.13)

170 (27.20), 1.00

1.00

1.16 (0.94–1.43)

1.15 (0.85–1.54)

90 (15.1)

1.16 (0.94–1.43)

470 (0.39)

1.09 (0.95–1.26)

Table 1. The association of the functional FcγRIIa*A519G and FcγRIIIa*A559C variants to T1D, CD and RA

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Controls (number, %)</th>
<th>T1D (number, %)</th>
<th>CD (number, %)</th>
<th>RA (number, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FcγRIIa*A519G</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-carrier (A/A)</td>
<td>382 (28.85)</td>
<td>792 (24.38)</td>
<td>118 (23.20)</td>
<td>170 (27.20)</td>
</tr>
<tr>
<td>Heterozygote (A/G)</td>
<td>642 (48.50)</td>
<td>151 (46.60)</td>
<td>225 (44.20)</td>
<td>338 (54.08)</td>
</tr>
<tr>
<td>Homozygote (G/G)</td>
<td>300 (22.65)</td>
<td>94 (29.01)</td>
<td>557 (0.55)</td>
<td>572 (0.46)</td>
</tr>
<tr>
<td>Allele G6</td>
<td>1242 (0.47)</td>
<td>339 (0.52)</td>
<td>1.24 (0.93–1.55)</td>
<td>1.05 (0.85–1.31)</td>
</tr>
<tr>
<td><strong>FcγRIIIa*A559C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-carrier (A/A)</td>
<td>353 (40.2)</td>
<td>131 (41.1)</td>
<td>209 (41.0)</td>
<td>221 (36.8)</td>
</tr>
<tr>
<td>Heterozygote (A/C)</td>
<td>604 (45.6)</td>
<td>132 (41.4)</td>
<td>238 (46.8)</td>
<td>290 (48.3)</td>
</tr>
<tr>
<td>Homozygote (C/C)</td>
<td>189 (14.3)</td>
<td>56 (17.6)</td>
<td>63 (12.4)</td>
<td>90 (15.1)</td>
</tr>
<tr>
<td>Allele C6</td>
<td>982 (0.37)</td>
<td>248 (0.83)</td>
<td>1.06 (0.89–1.27)</td>
<td>1.09 (0.95–1.26)</td>
</tr>
</tbody>
</table>

N, number of subjects. Significance: overall genotype test (cases were compared with controls)–**P = 0.04. Homozygotes compared with reference: **P = 0.00004. Allotype compared with reference: **P = 0.00002.

The number of chromosomes with the specified alleles (i.e. G or C), and figures within the brackets represent the relative frequencies.

RESULTS

**FcγRIIa**

Overall, the frequencies of FcγRIIa*A519G genotypes differed significantly between controls and T1D patients (P = 0.04; Table 1). Individuals homozygous for the FcγRIIa*A519G variant (corresponding to the high-binding 131H isoform) were more frequent in T1D patients (29.01%) than in controls (22.65%), yielding a 1.51-fold increased risk for T1D in carriers (95% confidence interval (95% CI) 1.08–2.12; P = 0.015, Table 1). Similarly, we found a significant difference in the frequency of FcγRIIIa*T519G genotypes in controls and CD patients (P = 0.000005). CD patients were also more frequently (32.61%) homozygous for the high-binding FcγRIIIa*T519G than controls, leading to a 1.81-fold (95% CI 1.35–2.37; P = 0.000004) increase in risk for CD in carriers (Table 1).
Overall, the frequency of FcgRIIa*A519G genotypes differed significantly between controls and RA patients \((P = 0.047; \text{Table 1})\). RA patients were more frequently heterozygous for this SNP than controls (54.08 versus 48.50%) and were less often homozygous for both A (27.20%) and G (18.72%) alleles than the controls (28.85 and 22.65%, respectively). When the data were analyzed per chromosome, the high-binding FcgRIIa*A519G allele was significantly more frequent in patients with T1D (frequency 0.52; \(P = 0.047\)) and CD (0.35), whereas it was significantly associated with T1D and CD, suggesting an FcgRIIa-independent association of FcgRIIa*A519G variant with T1D and CD.

### FcgRIIIa

Overall, there was no significant difference of FcgRIIIa*A559C genotypes between controls and T1D, CD or RA (Table 1). Also, the frequency of the FcgRIIIa*A559C allele did not differ significantly among the controls and patients with T1D, CD or RA (Table 1).

### Linkage disequilibrium and haplotype analysis

Since the FcgRIIa and FcgRIIIa genes reside close to each other in the same chromosomal region, we tested whether there was an allelic association between these two genes in our cohorts (Table 2). We found that FcgRIIa*A519G and FcgRIIIa*A559C were significantly associated in the controls \((P = 5.97 \times 10^{-19}\), T1D \((7.06 \times 10^{-8}\)), CD \((1.70 \times 10^{-5}\)) and RA patients \((5.30 \times 10^{-5}\)). To quantify the strength of LD, we calculated the \(D'\) between the two loci (Table 2). The \(D'\) as well as \(R^2\) between the FcgRIIa*A519G and FcgRIIIa*A559C variants were low in our cohorts, indicating that there is a low LD between the variants studied.

Next, we tested whether there was a specific disease-associated FcgRIIa*A519G, FcgRIIIa*A559C haplotype for T1D and CD. Overall, we found significant differences in the frequencies of FcgRIIa*A519G_FcgRIIIa*A559C haplotypes between controls and T1D \((P = 0.03\)), and CD \((P = 0.00038\), but not with RA patients \((P = 0.58; \text{Table 2})\). The haplotype specific risks indicate that it is mainly the FcgRIIa*A519G allele that explains the association of the FcgRIIa–FcgRIIIa haplotypes to T1D and CD, whereas there were no differences in the frequencies of these haplotypes between RA patients and controls (Table 2).

We further fitted the statistical model with interaction terms between FcgRIIa*A519G and FcgRIIIa*A559C genotypes. We found no significant evidence of interaction between the two loci in susceptibility to T1D or CD, suggesting an FcgRIIa-independent association of FcgRIIa*A519G variant with T1D and CD.

### Meta-analysis of FcgRIIIa genotypes

To further clarify the inconclusive association between FcgRIIIa and RA, we conducted a meta-analysis of 11 studies together with our current data (Fig. 1). The heterogeneity test was not statistically significant in the analysis of the FcgRIIIa*A559CC genotype \((P = 0.35\), whereas it was significant in the analysis of the FcgRIIIa*A559CA genotype \((P = 0.03\). In Caucasians, we found that the FcgRIIIa*A559CC genotype was associated with a significant 1.37 (95% CI 1.14–1.66; \(P = 0.001\)) fold increased risk of RA in carriers (Fig. 1), whereas the FcgR*A559CA genotype was not associated with RA \((P = 0.33\). We found no association of FcgRIIIa*A559CC or of FcgRIIIa*A559CA with RA in Asians (Fig. 1).

### Discussion

We demonstrated that the FcgRIIa*A519G variant is associated with both T1D and CD. We found a relatively low LD between FcgRIIa*A519G and FcgRIIa*A559C, and hence the FcgRIIa–FcgRIIa haplotypes showed different frequencies in the healthy controls and T1D or CD patients, which is mainly explained by FcgRIIa*A519G, but not between RA patients and controls. Our meta-analysis showed that
FcgRIIIA*559CC genotype is significantly associated with a mild increase in the risk of RA.

In our cohorts, homozygosity for the FcgRIIa/C3*519G variant was consistently associated with T1D and CD, which agrees with the studies that demonstrated that homozygosity for the FcgRIIa/C3*519G variant is consistently associated with other autoimmune disorders (16,20,24–26). A meta-analysis of a large number of SLE patients and controls confirmed the FcgRIIa/C3*519G variant as a genetic risk factor to SLE (16). Others have found an association between this SNP and Guillain–Barre syndrome (GB), and RA (1,4,17–30). Our findings also fit with the functional characteristics of this stimulatory variant in the determination of immune hyper-reactivity and thus suggest that the FcgRIIa gene is a predisposing factor for several autoimmune diseases (1,4,16–29).

Some reports on the association of this SNP with RA were contradictory. Some have shown an association of this marker with RA in the English, Indian and Pakistani populations (26,31,42), but several other studies found no association between the FcgRIIIa/C3*559C variant and RA in Japanese, Taiwanese, Norwegian, Dutch or Spanish populations (22,25,43–45). However, our meta-analysis confirmed an association of FcgRIIIa/C3*559CC genotypes with RA in Caucasians. This finding agrees with the results of an earlier pivotal meta-analysis that confirmed the association between FcgRIIIa/C3*559CC and SLE (15). It should be noted that several studies reported a positive association between FcgRIIIa and RA in subgroups of patients who shared a particular clinical characteristic such as shared anti-GPI positivity.
observations fit with the finding that the FcgRIIIa variant is associated with lupus nephritis (1), a disease complication, implying that the FcgRIIIa gene may be involved in the course of autoimmune diseases. This would partly explain the contradictory findings in RA, including those in the present study. Altogether, and given the findings of our meta-analysis, we concluded that FcgRIIa is associated with RA in Caucasians, most likely in those with a severe form of RA.

Our study focused on the FcgRIIa and FcgRIIIa genes, two activating receptors for immuno-effector cells. However, there are effective classes of inhibitory FcgRs, such as FcgRIIb and FcgRIIIb, which were also clustered to chromosome 1q21–q24, often present as pairs on the cell surface, and associated with several autoimmune diseases (3,15,47). The inhibitory receptors nullify stimulation signals from FcgRIIa and FcgRIIIa. Interestingly, association between copy number variation of FcgRIIb and lupus nephritis, SLE and Wegner’s granulomatosis has been reported (48,49). The low LD FcgRIIa and FcgRIIIa and the close proximity of FcgRIIIb to the latter indicate that FcgRIIIb may be an independent risk locus in this region, which warrants further investigation (48). Our risk estimates may therefore be skewed due to the modifying effects of ‘functionally’ interacting variants in neighboring inhibitory FcgRs.

We have tested FcgRIIa*A519G and FcgRIIIa*A559C alleles on the basis of prior evidence of previously reported association in other autoimmune diseases. Thus, multiple testing may not be applicable to our study. Nevertheless, significant association would still remain for the CD study when a conservative 6-fold Bonferroni correction for multiple-hypothesis testing is used.

It has been shown that the efficacy and compliance to the immunomodulatory monoclonal antibody against CD3 (anti-CD3 mAb) therapy differs according to immunoglobulin FcgRIIa, and FcgRIIIa isoforms due to variation in the encoding genes (39,40,50,51), whereas the non-FcR-binding anti-CD3 is less immunogenic than FcR-binding forms (52–54). Furthermore, it has been shown that FcgRs variations influence the release of cytokines, which may underlie the occurrence of side effects after the initiation of anti-CD3 mAb (54). On the basis of these observations, several studies investigated the role variations in the FcgRIIa and FcgRIIIa genes in the efficacy of anti-CD3 therapy in different immune diseases, and they found conflicting and non-consistent results (55–57). Therefore, our findings may further delineate that the disease risk genotype of FcgRIIa may modify the efficacy of anti-CD3 Ab-oriented therapies, a hypothesis which remains to be tested.

In conclusion, this is the first report of homozygosity for FcgRIIa*A519G as a predisposing factor to T1D and CD but not to RA. Our novel findings need to be replicated by others. We also found that homozygosity for FcgRIIIa*559C was associated with RA in the meta-analysis. Our findings provide basic insight into the possible mechanism of AIDS and may well have clinical implications with respect to the efficacy, and side effects of immuno-modulator interventions such as anti-CD3 therapy.

MATERIALS AND METHODS

Study populations

Type 1 diabetes Patients were retrieved from the Kolibri T1D cohort that included 350 Dutch patients with juvenile onset T1D (median 8.7 years, range 1–17 years). The cohort was selected consecutively after diagnosis by pediatricians in the southwestern part of the Netherlands between 1995 and 1999. The diagnosis was made according to the International Society of Pediatric and Adolescent Diabetes (ISPAD) and WHO criteria.

Celiac disease Patients were included from cohorts of Dutch CD patients that included children and adults. All the 519 CD patients have been diagnosed according to the revised ESPGHAN criteria (58). More than 90% of the patients were HLA-DQ2 positive. The patients’ initial biopsy specimens were retrieved and all showed a Marsh III lesion on re-evaluation by experienced pathologists.

Rheumatoid arthritis The characteristics of patients with RA have been described elsewhere (59). In brief, the RA patients included in our study were recruited from an ongoing early-RA inception study that was started in 1985 at the Department of Rheumatology, Radboud University Nijmegen Medical Center (RUNMC) in the Netherlands. All the patients were diagnosed according to the American College of Rheumatology criteria for RA (60), had a disease duration of less than 1 year and had no prior use of disease-modifying anti-rheumatic drugs or biological agents before presentation. All patients in the early-RA inception cohort are regularly monitored for disease phenotype, severity and outcome. In total, 639 Dutch patients with RA were included in our study.

The T1D, CD and RA patients were also born in the Netherlands and had at least three out of four grandparents also born in the Netherlands.

Control subjects A total of 1359 unrelated Dutch individuals were selected for being born in the Netherlands and had at least three out of four grandparents also born in the Netherlands.

All the patients and controls gave their informed consent and the medical ethical committee of the University Medical Center Utrecht or the Radboud University Nijmegen Medical Center approved this study.

Genotyping

We genotyped our study cohorts for the FcgRIIa*A519G SNP (rs1801274) and the FcgRIIIa*A559C SNP (rs396991). The genotyping of FcgRIIa*A519G SNP was successful for 1324 controls, 319 T1D patients, 509 CD and 601 RA patients, whereas the genotyping for FcgRIIIa*A559C succeeded in 1326 controls, 319 T1D patients, 510 CD and 601 RA patients. Participants’ genotypes for both the FcgRIIa*A519G and FcgRIIIa*A559C variants were available for 1290 controls, 314 T1D patients, 503 CD patients and 587 RA patients. Genotype frequencies of FcgRIIa*A519G and FcgRIIIa*A559C variants were in Hardy–Weinberg proportions in controls.
The Taqman® SNP genotyping assays for PCR were supplied by Applied Biosystems (Nieuwerkerk a/d IJssel, the Netherlands) for FcgRIIa*A159G (ABI assay identification number C_9077561_20) or for FcgRIIa*A559C (C_25815666_10).

Meta-analysis We searched Medline for all publications relating to association studies, using the combinations of ‘FcgRIIa*A159G’, ‘FcgRIIa*A559C’, ‘IIA’, ‘Ia’, ‘RA’, ‘Rheumatoid’, ‘Arthritis’, ‘FcgR’, ‘FcgammaRIIA’, ‘FcgammaRIIa’ and checked the references from retrieved publications for additional studies. We identified 14 articles: two performed analysis in two different ethnic populations, i.e. Caucasians and Indians (26,27), of which each analysis was treated as a separate entity in the meta-analysis. One study provided family-based association study (44), and one study presented data only for patients (61). These studies were not included in our meta-analysis. All the studies used the same diagnostic criteria for RA, and patients were diagnosed according to the American College of Rheumatology criteria (66). In total, we included 11 studies in our meta-analysis, of which eight were conducted in Caucasian populations (25–27,42,45,62,63), including two analyses in Indians (26,27), and three in Asians (22,30,43). In total, the meta-analysis covered chromosomes from 3341 patients and 4161 controls.

Data analyses
Genotype and allele frequencies were calculated by direct counting. Hardy–Weinberg equilibrium was checked using GenePOP software. First, the data were analyzed overall by genotypes. χ² tests were used to compare frequencies. Regression analysis was used to estimate first genotypic main-effect odds ratio (OR) and the corresponding 95% CI, and then the main effect of the risk allele of the two SNPs and the interaction-effect OR (95% CI) between variants of the FcgRIIa included according to association studies, using the combinations of ‘FcgRIIa*A159G’, ‘FcgRIIa*A559C’, ‘IIA’, ‘Ia’, ‘RA’, ‘Rheumatoid’, ‘Arthritis’, ‘FcgR’, ‘FcgammaRIIA’, ‘FcgammaRIIa’ and checked the references from retrieved publications for additional studies. We identified 14 articles: two performed analysis in two different ethnic populations, i.e. Caucasians and Indians (26,27), of which each analysis was treated as a separate entity in the meta-analysis. One study provided family-based association study (44), and one study presented data only for patients (61). These studies were not included in our meta-analysis. All the studies used the same diagnostic criteria for RA, and patients were diagnosed according to the American College of Rheumatology criteria (66). In total, we included 11 studies in our meta-analysis, of which eight were conducted in Caucasian populations (25–27,42,45,62,63), including two analyses in Indians (26,27), and three in Asians (22,30,43). In total, the meta-analysis covered chromosomes from 3341 patients and 4161 controls.

Meta-analysis For each study, the frequency of FcgRIIIa genotypes was derived from the counting method in patients and controls. In all the studies, allele frequencies were consistent with Hardy–Weinberg equilibrium. In addition to the total group, we classified the studies into Caucasians and Asian. The effect of the FcgRIIIa*A559C genotypes were assessed by comparing the frequency of the FcgRIIIa*A559AC and FcgRIIIa*A559CC genotype versus the FcgRIIIa*A559AA genotype in patients and controls. We used funnel plots to examine publication bias of reported associations. The study of Millicic et al. (27) had a very skewed frequency for the FcgRIIIa*559CC genotype that led us to consider this study as an outlier for the analysis of FcgRIIIa*A559AC analysis. To accommodate the effect of different ethnic backgrounds on the association between FcgRIIIa*A559AC and RA, heterogeneity between studies was tested using the χ² test, and the CI for the OR was estimated using a random effect model. We included FcgRIIIa*A559C only in the meta-analysis since there were not enough data on the association of FcgRIIIa*A159G variant and RA. The meta-analysis was conducted using the Cochrane Review Manager, version 4.1.2.

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Conflict of Interest statement. None declared.

REFERENCES


