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Ann Rheum Dis 2010 69: 1054-1057 originally published online August 20, 2009

doi: 10.1136/ard.2009.110874

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Dissection of the *FCGR3A* association with RA: increased association in men and with autoantibody positive disease

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Accepted 24 July 2009
Published Online First
20 August 2009

ABSTRACT

Objectives Genome-wide association studies in rheumatoid arthritis (RA) have failed to examine the *FCGR* gene cluster because of the confounding effects of segmental duplication. This study aimed to replicate previous candidate gene studies that had identified a significant association between the *FCGR3A*-158V allele and RA and then sought to estimate specific subgroup effects.

Methods *FCGR3A*-158F/V genotyping was undertaken in a UK Caucasian replication cohort comprising 2049 patients with RA and 1156 controls. Subgroup analyses assessing the magnitude of association according to gender and autoantibody (rheumatoid factor (RF) and cyclic citrullinated peptide (CCP)) status were undertaken in a pooled cohort of 2963 patients with RA and 1731 controls. Logistic regression was used to test for interaction between *FCGR3A* and *HLA-DRB1* shared epitope (SE) alleles.

Results In the combined RA cohort, borderline association with homozygosity was found for the *FCGR3A*-158V allele (OR 1.2, $p=0.05$), which was stronger in men (OR 1.7, $p=0.01$). Stratification by autoantibody status showed an increased risk in RF and CCP positive RA. Analysis of the *FCGR3A*-158V and *HLA-DRB1* SE interaction revealed roles for both genes in susceptibility to autoantibody positive RA, with no evidence of interaction.

Conclusions *FCGR3A* is a risk factor for the development of autoantibody positive RA, particularly in men, with evidence of a multiplicative effect with *HLA-DRB1* SE.

INTRODUCTION

Recent genome-wide association studies (GWAS) in rheumatoid arthritis (RA) have been highly successful in both the confirmation of known genetic associations and in highlighting new loci/immunological pathways that warrant further investigation.¹ However, GWAS are limited by the technologies they employ. Those technologies based on probe hybridisation rely on each single nucleotide polymorphism (SNP) having a unique flanking sequence. SNPs in segmental duplications, which comprise ~5% of the genome,² frequently fail quality controls owing to the presence of multiple paralogs, leading to poor coverage of duplicated regions in GWAS.

The *FCGR* locus on chromosome 1q23 has arisen through a series of duplication events.³ The best characterised SNP in terms of the pathogenesis of

RA is the non-synonymous 158 V/F polymorphism in *FCGR3A* (rs396991), which substitutes a valine for a phenylalanine at amino acid position 158, thereby increasing the affinity of FcγRIIIa for IgG1 and IgG3.⁴ We and other groups have previously found an association between the higher affinity V allele and RA,⁵⁻⁹ particularly in subgroups with more severe disease marked by nodules,⁸⁻¹⁰ and have discussed potential biological pathways that may be upregulated by this SNP. Some groups have been either unable to detect similar associations, possibly due to cohort size, or have identified an association with the alternative allele.¹¹⁻¹⁴

The *FCGR* locus has poor SNP coverage on the main commercial arrays owing to the presence of homologous sequence paralogs. For *FCGR3A* and *FCGR3B*, none of the array-based assays on the commonly used commercial arrays (Affymetrix 5.0, 6.0, Illumina Human Map 300, 550, 650) are gene-specific. Thus, despite advances in genotyping technologies, the *FCGR* locus can currently only be investigated using well-designed conventional low to medium throughput technologies.

The aim of the current study was to re-evaluate the association of *FCGR3A* with RA by undertaking a replication study in a large UK Caucasian population and a meta-analysis of previous work. We previously demonstrated a multiplicative joint effect with the *HLA-DRB1* shared epitope (SE). Given the strong association between *HLA-DRB1* SE and cyclic citrullinated peptide (CCP) autoantibodies,¹⁵ we also undertook a series of subgroup analyses stratifying for autoantibody status.

PATIENTS AND METHODS

Patients with RA aged ≥18 years of age at disease onset ($n=834$) were recruited from the Yorkshire Early Arthritis Register (YEAR). An additional 1215 patients were recruited from the general rheumatology outpatients in Leeds or through the Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate (BRAGGSS), as described previously.¹⁶ All cases were Caucasian of Northern European descent and fulfilled the 1987 American College of Rheumatology classification criteria.¹⁷ Healthy controls were a combination of blood donors ($n=235$) and general population controls recruited from Sheffield ($n=921$).^{18,19} An additional 914 patients with RA and 575 control subjects from our previous studies were included in the combined and subgroup analyses.^{8,9}

Genotyping

FCGR3A genotyping (rs396991) was carried out using a validated single-stranded conformational polymorphism (SSCP) assay or, alternatively, by the direct sequencing of the same PCR product.⁹ *HLA-DRB1* typing and SE classification was undertaken as previously described.⁹

Immunoassays

The majority of RA cases were recruited from NHS rheumatology clinics throughout the UK and IgM rheumatoid factor (RF) status was measured using standard nephelometric assays. Patients who had ever had titres ≥ 40 units/ μ l were defined as RF positive. The presence of CCP antibodies was documented at a single time point for a proportion of the patients (n=1452) included in both the original and replication studies using the commercially available DIASTAT anti-CCP ELISA (Axis-Shield Diagnostics, Cambridgeshire, UK) or the ELiA CCP kit on an ImmunoCAP 100 (Phadia AB, Uppsala, Sweden). Patients who had a titre of ≥ 5.5 units/ml or ≥ 10 units/ml for the two assays, respectively, were defined as CCP positive.

Statistical analysis

Power calculations (power 80%, significance 5%) indicated that the replication study had sufficient power to detect an association between *FCGR3A*-158V and RA with an OR of 1.45, assuming a recessive model. Statistical analyses were performed using the SPSS 13.0 statistical package for Windows (Chicago, Illinois, USA). Data from the combined cohorts were stratified according to gender and the presence of autoantibodies (RF and CCP). ORs and their 95% CI were calculated for the effect of the *FCGR3A*-158VV genotype on risk compared with alternative genotypes (ie, recessive model). The χ^2 test was used for statistical comparisons and p values < 0.05 were considered statistically significant throughout.

The possibility of an interaction between *FCGR3A* and the *HLA-DRB1* SE was formally tested, within a logistic regression framework, by performing a test for interaction (departure from a multiplicative joint effect) using the Stata statistical software release 10.0 (Stata Corporation, College Station, Texas, USA). Departure from an additive joint effect was investigated by calculating the relative excess risk due to interaction (RERI) and hence (if RERI > 0) the attributable proportion (AP) of risk due to interaction. The test was based on standard error estimates obtained from Taylor series expansion.²⁰

For the meta-analysis, we searched PubMed for association studies using the terms 'FCGR3A', 'RA', 'Rheumatoid', 'Arthritis', 'FCGR', 'Fcgamma' and using references retrieved from these manuscripts. The meta-analyses were restricted to studies that specified Caucasian ethnicity in the patients and controls. Seven studies fitted the criteria for the meta-analysis, resulting in a total of 5320 cases of RA and 4458 controls. Random effects models were used to perform the meta-analyses under a recessive model. Heterogeneity was investigated using I^2 , which describes the proportion of total variation in study estimates that is due to heterogeneity and by Cochran's Q-statistic. A meta-analysis stratified by autoantibody status was performed for studies which reported the relevant data.

RESULTS

The replication study comprised 2049 subjects with RA and 1156 controls who had not previously been genotyped. The sub-analyses were undertaken on a combined cohort that included previously genotyped subjects and comprised 2963 patients

with RA and 1731 controls. For the combined RA cohort, 69% of the patients were women, 76% were *HLA-DRB1* SE positive, 75% were IgM RF positive and 73% were positive for CCP antibodies. The gender was known for 1551 control subjects, 60% of whom were women.

Association of *FCGR3A* with rheumatoid arthritis

There was no departure from Hardy-Weinberg equilibrium in the control group. Assuming a recessive model, the effect size for *FCGR3A*-158V was reduced in the replication cohort and failed to reach statistical significance (OR 1.1, 95% CI 0.9 to 1.4; table 1), but the estimate was consistent with an increase in risk.

When the replication study was combined with the original cohort, homozygosity for the *FCGR3A*-158V allele showed borderline significance with RA (OR 1.2, 95% CI 1.0 to 1.5; table 1). The strength of the association was found to be greater in men (table 1), as previously reported by others.⁷ The association was also greater in those patients harbouring autoantibodies (CCP and/or IgM RF). An increased frequency of homozygosity for the *FCGR3A*-158V allele was observed in both RF positive and negative RA, but this was only statistically significant in the RF positive subgroup (OR 1.3, 95% CI 1.0 to 1.6, p=0.03), most likely as a result of increased sample size and statistical power. However, an increased frequency of *FCGR3A*-158V homozygotes was only observed in the CCP positive RA group (OR 1.3, 95% CI 1.0 to 1.6, p=0.05), with an estimated OR of 1.0 in the CCP negative group (table 1). No statistically significant difference in autoantibody status between men and women was observed.

Meta-analysis

Data from 5320 cases of RA and 4558 healthy controls were available. There was no evidence of significant population heterogeneity. The I^2 statistic of 0% suggested that all variability in effect estimates was due to sampling error within studies. We found that homozygosity for the V allele was significantly associated with disease susceptibility (figure 1A, p=0.003), particularly in CCP positive RA (figure 1B, p=0.004).

Table 1 *FCGR3A* genotype frequencies and subgroup analyses

Cohort	<i>FCGR3A</i> 158			OR (95% CI, p value)*
	FF	FV	VV	
Replication				
Controls (n=1156)	525 (0.45)	515 (0.45)	116 (0.10)	
RA (n=2049)	918 (0.45)	903 (0.44)	228 (0.11)	1.1 (0.9 to 1.4, p=0.3)
Combined				
Controls (n=1731)	798 (0.46)	760 (0.44)	173 (0.10)	
RA (n=2963)	1311 (0.44)	1300 (0.44)	352 (0.12)	1.2 (1.0 to 1.5, p=0.05)
Subgroup				
RF+ (n=2005)	881 (0.44)	878 (0.44)	246 (0.12)	1.3 (1.0 to 1.6, p=0.03)
RF- (n=650)	283 (0.44)	292 (0.45)	75 (0.12)	1.2 (0.9 to 1.6, p=0.3)
CCP+ (n=1071)	469 (0.44)	469 (0.44)	133 (0.12)	1.3 (1.0 to 1.6, p=0.05)
CCP- (n=381)	164 (0.43)	180 (0.47)	37 (0.10)	1.0 (0.7 to 1.4, p=0.9)
Women				
Controls (n=922)	425 (0.46)	393 (0.43)	104 (0.11)	
RA (n=2060)	888 (0.43)	926 (0.45)	246 (0.12)	1.1 (0.8 to 1.4, p=0.6)
Men				
Controls (n=629)	295 (0.47)	284 (0.45)	50 (0.08)	
RA (n=755)	349 (0.46)	311 (0.41)	95 (0.13)	1.7 (1.2 to 2.4, p=0.01)

*A recessive model was used to estimate the relative risk of homozygosity for the *FCGR3A*-158V allele.
CCP, cyclic citrullinated peptide; RA, rheumatoid arthritis; RF, rheumatoid factor.

Extended report

Joint effect of *FCGR3A* and the *HLA-DRB1* shared epitope

We calculated the risk of developing RA conferred by combinations of homozygosity for the *FCGR3A*-158V allele and the presence of a *HLA-DRB1* SE allele (table 2). The data were consistent with either an additive or multiplicative joint effect, with no evidence of an interaction between these two genetic loci (table 2). The increased risk to *FCGR3A* and *HLA-DRB1* SE positive individuals was strong (OR 5.1, 95% CI 3.6 to 7.1, $p < 0.0001$), particularly in the RF positive (OR 7.0, 95% CI 4.9 to 9.9, $p < 0.0001$) and CCP positive (OR 8.4, 95% CI 5.7 to 12.4, $p < 0.0001$) subgroups.

DISCUSSION

We have previously demonstrated and confirmed an association with homozygosity for the *FCGR3A*-158V allele in established RA cohorts. Our replication study showed the same trend towards an increase in *FCGR3A*-158VV genotype frequency and the combined analysis demonstrated borderline statistical significance, with an OR of 1.2 compared with 1.5 in our previous studies.⁹ To detect a more modest association with an OR of 1.2, which is comparable with other non-MHC genetic loci in RA, 4794 cases and controls would have been required which were not available for the current study. A meta-analysis of 5320 patients with RA and 4558 healthy controls was therefore undertaken (figure 1) and confirmed the association of *FCGR3A*-158VV with RA (OR 1.2).

Emerging data suggest that different genetic and environmental associations and thus pathogenic mechanisms may be observed in autoantibody positive and negative RA.²¹ This is of particular importance with respect to *FCGR3A*, since homozygosity for the high affinity variant may only be of functional significance in the subgroup of patients with RA with either IgG antibodies (CCP) or IgG-containing immune complexes (RF). The presence of a higher affinity activating receptor may enhance immune complex binding, thereby stimulating proinflammatory mediator release at lower concentrations. Indeed, FcγRIIIa has been implicated as a major trigger for release of tumour necrosis factor from human macrophages following the binding of small immune complexes.²² Correspondingly, *FCGR3A* was significantly associated with RA in the subgroup of patients positive for either CCP or RF antibodies (OR 1.3, $p = 0.02$) and with CCP antibodies in the meta-analysis (OR 1.3, $p = 0.004$), but not those patients negative for each antibody. Further subgroup analyses

revealed that RA subgroups with either RF or CCP autoantibodies demonstrated an additive/multiplicative joint effect between both *FCGR3A* and *HLA-DRB1* SE alleles, with no evidence of statistical interaction. Much larger studies will be required to identify sufficient numbers of subjects with RA positive for a single autoantibody to determine whether the primary association is downstream of RF or CCP autoantibodies, which may ligate different FcγRs.

Segmental duplications and copy number variants are mechanistically linked.²³ A recent report has indicated that both *FCGR3A* and *FCGR3B* are subject to independent copy number variation.²⁴ To date, there is no evidence that *FCGR3A* copy number is associated with RA per se. The impact of copy number variation on *FCGR3A* allelic association has been shown to be slight in a Dutch

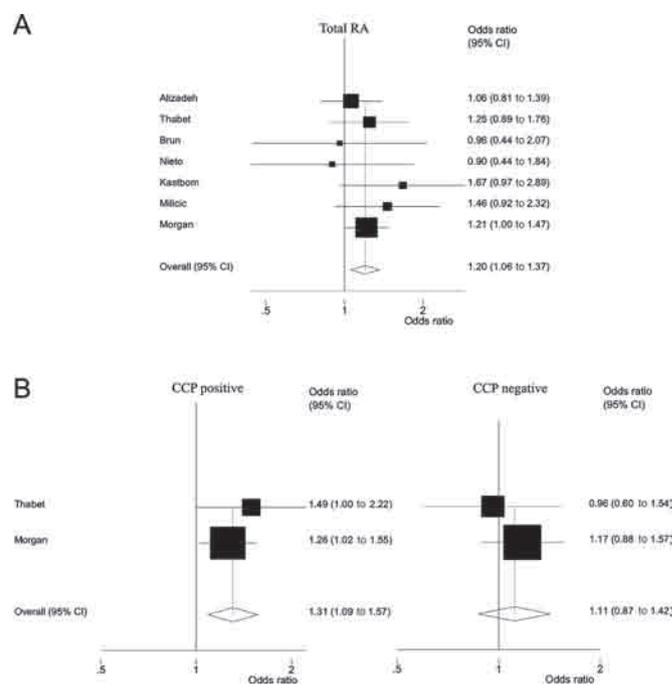


Figure 1 (A, B) Meta-analysis of susceptibility to rheumatoid arthritis (RA) conferred by homozygosity for the *FCGR3A*-158 V allele compared with all other genotypes. CCP, cyclic citrullinated peptide.

Table 2 ORs for developing RA according to the presence or absence of homozygosity for the *FCGR3A*-158V and carriage of the *HLA-DRB1* SE alleles

Cohort	N	<i>FCGR3A</i> 158 genotype (<i>HLA-DRB1</i> SE)				p Value for departure from multiplicity*	p Value for departure from additivity†
		FForFV(+SE)	FForFV(-SE)	VV(+SE)	VV(-SE)		
Controls	1093	454 (0.42)	532 (0.49)	49 (0.04)	58 (0.05)		
Total RA	2363	1577 (0.67)	484 (0.20)	226 (0.10)	76 (0.03)	0.7	0.4 (0.16)
OR (95% CI)		3.8 (3.6 to 4.5)	1.0	5.1 (3.6 to 7.1)	1.4 (1.0 to 2.1)		
p Value		<0.0001	-	<0.0001	0.05		
RF+ RA	1693	1203 (0.71)	272 (0.16)	175 (0.10)	43 (0.03)	0.8	0.3 (0.19)
OR (95% CI)		5.2 (4.3 to 6.2)	1.0	7.0 (4.9 to 9.9)	1.5 (1.0 to 2.2)		
p Value		<0.0001	-	<0.0001	0.08		
RF- RA	528	292 (0.55)	169 (0.32)	37 (0.07)	30 (0.06)	0.3	0.7
OR (95% CI)		2.0 (1.6 to 2.6)	1.0	2.4 (1.5 to 3.9)	1.6 (1.0 to 2.7)		
p Value		<0.0001	-	0.0002	0.04		
CCP+ RA	899	655 (0.73)	127 (0.14)	98 (0.11)	19 (0.02)	1.0	0.2 (0.23)
OR (95% CI)		6.0 (4.8 to 7.6)	1.0	8.4 (5.7 to 12.4)	1.4 (0.8 to 2.4)		
p Value		<0.0001	-	<0.0001	0.3		
CCP- RA	316	152 (0.48)	130 (0.41)	13 (0.04)	21 (0.07)	0.1	0.2
OR (95% CI)		1.4 (1.0 to 1.8)	1.0	1.1 (0.5 to 2.1)	1.5 (0.8 to 2.6)		
p Value		0.02	-	0.8	0.15		

*Departure from a multiplicative joint effect on risk was tested using logistic regression, comparing a model with and without the interaction term using the likelihood ratio test.

†Attributable proportion (AP) is not calculated where the estimated joint effect is less than additive.

CCP, cyclic citrullinated peptide; RA, rheumatoid arthritis; RF, rheumatoid factor.

cohort,⁶ but the study was underpowered to exclude the association of *FCGR3A* copy number variation with disease.

It is likely that quantitative and qualitative analyses of all low-affinity *FCGR* genes used in a combined approach will ultimately be needed to fully elucidate the complex contribution of *FCGR* genetic variation to the pathogenesis of RA. This study highlights the inherent difficulties in studying genes contained within segmental duplications and the fact that some genetic loci cannot be adequately assayed using modern high-throughput genotyping technologies. The study supports the ongoing investigation of the *FCGR* genetic locus in the pathogenesis of RA. Future efforts directed towards obtaining clearly defined patient cohorts with longitudinal clinical data from disease onset will ultimately determine whether this locus also contributes to disease severity and may serve as a prognostic marker.

Funding This work was supported by the Arthritis Research Campaign, UK and the NIHR-Leeds Musculoskeletal Biomedical Research Unit.

Competing interests None.

Ethics approval This study was conducted with the approval of the Northern and Yorkshire multicentre research ethics committee and all participants gave informed consent.

Provenance peer review Not commissioned; externally peer reviewed.

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