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ORIGINAL INVESTIGATION

FCGR3A and *FCGR3B* copy number variations are risk factors for sarcoidosis

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Abstract Sarcoidosis is a multisystem granulomatous disorder that causes significant morbidity. Genetic factors contribute to sarcoidosis risks. In this study, we investigated whether copy number variations (CNVs) of *FCGR3A* (coding for FcγRIIIA) and *FCGR3B* (coding for FcγRIIIB) genes are associated with sarcoidosis susceptibility and whether the expressions of FcγRIIIA on NK cells and FcγRIIIB on neutrophils are altered in sarcoidosis patients. TaqMan real-time PCR assays were used to analyze the CNV of *FCGR3A* and *FCGR3B* genes. *FCGR3A* and *FCGR3B* CNV genotypes were compared between 671 biopsy-proven sarcoidosis patients and the same number of healthy controls matched with age, sex, race, and geographic area from the ACCESS (A Case Control Etiologic Study of Sarcoidosis) cohort. Flow cytometry analyses were used to determine expressions of FcγRIIIA on NK cells and FcγRIIIB on neutrophils in phenotype analyses. We found that *FCGR3A* CNVs were significantly associated with sarcoidosis in females (CN = 1 vs. CN = 2 logistic regression adjusted for sex and race, OR 4.0156, SE = 2.2784, $P = 0.0143$; CN = 3 vs. CN = 2 logistic regression adjusted for sex and race, OR 2.8044, SE = 1.1065, $P = 0.0090$), suggesting that *FCGR3A* gene abnormality influences sarcoidosis development in a gender-specific manner. Furthermore, FcγRIIIA expressions were significantly decreased on NK cells from sarcoidosis patients compared to those from healthy controls ($P = 0.0007$). Additionally, low *FCGR3B* CN was associated with sarcoidosis (CN <2 vs. CN = 2 logistic regression adjusted for sex and race, OR 1.5025, SE = 0.2682, $P = 0.0226$), indicating that the functions of *FCGR3B* gene may also contribute to the pathogenesis of sarcoidosis. We conclude that *FCGR3A* CNVs are a major risk factor for female sarcoidosis and *FCGR3B* CNVs may also affect the development of sarcoidosis.

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Introduction

Sarcoidosis is a multisystem granulomatous disorder of unknown etiology. Sarcoidosis frequently affects the lungs and may cause significant morbidity (Gerke 2014). Infectious and non-infectious factors may induce autoimmune responses leading to the development of sarcoidosis (Dubaniewicz 2010, 2013; Lazarus 2009; Morgenthau and Iannuzzi 2011). The critical role for genetic factors contributing to sarcoidosis etiology is strongly supported by twin studies, disease clustering in families, and racial differences in incidence rates (Rybicki et al. 1997, 2001a, b; Smith et al. 2008; Sverrild et al. 2008). The adjusted annual incidence among African Americans is roughly three times of that among Caucasian Americans (35.5 cases per 100,000 for African Americans as compared to 10.9 per 100,000 in Caucasian Americans) (Iannuzzi et al. 2007). Genetic factors contribute to sarcoidosis risks (Sverrild et al. 2008). Multiple genes could influence sarcoidosis (Grunewald 2008; Iannuzzi and Rybicki 2007; Iannuzzi et al. 1997, 2007). While the human MHC region is strongly associated

with sarcoidosis (Grunewald 2008; Smith et al. 2008), non-MHC genes also contribute to the pathogenesis of sarcoidosis (Hofmann et al. 2011, 2008; Iannuzzi et al. 2005; Rybicki et al. 2005).

Sarcoidosis is frequently associated with humoral abnormalities such as hypergammaglobulinemia (Bell et al. 1986; Hedfors and Norberg 1974; Hunninghake and Crystal 1981), autoantibody production (Weinberg et al. 2000), and the presence of circulating immune complexes (Daniele et al. 1978; Dubaniewicz et al. 2012, 2013). Additionally, IgG Fc receptor (FcγR) expressions on immune cells were significantly different between sarcoidosis patients and healthy controls (Dubaniewicz et al. 2012; Heron et al. 2008; Okamoto et al. 2003; Rossman et al. 1986; Yoshida et al. 1991), suggesting FcγRs may be involved in the pathogenesis of sarcoidosis. FcγRs mediate a range of immune functions (immune complex clearance, phagocytosis, antigen presentation, degranulations, ADCC, and cytokine production) and serve as the essential link between the humoral and cellular immunities (Ravetch and Bolland 2001; Ravetch and Lanier 2000). The classical human low-affinity Fcγ receptor family has five members with high degrees of sequence homology (Qiu et al. 1990). Three family members, FcγRIIIA (*FCGR3A* or CD16A), FcγRIIA (*FCGR2A* or CD32A), and FcγRIIC (*FCGR2C* or CD32C), have either a tyrosine activation motif (ITAM) in their cytoplasmic domains (FcγRIIA and FcγRIIC) or an ITAM in the associated FcR γ-chain. FcγRIIIB (*FCGR3B* or CD16B), anchored to the membrane by a glycosylphosphatidyl inositol moiety, mediates activation signals through its co-receptor Mac-1 (Krauss et al. 1994; Lei et al. 2001; Stockl et al. 1995). FcγRIIB (*FCGR2B* or CD32B) has a tyrosine inhibitory motif and functionally counterbalances the activation signals from activating receptors (Ravetch and Bolland 2001; Ravetch and Lanier 2000). Interaction between IgG immune complexes (ICs) and FcγRs critically affects the functions of human immune system.

Although several genes have been identified to associate with sarcoidosis in genome-wide association studies (GWAS) (Hofmann et al. 2008, 2011), investigations have failed to identify a unifying genetic signature associated with sarcoidosis thus far (Rossman and Kreider 2007; Smith et al. 2008). Technically, homologous *FCGR* genes are not suitable for GWAS assays and, therefore, the genetic markers within the human *FCGR* gene cluster are not included in any GWAS assays. The expressions of FcγRs were altered on monocytes and the functional polymorphisms of *FCGR2A*, *FCGR2C*, and *FCGR3A* were associated with sarcoidosis phenotypes (Dubaniewicz et al. 2012; Typiak et al. 2014, 2016), suggesting that FcγRs may be involved in the development of sarcoidosis. *FCGR* genes have copy number variations (CNVs, or gene deletion and duplication polymorphisms), which lead to the gene deficiency or gain-of-functions. It has been demonstrated that gene deletions or low copy numbers (<2 copy) of *FCGR3B* gene are significantly associated with SLE (systemic lupus erythematosus) (Chen et al. 2014; Fanciulli et al. 2007; McKinney and Merriman 2012; Niederer et al. 2010; Willcocks et al. 2008), with which sarcoidosis often coexists (Chatham 2010). Nevertheless, it remains unknown whether *FCGR* CNVs have a role in the development of sarcoidosis. The current study revealed that *FCGR3A* and *FCGR3B* CNVs are risk factors for sarcoidosis susceptibility.

Patients and methods

ACCESS study cohort characteristics

The ACCESS (A Case Control Etiologic Study of Sarcoidosis) cohort DNA samples and data (672 pairs of biopsy-proven sarcoidosis patients and healthy controls matched by age, sex, race, and geographic area) were provided by the NHLBI Biologic Specimen and Data Repository. The original goal of ACCESS was to generate hypotheses about the etiology of sarcoidosis (Group 1999; Rossman and Kreider 2007). The major hypothesis of the ACCESS was that sarcoidosis occurs in genetically susceptible individuals through alteration in immune response after exposure to an environmental, occupational, or infectious agent. Cases of sarcoidosis were recruited prospectively within geographic regions surrounding the ten participating clinical centers between 1996 and 1999 (Group 1999). Sarcoidosis subjects met the following inclusion criteria: (1) first tissue confirmation of non-caseating granulomas on biopsy within 6 months of enrollment, (2) clinical signs or symptoms consistent with sarcoidosis, and (3) age >18 years. Specific phenotypes of sarcoidosis were determined with an instrument developed by the ACCESS group (Judson et al. 1999). The clinical characteristics of the study patients have been described previously (Baughman et al. 2001). Since strict criteria were used for the diagnosis of sarcoidosis and definitions of specific organ involvement, the patients recruited for the ACCESS represent the best clinical description of sarcoidosis at presentation in the United States (Group 1999; Rossman and Kreider 2007). Controls were recruited by random digit dialing methods from within the same geographic region as cases. Controls were matched to cases on the basis of age (within 5 years), gender, and self-reported race and ethnicity. Controls were excluded if they reported a history of sarcoidosis or medical conditions that made the determination of sarcoidosis uncertain (e.g., granulomatous

Table 1 Distribution of cases and controls by gender and ethnic origin

	Ethnic origin			Total (%)
	Caucasians (%)	Africans (%)	Others (%)	
ACCESS cases				
Male	158 (23.5)	79 (11.8)		237 (35.3)
Female	213 (31.8)	221 (32.9)		434 (64.7)
Total	371 (55.3)	300 (44.7)		671 (100.0)
ACCESS controls				
Male	158 (23.5)	79 (11.8)		237 (35.3)
Female	213 (31.8)	221 (32.9)		434 (64.7)
Total	371 (55.3)	300 (44.7)		671 (100.0)
Phenotyped cases				
Female	11 (40.8)	1 (3.7)	2 (7.4)	14 (51.9)
Male	9 (33.3)	2 (7.4)	2 (7.4)	13 (48.1)
Total	20 (74.1)	3 (11.1)	4 (14.8)	27 (100.0)
Phenotyped controls				
Female	12 (41.4)	1 (3.4)		13 (44.8)
Male	14 (48.3)	2 (6.9)		16 (55.2)
Total	26 (89.6)	3 (10.4)		29 (100.0)

hepatitis or idiopathic uveitis). Table 1 lists the distribution of cases and controls by gender and ethnic origin of ACCESS cohort. The clinical characteristics and organ involvement of ACCESS subjects were described in detail previously (Baughman et al. 2001).

Human subjects for phenotype analysis

Sarcoidosis patients were recruited at the University of Minnesota Medical Center Interstitial Lung Disease Clinic. The ages of 27 sarcoidosis patients (13 males and 14 females) ranged from 28.7 to 79.2 years with the mean age of 55.4 ± 13.7 years. Eleven patients were not treated and 16 patients were undergoing treatment when the patients were enrolled for phenotype analysis. Healthy control donors were recruited through Memorial Blood Centers in Minnesota as described previously (Li et al. 2015). The ages of 29 healthy controls (16 males and 13 females) ranged from 18 to 85 years with the mean age of 64 ± 13.5 years. The majority of sarcoidosis cases (74 %) and healthy controls (89.6 %) for phenotype analysis were Caucasians (Table 1). The human study was approved by the Institutional Review Board for Human Use at the University of Minnesota.

Nucleic acid isolation

Genomic DNA samples were isolated from anti-coagulated peripheral blood using the Wizard Genomic DNA Purification kit (Promega, Madison, WI).

Determination of *FCGR3* CNVs

CNV assay probes containing FAM, MGB, and non-fluorescent quencher were produced at Applied Biosystems (Foster City, CA, USA). The CNVs of *FCGR3A* and *FCGR3B* were genotyped using custom TaqMan CNV real-time, quantitative PCR assays with the labeled probes as previously described (Chen et al. 2014). Briefly, duplex quantitative real-time PCR reactions were carried out on an Applied Biosystems 7500 Real-Time PCR System (Life Technology) with Copy Number Reference Assay RNase P with VIC-TAMRA dual-labeled probe (Applied Biosystems, cat#4403328) as the internal control of CN reference, according to the manufacturer's instructions. Fluorescence signals of duplicate samples were normalized to ROX. The quantitative PCR amplification curves were analyzed using 7500 Software on a plate by plate basis, and the CN was assigned from the raw Cq values using CopyCaller™ software (version 2.0; Applied Biosystems). This software employs a clustering algorithm and assigns the cluster with the most samples as CN = 2. The CopyCaller™ software also provides extensive diagnostics for the validity of the results, which were set to accept the CN assignment only when confidence was >95 %, the standard deviation of the sample replicate ΔCq estimates was <0.20, and a reference gene Cq was <32. Overall, our methodology resulted in clear assignment of *FCGR3* CN for 99.9 % (1343/1344) samples.

Evaluation of FcγRIII (CD16) expression levels

To determine the expression of CD16 (FcγRIII) on NK cells and neutrophils, 100 µl fresh whole blood samples were stained with FITC-conjugated anti-human CD16 mAb (clone 3G8) (Life Technologies) and APC-conjugated anti-human CD56 (BD Biosciences). Whole blood samples stained with FITC-conjugated mIgG1 and APC-conjugated anti-human CD56 in separate tubes were used as isotype controls. After incubation at room temperature for 30 min, blood samples were treated with 1 × FACS Lysing Solution (BD Biosciences) to lyse red blood cells, followed by analysis on a Canto Flow cytometer (BD Biosciences). NK cells were identified within the lymphocyte population as $CD56^+$ cells. Characteristic light-scatter properties were used to identify neutrophils in flow cytometry. Expression of FcγRIII (CD16) was analyzed with FlowJo software (Tree Star Inc., http://www.flowjo.com/).

Statistical analysis

Conditional logistic regression was used to test for association between *FCGR3* CN and sarcoidosis, incorporating the age-, sex-, and race-matching in the matched

case–control design. The null hypothesis was rejected at 2.5 % level of significance ($P < 0.025$) as two genes were analyzed and that the Bonferroni corrections (0.05/2) were applied. We further carried out race- and gender-specific analyses. Fisher's exact test was used since the tests were performed within each race group and the small counts of CN <2 and CN >2 groups. The distributions of *FCGR3* CN genotypes between sarcoidosis patients and healthy controls within each ethnic group (Caucasians or Africans) were compared using χ^2 test. The *P* value (*P*), odds ratio (OR), and 95 % confidence interval (CI) were calculated assuming CN = 2 (two copy carrier) as the neutral genotype. Bonferroni correction was applied for the total of eight hypothesis tests; a *P* value less than 0.00625 (0.05/8) in χ^2 test and Fisher's exact test was considered as a significant association between a specific *FCGR3* CN and sarcoidosis susceptibility. Mann–Whitney *U* tests was used to analyze FcγRIII (CD16) expression changes on NK and neutrophils. A *P* value less than 0.05 was considered statistically significant in the study.

Results

FCGR3A CNVs are associated with sarcoidosis susceptibility

We examined the single-locus association between the *FCGR3A* CNVs and the susceptibility to sarcoidosis by stratified human subjects by race and gender (Table 2). As shown in Table 2, the *FCGG3A* CNV genotypes were significantly associated with sarcoidosis in Caucasian females ($\chi^2 = 10.74$, $P = 0.0047$). Conditional logistic regression analyses revealed that both low *FCGR3A* CN (CN = 1 vs. CN = 2, OR 4.0256, SE 2.2784, $P = 0.0143$) and high *FCGR3A* CN (CN = 3 vs. CN = 2, OR 2.8044, SE = 1.1065, $P = 0.0090$) were significantly associated with sarcoidosis susceptibility in ethnically combined females. Specifically, the high *FCGR3A* CN (CN = 3) was significantly associated with sarcoidosis disease susceptibility in female Caucasians (CN = 3 vs. CN = 2, $P = 0.0042$, OR 3.676, 95 % CI: 1.444–9.363). Although low *FCGR3A* CN (CN = 1) was enriched in female African sarcoidosis patients compared to the respective controls, the difference was not statistically significant after Bonferroni correction (CN = 1 vs. CN = 2, $P = 0.0371$, OR 7.233, 95 % CI 0.8819–59.33). In contrast, *FCGR3A* CNVs were not associated with sarcoidosis susceptibility in males, suggesting a gender-dependent effect of *FCGR3A* CNVs on sarcoidosis susceptibility. Our data indicate a role of *FCGR3A* gene abnormality in the development of sarcoidosis.

FCGR3B CNV is also a risk factor for sarcoidosis susceptibility

As shown in Table 3, conditional logistic regression analysis adjusted for sex and race revealed that the low *FCGR3A* CN (CN <2 vs. CN = 2, OR 1.5025, SE = 0.2582, $P = 0.0226$) was significantly associated with sarcoidosis susceptibility in combined subjects after Bonferroni correction. On the other hand, the high *FCGR3B* CN (CN = 3) was not associated with sarcoidosis disease susceptibility (CN = 3 vs. CN = 2, OR 1.0907, SE = 0.2177, $P = 0.6634$). After stratification, the low *FCGR3B* CN (CN <2) genotype frequencies tend to be increased in both Caucasian sarcoidosis patients (10.8 %) and African sarcoidosis patients (17.7 %) compared to respective healthy controls (6.7 % for Caucasians and 14.0 % for Africans), but the difference was not significant, likely due to the decreased sample sizes after the stratification. Our data suggest that the lower expression of FcγRIIIB may contribute to the pathogenesis of sarcoidosis.

FcγRIIIA (CD16A) expression on NK cells is abnormal in sarcoidosis patients

The FcγRIIIA (CD16A) encoded by *FCGR3A* is the sole activating IgG Fc receptor on human NK cells capable of mediating ADCC (Perussia et al. 1983a, b). Since *FCGR3A* CNVs were associated with sarcoidosis in females (Table 2), we subsequently determined whether the FcγRIIIA (CD16A) expressions on peripheral blood NK cells from sarcoidosis patients were different from those of healthy controls. As shown in Fig. 1a, compared to NK cells from healthy controls ($N = 29$), significant low percentages of $CD56^+$ NK cells from sarcoidosis patients ($N = 27$) expressed CD16A ($P = 0.0007$). After stratifying sarcoidosis patients and healthy controls into male and female groups, female and male healthy controls had very similar percentages of $CD56^+CD16^+$ NK cells ($P = 0.9301$) (Fig. 1b). However, female sarcoidosis patients ($N = 14$) had significant lower percentages of $CD56^+CD16^+$NK cells compared to the female healthy controls ($N = 13$) ($P = 0.0033$). Male sarcoidosis patients ($N = 13$) tended to have lower percentages $CD56^+CD16^+$ NK cells than male healthy controls ($N = 16$), but the difference was not significant ($P = 0.0832$) (Fig. 1b). Furthermore, treatment conditions did not significantly affect the percentages of $CD56^+CD16^+$ NK cells in sarcoidosis patients ($P = 0.7219$) (Fig. 1c). Our data suggest that abnormal CD16A expression on NK cells may contribute to the development of sarcoidosis. The genotype distribution of *FCGR3A* CNVs was very similar between sarcoidosis patients and healthy controls ($P = 0.8029$) in the phenotyped subjects (Table 4), indicating that the FcγRIIIA

Table 2 Association of copy number variations of FcγRIIIA gene (*FCGR3A*) with sarcoidosis

Race	Male (%)		Female (%)		Total (%)	
	Cases	Controls	Cases	Controls	Cases	Controls
Caucasians						
CN = 1	3 (1.9)	5 (3.1)	8 (3.8)	3 (1.4)	11 (3.0)	8 (2.1)
CN = 2	145 (91.8)	142 (89.9)	185 (86.8)	204 (95.8)	330 (88.9)	346 (93.3)
CN = 3	10 (6.3)	11 (7.0)	20 (9.4)	6 (2.8)	30 (8.1)	17 (4.6)
Total subjects	158 (100)	158 (100)	213 (100)	213 (100)	371 (100)	371 (100)
χ^2	0.5790		10.74		4.448	
P	0.7486		0.0047**		0.1082	
Fisher's exact *P* (CN = 1 vs. CN = 2)	0.5010		0.1292		0.4908	
OR (95 % CI)	0.5876 (0.1378–2.506)		2.941 (0.7684–11.25)		1.442 (0.5726–3.630)	
Fisher's exact *P* (CN = 3 vs. CN = 2)	0.8250		0.0042**		0.0506*	
OR (95 % CI)	0.8903 (0.3666–2.162)		3.676 (1.444–9.363)		1.850 (1.001–3.3419)	
Africans						
CN = 1	0 (0.0)	2 (2.5)	7 (3.2)	1 (0.5)	7 (2.3)	3 (1.0)
CN = 2	77 (97.5)	75 (95.0)	210 (95.0)	217 (98.2)	287 (95.7)	292 (97.3)
CN = 3	2 (2.5)	2 (2.5)	4 (1.8)	3 (1.3)	6 (2.0)	5 (1.7)
Total subjects	79 (100)	79 (100)	221 (100)	221 (100)	300 (100)	300 (100)
χ^2	2.111		4.758		1.734	
P	0.3481		0.0927		0.4202	
Fisher's exact *P* (CN = 1 vs. CN = 2)	0.4967		0.0371*		0.222	
OR (95 % CI)	0.1948 (0.0092–4.129)		7.233 (0.8819–59.33)		2.2374 (0.6077–9.274)	
Fisher's exact *P* (CN = 3 vs. CN = 2)	1.000		0.7210		0.7710	
OR (95 % CI)	0.9740 (0.1337–7.098)		1.378 (0.3046–6.232)		1.221 (0.3684–4.046)	
Combined races						
CN = 1	3 (1.3)	7 (2.9)	15 (3.4)	4 (0.9)	18 (2.7)	11 (1.6)
CN = 2	222 (93.7)	217 (91.6)	395 (91.0)	421 (97.0)	617 (92.0)	638 (95.1)
CN = 3	12 (5.0)	13 (5.5)	24 (5.6)	9 (2.1)	36 (5.3)	22 (3.3)
Total subjects	237 (100)	237 (100)	434 (100)	434 (100)	671 (100)	671 (100)
Logistic regression						
OR for CN = 1	0.4157		4.0156		1.7311	
SE	0.2892		2.2784		0.6667	
P value	0.2070		0.0143**		0.1542	
OR for CN = 3	0.8624		2.8044		1.6855	
SE	0.3502		1.1065		0.4584	
P value	0.7155		0.0090**		0.0549	

* Indicates an association between *FCGR3A* CN and sarcoidosis before the Bonferroni correction

** Indicates a significant association between *FCGR3*A CN and sarcoidosis after the Bonferroni correction

expression difference between sarcoidosis patients and healthy controls were not due to the difference of *FCGR3A* CNV genotypes. On the other hand, peripheral blood neutrophil CD16B expressions were not significantly different between sarcoidosis patients and healthy controls when compared in groups ($P = 0.9608$) (Fig. 1d). Additionally, one out of 27 sarcoidosis patients completely lacked FcγRIIIB (CD16B) expression on neutrophils (Fig. 1d). Genotyping analysis confirmed that the sarcoidosis patient with the null FcγRIIIB expression on neutrophils carried the *FCGR3B* CN = 0 genotype, which predicts the FcγRIIIB expression deficiency (Chen et al. 2014).

Discussion

Gene copy number variations (CNVs) contribute significantly to the development of human diseases (McKinney and Merriman 2012). Human *FCGR* locus at 1q23 region has two types of CNVs involving either *FCGR3A*

Table 3 Association of copy number variations of FcγRIIIB gene (*FCGR3B*) with sarcoidosis

Ethnicity	Male (%)		Female (%)		Total (%)	
	Cases	Controls	Cases	Controls	Cases	Controls
Caucasians						
CN < 2	14 (8.9)	11 (7.0)	26 (12.2)	14 (6.6)	40 (10.8)	25 (6.7)
CN = 2	130 (82.2)	134 (84.8)	167 (78.4)	185 (86.8)	297 (80.0)	319 (86.0)
CN = 3	14 (8.9)	13 (8.2)	20 (9.4)	14 (6.6)	34 (9.2)	27 (7.3)
Total	158 (100)	158 (100)	213 (100)	213 (100)	371 (100)	371 (100)
χ^2	0.4576		5.579		5.051	
P	0.7955		0.0614		0.080	
Fisher's exact *P* (CN <2 vs. CN = 2)	0.5238		0.0446*		0.0500*	
OR (95 % CI)	1.312 (0.5744–2.996)		2.057 (1.039–4.072)		1.719 (1.017–2.903)	
Fisher's exact P (CN = 3 vs. CN = 2)	0.8419		0.2143		0.2845	
OR (95 % CI)	1.110 (0.5024–2.453)		1.583 (0.7746–3.233)		1.363 (0.7965–2.297)	
Africans						
CN <2	12 (15.2)	8 (10.1)	41 (18.6)	34 (15.4)	53 (17.7)	42 (14.0)
CN = 2	60 (75.9)	64 (81.0)	164 (74.2)	166 (75.1)	224 (74.7)	230 (76.7)
CN = 3	7 (8.9)	7 (8.9)	16 (7.2)	21 (9.5)	23 (7.6)	28 (9.3)
Total	79 (100)	79 (100)	221 (100)	221 (100)	300 (100)	300 (100)
χ^2	0.9290		1.341		1.843	
P	0.6284		0.5114		0.3979	
CN <2 vs. CN = 2 Fisher's exact *P*	0.4705		0.4461		0.2614	
OR (95 % CI)	1.600(0.6116–4.186)		1.221 (0.7378–2.019)		1.296 (0.8304–2.022)	
CN = 3 vs. CN = 2 Fisher's exact *P*	1.000		0.4917		0.6581	
OR (95 % CI)	1.067 (0.3531–3.222)		0.7712 (0.3886–1.531)		0.8434 (0.4715–1.509)	
Combined races						
CN <2	26 (11.0)	19 (8.0)	67 (15.4)	48 (11.1)	93 (13.9)	67 (10.0)
CN = 2	190 (80.2)	198 (83.5)	331 (76.3)	351 (80.9)	521 (77.6)	549 (81.8)
CN = 3	21 (8.8)	20 (8.5)	36 (8.3)	35 (8.0)	57 (8.5)	55 (8.2)
Total	237 (100)	237 (100)	434 (100)	434 (100)	671 (100)	671 (100)
Logistic regression						
OR for CN <2	1.4940		1.5067		1.5025	
SE	0.5067		0.3165		0.2682	
P value	0.2366		0.0510		0.0226**	
OR for CN = 3	1.1195		1.0731		1.0907	
SE	0.6641		0.2717		0.2177	
P value	0.7287		0.7807		0.6634	

* Indicates an association between *FCGR3B* CN and sarcoidosis before the Bonferroni correction

** Indicates a significant association between *FCGR3B* CN and sarcoidosis after the Bonferroni correction

or *FCGR3B* gene (Nagelkerke et al. 2015). Among five *FCGR* genes (*FCGR2A, FCGR2B, FCGR2C, FCGR3A,* and *FCGR3B*) in the *FCGR* cluster, three genes (*FCGR3A*, *FCGR2C,* and *FCGR3B*) have CNVs. *FCGR3B* CNVs are in strong linkage disequilibrium with the pseudogene *FCGR2C* CNVs while *FCGR3A* CNVs exist independently (Machado et al. 2012; Mueller et al. 2013). CNVs are absent for *FCGR2A* and *FCGR2B* (Breunis et al. 2009; Machado et al. 2012; Nagelkerke et al. 2015). In the current study, we observed that abnormal *FCGR3A* CN (CN ≠ 2) is significantly associated with sarcoidosis susceptibility in females, who have significantly different immune responses against infections and environmental insults compared to males (Bouman et al. 2005; McClelland and Smith 2011). In addition, we observed that the low *FCGR3B* CN (CN <2) was significantly associated with sarcoidosis, suggesting that defective FcγRIIIB gene function is also a risk factor for sarcoidosis. Our study was the first to demonstrate that both *FCGR3A* and *FCGR3B* CNVs are risk factors for

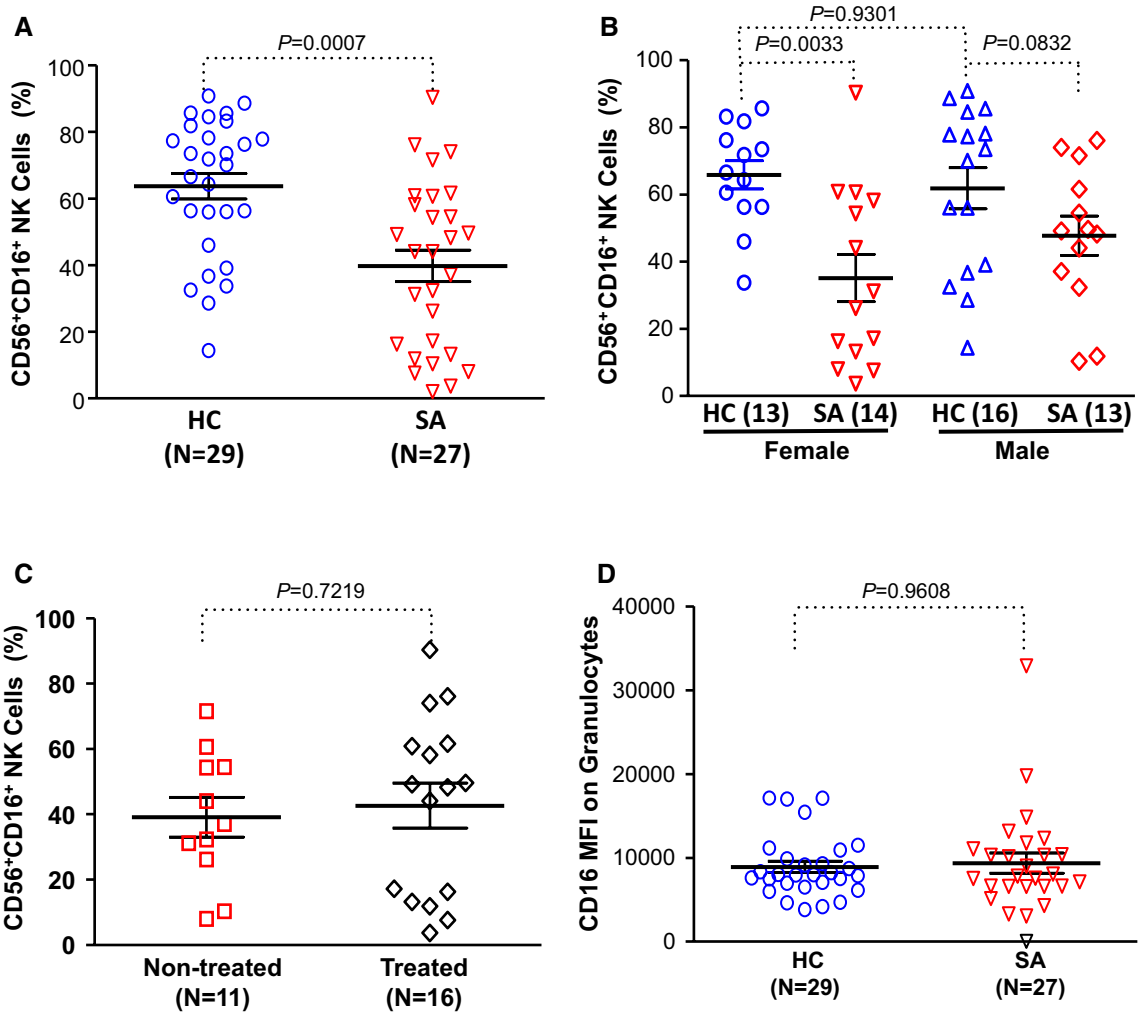

Fig. 1 FcγRIIIA and FcγRIIIB expressions on NK cells and neutrophils. CD16 expressions on NK cells and neutrophils were detected with FITC-conjugated anti-CD16 mAb. Each *empty circle* or *triangle* represents one human subject in respective groups. **a** Percentages of $CD56^+$ NK cells expressing CD16 in sarcoidosis patients (SA) and healthy controls (HC). Sarcoidosis patients had significantly lower percentages of $CD56^+CD16^+$ NK cells than healthy controls ($P = 0.0007$). **b** Percentages of $CD56^+$ NK cells expressing CD16 in subgroups of sarcoidosis patients (SA) and healthy controls (HC). Female sarcoidosis patients ($N = 14$) had significantly lower percentages of $CD56^+CD16^+$ NK cells than female healthy controls ($N = 13$) ($P = 0.0033$) while the percentages of $CD56^+CD16^+$ NK cells were not significantly different between male sarcoidosis patients ($N = 13$) and male healthy controls ($N = 16$) ($P = 0.0832$). **c** Percentages of $CD56^+$ NK cells expressing CD16 in sarcoidosis patients without treatment (non-treated) and with the treatment (treated). Medical treatment had no effect on the percentages of $CD56^+CD16^+$ NK cells in sarcoidosis patients ($P = 0.7219$). **d** CD16 expression on neutrophils. CD16 expression is presented as MFI (mean fluorescent intensity). Neutrophils from sarcoidosis patients (SA) tended to have a wider range of CD16 expressions than those from healthy donors (HC). Nevertheless, the expression levels of CD16 on neutrophils were not significantly different sarcoidosis patients and healthy controls ($P = 0.9608$)

sarcoidosis susceptibility, offering critical insights into the role of FcγRIII family members in the development of sarcoidosis.

We found that high *FCGR3A* CN (CN = 3) was significantly enriched in female sarcoidosis patients with a high odds ratio (OR = 2.8044) (Table 2). Several studies showed a significant expansion of $CD14^+CD16^+$ monocyte subpopulation in sarcoidosis compared to healthy controls (Dubaniewicz et al. 2012; Heron et al. 2008; Okamoto et al. 2003), which also occurs in a number of inflammatory diseases (Ziegler-Heitbrock 2007). The proinflammatory $CD14^+CD16^+$ monocytes display higher phagocytic capacity, produce larger amounts of inflammatory cytokines (TNF-α, IL-6, IL-1 and IL-12), and have higher potency in antigen presentation than the classical $CD14^+CD16^-$ monocytes (Ziegler-Heitbrock 2007). The high *FCGR3A* CN may lead to the higher expression of the activating FcγRIIIA (CD16A) on the surface of those non-classical

Table 4 Distributions of *FCGR3A* and *FCGR3B* CNV genotypes in the phenotyped subjects

Genotype	*FCGR3A*		*FCGR3B*	
	Sarcoidosis (%) $N = 27$	Controls (%) $N = 29$	Sarcoidosis (%) $N = 27$	Controls (%) $N = 29$
CN = 0	0 (0.0)	0 (0.0)	1 (3.7)	0 (0.0)
CN = 1	1 (3.7)	1 (3.4)	3 (11.1)	4 (13.8)
CN = 2	24 (88.9)	27 (93.1)	23 (85.2)	22 (75.9)
CN = 3	2 (7.4)	1 (3.4)	0 (0.0)	3 (10.3)
χ^2	0.4398		4.099	
P	0.8029		0.2510	

The distributions of *FCGR3A* and *FCGR3B* CNV genotypes were not significantly different between sarcoidosis patients and healthy controls

proinflammatory monocytes, which could result in excessive inflammation and the development of sarcoidosis.

Interestingly, low *FCGR3A* CN (CN = 1) was also significantly enriched in female sarcoidosis patients with a very high odds ratio (OR = 4.0156). Our previous study demonstrated a correlation between low *FCGR3A* CN (CN = 1) and low CD16A expression on NK cells (Chen et al. 2014), suggesting that low *FCGR3A* expression may have physiological implications in the NK cell functions and in the development of sarcoidosis. Consistent with the speculation, we found significantly lower percentages of $CD56^+CD16^+$ NK cells from sarcoidosis patients than those from healthy controls, indicating that defective CD16A functions of NK cells may have a role in the pathogenesis of sarcoidosis, especially in females (Fig. 1b). While multiple FcγRs could be expressed on some immune cells, NK cells only express CD16A (Nimmerjahn and Ravetch 2008). Also, CD16A undergoes very rapid and efficient down-regulation from the surface of NK cells by a proteolytic process upon cell activation (Harrison et al. 1991; Romee et al. 2013). The metalloproteinase ADAM17 cleaves CD16A upon NK cell activation by diverse stimuli including CD16A engagement, pro-inflammatory cytokines, and target cell interactions (Jing et al. 2015; Lajoie et al. 2014; Romee et al. 2013; Wiernik et al. 2013). Therefore, there could be two possible explanations for the down-regulation of CD16A on NK cells from sarcoidosis patients: (1). the activation-induced CD16A shedding may lead to the loss of receptor from NK cells; and (2) the development of $CD56^+CD16^+$ NK cells in sarcoidosis patients were altered due to the cytokine milieu in patients. Lower FcγRIIIA-expression could result in impairment of ADCC functions of NK cells, which may contribute to the development of sarcoidosis. The association between the lower percentages of $CD56^+CD16^+$ NK cells and sarcoidosis suggests that modulating FcγRIIIA function may be an important avenue for therapeutic option for sarcoidosis as blocking CD16A shedding with a selective ADAM17 inhibitor could restore the functionality of peripheral blood NK cells, including cytokine production and ADCC (Romee et al. 2013; Wiernik et al. 2013).

Low *FCGR3B* copy number has been reported as the risk factor for a number of autoimmune diseases including systemic lupus erythematosus (SLE) (Aitman et al. 2006; Chen et al. 2014; Fanciulli et al. 2007; Willcocks et al. 2008), Sjogren's syndrome (Nossent et al. 2012), and systemic sclerosis (McKinney et al. 2012). However, our group demonstrated that the low *FCGR3B* copy number is not a risk factor for rheumatoid arthritis (RA) (Chen et al. 2014), highlighting the distinctive genetic effects of *FCGR3B* CNVs on different autoimmune diseases. Similar to the findings in most of autoimmune diseases aforementioned, we found that the *FCGR3B* deficiency was associated with sarcoidosis susceptibility with the odds ratio of 1.503, which is similar to the odds ratio for prototypical autoimmune disease SLE (McKinney and Merriman 2012). However, high *FCGR3B* CN (CN >2) does not seem to have a role in the development of sarcoidosis (Table 3). Our findings provide the growing evidence that FcγRIIIB functions play an important role in the pathogenesis of multiple autoimmune diseases. Previously, we reported a distinctive correlation between *FCGR3B* CN and CD16B cell surface expression (Chen et al. 2014), demonstrating that *FCGR3B* CNVs have physiological implications in the neutrophil functions. FcγRIIIB promotes the adherence of neutrophils to immune complexes and their subsequent clearance (Tsuboi et al. 2008; Willcocks et al. 2008). FcγRIIIB could efficiently capture and internalize ICs with minimal neutrophil activation (Coxon et al. 2001; Tsuboi et al. 2008). Thus, FcγRIIIB on neutrophils may have an important role in autoimmunity and expression levels of FcγRIIIB might have significant impact on the pathogenesis of autoimmune diseases. Insufficient FcγRIIIB-mediated immune complex clearance and immune modulation may be the underlying mechanism with which the low *FCGR3B* CN carriers predispose to sarcoidosis. In our phenotype analysis, we did identify one patient among 27 sarcoidosis patients as FcγRIIIB (CD16B) deficient (Fig. 1d). However, the

expressions of peripheral blood neutrophil FcγRIIIB were not different between the sarcoidosis patient group and the healthy control group. We speculate that the proinflammatory environment in sarcoidosis patients may promote the *FCGR3B* gene expression in neutrophils, which may lead to the minimal difference of surface FcγRIIIB expression on circulating peripheral neutrophils between patients and healthy control. We failed to detect association between any functional *FCGR* SNPs and sarcoidosis susceptibility, suggesting that *FCGR* SNPs may have insignificant roles in the development of sarcoidosis.

FCGR CNVs are caused by independent and recurrent non-allelic homologous recombination (NAHR) between the two segments containing either *FCGR3A* or *FCGR3B* (Machado et al. 2012; Mueller et al. 2013). Deletions or insertions of *FCGR3A* or *FCGR3B* differ only in the NAHR breakpoint position on the chromosome. The mutation rate of *FCGR* CNVs is around 1.008×10^{-3} per generation (Machado et al. 2012). Additionally, linkage disequilibrium between *FCGR3A* CNVs and *FCGR3B* CNVs is improbable based on the model (Machado et al. 2012; Mueller et al. 2013). Consistent with the model, no linkage disequilibrium between *FCGR3A* CNVs and *FCGR3B* CNVs was observed in our populations including Caucasians and Africans. Therefore, the association of *FCGR3A* and *FCGR3B* CNVs with sarcoidosis most likely reflects the independent *FCGR3* gene effects on the disease.

In conclusion, the present study demonstrated a role of *FCGR3* CNVs in sarcoidosis. The abnormality of *FCGR3* gene family members is one of mechanisms in the development of sarcoidosis.

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Compliance with ethical standards

Conflict of interest The authors declare no any financial support or other benefits from commercial sources for the work reported on in the manuscript, or any other financial interests that any of the authors may have, which could create a potential conflict of interest with regard to the work.

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