

## Polymorphism of *FCGR2A*, *FCGR2C*, and *FCGR3B* Genes in the Pathogenesis of Sarcoidosis

M. Typiak, K. Rębała, M. Dudziak, J.M. Słomiński, and A. Dubaniewicz

### Abstract

We have previously presented evidence that the polymorphism of the *FCGR3A* gene, encoding the receptor for Fc fragment of immunoglobulin G IIIa (FcγRIIIa) plays a role in the enhancement of circulating immune complexes (CIs) with the occurrence of *Mycobacterium tuberculosis* heat shock proteins in patients with sarcoidosis (SA). The immunocomplexemia might be caused by decreased affinity of CIs to Fcγ receptors, with the subsequently decreased receptor clearance by immune cells. In the present study we examined whether the polymorphisms of other related genes (*FCGR2A*, *FCGR2C*, *FCGR3B*) encoding other activatory Fcγ receptors, could have a similar effect. To this end, we genotyped 124 patients with sarcoidosis and 148 healthy volunteers using polymerase chain reaction with sequence-specific primers. We revealed a significant decrease in the percentage of the *FCGR2A* and *FCGR2C* variants that ensure effective CIs clearance, with a concomitant increase of less functional variants of these genes in Stages I/II, compared with Stages III/IV of SA. There was no aberration in *FCGR3B* allele/genotype frequencies. We conclude that the *FCGR2A* and *FCGR2C* polymorphisms may also contribute to immunocomplexemia present in SA. The assessment of *FCGR* genes could become a tool in presaging a clinical course of sarcoidosis and in its personalized therapy.

### Keywords

*FCGR* genes • Immunoglobulin G • Polymorphism • Receptor • Sarcoidosis

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## 1 Introduction

Sarcoidosis (SA) is a multisystem, granulomatous disorder of unknown etiology. Infectious, genetic factors, and autoimmunity have been explored as potential causes of SA (American Autoimmune Related Diseases Association 2015; Spagnolo and Grunewald 2013; Baughman et al. 2011; American Thoracic Society et al. 1999). Due to similarities between SA and tuberculosis, *Mycobacterium tuberculosis* (Mtb) and its antigens, e.g., KatG, A85, SOD2, and heat shock proteins (Mtb-hsps), have been studied as causative factors for sarcoidosis (Dubaniewicz 2013; Dubaniewicz et al. 2012a; Dubaniewicz 2010; Kivity et al. 2009).

Mtb-hsps, involved in the formation of immune complexes (CIs), may be crucial in connecting infection and autoimmunity, both considered in sarcoidosis. Recently, we have shown the presence of immunocomplexemia with the involvement of Mtb-hsps in SA patients, especially of Mtb-hsp16 - the main marker of a dormant stage of mycobacteria. A high level of CIs suggests the occurrence of antigenemia, which may result from the persistent presence of phagocytosed mycobacteria releasing Mtb-hsps. Mtb-hsps, in turn, presented through the human leukocyte antigen (HLA) system to T and B lymphocytes would trigger the cellular and humoral immune response (Dubaniewicz 2010; 2013; Dubaniewicz et al. 2012a; Baughman et al. 2011; Kivity et al. 2009). The immunocomplexemia may also be caused by altered elimination of antigen(s) by monocytes/macrophages and neutrophils. Both phagocytosis and clearance of CIs may be disrupted due to dysfunction of receptors, especially for Fc fragments of immunoglobulin G (FcγR): FcγRI, FcγRII, and FcγRIII. We have recently revealed an increased number of blood FcγRI<sup>-</sup>, FcγRII<sup>-</sup>, and FcγRIII<sup>+</sup>-monocytes with a higher phagocytic activity in SA patients (Dubaniewicz 2013; Dubaniewicz et al. 2012b). Increased phagocytosis and immunocomplexemia in SA patients may result from dysfunction of FcγRI-III receptors

due to their functional polymorphism. To this end we have revealed the *FCGR3A*-V158F polymorphism, responsible for decreased affinity of FcγRIIIa to CIs and decreased FcγRIIIa clearance in SA (Typiak et al. 2014). Similar findings have been presented by Maertzdorf et al. (2012) who report increased expression of genes connected with FcγR-mediated phagocytosis in SA patients, although the authors did not specify the receptors involved in the process.

The immunocomplexemia also may result from aberrant interaction of neutrophils with FcγRIIIa, FcγRIIc and FcγRIIIb receptors, encoded by the polymorphic *FCGR2A*, *FCGR2C*, and *FCGR3B* genes (Behnen et al. 2014; Li et al. 2009; Tutor-Ureta et al. 2006; Schmekel et al. 1985). These receptors are important for the recognition and phagocytosis of IgG-opsonized pathogens, CIs, degranulation, antibody-dependent cell-mediated cytotoxicity, release of proinflammatory cytokines, oxidative burst, and apoptosis. It has also been reported that neutrophils are among the first cells to arrive at the site of mycobacterial infection; often an underlying pathogenetic mechanism of sarcoidosis. Therefore, dysfunction of FcγRs on neutrophils may play a role in the CIs-mediated inflammatory process and thus in the induction of autoimmunity in sarcoidosis.

The *FCGR2A*-H131R, *FCGR2C*-X57Q, and *FCGR3B*-NA1/NA2/SH polymorphisms have been detected in different autoimmunological disorders, such as systemic lupus erythematosus, rheumatoid arthritis, and idiopathic thrombocytopenic purpura (Li et al. 2009), but not yet in SA. Therefore, the aim of the current study was to evaluate the polymorphism of these genes in patients with sarcoidosis.

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## 2 Methods

Ethical approval for the study was granted by the Bioethics Committee for Scientific Research of the Medical University of Gdansk, Poland (NKEBN/337/2009). Written informed consent

was obtained from the study participants. Recruitment of patients, collection of blood samples, and genotyping all were conducted in the period January 2007 to June 2014.

## 2.1 Patients

Patients with sarcoidosis were followed by for at least three years to ensure the acquisition of data about disease recurrence and its chronic character (the average follow-up duration amounted to four years). There were 124 untreated patients (69 smokers, 55 non-smokers) with newly diagnosed pulmonary sarcoidosis enrolled into the study (Table 1). The diagnosis of SA was based on histological (scaleniobiosy of lymph nodes), clinical, and radiological evidence. Disease staging was performed using high resolution computed tomography according to the classification of Scadding (1961): Stage I – bilateral hilar lymphadenopathy (38 patients); Stage II – bilateral hilar lymphadenopathy and diffuse pulmonary infiltrations (60 patients); Stage III – diffuse pulmonary infiltrations (20 patients); Stage IV – fibrosis and cavities (6 patients). Twenty five patients had Löfgren’s syndrome. Microbiological and cytological examination of lymph nodes and sputum samples revealed no acid-fast bacilli (PCR, culture of the *M. tuberculosis* strain), fungi, or atypical cells.

The control group consisted of 148 unrelated healthy volunteers (80 smokers, 68 non-smokers) recruited in the same time period as that for sarcoidosis patients (Table 1). All these individuals had normal chest radiographs, blood and serum analysis, as well as no acid-fast bacilli in sputum smears or sputum cultures of the *M. tuberculosis* strain. None of the controls or SA patients had a familial history of tuberculosis, sarcoidosis, or autoimmune disease. All participants of the study were free of HIV infection.

## 2.2 Sample Collection and DNA Isolation

Peripheral blood samples (10 ml) were collected into tubes with EDTA (Becton Dickinson Company, Franklin Lakes, NJ), and were stored at 4 °C for a maximum of 4 h and then at –20 °C for a maximum of one month. In case of patients, blood collection took place before treatment. Subsequently, samples were thawed in room temperature and DNA was isolated using a non-enzymatic method, according to the method of Lahiri and Nurnberger (1991). Analyzes were performed always by the same persons at the Department of Forensic Medicine of the Medical University of Gdansk in Poland.

### 2.2.1 Polymerase Chain Reaction for *FCGR2* Genes Polymorphism

Polymerase chain reaction with the sequence specific primers (PCR-SSP) was used for the

**Table 1** Comparative characteristics of patients with pulmonary sarcoidosis (SA) and healthy individuals (Controls)

	SA patients n = 124 (%)	Controls n = 148 (%)
Age (year)		
Mean	41	42
Range	21–68	18–79
Gender		
Female	52 (42)	67 (45)
Male	72 (58)	81 (55)
BCG vaccination	124 (100)	148 (100)
Positive PPD skin test	0	0
Relapses	0	0
Symptoms		
Cough	58 (47)	0
Dyspnea	12 (10)	0
Fever	21 (17)	0
Night sweats	2 (2)	0
Weight loss	6 (5)	0
Erythema nodosum	25 (20)	0
Arthritis	25 (20)	0

gene polymorphism analysis in both groups (Table 2). The *FCGR2A*-H131R polymorphism, involving A > G at 519 nucleotide position, causing a change in H131R aminoacid in the second extracellular domain of FcγRIIIa, resulting in the presence of two variants of the protein: 131H and 131R (rs1801274) was analyzed. To determine the genotype of a person, two separate PCR-SSP reactions were conducted for the two *FCGR2A* gene alleles according to the method of Edberg et al. (2002) with modifications, due to the same length of amplified products for 131H and 131R variants (371 bp). We used a reverse primer (5'-TCAAAGTGAAACAACAGCCTGACT-3') that ensured gene specificity for the *FCGR2A* and either a forward 131H allele-specific (5'-GGAAAATCCCAGAAATTCACACA-3') or 131R allele-specific primer (5'-GGAAAATCCAGAAATTCACACG-3'). To eliminate false negatives, each reaction was supplemented with an internal control of amplification. For this purpose, primers specific for human growth hormone (*hGH*) gene fragments were used after checking for and excluding unwanted primer interactions. Sequences of the primers for the amplification of the internal control (439 bp) were as follows: a forward primer 5'-CAGTGCCTTCCCAACCATTCCCTTA-3' and a reverse primer 5'-ATCCACTCACGGATTTCTGTTGTGTTTC-3'.

The PCR-SSP reaction for 131H (519A) allele was performed with 20 ng of genomic DNA in 1x Pol Buffer B, 1.75 mM MgCl<sub>2</sub>, 0.3 mM of each dNTP, 0.2 μM of the *FCGR2A* gene-specific and 131H-specific primers, 0.16 μM of both *hGH*-specific primers and 1.0 U of Taq polymerase (EURx; Gdańsk, Poland) in a total volume of 10 μl. The PCR-SSP reaction for 131R (519G) allele was performed similarly, but with 30 ng of genomic DNA with 0.25 μM of both *FCGR2A* gene-specific and 131R-specific primers, and 0.32 μM of both *hGH*-specific primers. A temperature profile for the 131H allele comprised 2 min at 95 °C, 35 amplification cycles (1 min at 94 °C, 1 min of annealing at 56 °C, and 2 min at 72 °C), followed by a final elongation at 72 °C for 10 min. A temperature profile for the amplification of the 131R(519G) allele was identical except for a higher annealing temperature of 57 °C.

The *FCGR2C*-X57Q polymorphism involving T > C at 202 nucleotide position causing a change from STOP codone to a triplet coding for glutamine in the first extracellular domain of FcγRIIc, resulting in the presence of two variants of the protein: 57X (a shortened protein in the absence of the other variant) and 57Q (a full-length, functional receptor protein) was analysed (rs1801274). To determine the genotype of a person, two separate PCR-SSP reactions were performed for the two *FCGR2C* gene alleles according to the

**Table 2** Location of single nucleotide polymorphism (SNPs) in *FCGR2A*, *FCGR2C*, and *FCGR3B* genes

Gene	Nucleotide position	Major allele	Minor allele	dbSNP ID	Aminoacid change	Allele esignation	Change in receptor protein
<i>FCGR2A</i>	519	A	G	rs1801274	H131R	131H/R	Structural change in the second extracellular domain.
<i>FCGR2C</i>	202	T	C	rs1801274	X57Q	57X/Q	Production of nonfunctional/functional receptor protein.
<i>FCGR3B</i>	147	C	T	rs447536	L38L	NA1/ NA2	Structural change in the first extracellular domain
	141	G	C	rs403016	R36S		
	227	A	G	rs448740	N65S		
	277	G	A	rs428888	D82N		
	349	G	A	rs2290834	V106I		
	266	C	A	rs5030738	A78D	SH/NA2	

method of Su et al. (2002) with modifications, due to the same length of amplified products for 57X (202 T) and 57Q (202C) variants (124 bp). We used a reverse primer (5'-GAGATTCCTATTGTGGACCTACG-3') that ensured gene specificity for *FCGR2C* and either a forward 57X allele-specific (5'-GGCTGTGCTGAAACTGGAGACCT-3') or 57Q allele-specific primer (5'-GGCTGTGCTGAAACTGGAGCCAC-3'). To eliminate false negatives, we supplemented reactions with an internal control of amplification (439 bp). For this purpose, primers specific for the *hGH* gene fragments were used after checking for and excluding unwanted primer interactions (sequences of the *hGH*-specific primers were outlined above in the section describing the PCR-SSP for *FCGR2A*).

The PCR-SSP reaction for 57Q (202C) allele consisted of 10 ng of genomic DNA in 1x Pol Buffer B, 3 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 1 μM of *FCGR2C* gene-specific and 57Q-specific primers and 1.25 U of Taq polymerase (EURx; Gdansk, Poland), in a total volume of 10 μl. The PCR-SSP for 57X (202 T) allele was performed similarly, but with 0.15 μM of both *FCGR2C* gene-specific and 57X-specific primers and 1.0 U of Taq polymerase. Both primers for the internal control of amplification (*hGH*) were added in the proper amount to reach the concentration of 0.1 μM. A temperature profile for the 57Q (202C) allele comprised 5 min at 95 °C, 37 amplification cycles (30 s at 94 °C, 30 s of annealing at 55 °C and 10 s at 72 °C), followed by a final elongation at 72 °C for 7 min. A temperature profile for the amplification of the 57X (202 T) allele was identical except for a higher annealing temperature of 57 °C and a lower number of amplification cycles of 35.

The *FCGR3B*-NA1/NA2/SH (HNA-1a/HNA-1b/HNA-1c) polymorphism was also analyzed. The NA1 and NA2 alleles differ in five nucleotide positions (C147T/L38L, rs447536 and four mutations leading to the following aminoacid changes: G141C/R36S, rs403016; A227G/N65S, rs448740; G277A/D82N, rs428888;

G349A/V106I, rs2290834) in the sequence coding for the first extracellular domain of FcγRIIIb. The SH allele is similar to NA2, except for one nucleotide (C266A, rs5030738), which, however, changes the sequence of the receptor protein (A78D) in the above mentioned domain.

To determine the genotype of a person, one PCR-SSP reaction was performed for the three *FCGR3B* gene alleles according to the method of Siriboonrit et al. (2003) with modifications, due to different lengths of amplified products for the NA1 (140 bp), NA2 (219 bp), and SH variants (102 bp). Therefore, the internal control of amplification was unneeded. In every reaction we used a common reverse primer (5'-ATGGACTIONTCTAGCTGCAC-3') that ensured gene specificity for *FCGR3B*, and three forward primers: NA1-specific (5'-CAGTGGTTTCA-C AATGAGAA-3'), NA2/SH-specific (5'-CAATGGTACAGCGTGCTT-3'), and SH allele-specific primer (5'-TCGAGCTACTTCAT TGACGA-3').

The PCR-SSP reaction for all three alleles was performed with 10 ng of genomic DNA in 1x Pol Buffer B, 3 mM MgCl<sub>2</sub>, 0.32 mM of each dNTP, 0.5 μM *FCGR3B* gene-specific primer, 0.3 μM of NA1-specific, 0.08 μM of NA2/SH-specific and 0.16 μM of SH-specific primers, and 0.5 U of Taq polymerase (EURx; Gdańsk, Poland) in a total volume of 10 μl. A temperature profile for the tested alleles comprised 2 min at 96 °C, 32 amplification cycles (30 s at 96 °C, 1 min of annealing at 63 °C, and 30 s at 72 °C), followed by a final elongation at 72 °C for 5 min.

Due to a close connection of the SH allele to NA2, which differ in only one of six nucleotides that define the allele, the PCR-SSP method used for the *FCGR3B* gene has a specific limitation. The NA2/SH-specific and *FCGR3B* gene-specific primers are substrates for the PCR product, which is specific for both NA2 and SH allele (NA2/SH product). It is worth emphasizing that supplementation of the PCR reaction with SH-specific primer allows obtaining a product that is specific only for the SH, and not NA2, variant. In the conjoint presence of both

NA2/SH- and SH-specific products, the method cannot differentiate the NA2/SH and SH/SH genotypes. Therefore, two values were given for the frequencies of NA2 and SH alleles, as well as of NA2/SH and SH/SH genotypes.

Electrophoresis of the amplified DNA samples was performed in 14 % polyacrylamide gels (acrylamide-bisacrylamide, 3 % of cross-linking). GeneRuler 100 bp DNA Ladder (Thermo Fisher Scientific, Waltham, MA) was used as a molecular weight marker (range 100–1000 bp). Gels were stained in 0.1 % silver nitrate solution.

### 2.2.2 Optimization of Methods

Our own contribution to the presented molecular methodology was a creation of a primer for the 57Q (202C) allele of *FCGR2C* and a changed sequence of the primer for the NA1 allele of *FCGR3B*, to enhance the specificity of connection of primers with DNA matrix. Further, to eliminate false negatives, we selected an internal control of amplification for the alleles of the *FCGR2A* and *FCGR2C* genes. The primers for an internal control were chosen on the basis of similarity of their length and annealing temperature to the primers amplifying the *FCGR* genes fragments and after excluding unwanted primer interactions (primer-dimer, hairpin structure). The use of internal control of amplification was unneeded in the method created for the *FCGR3B* gene analysis due to performing the amplification of all three gene alleles in one reaction (a lack of PCR product only in case of amplification error or gene deletion, which did not take place). In the PCR-SSP methods used we optimized the composition of reaction mixture (reaction buffer with ammonium sulfate, suitable concentrations of MgCl<sub>2</sub>, dNTP, Taq polymerase, DNA, and primers for *FCGR* genes fragments and internal control of amplification) and temperature profile (annealing temperature, time of amplification steps, and number of amplification cycles). Finally, we also optimized the polyacrylamide gels concentration to achieve a clear distinction

of the obtained PCR-products in the range of 100–1000 bp.

### 2.2.3 Statistical Analysis

The  $\chi^2$  test was used to compare the genotype and allele frequencies in the groups studied. Yates's correction was implemented when a number lower or equal to ten was included in the comparison. Odds ratios with 95 % confidence intervals were calculated for the *FCGRs* alleles and genotypes tested. Concordance with Hardy-Weinberg equilibrium was confirmed for each *FCGR* genotype using online software (Rodriguez et al. 2009). A p-value  $\leq 0.05$  was considered to indicate significant differences. Statistical elaboration was performed using STATISTICA for Windows ver.10.0 (StatSoft, Tulsa, OK).

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## 3 Results

### 3.1 *FCGR2A* Gene

There was no significant difference in the *FCGR2A* allele or genotype frequency between the SA and control groups. Nor was there any difference in the *FCGR2A* allele frequency between the particular stages of SA and controls. However, in Stage III of SA, there was a significant increase in the occurrence of 131HH homozygotes and a decrease in 131HR heterozygotes compared with healthy individuals. In Stage II, in contrast, there was a significant decrease in 131HH homozygotes and an increase in the frequency of 131HR heterozygotes. In Stage I, we also found a lower presence of 131HH homozygotes and a higher percentage of 131HR heterozygotes than those in Stage III. Concerning the occurrence of particular genotypes of *FCGR2A* in patients with Stages I-IV of SA, we found a significant decrease in the percentage of 131HH homozygotes and an increase in 131HR heterozygotes in Stages I/II compared with Stages III/IV (Table 3).



**Table 3** Frequency of alleles and genotypes of *FCGR2A* gene in patients with sarcoidosis (SA) and healthy controls; both SA ( $p = 0.43$ ) and control ( $p = 0.70$ ) groups were in Hardy–Weinberg equilibrium

Groups	Number of alleles (%)		Number of genotypes (%)		
	131H	131R	131HH	131HR	131RR
Sarcoidosis (n = 114)	130 (57)	98 (43)	35 (31)	35 (31)	19 (17)
Stage I (n = 36)	42 (58)	30 (42)	10 (28) <sup>a</sup>	22 (61) <sup>b</sup>	4 (11)
Stage II (n = 53)	56 (53) <sup>c</sup>	50 (47)	13 (24) <sup>d</sup>	30 (57) <sup>e</sup>	10 (19)
Stage III (n = 19)	27 (71)	11 (29)	11 (58) <sup>f</sup>	5 (26) <sup>c</sup>	3 (16)
Stage IV (n = 6)	5 (42)	7 (58)	1 (17)	3 (50)	2 (33)
Stage I/II (n = 89)	98 (55)	80 (45)	23 (26) <sup>a</sup>	52 (58) <sup>e</sup>	14 (16)
Stage III/IV (n = 25)	32 (64)	18 (36)	12 (48)	8 (32)	5 (20)
Controls (n = 142)	167 (59)	117 (41)	48 (34)	71 (50)	23 (16)

<sup>a</sup> $p = 0.03$  for Stage I vs. Stage III, Stage I/II vs. Stage III/IV

<sup>b</sup> $p = 0.01$  for Stage I vs. Stage III

<sup>c</sup> $p = 0.05$  for Stage III vs. controls, Stage II vs. Stage III

<sup>d</sup> $p = 0.008$  for Stage II vs. Stage III

<sup>e</sup> $p = 0.02$  for Stage II vs. Stage III, for Stage I/II I. Stage III/IV

<sup>f</sup> $p = 0.04$  for Stage III vs. controls

### 3.2 *FCGR2C* Gene

There was no significant difference in the *FCGR2C* allele or genotype frequency between the SA and control groups. In Stage III of SA, there were a significant increase in the occurrence of the 57Q allele and a decrease in the 57X allele compared with healthy individuals. Likewise, in Stages III/IV an increase in the 57Q allele and a decrease in the frequency of the 57X allele were found compared with healthy individuals. In Stages III and III/IV, we found a significant decrease in the percentage of 57XX homozygotes and an increase of 57XQ heterozygotes, which was in contrast to the findings in healthy individuals. In Stages I/II and II, there was a significant decrease in the percentage of 57XQ heterozygotes compared with Stage III. A significant decrease in the frequency of 57XQ heterozygotes was also detected in Stages I/II versus Stages III/IV (Table 4).

### 3.3 *FCGR3B* Gene

There were no significant differences in the *FCGR3B* allele or genotype frequency between the SA and control groups, particular stages of SA and controls, or between SA stages themselves (Table 5).

## 4 Discussion

In the current study, we found significant differences in the occurrence of alleles and genotypes of the *FCGR2A* gene in SA patients in Stages III and IV compared with Stages I and II, and with healthy individuals. In Stages III and IV there was a decrease in the frequency of *FCGR2A*-131HR heterozygotes and an increase in *FCGR2A*-131HH homozygotes compared with Stages II and II. We also found a lower frequency of *FCGR2A*-131H allele in Stage II than that in Stage III or IV. Further, a comparative analysis of the *FCGR2A*-H131R polymorphism in particular stages of SA versus healthy controls revealed an increased frequency of *FCGR2A*-131H allele and 131HH homozygotes in Stages III and IV. Concerning the *FCGR2C*-X57Q polymorphism, we found a decrease in the frequency of the 57XQ genotype with 57Q allele in Stages I and II compared with Stages III and IV. Moreover, in contrast to controls, a significant decrease in the frequency of the 57X allele and 57XX genotype, and an increase in the 57Q allele and 57XQ genotype were found in Stages III and IV. However, there were no appreciable differences in the frequency of *FCGR3B*-NA1/NA2/SH alleles or genotypes of the gene between SA patients and controls.

**Table 4** Frequency of alleles and genotypes of *FCGR2C* gene in patients with sarcoidosis (SA) and healthy controls; both SA ( $p = 0.89$ ) and control ( $p = 0.79$ ) groups were in Hardy–Weinberg equilibrium

Groups	Number of alleles (%)		Number of stages (%)		
	57X	57Q	57XX	57XQ	57QQ
Sarcoidosis (n = 119)	193 (81)	45 (19)	78 (66)	37 (31)	4 (3)
Stage I (n = 37)	61 (82)	13 (18)	25 (67)	11 (30)	1 (3)
Stage II (n = 57)	94 (82)	20 (18)	40 (70)	14 (25) <sup>a</sup>	3 (5)
Stage III (n = 19)	28 (74) <sup>b</sup>	10 (26) <sup>b</sup>	9 (47) <sup>c</sup>	10 (53) <sup>d</sup>	0
Stage IV (n = 6)	10 (83)	2 (17)	4 (67)	2 (33)	0
Stage I/II (n = 94)	155 (82)	33 (18)	65 (69)	25 (27) <sup>e,f</sup>	4 (4)
Stage III/IV (n = 25)	38 (76) <sup>g</sup>	12 (24) <sup>g</sup>	13 (52) <sup>d</sup>	12 (48) <sup>h</sup>	0
Controls (n = 144)	249 (86)	39 (14)	108 (75)	33 (23)	3 (2)

OR odds ratio, CI confidence intervals

<sup>a</sup> $p = 0.046$  for Stage II vs. Stage III

<sup>b</sup> $p = 0.038$  for Stage III vs. controls (OR = 2.28, 95 % CI 1.03–5.06 for 57Q allele; OR = 0.44, 95 % CI 0.20–0.97 for 57X allele)

<sup>c</sup> $p = 0.02$  for Stage III vs. controls (OR = 0.27, 95 % CI 0.10–0.73, for 57XX to 57XQ proportion); for Stage III/IV vs. controls (OR = 0.33, 95 % CI 0.14–0.79, for 57XX to 57XQ proportion)

<sup>d</sup> $p = 0.01$  for Stage III vs. controls (OR = 3.64, 95 % CI 1.36–9.70, for 57XQ to 57XX proportion)

<sup>e</sup> $p = 0.049$  for Stage I/II vs. Stage III

<sup>f</sup> $p = 0.04$  for Stage I/II vs. Stage III/IV

<sup>g</sup> $p = 0.056$  for Stage III/IV vs. controls (OR = 2.02, 95 % CI 0.97–4.19 for 57Q allele; OR = 0.50, 95 % CI 0.24–1.03 for 57X allele)

<sup>h</sup> $p = 0.009$  for Stage III/IV vs. controls (OR = 3.02, CI 1.26–7.26, for 57XQ to 57XX proportion)

**Table 5** Frequency of alleles and genotypes of *FCGR3B* in patients with sarcoidosis (SA) and healthy controls; both SA ( $p = 0.30$ ) and control ( $p = 0.84$ ) groups were in Hardy–Weinberg equilibrium. Due to similarities between NA2 and SH alleles two values of results were provided for them

Groups	Number of alleles (%)					
	NA1	NA2	SH			
Sarcoidosis (n = 124)	91 (37)	144/140 (58/56)	13/17 (5/7)			
Stage I (n = 38)	32 (42)	38/36 (50/47)	6/8 (8/11)			
Stage II (n = 60)	38 (32)	75/73 (62/61)	7/9 (6/7)			
Stage III (n = 20)	15 (37.5)	25/25 (62.5/62.5)	0/0			
Stage IV (n = 6)	6 (50)	6/6 (50/50)	0/0			
Stage I/II (n = 98)	70 (35.70)	113/109 (57.65/55.60)	13/17 (6.65/8.70)			
Stage III/IV (n = 26)	21 (40)	31/31 (60/60)	0/0			
Controls (n = 148)	113 (38)	168/167 (57/57)	15/16 (5/5)			
Groups	Number of genotypes (%)					
	NA1/NA1	NA2/NA2	SH/SH	NA1/NA2	NA1/SH	NA2/SH
Sarcoidosis (n = 124)	14 (11)	43 (35)	0/4 (0/3)	54 (44)	9 (7)	4/0 (3/0)
Stage I (n = 38)	5 (13)	9 (24)	0/2 (0/5)	18 (47)	4 (11)	2/0 (5/0)
Stage II (n = 60)	5 (8)	25 (42)	0/2 (0/3)	23 (39)	5 (8)	2/0 (3/0)
Stage III (n = 20)	3 (15)	8 (40)	0/0	9 (45)	0/0	0/0
Stage IV (n = 6)	1 (17)	1 (17)	0/0	4 (66)	0/0	0/0
Stage I/II (n = 98)	10 (10)	34 (35)	0/4 (0/4)	41 (42)	9 (9)	4/0 (4/0)
Stage III/IV (n = 26)	4 (15)	9 (35)	0/0	13 (50)	0/0	0/0
Controls (n = 148)	21 (14)	55 (37)	0/1 (0/1)	57 (39)	14 (9)	1/0 (1/0)



To the best of our knowledge, it is the first study of the polymorphism of the *FCGR2A*, *FCGR2C*, and *FCGR3B* genes in sarcoidosis and its particular stages; the stages that are, by some authors, considered as composing separate disease entities. However, studies of these genetic variations were performed in other autoimmune disorders, such as systemic lupus erythematosus (SLE), idiopathic thrombocytopenic purpura (ITP), systemic sclerosis, rheumatoid arthritis (RA), idiopathic pulmonary fibrosis (IPF), granulomatosis with polyangiitis, giant cell arthritis (GCA), anti-glomerular basement membrane antibody disease, Kawasaki disease, microscopic polyangiitis, antiphospholipid syndrome, Sjögren's syndrome, Behçet's disease, Guillain-Barré syndrome (GBS), colitis ulcerosa, Crohn's disease, and myasthenia gravis (Li et al. 2014; Vigato-Ferreira et al. 2014; Wang et al. 2014; Haldorsen et al. 2013; Ji et al. 2013; Sanchez et al. 2011; Bournazos et al. 2010; Weersma et al. 2010; Asano et al. 2009; Bournazos et al. 2009; Li et al. 2009; Aksu et al. 2008; Morgan et al. 2006a; Morgan et al. 2006b; Hughes et al. 2004; Radstake et al. 2003; Manger et al. 2002).

In line with the present results concerning Stages I and II of SA, a decrease in the frequency of the *FCGR2A*-131HH genotype, with a simultaneous increase in 131RR homozygotes, was reported in SLE and GCA. The *FCGR2A*-131RR genotype has also been linked to earlier development of SLE and lupus nephritis. Further, the *FCGR2A*-131RR genotype, along with the presence of HLA-DRB1\*04 allele, has been associated with a six-fold increase in the risk of developing GCA in Spanish patients, compared with individuals with the *FCGR2A*-131HH and 131HR genotype and a lack of DRB1\*04 allele (Morgan et al. 2006a). Increased risk of disease development has also been reported in individuals having the *FCGR2A*-131R in GCA, SLE, antiphospholipid syndrome, ITP, granulomatosis with polyangiitis, and in RA (Vigato-Ferreira et al. 2014; Sanchez et al. 2011; Bournazos et al. 2009; Li et al. 2009; Morgan et al. 2006b; Manger et al. 2002), akin to what we found in Stage II of SA patients of the present study. In

contrast to our results, however, the presence of *FCGR2A*-131R allele has been linked to a more severe course of disease in RA, myasthenia gravis, and in some patients with SLE. Increases in the frequency of 131H variant, like in our patients with Stage III of SA, and in 131HH homozygotes, like in our patients with Stages III and IV of SA have been reported in colitis ulcerosa, Crohn's disease, GBS, childhood-onset ITP, and in Chinese patients with Kawasaki disease (Wang et al. 2014; Ji et al. 2013; Weersma et al. 2010; Asano et al. 2009; Bournazos et al. 2009). In patients with Guillain-Barré syndrome having the *FCGR2A*-131HH genotype, increased risk of disease development has been reported, compared with individuals having the *FCGR2A*-131RR or 131HR genotype, which corresponds to our patients in advanced Stages III and IV of SA. However, some investigations of a genetic predisposition to develop RA in Caucasian and Asian populations, in European patients with systemic sclerosis, Japanese patients with microscopic polyangiitis, Greek patients with Kawasaki disease, and in Scandinavian patients with Sjögren's syndrome failed to connect the *FCGR2A*-H131R polymorphism to risk of disease development (Haldorsen et al. 2013; Bournazos et al. 2009; Radstake et al. 2003).

In a study on the *FCGR2C*-X57Q polymorphism in ITP patients *versus* healthy controls, increased percentage of the 57Q allele and 57XQ genotype has been reported (Bournazos et al. 2009), which corresponds to our patients with advanced Stages III and IV of SA. Likewise, the present results are in line with those concerning the *FCGR3B*-NA1/NA2/SH polymorphism reported in Chinese patients with anti-GBM disease, the majority of patients with RA and SLE from Europe and Asia, in Indian patients with GBS, and in Scandinavian patients with Sjögren's syndrome, where no association was revealed between this genetic variation and risk of disease development (Haldorsen et al. 2013; Li et al. 2009; Radstake et al. 2003; Manger et al. 2002). On the other hand, in Dutch and Norwegian patients with GBS, Spanish

patients with RA, in Turkish patients with Behçet's disease, or in Asian populations suffering from SLE, higher risk of disease development has been reported in those having the NA2 allele or NA2/NA2 genotype (Bournazos et al. 2009; Aksu et al. 2008; Morgan et al. 2006a). Some authors, however, have reported a decrease in the frequency of this genotype in Korean patients with SLE (Li et al. 2009). The *FCGR3B*-NA2 allele is linked to increased susceptibility to concomitant respiratory infections in some RA patients (Hughes et al. 2004). In contrast, increased frequency of the NA1 allele and NA1/NA1 genotype reported in patients with IPF plays a mitigating role in disease progression. A similar advantageous predisposition is reported in ITP patients having the *FCGR3B*-NA1 variant, whereas in patients with myasthenia gravis this variant seems associated with more severe disease course (Bournazos et al. 2010).

In the autoimmune disorders above outlined, the *FCGR2A*-H131R polymorphism affects FcγRIIa affinity to IgG2, phagocytosis, and CIs clearance. The FcγRIIa, with histidine (131H) in the second extracellular domain of the receptor protein, binds IgG2-CIs more effectively than the FcγRIIa with arginine in this site (131R). Therefore, decreased presence of the 131H allele and 131HH genotype in Stages I and II of SA may lead to lower binding of IgG2 with bacteria or antigen in immune complexes, lower phagocytosis and clearance of CIs by monocytes/macrophages and neutrophils, and in consequence antigenemia and immunocomplexemia. As a result, an excessive (auto)immune, granulomatous reactions may arise in affected organs. Likewise, these reactions can be triggered by lower frequency of the 57Q allele of *FCGR2C* noted in our patients in Stages I and II of SA, since this allele shapes the expression of a full-length, functional FcγRIIc receptor and effective CIs binding. In contrast, 57X allele produces a truncated, nonfunctional receptor protein. On the other hand, increased frequency of the *FCGR2C*-131H and *FCGR2C*-131HH variants in advanced Stages III and IV of SA may enhance FcγRIIa affinity to IgG2, its binding to bacteria or

antigen, which accelerates CIs clearance. All these phenomena have been previously reported in SA patients (Dubaniewicz 2010; 2013; Dubaniewicz et al. 2012a; b; Bournazos et al. 2009; Kivity et al. 2009; Li et al. 2009).

After absorption of bacteria or antigen through FcγRIIa, their presence in phagocytes of SA patients is extended due to lower bactericidal activity of phagocytes, compared with tuberculosis patients. A dormant presence of *M. tuberculosis* in a phagocyte would subsequently cause heat shock protein secretion from both bacterial and host cells, which may cause extended antigenemia and immunocomplexemia with the subsequent (auto)antigen presentation to T and B lymphocytes in the context of HLA, their increased activation, proliferation, and formation of a sarcoid granuloma (Dubaniewicz et al. 2012a; Dubaniewicz 2010). Further, IgG2, through C1q binding, would initiate a classical pathway of complement activation, which greatly accelerates inflammatory reactions, present especially in patients with Stages III and IV of SA (Li et al. 2009). Increased occurrence of the 57Q allele and 57XQ genotype of *FCGR2C*, with the 57Q functional allele, in our patients with advanced Stages III and IV of SA, may also enhance phagocytosis of CIs by monocytes/macrophages and neutrophils and lead to excessive Hsp production, antigen presentation, and lymphocyte over-proliferation, causing granulomatous formation (Dubaniewicz et al. 2012b). Increased frequency of the NA1 allele of *FCGR3B*, with higher affinity to IgG1 and IgG3 bound in CIs, compared with the NA2 and SH alleles, could also enhance inflammatory processes accompanied by abundant neutrophil involvement (Bournazos et al. 2009; Li et al. 2009). Since the distribution of the NA1/NA2/SH variants of *FCGR3B* remained basically unchanged in the patients of the present study, the *FCGR2A* and *FCGR2C* polymorphisms found, and that of *FCGR3A* previously reported, may drive the excessive (auto) immune response in sarcoidosis (Typiak et al. 2014). This plausibility is supported by the finding that FcγRIIa, but not FcγRIIb, induces L-selectin shedding of neutrophils to

enable their arrival at the site of infection (Kocher et al. 1997). Additionally, solely FcγRIIa-mediated neutrophil interactions with CIs result in the formation of neutrophil extracellular traps in tissues, a proinflammatory process that is linked to autoimmunity (Chen et al. 2012).

Differences in the allele and genotype distribution concerning the *FCGR2A* and *FCGR2C* genes may suggest the presence of different pathomechanisms underlying less severe Stages I/II and more advanced, parenchymal Stages III/IV of sarcoidosis. Furthermore, variations in the distribution of polymorphic *FCGR2A*, *FCGR2C*, along with the previously reported *FCGR3A*, may explain the immunocomplexemia combined with the presence of Fcγ receptors on the surface of phagocytes, observed in patients with sarcoidosis. The genotyping analysis may help anticipate the clinical run of sarcoidosis, i.e., the transition from initial mild to later advanced phases, and thus may help personalize therapy.

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