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No association between *FCGR2A* and *FCGR3A* polymorphisms in Guillain-Barré Syndrome in a Brazilian population

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ABSTRACT

The pathogenesis of Guillain-Barré Syndrome (GBS) is not entirely understood, but includes infection-induced aberrant immune responses. Genetic polymorphisms in Fc gamma receptor genes (*FCGR)* have been associated with GBS. We assessed whether polymorphisms rs1801274 in *FCGR2A* and rs396991 in *FCGR3A* were associated with GBS in a Brazilian population. We genotyped 141 GBS cases and 364 healthy controls from Brazil for both polymorphisms. The *FCGR* genotypes and alleles frequencies did not differ significantly between GBS and controls. In addition, there was no genetic association with either severity or clinical outcomes. We conclude that these *FCGR* polymorphisms are not associated with susceptibility to Guillain-Barré Syndrome in this Brazilian population.

1. Introduction

Guillain-Barré Syndrome (GBS) is an immune-mediated polyneuropathy and is currently the principal cause of acute neuromuscular paralysis. The three main GBS subtypes are acute motor axonal neuropathy (AMAN), Miller-Fisher Syndrome (MFS), and acute inflammatory demyelinating polyneuropathy (AIDP) (Hughes and Cornblath, 2005). In Brazil, Europe and North America the demyelinating GBS accounts for 80 to 90% of cases (Hughes and Cornblath, 2005; Dourado et al., 2012; Yuki and Hartung, 2012), whereas in China, Japan, Bangladesh and Mexico the axonal GBS is more prevalent (Yuki and Hartung, 2012).

Infection- and vaccination-induced aberrant immune responses along with complement activation have been associated with the pathogenesis of GBS (van Doorn et al., 2008). About two-thirds of patients have a prior history of infections with pathogens such as *Campylobacter jejuni*, cytomegalovirus and Epstein-Barr viruses (Hughes and Cornblath, 2005; Yuki and Hartung, 2012). *C. jejuni* is the best-studied triggering agent for GBS of the axonal subtype. *C. jejuni* has ganglioside-like structures in its lipopolysaccharide coat (Yuki et al., 1990; Yuki et al., 2004; Ho et al., 1995; Odaka et al., 2001; Dourado et al., 2003; Susuki et al., 2007). Other factors influence the development of GBS after infection, in part due to molecular mimicry, as well as the type of the host response. (Sheikh et al., 1998; Nachamkin et al. 2008, Godschalk et al., 2006; McCarthy and Giesecke, 2001).

Fc gamma receptor gene (*FCGR)* polymorphisms have been associated with human diseases (Takai, 2002). Three classes of human *FCGR* (*FCGR2A, FCGR3A* and *FCGR3B*) contain allelic polymorphisms that influence their affinity for IgG subclasses (Takai, 2002; Gillis et al., 2014). Anti-ganglioside antibodies via immunoglobulin receptor form a complex that activate macrophages and lead to damage in myelin or axons, supporting the role of *FCGR* in the pathogenesis of GBS (He et al., 2015; van Sorge et al., 2003; Zhang et al., 2014). In addition, intravenous IgG, which is effective in GBS treatment, acts by blocking receptors for the Fc region of IgG (FcγR) and improves recovery time (van der Meche and Schmitz, 1992; Dalakas, 1998). The polymorphisms rs1801274 and rs396991 alter the receptor binding affinity for IgG (Takai, 2002; van der Pol et al., 2000; Sinha et al., 2010).

Although there are numerous reports suggesting an association between polymorphisms in genes for *FCGR2A* and *FCGR3A* and susceptibility to GBS, the results have been inconsistent. Some studies have shown an association (van der Pol et al., 2000; Sinha et al., 2010), whereas others showed no association (Vedeler et al., 2000; van Sorge et al., 2005). In this study, we determined *FCGR2A* and *FCGR3A* genotypes in 141 GBS patients and 364 healthy controls in the state of Rio Grande

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do Norte, Brazil, prior to the introduction of Zika and Chikungnya viruses (Araujo et al., 2016; Campos et al., 2015).

2. Materials & methods

2.1. Subjects

From 1994 to 2013, a total of 141 individuals with GBS were recruited from the state of Rio Grande do Norte, Brazil. GBS was defined by the Asbury and Cornblath criteria (Asbury and Cornblath, 1990). Electrophysiological studies were used to classify the cases as either demyelinating or axonal, according to criteria described previously (Ho et al., 1995). The Miller-Fisher variant included those subjects presenting with a triad of ataxia, areflexia, and ophthalmoplegia in the absence of limb weakness (Fisher, 1956).

The degree of severity was assessed at the peak of illness. The GBS Disability Scale grading system, Hughes scale, of 0–6 was used (state 0, normal; state 1, minimal signs and symptoms, able to run; state 2, able to walk without assistance; state 3, able to walk 5 m with aid; state 4, confined to bed or chair bound; state 5, needs ventilatory support; state 6, deceased (Hughes et al., 1978). Cases were classified as severe (Hughes grade of 4 or more) or mild (Hughes grade of 3 or less).

The outcome measure for the study was Hughes grade at 6 months and the number of days to recover the ability to walk (days to walk). Poor outcome was defined as a Hughes grade of 3 or more at 6 months, which corresponded to the inability to walk 5 m independently.

2.2. Anti-ganglioside antibodies

Blood samples were collected from GBS patients during the acute phase of disease. Serum samples were used to test for the presence of GM1 and GD1a antibodies using ELISA, as described in a previous study (Dourado et al., 2003).

2.3. Polymorphism genotyping

Genomic DNA was extracted from leucocytes isolated from whole blood using a standard protocol (Grimberg et al., 1989). *FCGR2A* rs1801274 and *FCGR3A* rs396991 genotypes were determined by the Real-Time PCR TaqMan® SNP Genotyping Assays (the assay IDs were C_9077561_20 and C_25815666_10, respectively) using a 7500 Real-Time PCR System (Applied Biosystems, CA, USA) in accordance with manufacturer's instruction.

2.4. Statistical analysis

Categorical variables, including genotype and allele distributions, were compared by chi-square test. To estimate the genetic effect, genotypes were coded assuming a log-additive model and including them, as explanatory variables, in a logistic regression model adjusting for age and sex. The days to walk were compared among genotypic groups by the Kruskal-Wallis test. The analyses were performed in Stata 11.2 assuming a significance level of $\alpha = 0.05$.

2.5. Ethical considerations

The protocol was reviewed and approved by the Universidade Federal do Rio Grande do Norte Ethical Committee (CEP-UFRN 046/03 and CEP-UFRN 198/09) and by the Brazilian National Ethical Committee (CONEP: 9061). The certificate of ethical approval (CAAE) is 0215.0.051.000-09. All subjects or their legal guardian signed the informed consent.

3. Results

3.1. Characteristics of the study population

Of the 141 SGB patients, 82 (58.2%) were males. The mean age was 29.8 years (±20.3). A group of 364 healthy blood donors from the same region were recruited as controls (310 males, 54 females; median age = 35.8 years and male/female = 54/310, both with $p < 0.05$ when compared to SGB group). Controls were purposely selected from an older population from the same geographic region to allow for potential exposure to environmental factors, such as infections.

The GBS patient characteristics are presented in Table 1. Evidence of recent infection, based on oral history was detected in 91 (64.5%) patients. Of those, 69 (49%) presented a previous episode of respiratory tract infection, the majority had a severe score at diagnosis. Of the 141 GBS cases, 100 (70.9%) were classified as AIDP.

3.2. Anti-GM1 comparisons

Anti-GM1 antibodies, in our study, were more frequently present in the AMAN than in the demyelinizing variant, ($p < 0.001$). Patients with Hughes scale at 6 months >3 had more positive GM1 antibodies ($p = 0.02$) than the ones that were negative.

3.3. Genetic analysis

Genotype and allele distributions did not differ among healthy blood donors and GBS patients, even when compared with mild and severe GBS groups (Table 2). We applied several logistic regression models in order to test for genetic association with distinct clinical forms and outcomes, with the advantage of controlling for potential confounders such as age and sex. Neither *FCGR2A* rs1801274 (R/H) nor *FCGR3A* rs396991 (F/V) were associated with GBS phenotypes, as summarized in Table 3. Lastly, the time to walk did not differ among genotypic groups (Fig. 1A and B). *FCGR* genotypes did not correlate with positive or negative antiganglioside antibodies (*FCGR2A*, $p = 0.907$; *FCGR3A*, $p = 0.583$).

4. Discussion

In this study, the *FCGR* genotypes and allele frequencies did not differ significantly between the GBS patients and the controls. In addition, *FCGR* genotypes did not correlate with disease outcome. The most

Table 1
Characteristics of patients with Guillain-Barré Syndrome.

Characteristics	GBS
Number	141
Male/female	82/59
Age, y, mean (SD)	29.8 (±20.3)
Previous infection, n (%)	
Respiratory tract infection	69 (48.9)
Diarrhea	21 (14.9)
Diarrhea and Respiratory tract infection	1 (0.70)
Vaccine	3 (2.1)
None	47 (33.3)
Severity score, n (%)	
Mild (1–3)	27/135 (20)
Severe (4–5)	108/135 (80)
Antiganglioside antibodies, n(%)	
Anti-GM1	24/116 (20.6)
Electrophysiological classification, n (%)	
Demyelinating	100 (70.9)
Axonal	36 (25.5)
Miller Fisher	2 (1.4)
Unclassified	3 (2.2)
Clinical outcomes at 6 months	
Hughes grade < 3	103 (81.1)
Hughes grade ≥ 3	24 (18.9)

SD, standard deviation; n, number; y, age in years.