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*559C genotypes or allele types 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 = 0.000038) 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*559CC (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*559CC 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 (FcγRI, FcγRII and FcγRIII) 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 FcγRs that are expressed at the cell surface, namely the high-affinity receptor FcγRI (CD64), which binds monomeric IgG, and FcγRII (CD32) and FcγRIII (CD16), which bind to multivalent IgG. FcγRII and FcγRIII have different subclasses. FcγRIIa and FcγRIIIa associate with the common FcγRgamma-chain containing a

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Table 1. The association of the functional FcgRIIa*519G and FcgRIIa*559C 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></td>
<td></td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>FcgRIIa*519G</td>
<td>N = 1324</td>
<td>N = 324</td>
<td>N = 509</td>
<td>N = 625</td>
</tr>
<tr>
<td>Non-carrier (A/A)</td>
<td>382 (28.85)</td>
<td>079 (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 (50.30)</td>
</tr>
<tr>
<td>Homozygote (G/G)</td>
<td>300 (22.65)</td>
<td>94 (29.01)</td>
<td>158 (32.61)</td>
<td>117 (18.72)</td>
</tr>
<tr>
<td>Allele G⁶</td>
<td>1242 (0.47)</td>
<td>339 (0.52)</td>
<td>557 (55.5%)</td>
<td>572 (46.4%)</td>
</tr>
<tr>
<td>FcgRIIa*559C</td>
<td>N = 1326</td>
<td>N = 319</td>
<td>N = 510</td>
<td>N = 601</td>
</tr>
<tr>
<td>Non-carrier (A/A)</td>
<td>533 (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 (0.80)</td>
<td>375 (0.36)</td>
<td>470 (0.39)</td>
</tr>
</tbody>
</table>

N, number of subjects. Significance: overall genotype test (cases were compared with controls)—**P < 0.000004. Homozygotes compared with reference: +P < 0.05, +P = 0.000004. Allotype compared with reference: ++P = 0.01, ++P = 0.000002.

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

NcgRIIa can have either histidine (H131) or arginine (R131) at amino acid position 131 located in the IgG-binding site, which is encoded as FcgRIIa*519G SNP (8,9). The FcgRIIa*519G allele encodes the H131 high-binding isofrom to IgG2, and the FcgRIIa*519A encodes the low-binding R131 isoform (3,9). The FcgRIIIa gene expresses two isoforms, namely V158 (or V176) and F158 (or F176), which differ at amino acid 158 (or 176) in the extracellular domain and are encoded by FcgRIIa*559C SNP. The FcgRIIIa*559C allele encodes the high-binding isoform to IgG1 and IgG3 valine isoform (i.e. V158), and the FcgRIIIa*559A encodes the 158 low-binding phenylalanine isoform (i.e. F158) (10,11). It is interesting that several independent genetic studies have linked chromosome 1q22–q24 to RA and SLE (7,12–14). However, extensive studies yielded different results on the relationship between genetic polymorphisms in the FcgR genes, in particular, the FcgRIIa and FcgRIIIa high/low-binding polymorphisms, to autoimmune diseases. On the one hand, meta-analyses have confirmed the association of the FcgRIIa*519G variant with susceptibility to SLE worldwide (1,15) and an association of FcgRIIIa*559C with SLE nephritis (16) and several other inflammatory diseases (1,4,16–29). On the other hand, the association of these two genes with RA remains interesting, but inconclusive for FcgRIIa*559C, whereas no association was found in multiple sclerosis (MS) (22,25,27,30–32). Similar to SLE and RA, type 1 diabetes (T1D) and celiac disease (CD) are common autoimmune diseases, which are characterized clinically by the presence of autoantibodies: against insulin-producing beta cells in T1D or intestinal mucosal cells in CD (33–37). The experimental–clinical studies are of great interest and have implied a role for FcgRs in the efficacy and side-effects of anti-CD3 immunomodulatory therapy in T1D (30,38–40). However, the relevance of the FcgRI or FcgRIIa genes to the pathogenesis of T1D and CD has not been investigated so far. In this study, we investigated FcgRIIa*519G and FcgRIIa*559C and their joint effects on susceptibility to T1D, CD and RA and performed a meta-analysis on the association between FcgRIIa*559C and RA.

RESULTS

FcgRIIa

Overall, the frequencies of FcgRIIa*519G genotypes differed significantly between controls and T1D patients (P = 0.04; Table 1). Individuals homozygous for the FcgRIIa*519G variant (corresponding to the high-binding 131H isoform) were more frequent in T1D patients (29.01%) than in controls (22.65%), yielding a 1.5-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 FcgRIIa*T519G genotypes in controls and CD patients (P = 0.000005). CD patients were also more frequently (32.61%) homozygous for the high-binding FcgRIIa*519G 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*519G genotypes differed significantly between controls and RA patients ($P = 0.047$; 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*519G allele was significantly more frequent in patients with T1D (frequency $0.52$; $P = 0.01$) and CD (0.35; $P = 0.000002$) than in controls (0.47), yielding an increased risk of 1.24 (95% CI 0.93–1.55) and 1.39 (95% CI 1.04–1.74) for T1D and CD, respectively. We found no significant difference in the frequencies of the FcgRIIa*519G allele between controls and RA patients.

**FcgRIIa**

Overall, there was no significant difference of FcgRIIa*559C genotypes between controls and T1D, CD or RA (Table 1). Also, the frequency of the FcgRIIa*559C 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*519G and FcgRIIa*559C 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 ($5.30 \times 10^{-9}$). 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*519G and FcgRIIa*559C 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*519G_FcgRIII*559C haplotype for T1D and CD. Overall, we found significant differences in the frequencies of FcgRIIa*519G_FcgRIII*559C haplotypes between controls and T1D ($P = 0.03$), and CD ($P = 0.00038$), but not with RA patients ($P = 0.58$; Table 2). The haplotype specific risks indicate that it is mainly the FcgRIIa*519G 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*519G and FcgRIIa*559C 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*519G 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*559CC genotype ($P = 0.35$), whereas it was significant in the analysis of the FcgRIIIa*559CA genotype ($P = 0.03$). In Caucasians, we found that the FcgRIIIa*559CC 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 FcgRIIIa*559CA genotype was not associated with RA ($P = 0.33$). We found no association of FcgRIIIa*559CC or of FcgRIIIa*559CA with RA in Asians (Fig. 1).

**DISCUSSION**

We demonstrated that the FcgRIIa*519G variant is associated with both T1D and CD. We found a relatively low LD between FcgRIIa*519G and FcgRIIa*559C, and hence the FcgRIIa–FcgRIIIa haplotypes showed different frequencies in the healthy controls and T1D or CD patients, which is mainly explained by FcgRIIa*519G, 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 hyperreactivity and thus suggest that the FcgRIIa gene is a predisposing factor for several autoimmune diseases (1,4,16–29). This finding seems to be similar to the associations of CTLA4 and PTPN22 with autoimmunity in general (41). PTPN22 and CTLA4 are functionally completely different molecules and are involved in the regulation of T cell function (41). There may be also a point of concern: the effect of FcgRIIa varies with autoimmune diseases, in the sense that the FcgRIIa*519G variant is associated with SLE and GB, and in our study, with CD and T1D, but it shows no consistent association with MS or RA (22). Also in our study, the association of FcgRIIa*G with RA was weaker and not convincing. The association was due to an excess of heterozygosity, which lacks a plausible biological meaning. In contrast, we detected novel association of FcgRIIa with CD and T1D with excess of homozygosity, which supports the hypothesis that FcgRIIa is a crucial determinant of susceptibility to several autoimmune diseases. Thus, FcgRIIa genotypes may serve as a marker for distinguishing the underlying basic pathologic heterogeneity in autoimmune diseases.

We could not confirm an association of the FcgRIIIa/C3*559C genotypes with RA in our study. Indeed, reports on this association are 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 FcgRIII*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*559CC genotypes with RA in Caucasians. This finding agrees with the results of an earlier pervious meta-analysis that confirmed the association between FcgRIIIa*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 FcγRIIIa (46), severe in hands RA (25) nodular arthritis (26) or only in men (25), which are indicators of disease severity. These observations fit with the finding that the FcγRIIIa variant is associated with lupus nephritis (1), a disease complication, implying that the FcγRIIIa 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 FcγRIIIa is associated with RA in Caucasians, most likely in those with a severe form of RA.

Our study focused on the FcγRIIa and FcγRIIIa genes, two activating receptors for immuno-effector cells. However, there are effective classes of inhibitory FcγRs, such as FcγRIIB and FcγRIIBb, 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 FcγRIIa and FcγRIIIa. Interestingly, association between copy number variation of FcγRIIB and lupus nephritis, SLE and Wegner’s granulomatosis has been reported (48,49). The low LD FcγRIIa and FcγRIIIa and the close proximity of FcγRIIB to the latter indicate that FcγRIIB 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 FcγRs.

We have tested FcγRIIa*A519G and FcγRIIIa*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 FcγRIIa, and FcγRIIIa 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 FcγRs 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 FcγRIIa and FcγRIIIa 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 FcγRIIa 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 FcγRIIa*519G 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 FcγRIIIa*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 Diabetics (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 antirheumatic 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 FcγRIIa*A519G SNP (rs1801274) and the FcγRIIIa*A559C SNP (rs396991). The genotyping of FcγRIIa*A519G SNP was successful for 1324 controls, 324 T1D patients, 509 CD patients and 625 RA patients, whereas the genotyping for FcγRIIIa*A559C succeeded in 1326 controls, 319 T1D patients, 510 CD and 601 RA patients. Participants’ genotypes for both the FcγRIIa*A519G and FcγRIIIa*A559C variants were available for 1290 controls, 314 T1D patients, 503 CD patients and 587 RA patients. Genotype frequencies of FcγRIIa*A519G and FcγRIIIa*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*A519G (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*A519G’, ‘FcgRIIa*A559C’, ‘IIA’, ‘Ila’, ‘RA’, ‘Rheumatoid’, ‘Arthritis’, ‘FcgR’, ‘FegammaRIIIa’, ‘FegammaRIIa’ and ‘FcgRIIa’ 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 (25–27,42,43,62,63), 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 and FcgRIIa/A559C SNPs. Coefficient (D’) and corresponding 95% confidence bound of pairwise linkage disequilibrium as well as R² between FcgRIIa*A519G and FcgRIIa*A559C using the LD plot module were implemented in the Haploview software, version 3.2. We tested for allelic association between these SNPs independently in the controls, T1D, CD and RA patients using an EM algorithm as described elsewhere (64). A two-tailed P-value < 0.05 was considered statistically significant. Data analysis was performed using UNPHASED (65,66), and STATA statistical software, version 8.0 for MS Windows.

**Meta-analysis** For each study, the frequency of FcgRIIa 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 FcgRIIa*A559C genotypes were assessed by comparing the frequency of the FcgRIIa*A559AC and FcgRIIa*559CC genotype versus the FcgRIIa*559AA genotype in patients and controls. We used funnel plots to examine publication bias of reported associations. The study of Milicic et al. (27) had a very skewed frequency for the FcgRIIa*559CC genotype that led us to consider this study as an outlier for the analysis of FcgRIIa*559AC analysis. To accommodate the effect of different ethnic backgrounds on the association between FcgRIIa*559AC 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 FcgRIIa*A559C only in the meta-analysis since there were not enough data on the association of FcgRIIa*A519G variant and RA. The meta-analysis was conducted using the Cochrane Reviewer Manager, version 4.1.2.

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**Conflict of Interest statement** None declared.

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