Fc-gamma IIIa-V158F receptor polymorphism contributes to the severity of Guillain-Barré syndrome

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Abstract

Objective: Guillain-Barré syndrome (GBS) is a rare, life-threatening disorder of the peripheral nervous system. Immunoglobulin G Fc-gamma receptors (FcγRs) mediate and regulate diverse effector functions and are involved in the pathogenesis of GBS. We investigated whether the FcγR polymorphisms FcγRIIa H/R131 (rs1801274), FcγRIIIa V/F158 (rs396991), and FcγRIIIb NA1/NA2, and their haplotype patterns affect the affinity of IgG-FcγR interactivity and influence GBS susceptibility and severity. Methods: We determined FcγR polymorphisms in 303 patients with GBS and 302 ethnically matched healthy individuals from Bangladesh by allele-specific polymerase chain reaction. Pairwise linkage disequilibrium and haplotype patterns were analyzed based on D’ statistics and the genotype package of R statistics, respectively. Logistic regression analysis and Fisher’s exact test with corrected P (Pc) values were employed for statistical comparisons. Results: FcγRIIIa-V158F was associated with the severe form of GBS compared to the mild form (P = 0.005, OR = 2.24, 95% CI = 1.28–3.91; Pc = 0.015); however, FcγR genotypes and haplotype patterns did not show any association with GBS susceptibility compared to healthy controls. FcγRIIIa-V/V158 and FcγRIIIb-NA2/2 were associated with recent Campylobacter jejuni infection (P ≤ 0.001, OR = 0.36, 95% CI = 0.23–0.56; Pc ≤ 0.003 and P = 0.004, OR = 1.70, 95% CI = 1.18–2.44; Pc ≤ 0.012, respectively). Haplotype 1 (FcγRIIa-H131R- FcγRIIIa-V158F- FcγRIIIb-NA1/2) and the FcγRIIIb-NA2/2 genotype were more prevalent among anti-GM1 antibody-positive patients (P = 0.031, OR = 9.61, 95% CI = 1.24–74.77, Pc = 0.279; P = 0.027, OR = 1.62, 95% CI = 1.06–2.5, Pc = 0.081, respectively). Interpretation: FcγR polymorphisms and haplotypes are not associated with susceptibility to GBS, though the FcγRIIIa-V158F genotype is associated with the severity of GBS.

Introduction

Guillain-Barré syndrome (GBS) is a post-infectious autoimmune disorder of the peripheral nervous system that can lead to significant morbidity, long-term disability or death.

Cross-reactive immune responses induced by molecular mimicry between the outer core structure of infectious agents that trigger GBS and host nerve gangliosides1 result in a blockade of nerve conduction.1,2 Campylobacter jejuni has been identified as the predominant causative microbial infectious agent in GBS.3–5 In addition to multifarious microorganism-derived factors, host immunogenic factors are likely to affect GBS susceptibility as only a subset of C. jejuni-infected individuals (1 in 1000–5000 cases) develop GBS.6–9 Natural variations in genetic host susceptibility factors have become a focus of research on the susceptibility and severity of disease pathogenesis in GBS.

Immunoglobulin G Fc-gamma receptors (FcγRs) are important immune-response modulating molecules that link the cellular and humoral immune system by interacting with IgG subtypes (IgG1–4). The most common autoantibodies in GBS are produced against GM1, GD1a...
These autoantigens may influence nerve disruption, demyelination or axonal degeneration via diverse mechanisms, including induction of inflammatory immune responses, by interacting with Fc receptors. FcγR polymorphisms can determine the vigor of inflammatory responses, affect downstream functions such as phagocytosis, antibody-dependent cellular cytotoxicity (ADCC) and the release of inflammatory mediators, and have been implicated in the development of autoimmune disease. Thus, FcγRs may represent important effector molecules in the pathogenesis of GBS. Three subclasses of FcγRs, namely FcγRIIa, FcγRIIIa and FcγRIIIb, exhibit allelic variation. The most widely distributed receptor, FcγRIIa, is expressed on all types of white blood cells and has two allelic forms: FcγRIIa-H131 and FcγRIIa-R131. These alleles differ by the replacement of histidine by arginine at position 131 due to an A → G single nucleotide exchange at position 494. FcγRIIa-H131 is reported to bind human IgG2 with a higher affinity than FcγRIIa-R131. FcγRIIIa is expressed on macrophages, dendritic cells, γ/δ T-cells and natural killer (NK) cells. A functional polymorphism at nucleotide 559 results in either a valine (V) or phenylalanine (F) at amino acid position 158, which affects the receptor binding capacity of IgG1, IgG3, and IgG4. FcγRIIIb is expressed on neutrophils and exhibits two allelic forms, neutrophil antigen 1 (NA1) and neutrophil antigen 2 (NA2). NA1 and NA2 differ by five base substitutions (nucleotides 141, 147, 227, 277, and 349) that lead to four amino acid changes (at positions 36, 65, 82, and 106) within exon 3. However, these allelic forms of FcγR (NA1/NA2) have different affinities for IgG1 and IgG3. Thus, the various allelic forms of FcγR may possibly determine the extent of inflammatory responses and thereby influence autoimmune diseases, including GBS.

Several studies have already evaluated the relationship between FcγR polymorphisms and the pathogenesis of GBS. FcγRIIa-H/H131 was significantly associated with susceptibility to GBS and was also a potent risk factor for the development of GBS in a Dutch population. These findings were consistent with a study of Indian patients with GBS, but not with a report on Norwegian Caucasian patients. One meta-analysis indicated that every FcγRIIIb-NA2 allele cumulatively increases the GBS severity score, though none of the genotypes or alleles were associated with susceptibility to GBS. However, consensus regarding the role of FcγR polymorphisms in the pathogenesis of GBS has not yet been established due to the inadequate statistical power of studies with small sample sizes and differences in the ethnicities of the populations tested. Thus, we aimed to evaluate whether candidate gene polymorphisms in FcγR are a major causative factor for GBS susceptibility or severity in Bangladeshi patients with C. jejuni-triggered GBS, which represents the world’s largest cohort.

### Materials and Methods

#### Research participants

The GBS cohort used in this study includes 303 patients with GBS (208 males, 95 females; median age: 30 years [interquartile range, 17–42]; Table 1) and 302 ethnically matched healthy controls (204 males, 99 females; median age: 34 years [interquartile range, 28–46]). Patients with GBS were diagnosed based on the previously established diagnostic criteria described by Asbury and Cornblath and enrolled from Dhaka Medical College and Hospital (DMCH), Dhaka, Bangladesh. No preference was given to race, religion, or socioeconomic status during study subject selection. Genetically unrelated healthy individuals who did not have neurological diseases, antecedent infections, recent surgery or other illnesses were included in this study following informed consent and matched with patients. Clinical, electrophysiological, and serological data were obtained from patients with informed consent.

Blood specimens were collected by venipuncture before patients received medication and disease outcome was

#### Table 1. Demographic and clinical characteristics of the patients with GBS.

| Characteristic                              | Number of patients, n = 303 (%) |
|--------------------------------------------|---------------------------------|
| Sex                                        |                                 |
| Male/female                                | 208/95                          |
| Age                                        |                                 |
| Median (IQR)                               | 30 (17–42)                     |
| Preceding illness, n = 303                  |                                 |
| Diarrhea                                    | 129/303 (43)                   |
| Respiratory tract infections                | 45/303 (15)                    |
| Fever                                      | 25/303 (8)                     |
| Other                                       | 28/303 (9)                     |
| None/unknown                               | 76/303 (25)                    |
| Electrophysiological classification, n = 247|                                 |
| Axonal                                     | 146/247 (59)                   |
| Demyelinating                              | 68/247 (27)                    |
| Unclassified                               | 33/247 (13)                    |
| MRC sum score (at entry)                   |                                 |
| Severely affected patients                 | 232/303 (77)                   |
| Mildly affected patients                    | 71/303 (23)                    |
| Serological characteristics                |                                 |
| Anti-GM1-Ab-seropositive                   | 118/303 (39)                   |
| C. jejuni-seropositive                     | 186/303 (61)                   |
| Disease prognosis at 6 months, n = 303     |                                 |
| Good outcome                               | 209/303 (69)                   |
| Poor outcome                               | 94/303 (31)                    |

GBS, Guillain-Barré syndrome; IQR, interquartile range; MRC, Medical Research Council; Ab, antibody; C. jejuni, Campylobacter jejuni.
evaluated by assessing clinical data at specific standard time-points (at entry, 2 weeks, 4 weeks and 6 months). In this cohort, 75% (227/303) patients had an antecedent illness; most frequently diarrhea (43%; 129/303), followed by respiratory infection (15%, 45/303), fever (8%, 25/303) or other illnesses (9%, 28/303); 25% (76/303) of patients had history of unknown infections or no infection. Serological tests, that is, antibodies against C. jejuni or GM1, GD1a and GQ1b gangliosides were measured using enzyme-linked immunosorbent assays (ELISAs).5,29

Electrophysiological studies of 82% (247/303) of the GBS patients indicated 59% (146/247) of patients had an axonal subtype of GBS, including acute motor axonal neuropathy (AMAN) and acute motor and sensory axonal neuropathy (AMSAN), 27% (68/247) of patients had acute inflammatory demyelinating polyradiculoneuropathy (AIDP) and 13% (33/247) of cases were unclassified with inexcitable nerves or equivocal findings.30 Severity of disease (degree of muscle weakness) was assessed using the Medical Research Council (MRC) sum score31,32 ranging from 0 to 60 at nadir (maximum muscle weakness); GBS patients at nadir with MRC sumscore < 40 were defined as severely affected patients and with MRC sumscore ≥ 40 were defined as mildly affected patients.33 The outcome of the disease was measured using the GBS disability score after 6 months of follow-up.34 This study was reviewed and approved by the Institutional Review Board (IRB) and ethical committees of the icddr, b, Dhaka, Bangladesh.

Genomic DNA isolation

Whole blood samples were collected from 605 study subjects into lithium heparin-coated anti-coagulation tubes for genomic DNA isolation. Genomic DNA was extracted using the QIAamp® DNA Blood Midi Kit (100) (Qiagen, Hilden, Germany), dissolved in 1 x TE buffer (10 mmol/L Tris-Cl, pH 8.0, 1 mmol/L EDTA), stored at −20°C, diluted to 10 ng/µL with Milli-Q water and then stored at −20°C until SNP detection.

FcγRIIA polymorphism detection and genotype analysis

The FcγRIIA polymorphisms FcγRIIA H/R131 (rs1801274), FcγRIIA V/F158 (rs3969991) and FcγRIIb NA1/NA2 were genotyped via a previously described allele-specific polymerase chain reaction (AS-PCR) method using published primer sequences and reaction conditions.18,21 Human growth hormone (HGH) primers (5'-GCTTTCCAAACACTTCCCTTTA-3' and 5'-CTTCAAGTTTCGTTGTTTC-3') were used as an internal positive control.18 The PCR products were visualized on 2% agarose gels using a Molecular Imager® Gel Doc230 XR + system (Bio-Rad Laboratories Inc).

**Statistical analysis**

Statistical analysis was performed using logistic regression analysis and Fisher’s exact test with Yates’ continuity correction to assess associations between the FcγR polymorphisms and disease susceptibility or subgroups. In the control group, all SNPs were within Hardy-Weinberg equilibrium. P values less than 0.05 were considered statistically significant. The Bonferroni method was applied to correct the P values for multiple comparisons: each P value was multiplied by the number of comparisons and denoted Pc (Pc, P corrected). Genotype/allelic frequencies were estimated by a simple counting method and the data were processed using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA), GraphPad prism (version 5.01, GraphPad software, Inc., La Jolla, CA) or SPSS (version 16.0, Company, Chicago, IL). Haplotype patterns and frequencies were analyzed using the genotype package of R statistics and their associations with GBS susceptibility and subgroups were assessed using logistic regression analysis.

**Results**

**FcγRIIa, FcγRIIIa, and FcγRIIib polymorphisms and haplotypes in patients with GBS and healthy individuals**

No significant associations were observed between the FcγRIIa, FcγRIIIa, and FcγRIIib polymorphisms and susceptibility to GBS compared to healthy controls (Table 2). The comparison of axonal variants of GBS versus healthy controls or demyelinating subtypes versus healthy subjects showed no relation with disease susceptibility (Table 3). The haplotype distributions of the three loci were compared between patients with GBS and healthy individuals. Haplotype analysis revealed 27 possible different patterns for the FcγRIIa, FcγRIIIa, and FcγRIIib polymorphic loci (Fig. 1). The nine most predominant patterns (haplotypes 1–9; frequency > 5%), representing 61.5% of total variation, were selected for further haplotype analysis (Fig. 2). No significant association was observed between any haplotype and GBS susceptibility when each haplotype was analyzed individually.

**FcγRIIa, FcγRIIIa, and FcγRIIib polymorphisms and haplotypes in anti-GM1 antibody-positive GBS**

The frequency of FcγRIIib-NA2/2 genotypes was predominant among anti-GM1 antibody-positive patients compared to healthy individuals but association was not significant (P = 0.051, OR = 1.93, 95% CI = 1.03–3.62;
Table 2. FcγR genotype and allelic distributions in Bangladeshi patients with GBS and healthy controls.

| FcγR genotype/allele | HC, $n = 302$ (%) | GBS patients, $n = 303$ (%) | $P$ | Odds ratio (95% CI) |
|----------------------|-------------------|-------------------------------|-----|-------------------|
| FcγR-IIa             |                   |                               |     |                   |
| H/H-131              | 116 (38.4)        | 114 (37.6)                    |     | Reference         |
| H/R-131              | 136 (45)          | 144 (47.3)                    | 0.283 | 0.93 (0.65–1.32)  |
| R/R-131              | 50 (16.6)         | 65 (21.5)                     | 1.32 | 0.84–2.08         |
| R-131                | 236 (79.1)        | 254 (41.9)                    | 0.320 | 0.89 (0.71–1.12)  |
| H-131                | 368 (60.9)        | 352 (58.1)                    |     | Reference         |
| FcγR-IIIa            |                   |                               |     |                   |
| F/F-158              | 110 (36.4)        | 120 (39.6)                    |     | Reference         |
| V/F-158              | 150 (49.7)        | 143 (47.2)                    | 0.723 | 0.87 (0.62–1.23)  |
| V/V-158              | 42 (13.9)         | 40 (13.2)                     | 0.87 | 0.53–1.45         |
| V-158                | 234 (38.7)        | 223 (36.8)                    | 1.09 | 0.86–1.37         |
| F-158                | 370 (61.3)        | 383 (63.2)                    | 0.514 | Reference         |
| FcγR-IIIb            |                   |                               |     |                   |
| NA1/1                | 69 (22.9)         | 56 (18.5)                     |     | Reference         |
| NA1/2                | 124 (41.7)        | 125 (41.2)                    | 0.311 | 1.22 (0.79–1.88)  |
| NA2/2                | 105 (35.4)        | 122 (40.3)                    | 1.41 | 0.91–2.18         |
| NA1                  | 264 (43.7)        | 237 (39.1)                    | 0.115 | 1.21 (0.96–1.52)  |
| NA2                  | 340 (56.3)        | 369 (60.9)                    |     | Reference         |

GBS, Guillain-Barré syndrome; HC, healthy controls; 95% CI, 95% confidence interval.

Table 3. Distribution of FcγR genotypes and alleles among axonal and demyelinating cases of GBS compared to healthy controls.

| FcγR Genotypes/Alleles | Axonal, $n = 146$ (%) | Demyelinating, $n = 68$ (%) | Healthy control (HC), $n = 302$ (%) | $P$ value | OR (95% CI) |
|------------------------|-----------------------|-----------------------------|----------------------------------|-----------|-------------|
| FcγR-IIa               |                       |                             |                                  |           |             |
| H/H-131               | 50 (34.2)             | 28 (41.2)                   | 116 (38.4)                       | 0.289     | 1.1 (0.69–1.68) |
| H/R-131               | 63 (43.2)             | 24 (35.3)                   | 136 (45)                         |           | Reference   |
| R/R-131               | 33 (22.6)             | 16 (23.5)                   | 50 (16.6)                        | 1.5       | 1.5 (0.88–2.66) |
| R-131                 | 129 (44.2)            | 56 (41.2)                   | 236 (39.1)                       |           | Reference   |
| H-131                 | 163 (55.8)            | 80 (58.8)                   | 368 (60.9)                       |           | Reference   |
| FcγR-IIIa             |                       |                             |                                  |           |             |
| F/F-158               | 57 (39)               | 33 (48.5)                   | 110 (36.4)                       | 0.542     | 0.9 (0.6–1.4)  |
| V/F-158               | 74 (50.7)             | 27 (39.7)                   | 150 (49.7)                       | 0.7       | 0.4 (0.1–1.3)  |
| V/V-158               | 15 (10.3)             | 8 (11.8)                    | 42 (13.9)                        | 0.4       | 0.7 (0.4–1.3)  |
| V-158                 | 104 (35.6)            | 43 (31.6)                   | 234 (38.7)                       |           | Reference   |
| F-158                 | 188 (64.4)            | 93 (68.4)                   | 370 (61.3)                       |           | Reference   |
| FcγR-IIIb             |                       |                             |                                  |           |             |
| NA1/1                 | 27 (18.5)             | 17 (25)                     | 69 (22.8)                        | 0.506     | 0.8 (0.5–1.4)  |
| NA1/2                 | 61 (41.8)             | 25 (36.8)                   | 126 (41.7)                       | 1.0       | 1.0 (0.5–2.0)  |
| NA2/2                 | 58 (39.7)             | 26 (38.2)                   | 107 (35.4)                       |           | Reference   |
| NA1                   | 115 (39.4)            | 59 (43.4)                   | 264 (43.7)                       |           | Reference   |
| NA2                   | 177 (60.6)            | 77 (56.6)                   | 340 (56.3)                       | 0.248     | 0.8 (0.6–1.1)  |

OR, odds ratio; 95% CI, 95% confidence interval.

Associations of FcγRIla, FcγRIIla, and FcγRIIib polymorphisms and haplotype patterns with disease severity and outcome

FcγRIla, FcγRIIla, and FcγRIIib genotypes and haplotype patterns were investigated in patients with severe and mild form of GBS (Table 5). The haplotype patterns were not associated with disease severity, though homozygous FcγRIIla-F158 was significantly associated with the mild form of disease before Bonferroni correction ($P = 0.03$, OR = 0.55, 95% CI = 0.32–0.94; $Pc = 0.09$; Table 5). Heterozygous FcγRIIla-V158F was significantly associated with the severe form of disease (compared to the mild form) after correcting the $P$ value ($P = 0.005$, OR = 2.24, 95% CI = 1.28–3.91; $Pc = 0.015$; Table 5).
NA1 was significantly predominant in the mild form of GBS than the severe form \( (P = 0.007, \text{OR} = 0.41, 95\% \text{ CI} = 0.22–0.77; \text{PC} = 0.021; \text{Table 5}) \). FcγRIIIa-NA1/NA2 tended to be more common in severe GBS \( (P = 0.054, \text{OR} = 1.75, 95\% \text{ CI} = 0.99–3.08; \text{PC} = 0.162; \text{Table 5}) \). However, the FcγRIIa-H131 and FcγRIIa-R131 alleles and genotypes were not associated with the severity of GBS. Individual FcγR genotypes were not associated with disease outcome at 6-month follow-up.

**FcγRIIa, FcγRIIIa, and FcγRIIIb genotypes in patients with recent *C. jejuni* infection**

The homozygous FcγRIIIb-NA2 and heterozygous FcγRIIIb-NA1/2 genotypes were associated with recent *C. jejuni* infection in patients with GBS; however, the association for the heterozygous FcγRIIIb-NA1/2 genotype lost significance after Bonferroni correction \( (P = 0.004, \text{OR} = 1.70, 95\% \text{ CI} = 1.18–2.44; \text{PC} = 0.012 \) and \( P = 0.026, \text{OR} = 1.48, 95\% \text{ CI} = 1.05–2.10; \text{PC} = 0.078 \); respectively; Table 5). Frequency of homozygous FcγRIIIb-NA2 and heterozygous FcγRIIIb-NA1/2 genotypes were significantly prevalent in *C. jejuni* infected patients with GBS compared to healthy controls. But P-value lost its significance after Bonferroni correction \( (P = 0.041, \text{OR} = 1.74, 95\% \text{ CI} = 1.03–2.94; \text{PC} = 0.123 \) and \( P = 0.048, \text{OR} = 1.74, 95\% \text{ CI} = 1.02–2.98; \text{PC} = 0.144 \); respectively; Table 4). The FcγRIIIa-V/V158 genotype was less frequent in *C. jejuni* -seropositive patients \( (P \leq 0.001, \text{OR} = 0.36, 95\% \text{ CI} = 0.23–0.56; \text{PC} \leq 0.003; \text{Table 5}) \); however, the FcγRIIIa-F/F158 and FcγRIIIa-V/F158 genotypes were significantly prevalent among *C. jejuni* -seropositive patients than seronegative patients before correcting the P values \( (P = 0.038, \text{OR} = 1.47, 95\% \text{ CI} = 1.02–2.11; \text{PC} = 0.114 \) and \( P = 0.025, \text{OR} = 1.49, 95\% \text{ CI} = 1.05–2.10; \text{PC} = 0.075 \), respectively; Table 5).

**Discussion**

This study investigated the association of three functionally relevant polymorphisms in FcγR and the resulting haplotype patterns with the susceptibility and severity of GBS among patients compared to healthy controls in a large cohort of GBS in Bangladesh. We found no significant associations between individual FcγR alleles or genotypes and susceptibility to GBS; however, the FcγRIIIa-V/F158 genotype influenced the severity of disease. Moreover, associations between the FcγRIIa and FcγRIIIb genotypes and haplotype patterns were evident in patients...
with an antecedent C. jejuni infection and anti-GM1 antibody-positive patients, respectively.

Associations between FcγR polymorphisms and susceptibility to GBS have previously been studied in patients with different ethnic backgrounds (Table 6). We observed no significant differences in the FcγR allele or genotype frequencies and haplotype patterns between Bangladeshi patients with GBS and healthy controls. These findings confirm a previous meta-analysis of British, Dutch, and Norwegian GBS cases, which suggested FcγR polymorphisms were not related to disease susceptibility, regardless of ethnic variation.

In addition, we found the FcγRIIIa-F/F158 genotype was associated with the mild form of GBS based on MRC sum score at nadir, while the FcγRIIIa-V/F158 genotype was associated with the severe form of GBS. As phagocytosis, cellular cytotoxicity, cytokine production, and other immune responses depend on efficient FcγR-IgG interactions, the higher frequency of FcγRIIIa-F/F158 among patients with the mild form of GBS may indicate this genotype reduces the affinity of IgG binding and in turn impairs immune complex clearance and decreases subsequent inflammation. Patients with FcγRIIIa-V/F158 genotypes may have better ability to clear immune complexes (ICs) via degranulation and phagocytosis more efficiently, resulting in more severe disease. We observed a higher frequency of FcγRIIIb-NA1/NA1 genotypes in patients with the mild form of GBS, similar to a previous study of Norwegian patients with GBS. The NA1/NA1 genotype has a high affinity for IgG1 and IgG3, which are the most common among the anti-GM1 and anti-GQ1b antibodies. Autoantibodies such as anti-ganglioside antibodies are neutralized in the circulation, thus cross-reaction of these auto-antibodies with the peripheral nerves may be partially prevented in patients with GBS who are homozygous for FcγRIIIb-NA1.

Ganglioside-specific IgG have been reported to damage nerve tissues by activating effector functions (e.g., phagocytosis and/or degranulation) via FcγR. Homozygous FcγRIIIb-NA1 was less frequent among both C. jejuni seropositive patients and anti-GM1 antibody-positive patients with the mild form of the disease. In contrast, FcγRIIIb-NA2/2 was associated with recent C. jejuni infection and anti-GM1 antibody production. In addition,
Table 4. Distribution of FcγR genotypes and alleles between healthy controls versus C. jejuni-seropositive patients and healthy controls versus Anti-GM1 antibody-seropositive patients with GBS.

| FcγR genotype/allele | Healthy controls (a), n = 302 (%) | C. jejuni seropositive patients (b), n = 186 (%) | Anti-GM1-Ab-seropositive patients (c), n = 119 (%) | a versus b | a versus c | Odds ratio (95% CI) | a versus b | a versus c | Odds ratio (95% CI) |
|---------------------|---------------------------------|---------------------------------|---------------------------------|-----------|-----------|---------------------|-----------|-----------|---------------------|
| FcγR-IIa            |                                 |                                 |                                 |           |           |                     |           |           |                     |
| H/H-131             | 116 (38.4)                      | 67 (36.0)                       | 42 (35.3)                       | Reference | Reference |                     |           |           | 1.08 (0.67–1.73)    |
| H/R-131             | 136 (45)                        | 81 (43.6)                       | 53 (44.5)                       | 0.917 na  | na        | 1.03 (0.69–1.55)    | 0.809 na  | na        | 1.32 (0.78–2.11)    |
| R/R-131             | 50 (16.6)                       | 38 (20.4)                       | 24 (20.2)                       | 0.351 na  | na        | 1.32 (0.78–2.11)    | 0.354 na  | na        | 1.33 (0.73–2.42)    |
| H-131               | 236 (39.1)                      | 157 (42.2)                      | 101 (42.4)                      | 0.88 (0.68–1.14) | na        | 0.87 (0.64–1.18)    |           |           |                     |
| FcγR-IIIa           |                                 |                                 |                                 |           |           |                     |           |           |                     |
| F/F-158             | 110 (36.4)                      | 70 (37.6)                       | 44 (37.3)                       | Reference | Reference |                     |           |           | 1.03 (0.69–1.55)    |
| V/F-158             | 150 (49.7)                      | 90 (48.4)                       | 55 (46.6)                       | 0.839 na  | na        | 0.94 (0.63–1.40)    | 0.722 na  | na        | 0.87 (0.57–1.38)    |
| V/V-158             | 42 (13.9)                       | 26 (14.0)                       | 20 (16.1)                       | 1.0 na    | na        | 0.97 (0.55–1.73)    | 0.623 na  | na        | 1.20 (0.63–2.25)    |
| F-158               | 234 (38.7)                      | 142 (38.2)                      | 95 (39.9)                       | 1.02 (0.78–1.34) | na        | 0.95 (0.70–1.29)    |           |           |                     |
| FcγR-IIIb           |                                 |                                 |                                 |           |           |                     |           |           |                     |
| NA1/1               | 69 (22.9)                       | 27 (14.3)                       | 17 (14.2)                       | Reference | Reference |                     |           |           | 1.03 (0.69–1.55)    |
| NA1/2               | 126 (41.7)                      | 86 (46.2)                       | 51 (42.9)                       | 0.041 0.123 | 1.74 (1.03–2.94) | 0.134 na  | na        | 1.64 (0.88–3.06)    |
| NA2/2               | 107 (35.4)                      | 73 (39.3)                       | 51 (42.9)                       | 0.048 0.144 | 1.74 (1.02–2.98) | 0.051 na  | na        | 1.93 (1.03–3.62)    |
| NA1                 | 264 (43.7)                      | 140 (37.6)                      | 85 (35.7)                       | 1.29 (0.98–1.68) | na        | 1.40 (1.02–1.91)    |           |           |                     |
| NA2                 | 340 (56.3)                      | 232 (62.4)                      | 153 (64.3)                      | 0.071 na  | Reference | 0.36 (0.23–0.56)    | 0.072 na  | Reference |                     |

Table 5. Associations between FcγR genotypes and haplotypes with severe disease, anti-GM1 antibody-seropositivity and C. jejuni-seropositivity among patients with GBS.

| Variables                   | FcγR genotype/haplotype | P value | Odds ratio | 95% CI   | P corrected (Pc) |
|-----------------------------|-------------------------|---------|------------|----------|------------------|
| Mildly affected (n = 71)     versus severely affected (n = 232) patients | FcγR-IIa |                     |          |          |                  |
| FcγR-IIa                    | 0.03                    | 0.55    | 0.32–0.94  | 0.09     |                  |
| FcγR-IIIa                   | 0.05                    | 2.24    | 1.28–3.91  | 0.015    |                  |
| FcγR-IIIb                   | 0.25                    | 0.68    | 0.32–1.41  | –        |                  |
| Anti-GM1 Ab-seropositive (n = 118) versus seronegative (n = 185) | FcγR-IIIb |                     |          |          |                  |
| NA1/1                       | 0.007                   | 0.41    | 0.22–0.77  | 0.021    |                  |
| NA1/2                       | 0.054                   | 1.75    | 0.99–3.08  | 0.162    |                  |
| NA2/2                       | 0.891                   | 1.06    | 0.62–1.82  | –        |                  |
| C. jejuni-seropositive (n = 186) versus seronegative (n = 117) | FcγR-IIIb |                     |          |          |                  |
| NA1/1                       | ≤0.001                  | 0.32    | 0.21–0.49  | ≤0.003   |                  |
| NA1/2                       | 0.026                   | 1.48    | 1.05–2.10  | 0.078    |                  |
| NA2/2                       | 0.004                   | 1.70    | 1.18–2.44  | 0.012    |                  |

OR, odds ratio; 95% CI, 95% confidence interval; MRC sum scores < 40 at nadir were defined as severely affected; MRC sum scores ≥ 40 were defined as mildly affected; Anti-GM1 Ab, Anti-GM1 antibody; na, not applicable.
C. jejuni -seropositive patients had higher frequencies of the FcγRIIIa-F/F158 and FcγRIIIa-V158F genotypes. These findings indicate C. jejuni -seropositive patients with higher frequency of the FcγRIIIa-V158F genotype may suffer severe muscle weakness.

One limitation of this study is that polymorphisms of FcγRIIIb receptor gene, FcγRIIIb-SH alleles were not investigated; however, it is not yet known whether FcγRIIIb-SH polymorphisms influence the function of FcγRIIIb or not.16,40

The present study strengthens the evidence that FcγR polymorphisms and haplotypes influence the clinical and serological subgroup of GBS, as well as the strength of the immune responses that ultimately trigger the development of GBS and affect disease severity. In addition, the FcγRIIIa-V158F genotype was more frequent among patients with recent C. jejuni infection and was found to contribute to disease severity. Variation in the FcγR gene differs greatly between populations of different ethnicities, thus it will be important and interesting to confirm our findings in a multiethnic population, such as the International GBS Outcome Study (IGOS) population.41

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### Authors’ Contributions

ZI and SH conceived and designed the study. SH and MGB contributed to data acquisition. SH, MGB, and AD performed data analysis and interpreted the data. ZI and SH drafted the manuscript, which was critically reviewed by MGB, AD, ZHH, and IM for intellectual content. All authors read and approved the final manuscript before submission.

### Conflict of Interest

ZI received funding from the Fogarty International Center, National Institute of Neurological Disorders and Stroke of the National Institutes of Health, USA (Award Number K43 TW011447) and Annexon Biosciences (South San Francisco, CA 94080, USA). SH, MGB, AD ZHH and IM have no conflicts of interest to declare.

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