Antecedent infections in Guillain-Barré syndrome: a single-center, prospective study

Yanlei Hao1, Weifang Wang1, Bart C. Jacobs2, Baojun Qiao1, Mengshi Chen3, Daiqiang Liu1, Xungang Feng1 & Yuzhong Wang1,4

1Department of Neurology, Affiliated Hospital of Jining Medical University, Jining, Shandong Province, China
2Department of Neurology and Immunology, Erasmus University Medical Centre, Rotterdam, The Netherlands
3Department of Epidemiology and Health Statistics, School of Public Health, Central South University, Changsha, Hunan Province, China
4Central Laboratory, Affiliated Hospital of Jining Medical University, Jining, Shandong Province, China

Abstract

Objective: To investigate the spectrum of antecedent infections in Chinese patients with Guillain-Barré syndrome (GBS) and analyze the infections-related clinical phenotypes locally. Methods: A prospective case-control study of 150 patients diagnosed with GBS and age- and sex-matched neurological and healthy controls was performed to investigate recent infections of 14 pathogens serologically and collect the clinical data during a follow-up of 12 months. Results: In total, 53% of patients with GBS had a positive serology for recent infection, including Campylobacter jejuni (27%), influenza A (17%) and B (16%), hepatitis A virus (5%), dengue virus (3%), cytomegalovirus (3%), Epstein–Barr virus (3%), Mycoplasma pneumoniae (2%), herpes simplex virus (2%), varicella-zoster virus (1%), and rubella virus (1%). Serology for infections of hepatitis E virus, Haemophilus influenzae, and Zika virus was negative. There was a higher frequency of C. jejuni, influenza A, influenza B, and hepatitis A virus infections in GBS patients than both the neurological and healthy controls. C. jejuni infection was more frequent in younger GBS patients and was associated with antibodies against GM1, GalNAc-GD1a, and GM1:galactocerebroside complex. Influenza B infection was associated with a pure motor form of GBS. Interpretation: C. jejuni, influenza A, influenza B, and hepatitis A virus serve as the most common cause of antecedent infections in GBS locally. Influenza B-related GBS may represent a pure motor phenotype. Differences in the infectious spectrum worldwide may contribute to the geographical clinical heterogeneity of GBS.

Introduction

Guillain-Barré syndrome (GBS) is an immune-mediated polyradiculoneuropathy characterized by a rapidly progressive flaccid paresis. Recent evidence supports GBS as a spectrum disorder with regional variation and significant heterogeneity including clinical presentation, electrophysiology, and outcome.1,2 Two thirds of the patients complained of antecedent infections before the onset of neurological signs.3 Some antecedent infections were associated with various clinical phenotypes in GBS. Typically, Campylobacter jejuni bearing the gangliosides-like lipooligosaccharides (LOS) accounts for the pathogenesis of axonal GBS, particularly acute motor axonal neuropathy.4 Cytomegalovirus (CMV) infection is associated with severe motor sensory deficits, demyelination, and antibodies to the ganglioside GM2.5 Mycoplasma pneumoniae infection is associated with anti-galactocerebroside (GalC) antibodies and pediatric GBS.5 Global variation in infection burden may at least in part explain the regional differences in clinical presentation and subtype of GBS. In the 1990s, a study from Northern China reported axonal...
GBS as the major subtype in China associated with a high frequency of *C. jejuni* infection. More recent studies, however, showed that currently demyelinating GBS was the predominant subtype in both Northeastern and Southern China. The rapid changes in the socioeconomic status of China may have influenced the exposure to infections and resulted in a shift of the predominant GBS subtype. Furthermore, many GBS patients developed liver dysfunction before treatment without obvious causes, that may be related to specific types of antecedent infections. The current study aimed to investigate the spectrum of GBS-related antecedent infections in a Chinese local area and analyze the infection-related clinical features.

**Methods**

**Patients and blood samples**

This study was performed in the Affiliated Hospital of Jining Medical University, a central hospital regionally in Southwest of Shandong Province, Northern China, where it has a population of 17.1 million with an urban–rural ratio of 1.19. Written informed consent was obtained from all participants, and study procedures were approved by the local Ethics Committee (reference 2013B017 and 2016B006). From October 2013 to June 2017, a total of 150 consecutive patients meeting the diagnostic criteria for GBS and its variants from the Affiliated Hospital of Jining Medical University were included in this study, of whom 19 also participated in the International GBS Outcome Study. For each participant, the pretreatment serum was collected and kept at −80°C until use. The clinical data include: age, sex, upper respiratory tract infection or gastrointestinal infection within 4 weeks before developing neurological signs, motor and sensory deficits, cranial nerve involvement, ataxia, tendon reflex, pain, mechanical ventilation, nerve conduction study (NCS) within 2 weeks after onset, albuminocytological dissociation in cerebrospinal fluid (CSF), and GBS disability score (GBS-DS) at nadir and 12 months. The disability score at 12 months was obtained from 146 (97%) of the patients by a telephone follow-up or outpatient revisit. Four patients were lost to follow-up. To explore the relation between antecedent infection and liver function, data of liver function tests from patients before treatment were also collected. For the study of pretreatment liver dysfunction, 18 of the patients were excluded because of one or more of the reasons below: with a previous diagnosis of liver diseases, alcohol abuse or recent intake of liver-toxic or liver enzyme-inducing drugs, with definite factors resulting in muscle damage, and elevation of transaminases. The liver dysfunction was defined as either alanine aminotransferase or aspartate aminotransferase becoming 1.5 times higher than the upper limit of normal values.

**Controls**

After the inclusion of each patient with GBS, a sex- and age-matched inpatient with other neurological diseases (OND) and sex- and age-matched healthy donors in the same period were, respectively, selected from the local biological sample bank of the hospital established from July 2012. Among the 150 OND controls were included patients with a cerebral infarction (n = 23), cerebral hemorrhage (n = 27), peripheral vertigo (n = 28), Bell’s palsy (n = 39), migraine (n = 15), myasthenia gravis (n = 4), meningitis (n = 3), Parkinson disease (n = 6), and epilepsy (n = 5). The 150 healthy controls were recruited from the same hospital and in all healthy controls, organic diseases were ruled out by the routine medical examination.

**Antecedent infectious spectrum detection**

A total of 14 infectious agents were selected according to previous report and unpublished data from Department of Infectious Diseases, Chinese Centre for Disease Control, including *C. jejuni*, *M. pneumoniae*, *Haemophilus influenzae*, influenza A and B virus, herpes simplex virus, varicella-zoster virus, dengue virus, rubella virus, CMV, Epstein–Barr virus (EBV), hepatitis A virus, hepatitis E virus, and Zika virus. The details of ELISA kits for detecting the infectious agents are shown in Table S1. The serum samples were tested according to the manufacturer's instructions. To detect IgM antibodies, the serum was pretreated to remove the IgG antibodies and prevent false positivity. *C. jejuni*, influenza A, and influenza B virus infections were defined as the presence of IgA and/or IgM antibodies. *H. influenzae* infection was defined as the presence of IgG antibodies. Infections of *M. pneumoniae*, hepatitis A virus, herpes simplex virus, varicella-zoster virus, EBV, dengue virus, rubella virus, CMV, hepatitis E virus, and Zika virus were defined as the presence of IgM antibodies. The serology for specific infections in the controls was detected only if more than five GBS patients were positive for this infection as previously described.

**Anti-glycolipid antibody assay**

The serum samples of patients with GBS were tested in duplicate for IgM and IgG antibodies against GM1, GM2, GM3, GM1b, GD1a, N-acetylgalactosaminyl GD1a (GalNAc-GD1a), GD1b, GT1a, GQ1b, GalC, sulfatide, GM1: GalC, GM1:sulfatide, GalC:cholesterol, and GalC:sulfatide complexes as previously described. In general, a vinyl ELISA plate (Corning, ME, USA) was coated with the
glycolipid or glycolipid complex (5 pmol per well; for glycolipid complex, 2.5 pmol each). The serum diluted in phosphate buffered saline (PBS) (0.1 M, pH 7.4) containing 0.5% casein (1:500) was added into the plates for incubation overnight at 4°C. After three times of washing with 0.1 M PBS containing 0.05% Tween 20, the plates were added with horseradish peroxidase-conjugated goat anti-human IgG (gamma chain) (Thermo Scientific, Rockford, IL, USA) (1:3000 in PBS containing 0.5% casein) or horseradish peroxidase-conjugated goat anti-human IgM (heavy chain) (Thermo Scientific) (1:10000) and incubated at 37°C for 1 h. After washing the plates with the same washing buffer, the binding of IgG or IgM antibodies was visualized by O-phenylenediamine (Sigma, MO, USA) developing solution in the darkness for 15 min and then the reaction was stopped by 2N hydrochloride. The absorbance at 492 nm/630 nm (as reference) was measured using a ChroMate/C226 Microplate Reader (Awareness Technology, Palm City, USA). Each sample was tested with a blank and negative control for quality control. With reference to a blank control, the optical density value over 0.1 was considered positive. A positive serology for anti-glycolipid antibodies was defined as the presence of either IgG or IgM or both.

Data availability

Our data will be shared by request from any qualified investigator for scientific purposes.

Statistical analysis

Normally distributed continuous data were presented as means and standard deviations. The categorical variables were shown as n (%). The difference in the frequency of infections in patients with GBS and the controls was compared by the Chi-square test or Fisher’s exact test. For patients with and without infections, the difference in demographic and clinical features, anti-glycolipid antibodies, and clinical subtypes were compared by Chi-square test or Fisher’s exact test. The analysis was performed with the SPSS 20.0 analysis software (IBM, Armonk, NY). A two-sided \( P < 0.05 \) was considered to be significant.

Results

Antecedent infectious spectrum in patients with GBS

The demographic and clinical features of the patients with GBS are shown in Table 1. There was no difference in age or sex between the patients with GBS and the controls. The mean age of patients with GBS was 51.0; interquartile range (IQR) was 41 to 64 and the male to female ratio was 1.2 (81/69). For patients with OND and the healthy controls, the mean age was 51.9 (IQR, 42–64) and 51.3 (IQR, 42–64), respectively; the sex ratio was both the same 1.2 (81/69). Of the 150 patients with GBS, 53% (80/150) had positive serology for either \( C. jejuni \) (\( n = 40, 27\% \)), influenza A (\( n = 26, 17\% \)), influenza B (\( n = 24, 16\% \)), hepatitis A virus (\( n = 7, 5\% \)), dengue virus (\( n = 4, 3\% \)), CMV (\( n = 4, 3\% \)), EBV (\( n = 4, 3\% \)), \( M. pneumoniae \) (\( n = 3, 2\% \)), herpes simplex virus (\( n = 3, 2\% \)), varicella-zoster virus (\( n = 2, 1\% \)), and rubella virus (\( n = 1, 1\% \)). There were significant higher

Table 1. Demographic and clinical characteristics of 150 patients with Guillain-Barré syndrome.

| Characteristic                                              | n (%)                           |
|-------------------------------------------------------------|---------------------------------|
| **Age, mean (standard deviation)**                         | 51.0 (16.1)                     |
| Male/female ratio                                           | 1.2 (81/69)                     |
| Antecedent infection within 4 weeks                        |                                 |
| Upper respiratory tract infection                          | 36 (24)                         |
| Gastrointestinal infection                                  | 19 (13)                         |
| **Motor deficits**                                         |                                 |
| Upper and lower limb weakness                               | 111 (74)                        |
| Upper limb weakness only                                    | 5 (3)                           |
| Lower limb weakness only                                    | 11 (7)                          |
| None                                                        | 23 (15)                         |
| Sensory deficits                                            | 62 (41)                         |
| Craniopharyngeal involvement                               |                                 |
| Oculomotor weakness                                        | 23 (15)                         |
| Facial weakness                                             | 30 (20)                         |
| Bulbar weakness                                             | 29 (19)                         |
| None                                                        | 68 (45)                         |
| Ataxia                                                      | 6 (4)                           |
| Tendon reflex at the nadir                                 | 128 (85)                        |
| Hyporeflexia or areflexia                                  | 22 (15)                         |
| Normal                                                      | 6 (4)                           |
| Disability score at the nadir                              |                                 |
| 1                                                          | 35 (23)                         |
| 2                                                          | 31 (21)                         |
| 3                                                          | 20 (13)                         |
| 4                                                          | 45 (30)                         |
| 5                                                          | 17 (11)                         |
| 6                                                          | 2 (1)                           |
| Albuminocytological dissociation in CSF                    | 95/123 (77)                     |
| Single nerve conduction study                              |                                 |
| Primary demyelinating                                      | 41/120 (34)                     |
| Primary axonal                                              | 35/120 (29)                     |
| Unclassified                                                | 27/120 (23)                     |
| Normal                                                      | 17/120 (14)                     |
| Disability score at 12 months                              |                                 |
| 0–1                                                        | 113/146 (77)                    |
| 2                                                          | 13/146 (9)                      |
| 3                                                          | 8/146 (6)                       |
| 4                                                          | 5/146 (3)                       |
| 6                                                          | 7/146 (5)                       |
| Pretreatment liver dysfunction                              |                                 |
| CSF, cerebrospinal fluid                                    | 13 (17/132)                     |
frequencies of *C. jejuni*, influenza A, influenza B, and hepatitis A virus infections in patients with GBS than in the controls (Table 2). Twenty-six (17%) patients had more than one infection (Fig. 1). Eighty percent of patients (120/150) were from rural areas. There was no difference in the frequency of antecedent infections in patients with GBS between urban and rural areas (Table S2). None of the patients had positive serology for hepatitis E virus, *Haemophilus influenzae*, and Zika virus.

### Infection-related clinical features

As shown in Table 3, the comparison was made among the GBS patients with infection of *C. jejuni*, influenza A, and influenza B as well as patients with no infections. There were significantly younger age and higher frequency of antecedent diarrhea complaints in patients with *C. jejuni* infection than the controls. All seven patients with preceding influenza B infection had a pure motor GBS without sensory deficits, which differed from the patients with infection of *C. jejuni*, influenza A, and patients without infection (*P* = 0.037). In addition, these patients with influenza B infection had no ataxia (0/7), no pain (0/7), no demyelinating subtypes (0/5) and high frequency of mechanical ventilation (2/7, 29%), and pretreatment liver dysfunction (2/7, 29%). There was no mechanical ventilation (0/11) in GBS patients following influenza A infection. There was no pretreatment liver dysfunction (0/25) in GBS patients following *C. jejuni* infection. With regard to patients with *C. jejuni*, influenza A and B virus as well as without infection, there was no difference in frequency of patients on cranial nerve involvement, ataxia, tendon reflex at nadir, pain, mechanical ventilation, electrophysiological classification, and pretreatment liver dysfunction.

### Antecedent infections and the clinical variants

The patients were classified into GBS (*n* = 125, 83%), Miller Fisher syndrome (MFS) (*n* = 4, 3%), acute ophthalmoparesis (*n* = 4, 3%), GBS/MFS overlap (*n* = 2, 1%), bifacial weakness with paraesthesias (*n* = 2, 1%), acute pharyngeal weakness (*n* = 2, 1%), and pure sensory subtype (*n* = 11, 7%). As shown in Figure 2, there was no association between these variants of GBS and the presence of specific types of preceding infection.

### Antecedent infections and anti-glycolipid antibodies

As shown in Table S3, the frequency of antibodies against glycolipids and glycolipids complex in 150 of patients

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**Table 2.** Frequency of antecedent infections in patients with Guillain-Barré syndrome and the controls.

|                     | GBS (n = 150) | OND controls (n = 150) | OR (95% CI) | *P* value | HC (n = 150) | OR (95% CI) | *P* value |
|---------------------|---------------|------------------------|-------------|-----------|-------------|-------------|-----------|
| No infection        | 70 (47)       | 128 (85)               | Reference   |           | 132 (88)    | Reference   |           |
| *Campylobacter jejuni* | 40 (27)     | 10 (7)                 | 7.3 (3.5, 15.5) | <0.001    | 13 (9)      | 5.8 (2.9, 11.6) | <0.001    |
| Influenza A         | 26 (17)       | 8 (5)                  | 5.9 (2.6, 13.8) | <0.001    | 10 (7)      | 4.9 (2.2, 10.7) | <0.001    |
| Influenza B         | 24 (16)       | 7 (5)                  | 6.3 (2.6, 15.3) | <0.001    | 9 (6)       | 5.0 (2.2, 11.4) | <0.001    |
| Hepatitis A virus   | 7 (5)         | 3 (2)                  | 4.3 (1.1, 17.0) | 0.027     | 0 (0)       | -           | -         |
| Dengue virus        | 4 (3)         |                        |             |           |             |             |           |
| Cytomegalovirus     | 4 (3)         |                        |             |           |             |             |           |
| Epstein–Barr virus  | 4 (3)         |                        |             |           |             |             |           |
| *Mycoplasma pneumoniae* | 3 (2)     |                        |             |           |             |             |           |
| Herpes simplex virus| 3 (2)         |                        |             |           |             |             |           |
| Varicella-zoster virus | 2 (1)     |                        |             |           |             |             |           |
| Rubella virus       | 1 (1)         |                        |             |           |             |             |           |

The data are shown as n (%). OR, odds ratio; CI, confidence interval; GBS, Guillain-Barré syndrome; OND, other neurological disease; HC, healthy controls. No infection of the hepatitis E virus, *Haemophilus influenzae*, and Zika virus was detected.

**Figure 1.** The number of patients with more than one infection.
with GBS was anti-GM1 (39%), anti-GM1b (1%), anti-GD1a (11%), anti-GD1b (19%), anti-GalNAc-GD1a (11%), anti-GQ1b (13%), anti-GM1:GalC complex (23%), anti-GM1:sulfatide complex (20%), and anti-GalC:sulfatide complex (1%), respectively. There was significantly higher frequency of antibodies against GM1, GalNAc-GD1a, and GM1:GalC complex in patients following C. jejuni infection than patients without infections (P = 0.002, P < 0.001, and P = 0.019, respectively).

The anti-GalC:sulfatide complex antibodies were IgG subtype and were detected in one patient with influenza A and M. pneumoniae infections. For patients with anti-GM1 IgG antibodies, 40% (23/58) had cross-reaction with GM1:GalC complex and 60% solely bound to GM1. For patients with anti-GM1:GalC complex IgG antibodies, 26% (9/34) showed complex-independent, 24% (8/34) showed complex-enhancement, 18% (6/34) showed complex-attenuated, and 32% (11/34) showed complex-dependent. For patients with anti-GM1:sulfatide complex IgG antibodies, 43% (13/30) showed complex-independent, 13% (4/30) showed complex-enhancement, 17% (5/30) showed complex-attenuated, and 27% (8/30) showed complex-dependent.

### Discussion

We hereby demonstrated that C. jejuni, influenza A, influenza B, and hepatitis A virus currently served as the most common cause of antecedent infections in GBS in the Southwest of Shandong Province, Northern China. The proportion of patients following infections of CMV, EBV, and M. pneumoniae only accounted for less than 5% of the total patients, respectively. Infections of dengue virus, herpes simplex virus, varicella-zoster virus, and rubella virus were also detected but only in a minority of patients and not higher than in controls. The proportion of patients with C. jejuni infection in our region (27%) was similar to those patients reported in Dutch (32%) and the UK (26%) but lower than those reported in Bangladesh (57%) and a previous study from Northern China (66%). From 2000 to 2018, the average life expectancy locally increased from 73.9 to 78.1 (unpublished data). The changes in the proportion of GBS patients with C. jejuni infection in China may reflect the improved healthy conditions of this country and be related to the rapid development of society and the economy.

The C. jejuni infection has a strong relation with axonal GBS.

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Table 3. Antecedent infections and clinical characteristics of patients with Guillain-Barré syndrome.

| Characteristic                              | No infection (n = 70) | Campylobacter jejuni (n = 25) | Influenza A (n = 11) | Influenza B (n = 7) | P value* |
|--------------------------------------------|-----------------------|-------------------------------|----------------------|---------------------|----------|
| Age, mean (standard deviation)             | 51.8 (15.3)           | 44.6 (15.3)                   | 60.6 (10.3)          | 54.3 (19.3)         | 0.03     |
| Antecedent infection within 4 weeks        |                       |                               |                      |                     |          |
| Upper respiratory tract infection          | 16 (23)               | 7 (28)                        | 3 (27)               | 2 (28)              | 0.032    |
| Gastrointestinal infection                 | 6 (9)                 | 8 (32)                        | 0 (0)                | 0 (0)               | 0.002    |
| None                                       | 48 (68)               | 10 (40)                       | 8 (73)               | 5 (72)              | 0.002    |
| Motor deficits                             | 61 (87)               | 21 (84)                       | 9 (82)               | 6 (86)              | 0.969    |
| Sensory deficits                           | 35 (50)               | 8 (32)                        | 6 (55)               | 0 (0)               | 0.037    |
| Cranial nerve involvement                  | 41 (59)               | 15 (60)                       | 6 (55)               | 6 (86)              | 0.766    |
| Ataxia                                     | 4 (6)                 | 1 (4)                         | 1 (9)                | 0 (0)               | 0.846    |
| Hyporeflexia or areflexia at nadir         | 62 (89)               | 24 (96)                       | 9 (82)               | 5 (71)              | 0.276    |
| Pain                                       | 3 (4)                 | 3 (12)                        | 2 (18)               | 0 (0)               | 0.227    |
| Mechanical ventilation                     | 9 (13)                | 3 (12)                        | 0 (0)                | 2 (29)              | 0.353    |
| Disability score at nadir                  |                       |                               |                      |                     |          |
| <4                                         | 35 (50)               | 13 (52)                       | 8 (73)               | 3 (43)              | 0.524    |
| ≥4                                         | 35 (50)               | 12 (48)                       | 3 (27)               | 4 (57)              |          |
| Albuminocytological dissociation in CSF     | 41/58 (71)            | 18/20 (90)                    | 9/9 (100)            | 5/5 (100)           | 0.053    |
| Single nerve conduction study              |                       |                               |                      |                     |          |
| Primary demyelinating                      | 21/57 (37)            | 5/17 (29)                     | 3/9 (33)             | 0/5 (0)             | 0.661    |
| Primary axonal                             | 14/57 (25)            | 7/17 (41)                     | 2/9 (22)             | 2/5 (40)            |          |
| Unclassified                               | 13/57 (23)            | 2/17 (12)                     | 3/9 (33)             | 1/5 (20)            |          |
| Normal                                     | 9/57 (16)             | 3/17 (18)                     | 1/9 (11)             | 2/5 (40)            |          |
| Disability score at 12 months              |                       |                               |                      |                     |          |
| <2                                         | 52/68 (76)            | 18 (72)                       | 9/10 (90)            | 5 (71)              | 0.708    |
| ≥2                                         | 16/68 (24)            | 7 (28)                        | 1/10 (10)            | 2 (29)              |          |
| Pretreatment liver dysfunction             | 12/63 (19)            | 0/25 (0)                      | 2/11 (18)            | 2/7 (29)            | 0.109    |

CSF, cerebrospinal fluid. If not specified, the data are shown as n (%).

*The P value showed the difference among the four groups.
Reduced proportion of patients with C. jejuni infection may contribute to the predominant GBS subtype in China changed from axonal GBS in the 1990s to demyelinating GBS in 2010s. Notably, seven patients with C. jejuni infection complained of upper respiratory tract symptoms. The infection of C. jejuni may breakdown the host immune balance and increase the host susceptibility to the infectious diseases.19 It is possible that C. jejuni infection may cause other infections beyond our study, which accounts for the upper respiratory tract symptoms in the patients.

Patients with GBS in our cohort study displayed infection-related clinical features. Being similar to the Dutch study,3 patients with C. jejuni infection had more often complaints of diarrhea than other patients. Although a cohort study from Bangladesh showed no association between the age of patients with GBS and C. jejuni infection,17 our study showed that C. jejuni infection was more frequent in younger patients with GBS, which may reflect a special dietary construct increasing the risk of C. jejuni infection in the youth population. Similarly, a recent international study in GBS showed that patients with the axonal subtype are younger than other patients.2 However, in our study, there was no difference in frequency of electrophysiological subtypes between patients with C. jejuni and other infections. Notably, GBS patients following influenza B infections displayed pure motor deficits and high risk of needing mechanical ventilation (2/7, 29%) while GBS patients following influenza A infections had no need for mechanical ventilation (0/11) and relatively low frequency of patients with GBS-DS ≥ 4 at nadir. Our study supported influenza B-related GBS as a severe phenotype of pure motor deficits while influenza A-related GBS mainly presents as a mild clinical phenotype. Our results were similar to a previous study that none of influenza A-related GBS (0/8) needed mechanical ventilation while half of the GBS patients following influenza B infection (2/4) needed mechanical ventilation.20 The exposure to different types of preceding infection in combination with host factors among different regional or ethnic groups may result in the geographical clinical heterogeneity in GBS worldwide.

Previously, the relation between C. jejuni infection and antibodies against gangliosides, including GM1, GM1b, GD1a, GalNAc-GD1a, and GQ1b, has been well established in GBS.21,22 In our region, there was a strong correlation between C. jejuni infection and anti-GM1, anti-GalNAc-GD1a, and anti-GM1:GalC complex antibodies. The gene polymorphism of cst-II, encoding a sialyltransferase in C. jejuni, leads to the biosynthesis of different gangliosides-mimicking LOS.22,23 C. jejuni strains with cst-II (Asn51) regularly express the GQ1b-like LOS while the strains with cst-II (Thr51) express more GM1-like and GD1a-like LOS.22 Typically, IgG antibodies against GQ1b were associated with MFS while the IgG antibodies against GM1 and GD1a were associated with axonal GBS.24,25 High incidence of MFS was previously reported in East Asia, especially Japan (up to 26%);2,26 however, there was much lower frequency of MFS in both our current study (3%) and a large multicenter study from Southern China (12%).8 Our results supported C. jejuni with cst-II (Thr51), bearing GM1-like LOS, as one of the causative pathogens in our region, which deserves a further study using C. jejuni samples from the patients. Moreover, none of the patients in our cohort were detected with H. influenzae infection, which was frequently seen in patients with MFS.27 The regional infectious spectrum may partly explain why there was a low frequency of MFS in China.

Liver injury in patients following influenza A and B infections has been described in both the cohort study and case report.28,29 Some GBS-related pathogens including EBV, varicella-zoster virus, hepatitis A virus, and hepatitis E virus were able to trigger the autoimmune response against liver.30–32 Although pretreatment liver dysfunction has been reported for a long time,9 it remains unknown that the pretreatment liver dysfunction in GBS was caused by antecedent infections or GBS itself. In this study, 13% (17/132) of the patients were detected with pretreatment liver dysfunction at entry. Although the difference in frequency of pretreatment liver dysfunction among patients with and without infections was not significant, GBS patients following influenza B virus infection displayed more susceptibility to pretreatment liver dysfunction. Our study supported antecedent infection as one cause of pretreatment liver dysfunction in GBS patients. However, 19% of the patients without infection also had pretreatment liver dysfunction. It cannot be

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excluded that some other pathogens beyond our study were associated with liver dysfunction in patients with GBS. None of the GBS patients with C. jejuni infection had pretreatment liver dysfunction, which needs further confirmation by other cohort studies.

In conclusion, this is the first study to report the antecedent infectious spectrum in patients with GBS in China. We firstly reported influenza B-related GBS as a pure motor phenotype. C. jejuni serves as the predominant cause of antecedent infections in patients with GBS in our region, but the frequency is much lower than 30 years ago. The regional infectious spectrum contributed to the clinical heterogeneity of GBS. Our results deserve further confirmation by other larger cohort studies.

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Author Contributions
Y. Wang developed the concept and design of the article. Y. Hao, W. Wang and B. Qiao and D. Liu performed the experiment. W. Wang and X. Feng collected the clinical data. M. Chen and Y. Wang analyzed all of the data. Y. Hao and Y. Wang wrote the first draft. B.C. Jacobs and Y. Wang performed the critical revision and all the authors critically evaluated the manuscript.

Conflict of Interest
The authors report no disclosures relevant to the manuscript.

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Supporting Information
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Details of ELISA kits of antecedent infections assay.
Table S2. Urban and rural distribution of antecedent infections in patients with Guillain-Barré syndrome.
Table S3. Antecedent infections and antibodies to glycolipids and glycolipid complex in patients with Guillain-Barré syndrome.