Association of FcgR2a, but Not FcgR3a, with Inflammatory Bowel Diseases Across Three Caucasian Populations

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Background: The Fc receptors II and III (FcgR2a, and FcgR3a) play a crucial role in the regulation of the immune response. The *FcgR2a**519GG and *FcgR3a**559CC genotypes have been associated with several autoimmune diseases including systemic lupus erythematosus, rheumatoid arthritis, nephritis, and possibly to type I diabetes, and celiac disease. In a large multicenter, two-stage study of 6570 people, we tested whether the FcgR2a and *FcgR3a* genes were also involved in inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC).

Methods: We genotyped the *FcgR2a**A519G and *FcgR3a**A559C functional variants in 4205 IBD patients in six well-phenotyped Caucasian IBD cohorts and 2365 ethnically matched controls recruited from the Netherlands, Spain, and New Zealand.

Results: In the initial Dutch study we found a significant association of *FcgR2a* genotypes with IBD (P-genotype = 0.02); while the *FcgR2a**519GG was more common in controls (23%) than in IBD patients (18%; odds ratio [OR] = 0.75; 95% confidence interval [CI] 0.61–0.92; $P = 0.004$). This association was

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corroborated by a combined analysis across all the study populations (Mantel–Haenszel [MH] OR = 0.84; 0.74–0.95; $P = 0.005$) in the next stage. The *Fcgr2a**GG genotype was associated with both UC (MH-OR = 0.84; 0.72–0.97; $P = 0.01$) and CD (MH-OR = 0.84; 0.73–0.97; $P = 0.01$), suggesting that this genotype confers a protective effect against IBD. There was no association of *FcgR3a**A559C genotypes with IBD, CD, or UC in any of the three studied populations.

Conclusions: The *FcgR2a**519G functional variant was associated with IBD and reduced susceptibility to UC and to CD in Caucasians. There was no association between *FcgR3a**5A559C and IBD, CD or UC.

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Key Words: *FcgR2a*, *FcgR3a*, Crohn's disease, ulcerative colitis, genome-wide association study

The pathogenesis of inflammatory bowel disease (IBD) involves a disruption in the balance between host immune responses and luminal enteric bacteria.[1–3] Recent genome-wide linkage and association (GWA) studies have implicated alterations in autophagy, and in innate immunity, as among the most relevant risk factors for Crohn's disease (CD).[1,4] The CD-associated *NOD2, ATG16L1*, and *IRGM* genes are involved in microbial defense of innate and adaptive immune responses.[5–8] Here, a different class of molecules which have evolved as crucial immune factors in reactivity to environmental antigens are the Fc gamma receptors (FcgRs).[9–11] In fact, FcgR isoforms have been linked to the pathogenic consequences triggered in autoimmune diseases.[10,12]

Two of the main activating FcgRs are the high-affinity FcgRIIa (CD32) and FcgRIIIa (CD16a) receptors that are widely expressed by leukocytes.[10,11] Upon the binding of (auto)antibodies or immune complexes (ICs), FcgRIIa and FcgRIIIa activate phagocytosis, and the release of inflammatory mediators of a regulatory system that modulates cellular cytotoxicity against environmental pathogens, autoantigens, and ICs.[10,11,13] Since environmental pathogens and associated ICs are involved in the etiology of IBD,[14,15] one may speculate that FcgRIIa and FcgRIIIa and their corresponding genes play a role in the pathogenesis of IBD.

Eight genes clustered on chromosome 1q21-q24 encode FcgRs including the *FcgR2a* gene (encodes FcgRIIa) and the *FcgR3a* gene (encodes FcgRIIIa). Several meta-analyses of candidate gene association studies have confirmed the *FcgR2a**A519G functional variant as a genetic risk factor for systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and other autoimmune diseases across different populations.[16–25] Two GWA studies in autoimmune diseases have reported significant associations for *FcgR2a* in SLE[20] and RA.[26] Similarly, we have found that the *FcgR2a**519GG genotype was associated to celiac disease (CeD) and type 1 diabetes.[16] For the *FcgR3a**A559C functional variant, previous meta-analyses have confirmed that this variant is associated with RA, SLE, and nephritis.[16,18,27–30] Together, accumulating data confirm that *FcgR2a**A519G and *FcgR3a**A559C are common denominators for several autoimmune diseases. However, their role in IBD has been less studied.

A recent study[31] has shown a significant overexpression of both *FcgR2a* and *FcgR3a* in colon of UC patients compared to controls (UC patients $n = 129$, controls $n = 73$, $P = 1.30 \times 10^{-6}$). Likewise, a recent Japanese GWA study has reported *FcgR2a**A519G as the most significant non-HLA genetic variant associated with UC conferring a protective effect.[32] This study found no evidence of association of *FcgR3a* with UC, and a meta-analysis of GWA studies did not detect any significant association of CD with the *FcgR2a* locus.[33] In contrast, an Italian candidate gene study on a modest-sized sample set (*N* CD 343, *N* UC 306, and *N* controls 256) reported a significant association between *FcgR3a**A559C and CD.[34] As yet no independent replication of this association has been conducted. Therefore, the recent data suggest *FcgR2a* as a UC locus, whereas the data on CD remain less conclusive. In this study we investigated the role of *FcgR2a* in IBD and set out to confirm the association of *FcgR3a* with IBD in Caucasians. We tested whether the *FcgR2a**A519G and *FcgR3a**A559C variants were associated with IBD, UC, and CD in 6570 samples recruited from three populations.

MATERIALS AND METHODS

Study Design and Population

We performed a two-stage association study. For stage one we included two Dutch IBD cohorts and in stage two we expanded the study with a third Dutch IBD cohort, along with three independent IBD cohorts and a control group from Spain, and an IBD cohort and control group from New Zealand. We performed a final combined analysis for *FcgR3a* with our data and data reported by Latiano et al from Italy.[34]

Dutch Cohorts

The two initial Dutch cohorts compromised 547 IBD patients (222 UC patients and 325 CD) from the University Medical Center Groningen (UMCG), as described elsewhere,[7] and the cohort with 664 IBD patients (338 UC and 326 CD patients) from the VU University Medical Center (VUmc), Amsterdam, as described elsewhere.[35,36] The third Dutch cohort included 1139 IBD patients (477 UC and 662 CD patients) from the Academic Medical Center (AMC) Amsterdam, as described elsewhere.[37,38] These patients were diagnosed consecutively at the AMC. A total of 1401

unrelated Dutch individuals were selected as controls; they and at least three of their four grandparents were born in the Netherlands. The control set consisted of two groups: Leiden controls ($n = 681$) and Utrecht controls ($n = 720$) who have been genotyped at the UMC Utrecht, as described elsewhere.[16] After the preparations and quality checks of DNA samples, we had a total of 2208 patients with IBD (954 UC and 1254 CD patients) and 1360 controls for the present study.

Spanish Cohorts

The details of the three Spanish cohorts and the Spanish controls have been described previously.[39] The first cohort consisted of 535 IBD patients (262 UC and 273 CD patients), from the Virgen de las Nieves Hospital, Granada, which also provided 449 Spanish controls. These were blood donors and staff members, and were ethnically matched as described elsewhere.[39] The second cohort consisted of 305 IBD (117 UC and 188 CD patients) who were recruited from Hospital Universitario Central de Asturias, Oviedo. The third cohort included 210 IBD patients (87 UC and 123 CD patients) from several regions in Spain. After quality checks on the Spanish DNA samples, we included a total of 1021 IBD patients (448 UC and 573 CD patients) and 449 controls in this study. These subjects were genotyped in a research laboratory at CSIC, Granada, Spain.

New Zealand

The details of the population-based New Zealand Caucasian cohort have been described elsewhere.[40,41] This cohort included 987 IBD patients (481 UC and 506 CD patients). Diagnosis was confirmed in each patient by a review of the case notes. The New Zealand control group comprised of 556 Caucasians aged over 17 years, who had no history of inflammatory disorders, as described elsewhere.[42] They were all genotyped at the University of Otago, New Zealand.

Combining Studies

A diagnosis of IBD was made by clinicians in participating centers according to accepted clinical, endoscopic, radiologic, and histological criteria[43]; the diagnosis was based on the Montreal Classification for IBD. In each study, controls were ethnically matched with corresponding IBD patients. Nevertheless, the possible but undetected difference in ascertainment (bias) in controls among the participating studies is unlikely to affect the validity of our study.

Genotyping

FcgR2a has two isoforms, which are defined by the single nucleotide polymorphism (SNP) *FcgR2a* 519G>A (rs1801274).[44,45] The *FcgR2a**519G allele encodes the H131 high-binding allele to IgG2, whereas the *FcgR2a**519A encodes the low-binding R131 isoform.[10,45] The FcgR3a gene expresses two isoforms, namely, V158 (or V176) and F158 (or F176), which result from an A to C substitution at nucleotide 559 of the FcgR3a gene (rs396991). The *FcgR3a**559C allele encodes the valine isoform (i.e., V158) that is a high-binding allele to IgG1 and IgG3, whereas the *FcgR3a**559A allele encodes the 158 low-binding phenylalanine isoform (i.e., F158).[46,47]

Patients and controls were screened for *FcgR2a**A519G and *FcgR3a**A559C using the predesigned TaqMan SNP genotyping assays (*FcgR2a**A519G ABI assay identification number C_9077561_20, and FcgR3a*A559C was C_25815666_10; Applied Biosystems, Foster City, CA). Several samples, even after a second attempt, could not be clustered with a sufficient certainty to any of the genotype groups, in particular for FcgR3a. When we performed a quality check, the main reason for unsuccessful clustering was low DNA concentration, the low quality of DNA, i.e., presence of fragmentations, low intensity signals, and some due to unexplained failure of amplifications. Numbers of samples that were excluded from further analyses are 40 Dutch controls, 42 Dutch IBD patients, 29 Spanish cases, 11 New Zealand IBD cases. Genotyping was successful for at least one of the FcgR variants in 2208 (call rate 94%) Dutch IBD patients (954 UC and 1,254 CD) and 1360 (call rate 97%) Dutch controls, in 1021 (call rate 97%) Spanish IBD patients (448 UC and 573 CD patients) and 449 Spanish controls (call rate 100%), and in 976 (call rate 99%), New Zealand IBD patients (474 UC and 502 CD patients) and 556 (call rate 100%) New Zealand controls. Therefore, the rates of missing genotypes were comparable across the Dutch, Spanish, and New Zealand populations. Given the sample sizes in the present study, the genotyping failure would not have biased the results of the interaction analysis between the two SNPs tested.

In total, 4205 IBD patients and 2365 controls were included in the final analysis.

Study Power

We performed four independent tests, namely, comparing the frequency of *FcgR2a**A519G and *FcgR3a**A559C genotypes between controls and UC and CD patients in the initial screen, thus a significance level of less than 0.01 in our initial screen was considered statistically significant. The Dutch study had 80% power to detect an odds ratio (OR) of 0.70 at a *P*-value of 0.01 with a minor allele frequency of 0.23 in controls and recessive model of effect for *FcgR2a**519GG genotype, whereas the Spanish and the New Zealand studies were underpowered (≈20%) to detect any significant association. We decided a priori to improve the study power by two approaches: 1)

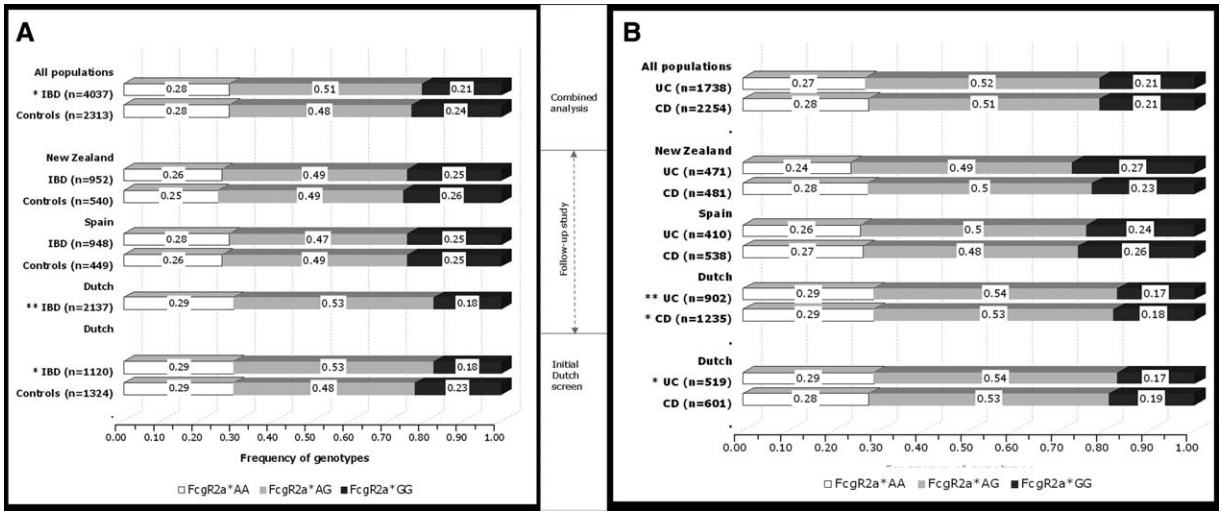

FIGURE 1. The genotype frequencies of *FcgR2a**A519G in controls and in patients with (A) IBD, and (B) UC, and CD by the study populations. Significance values represent the association of FcgR2a*A519G genotypes to IBD, UC, or CD. *$P = 0.01$; **$P = 0.005$.

Grouping CD and UC into a single disease category (i.e., IBD group) on the basis that they share clinical phenotypes and pathogenesis. This implies a common susceptibility for *FcgR2a*, as reported earlier for other IBD-related genes[2,3,48,49]; 2) Since a large number of previous studies and meta-analyses showed an association between *FcgR2a**519GG and *FcgR3a**559CC genotypes and susceptibility to different autoimmune disease,[16,17,19,21,27] we made an a priori decision to compare specifically the frequency of *FcgR2a**519GG and *FcgR3a**559CC genotypes between controls and patients; and 3) By performing a combined analysis of Dutch, Spanish, and New Zealand populations. Our study power improved to ≈80% to detect a 3% difference in allele frequency of tested variants between IBD patients and controls (control–case ratio = 0.70), at a significant level of 0.01 using a standard chi-squared (1 degree of freedom, *df*) test for a recessive effect (i.e., GG versus AG&AA).

Data Analysis

Genotype and allele frequencies were calculated by direct counting. Hardy–Weinberg equilibrium (HWE) was checked using a 1 *df* standard chi-squared test. We used the chi-square test to compare frequencies and the magnitude of genotypic risk for IBD was expressed as crude odds ratios (ORs). The Cochrane–Mantel–Hanszel (MH) test for stratified tables was used to estimate strata-weighted ORs, which were compared to crude ORs, and also to perform a meta-analysis.[50] The combined analysis was done using random effect model which allows for heterogeneity to estimate the confidence limit of combined ORs. The Breslow–Day test was used to check for the presence of heterogeneity. To test whether the genotypes of *FcgR3a* modify the effect of FcgR2a in IBD, we performed 1) an *FcgR3a* stratified analysis and compared the overall pooled ORs against the estimated MH OR weighted over FcgR3a strata, and 2) a logistic regression analysis to study the interaction of *FcgR2a* and *FcgR3a* while adjusting for the study population, as described elsewhere.[51] The analyses were performed using SPSS v. 15 for Windows (Chicago, IL). We also performed a haplotype analysis of these SNPs with disease susceptibility, using an expectation maximization algorithm implemented in Plink.[52]

Ethical Considerations

All the patients and controls gave informed consent and the review boards of the participating centers approved the use of the IBD cohorts for genetic studies.[7,35–40,53]

RESULTS

FcgR2a

Allele and genotype frequencies of *FcgR2a**A519G variant were in HWE controls of the Dutch ($P = 0.21$), Spanish ($P = 0.25$), and New Zealand ($P = 0.63$) studies. Figure 1 compares the frequency of *FcgR2a**A519G genotypes between controls and IBD patients in the initial and follow-up analyses. In the initial Dutch study there was a trend towards difference in the frequency of *FcgR2a**A519G genotypes in controls compared to IBD ($P = 0.02$; Fig. 1A). At the follow-up stage, when the third Dutch cohort (AMC) was included in this study, the difference in the frequency of *FcgR2a**A519G genotypes between controls and IBD patients was significant ($P = 0.005$; Fig. 1A). Next, when the Spanish and New Zealand studies were

TABLE 1. Association of FcgR2a and FcgR3a in Inflammatory Bowel Disease, Ulcerative Colitis, and Crohn's Disease, by Study Populations

	Controls		IBD			UC			CD		
	N	Freq.#	*N*	Freq.#	GG vs. (AG&AA)	*N*	Freq.#	GG vs. (AG&AA)	*N*	Freq.#	GG vs. (AG&AA)
FcgR2a											
Dutch	1324	$0.47/^{0.23}$	2137	$0.44/^{0.18}$	0.74 (0.62-0.87)^{*1}	902	$0.44/^{0.17}$	0.70 (0.57-0.87)^{*2}	1235	$0.45/^{0.18}$	0.76 (0.63-0.92)^{*3}
Spanish	449	$0.48/^{0.25}$	948	$0.49/^{0.25}$	1.01 (0.78-1.31)	410	$0.49/^{0.24}$	0.96 (0.70-1.31)	538	$0.50/^{0.26}$	1.05 (0.79-1.40)
New Zealand	541	$0.50/^{0.26}$	952	$0.49/^{0.25}$	0.92 (0.72-1.17)	471	$0.51/^{0.26}$	1.02 (0.77-1.35)	481	$0.47/^{0.23}$	0.83 (0.62-1.10)
Pooled- MH OR$^{*\ pvalue}$	2314	$0.50/^{0.24}$	4037	$0.50/^{0.21}$	0.84 (0.74-0.95)^{*3}	1783	$0.51/^{0.21}$	0.84 (0.72-0.97)^{*1}	2254	$0.47/^{0.21}$	0.84 (0.73-0.97)^{*4}
FcgR3a	*N*	Freq.&	*N*	MAF	CC vs. AC & AA	*N*	Freq.&	CC vs. AC & AA	*N*	Freq.&	CC vs. AC&AA
Dutch	1326	$0.37/^{0.14}$	2158	$0.37/^{0.14}$	0.97 (0.80-1.18)	928	$0.37/^{0.15}$	1.03 (0.81-1.31)	1230	$0.36/^{0.13}$	0.93 (0.74-1.16)
Spanish	446	$0.38/^{0.16}$	963	$0.36/^{0.14}$	0.80 (0.59-1.11)	424	$0.36/^{0.14}$	0.81 (0.56-1.19)	549	$0.36/^{0.13}$	0.79 (0.56-1.14)
New Zealand	457	$0.35/^{0.12}$	883	$0.33/^{0.11}$	0.89 (0.63-1.27)	434	$0.32/^{0.11}$	0.88 (0.58-1.34)	449	$0.34/^{0.11}$	0.89 (0.59-1.38)
Pooled - MH OR (This study)	2229	$/^{0.14}$	4014	$0.36/^{0.13}$	0.92 (0.79-1.07)	1786	$0.36/^{0.13}$	0.95 (0.79-1.14)	2228	$0.36/^{0.13}$	0.88 (0.74-1.06)
Latiano et al study[34]	256	$0.36/^{0.00}$	545	$0.27/^{0.004}$	—	202	$0.32/^{0.005}$	—	342	$0.23/^{0.002}$	—
Pooled - MH OR All studies	2485	$0.35/^{0.13}$	4559	$0.35/^{0.11}$	0.92 (0.79-1.07)	1988	$0.32/^{0.12}$	0.96 (0.80-1.15)	2571	$0.35/^{0.11}$	0.89 (0.75-1.06)

Significances: $*^1P = 0.02$; $*^2P = 0.001$; $*^3P = 0.005$; $*^4P = 0.01$. Freq.#; represents the allele frequency of FcgR*519G allele / $^{\text{the frequency of FcgR2aGG genotype}}$. Freq.$^{\&}$ represents the allele frequency of FcgR3a*559C allele/$^{\text{the frequency of FcgR*559CC genotype}}$. Figures in parentheses represent 95% confidence intervals for odds ratios (ORs).

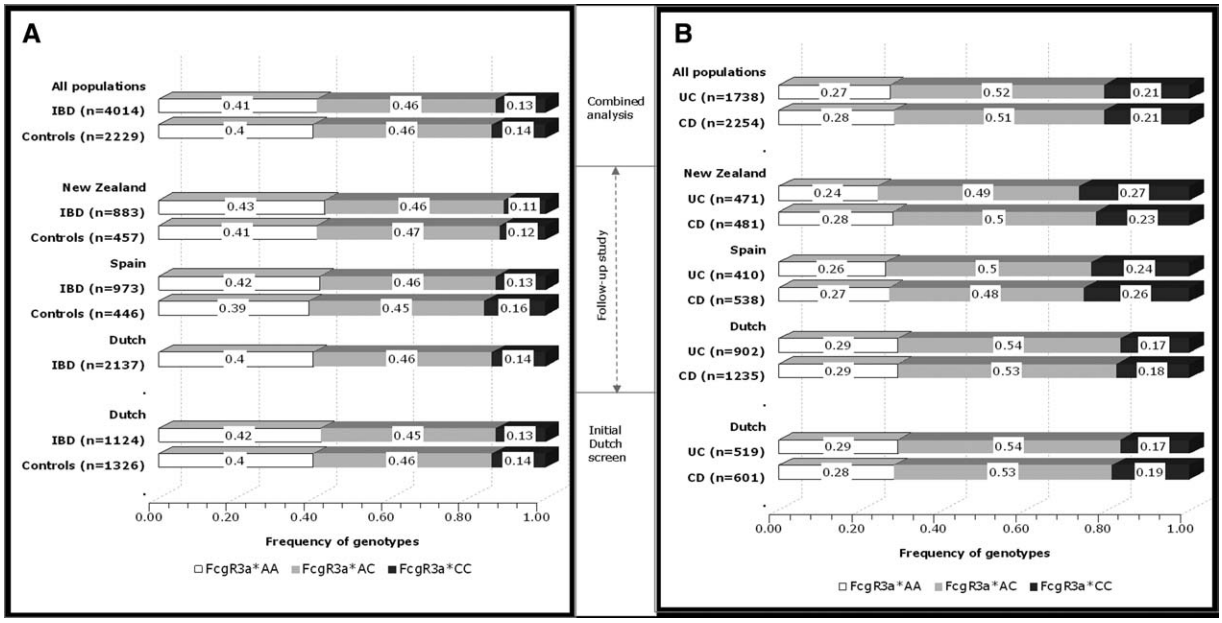

FIGURE 2. The genotype frequencies of *FcgR3a**A559C in controls and in patients with (A) IBD, and (B) UC, and CD by study population.

included, testing this polymorphism in two other populations did not reveal significant differences in the frequency of *FcgR2a**A519G genotypes (Fig. 1A), perhaps as a result of being underpowered in these specific cohorts. As identified previously,[16] *FcgR2a**519GG can be considered the most associated genotype with other inflammatory diseases. Our initial analysis showed *FcgR2a**519GG was significantly more common among controls (23%) than IBD patients (18%; OR = 0.75; 95% confidence interval [CI] 0.61–0.92; $P = 0.005$; Supporting Material, Table S1). Finally, when a combined analysis was performed across all the study populations, *FcgR2a**519GG was more common in controls than IBD patients (MH population-weighted OR 0.84; 95% CI 0.74–0.95; $P = 0.005$; Table 1). This MH-weighted OR did not differ from the estimated crude (pooled) OR for IBD, which was 0.83 (0.74–0.94; $P = 0.002$), suggesting a uniform association of *FcgR2a**519GG to IBD across our three Caucasian populations. Similarly, there was no significant heterogeneity for *FcgR2a**519GG associated OR among the study populations (Breslow–Day $P = 0.10$), though the Spanish study showed a slight shift to the right (i.e., towards OR = 1.00). We found no difference in the frequency of the *FcgR2a**519G allele between controls and IBD patients (0.94; 95% CI 0.88–1.01; $P = 0.11$) in a combined analysis, or when the data were analyzed population-wise, suggesting a lack of allele dosage (i.e., multiplicative) effect for this variant.

In the initial screen, the frequency of *FcgR2a**A519G genotypes differed between controls and patients with UC ($P = 0.04$). This difference was statistically significant for UC (2 *df* P genotype = 0.01), and trend towards significant for CD (2 *df* P genotype = 0.04), when the third Dutch IBD cohort was included in the analysis (Fig. 1B). Likewise, compared to controls, *FcgR2a**GG genotype was less common in patients with UC (0.72; 0.55–0.95; $P = 0.02$) or CD (0.77; 95% CI 0.60–0.99; $P = 0.04$), suggesting that this genotype confers protection against IBD, including UC and CD (Supporting Material, Table S1). This association remained significant in the combined analysis of all the study populations (Fig. 1B; Table 1). Again, the frequency of the *Fcgr2a**GG genotype was significantly lower in patients with UC (0.84; 0.72–0.97; $P = 0.01$) and patients with CD (MH OR 0.84; 95% CI 0.73–0.97; $P = 0.01$) compared to controls (Fig. 1b; Table 1). These ORs did not differ significantly from those of the crude ORs which were 0.85 (0.73–0.98; $P = 0.02$) for UC and 0.82 (0.72–0.95; $P = 0.005$) for CD, suggesting a uniform association across the populations studied (Breslow–Day test: P-UC = 0.08; P-CD = 0.20). Similarly, the frequency of the *FcgR2a**519G allele was not significantly different between controls and patients with UC (0.95; 0.87–1.04; $P = 0.25$) or CD (MH OR = 0.94; 0.86–1.02; $P = 0.12$).

FcgR3a

Allele and genotype frequencies of *FcgR2a**A559C variant were also in HWE in controls of the Dutch ($P = 0.64$), Spanish ($P = 0.33$), and New Zealand ($P = 0.54$) studies. There was no significant difference in the frequency of *FcgR3a**A559C genotypes in controls compared to IBD patients in the initial Dutch study (Fig. 2A;

Supporting Material, Table S1), or in the follow-up study across three populations (Fig. 2A; Table 1); or in the combined analysis that also included the data of Latiano et al[34] (Table 1). Also, we found no association between *FcgR3a**A559C with either UC or CD (Fig. 2B; Table 1). However, we did find a significant ($P < 1*10^{-8}$) difference between the frequency of *FcgR3a**A559C genotypes in controls reported by Latiano et al[34] and those of our Dutch, Spanish, and New Zealand populations in this study (Table 1).

Gene–Gene Interaction

*FcgR2a**A519G and *FcgR3a**A559C are both involved in promoting activation signals in effector cells, and they reside in close proximity on chromosome 1. They may therefore modify the effect that each conveys in IBD susceptibility, as has been reported earlier for other autoimmune diseases.[54,55] We therefore tested whether the effect of *FcgR2a**A519G on IBD varies significantly across the genotypes of the *FcgR3a**A559C variant. When controlling for a difference in the study population, we found no significant change in the estimated ORs for IBD across three different *FcgR3a**A559C genotypes (Breslow–Day *P*-IBD = 0.18; *P*-UC = 0.06; *P*-CD = 0.40). When the *FcgR2a**519GG associated OR was weighted with MH *FcgR3a* genotypes, it was 0.85 (0.74–0.96; $P = 0.01$), which did not differ significantly from the *FcgR2a**519GG associated crude OR (0.86; 0.76–0.97; *P* for difference = 0.97). When we performed a regression analysis including both the main effects of *FcgR2a**A519G and *FcgR3a**A559C, and the interaction terms, while adjusting for the study populations, the interaction factors were nonsignificant (data not shown). Likewise, the frequencies of the *FcgR2a**A519G-*FcgR3a**A559C haplotypes in controls did not differ significantly from those in any of the IBD patient cohorts or in our combined analysis (Supporting Material, Table S2). These results suggest that *FcgR3a**A519C genotypes do not substantially modify the effect of *FcgR2a**519GG on IBD.

Linkage Disequilibrium (LD)

In Caucasians the FcgR2a region on chromosome 1q23 is bounded by two confirmed CD-associated SNPs, namely, the SNP rs2274910 located in ITLN1 on 1q23 (*P* for CD = 1.4610^{-9}) and SNP rs9286879 located in a noncoding region on chromosome 1q24 (*P* for CD = 1.5310^{-9}).[33] The rs2274910 SNP had no LD with *FcgR2a**A519G, in HapMap European samples ($r^2 = 0.01$), in healthy Dutch controls ($n = 643$, $r^2 = 0.01$), or Spanish controls ($n = 421$; $r^2 = 0.01$). The rs9286879 SNP showed no LD with *FcgR2a**A519G in HapMap European samples ($r^2 = 0.01$), Dutch controls ($r^2 = 0.005$), or Spanish controls ($r^2 = 0.0008$).

DISCUSSION

FcgR2a has not yet been reported as a susceptibility gene for IBD in Caucasians, despite the fact that a large number of studies have reported association of *FcgR2a**519GG with other inflammatory disorders.[16,20,27] In this study we found a significant association between the *FcgR2a**519GG genotype with UC and CD in a combined analysis of 6570 subjects across three Dutch, Spanish, and New Zealand populations. Considering previous *FcgR2a**A519G data in other inflammatory diseases, our data suggest that *FcgR2a**519GG is likely to be associated with IBD, UC, and CD.

The *FcgR2a**519GG genotype also acts as a differential risk factor in inflammatory diseases. Previous studies have shown a consistent association of the *FcgR2a**519GG genotype with susceptibility to SLE worldwide,[17,18,56] to CeD, T1D, and RA,[16] and to other inflammatory diseases.[19,21–23] Our data suggest that the *FcgR2a**519GG genotype carries a protective effect for UC. This effect corroborates the findings from a preceding Japanese GWA study that found a genome-wide significant protective effect for *FcgR2a**519GG genotype against UC with an OR of 0.57.[32] In the present study we performed a candidate gene study with a focus only on one particular functional SNP within *FcgR2a*. The Japanese GWA study by Asano et al[32] performed a detailed genetic analysis of the *FcgR2a*-3a region in UC patients and concluded that the observed significant association of *FcgR2a**A519G with UC was not caused by LD with other SNPs or copy number variations (CNVs) within this region. In summary, accumulating data support our findings of a protective role for *FcgR2a**A519G in UC, and that the *FcgR3a* region may not be associated with UC.

One may question our findings about CD since neither a previous meta-analysis of GWA studies, nor a recent GWA study in early-onset IBD has reported any significant association for *FcgR2a* with CD.[33,57] There are several reasons for these inconsistent findings. First, it is possible the FcgR2a*A519G variant may not be involved in CD and our data represent a false-positive association. Second, the apparently conflicting data can be rationalized by a lack of LD between SNPs included in GWA arrays and functional variants i.e., in our case *FcgR2a**A519G.[58] For example, the *FcgR2a**A519G variant (i.e., rs1801274) was not included in some of the GWA platforms, and thus has not been genotyped directly by the GWA studies such as the Wellcome Trust Case Control Consortium (WTCCC) study or the study by UK IBD genetics consortium.[59,60] In fact, a weak association may exist between this SNP and CD, but this falls short when correcting for multiple testing in GWA studies.[33] In summary, the role of *FcgR2a**A519G in CD remains inconclusive. When it exists, this association is expected to be very small, or limited to clinical subtypes of CD.

The protective effect of the *FcgR2a**519G allele on UC is in contrast to the more than 1.30-fold increased risk this allele confers for SLE,[17] CeD, or T1D,[16] and antiphospholipid syndrome.[19] This concept is not unique to the *FcgR2a**A519G variant. The *PTPN22**620W variant has also been associated with no effect or a protective effect in IBD, while it is a risk factor for T1D and RA.[61,62] Whether a differential association of the FcgR2a*519GG genotype with autoimmune diseases has a biological or clinical implication remains to be established. Several studies have shown FcgRs to influence the efficacy and side effects of anti-CD3 immunomodulatory therapy.[63–65] The findings of an involvement of *FcgR2a* in UC, and perhaps in CD, could hint at the hypothesis that FcgRs might also modify the efficacy and tolerability of human monoclonal antibody strategies in IBD, such as anti-TNF,[66] anti-IL12-IL23,[67] anti-alpha-4 integrin,[68] or anti-CD3 monoclonal antibody.[69] However, our study lacks the clinical and experimental data to test this hypothesis.

FcgR3a

We found no association between the *FcgR3a**A559C polymorphism and IBD, CD, or UC in the Dutch, Spanish, or New Zealand populations, or in a combined analysis of all three populations. This is in contrast with the findings of Latiano et al[34] in the Italian population, who suggest there was an association between the FcgR3a*559AC genotype and CD. Of note, the study by Latiano et al[34] used controls who had a significantly lower frequency of *FcgR3a**559CC genotypes than our Dutch, Spanish, or New Zealand controls or those used in previous European studies.[16,27] Although the reason for this difference is unclear, it may explain why Latiano et al[34] found an association between the *FcgR3a**559C allele and CD that we could not confirm; perhaps the detectable effect size may have an inverse relation with the frequency of the risk allele. Nonetheless, we suggest that the association between *FcgR3a**A559C and IBD reported by Latiano et al should not be generalized to other Caucasian populations. Further, the recent GWA studies and meta-analyses have found no relationship between *FcgR3a* and UC or CD in general.[32,33,57,59] Taken together, our data and data of preceding studies do not suggest any association between *FcgR3a**A559C and IBD, UC, or CD.

The power of our study was sufficient to detect an association of *FcgR2a**A519G and *FcgR3a**A559C with IBD, CD, or UC. However, we cannot rule out the possibility of false-positives, although our findings are in concordance with recent GWA studies. Furthermore, the precise molecular mechanism underlying the role of *FcgR2a* in IBD needs to be elucidated. The current concept for the immunopathology of IBD assumes a complex interaction between dendritic cells and macrophages and CD4+ T-regulatory cells.[4] Induction of T-regulatory cells promotes autophagy and antimicrobial activity in macrophages, which involves abundant secretion of stimulatory cytokines,[4,70,13,71,72] a process in which FcgRs may contribute. This scenario fits with functional data that suggest that FcgRs constitute the link between innate and cell-mediated immunity, and identifies *FcgR2a* as a possible key modifier in the activation of immune cells triggered by cellular pathogenic components of microbiota.

In conclusion, our study and those of others suggest that the *FcgR2a**519G functional variant is associated with UC in the Dutch population, and the presence of this allele in Caucasians may lower their risk for UC, and perhaps for CD. Eventually this may well have clinical implications for our understanding of the pathogenesis of IBD and the use of monoclonal antibody-based therapies to treat it. We concluded that *FcgR3a**A559C is not associated with IBD.

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