


RESEARCH ARTICLE

Toll-like receptor-4 299Gly allele is associated with Guillain-Barré syndrome in Bangladesh

Israt Jahan¹, Rijwan U. Ahammad^{1,2}, Mir M. Khalid^{1,3}, Mohammad I. Rahman^{1,4}, Shoma Hayat¹, Badrul Islam^{1,5}, Quazi D. Mohammad⁶ & Zahirul Islam¹ ¹Laboratory Sciences and Services Division, icddr,b, Dhaka, Bangladesh²Graduate School of Medicine, Department of Neuroscience, Nagoya University, Nagoya, Japan³Gladstone Institutes, San Francisco, California⁴School of Molecular Sciences, Arizona State University, Tempe, Arizona⁵Department of Medical Microbiology and Infectious Diseases, Erasmus Medical Center, Rotterdam, The Netherlands⁶National Institute of Neurosciences and Hospital, Dhaka, Bangladesh

Correspondence

Zahirul Islam, Laboratory Sciences and Services Division, icddr,b, 68, Shaheed Tajuddin Ahmad Sarani, Mohakhali, Dhaka-1212, Bangladesh. Tel: +880 2 9886464; Fax: +880 2 8812529; E-mail: zislam@icddr.org

Funding Information

No funding information provided.

Received: 27 December 2018; Revised: 7 February 2019; Accepted: 7 February 2019

Annals of Clinical and Translational Neurology 2019; 6(4): 708–715

doi: 10.1002/acn3.744

Abstract

Objective: TLR4 plays an important role in the pathogenesis of Guillain-Barré syndrome (GBS). The relationships between *TLR4* polymorphisms and susceptibility to GBS are poorly understood. We investigated the frequency and assessed the association of two single nucleotide polymorphisms (SNPs) in the extracellular domain of *TLR4* (Asp299Gly and Thr399Ile) with disease susceptibility and the clinical features of GBS in a Bangladeshi cohort. **Methods:** A total of 290 subjects were included in this study: 141 patients with GBS and 149 unrelated healthy controls. The *TLR4* polymorphisms Asp299Gly and Thr399Ile were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. **Results:** The minor 299Gly allele was significantly associated with GBS susceptibility ($P = 0.0137$, OR = 1.97, 95% CI = 1.17–3.31), and was present at a significantly higher frequency in patients with the acute motor axonal neuropathy (AMAN) subtype of GBS ($P = 0.0120$, OR = 2.37, 95% CI = 1.26–4.47) than acute inflammatory demyelinating polyneuropathy (AIDP) subtype ($P = 0.961$, OR = 1.15, 95% CI = 0.38–3.48); when compared to healthy controls. The genotype frequency of the Asp299Gly polymorphism was not significantly different between patients with GBS and healthy controls. The Asp299-Thr399 haplotype was associated with a significantly lower risk of developing GBS ($P = 0.0451$, OR = 0.63, 95% CI = 0.40–0.99). No association was observed between the Thr399Ile polymorphism and GBS disease susceptibility. **Interpretation:** The *TLR4* minor 299Gly allele was associated with increased susceptibility to GBS and the axonal GBS subtype in the Bangladeshi population. However, no associations were observed between the genotypes of the Asp299Gly and Thr399Ile SNPs and antecedent *C. jejuni* infection or disease severity in Bangladeshi patients with GBS.

Introduction

The potentially life-threatening immune-mediated polyneuropathy Guillain-Barré syndrome (GBS) is frequently preceded by an infection that induces an aberrant autoimmune response targeting the peripheral nervous system (PNS).^{1,2} Several infections have been associated with the pathogenesis of GBS. *Campylobacter jejuni* is the most frequent antecedent infection in GBS,¹ and molecular mimicry between *C. jejuni*

lipopolysaccharides (LPS) and host nerve gangliosides can trigger GBS.³ However, only a very small proportion of *C. jejuni*-infected patients develop GBS, and the molecular mechanisms that trigger autoreactivity are still poorly understood.⁴ In addition to *C. jejuni* infection, the enthusiasm for investigation of host genetic factors involved in predisposition to disease has led to the identification of substantial roles for several polymorphisms in genes linked to immunogenicity. Several genetic polymorphisms are significantly associated

with the development of GBS. *IL10* gene polymorphisms are significantly more frequent among patients with GBS than healthy controls.⁵ Furthermore, a few genetic polymorphisms in genes encoding inflammatory mediators were significantly associated with higher risk of GBS disease in Bangladeshi population,^{6,7} which raises the question of whether the toll-like receptor-4 (*TLR4*) gene polymorphism also contribute to autoimmune diseases such as GBS.

The roles of the toll-like receptor (*TLR*) genes in autoimmune diseases have attracted significant attention. *TLR4* serves as the signal-transducing receptor in response to bacterial LPS binding to host cells and initiates cytokine and chemokine production cascades that trigger the host immune system to protect against microbial invasion.^{8–10} The polymorphic spectrum of the *TLR4* gene has been elucidated. Two co-segregating non-synonymous polymorphisms occur in the region encoding the extracellular domain of *TLR4*.¹¹ An A→G base transition occurs at nucleotide +896 (rs4986790), resulting in the exchange of aspartic acid to glycine at amino-acid position 299 (Asp299Gly). Moreover, a C→T transition at the +1196 position (rs4986791) causes substitution of threonine to isoleucine at amino acid position 399 (Thr399Ile).¹¹ Both of these substitutions alter the ligand-binding site of the receptor.¹² Therefore, these SNPs may possibly influence the response of *TLR4* to LPS and render cells hyper-susceptible to infection by gram-negative bacteria.^{13,14} Indeed, individuals with Asp299Gly or Thr399Ile *TLR4* polymorphisms exhibit blunted responses to bacterial LPS,¹⁵ which may be a consequence of conformational changes in the extracellular domain of *TLR4*.¹⁶

Very few studies have been conducted for identifying *TLR4* SNPs and its association with susceptibility to GBS. Indian patients with GBS had a higher prevalence of the Asp299Gly polymorphism compared to healthy controls.¹⁷ However, a similar association between the Asp299Gly polymorphism and GBS susceptibility was not observed in a Dutch population.¹⁸ A high prevalence of the Asp299Gly polymorphism was observed in African children,¹⁹ whereas this SNP was relatively rare in several Asian ethnic populations.^{20,21} The variations in the prevalence of *TLR4* genotypes in different populations suggest particular local infectious pressure and subsequent susceptibility to gram-negative infection in particular regions. Bangladesh has a high burden of *C. jejuni* infection and the clinical phenotype of patients with GBS from this country is quite severe.²²

To improve our understanding of the correlation between *TLR4* polymorphisms and the clinical manifestation of GBS, we determined the genotype and allele frequencies of the *TLR4* Asp299Gly and Thr399Ile polymorphisms in the Bangladeshi population and assessed the association of these polymorphisms with disease susceptibility and the clinical characteristics of GBS.

Methods and Materials

Study participants

The study cohort consisted of 300 individuals who were prospectively enrolled at Dhaka Medical College and Hospital (DMCH) between 2010 and 2013: 150 patients with GBS, and 150 healthy individuals with no history of neurological or chronic medical illnesses who were genetically unrelated to the patients with GBS. Subsequently, nine patients with GBS and one healthy control were excluded from the study due to poor quality of DNA samples. Finally, data for 141 patients with GBS and 149 healthy individuals was included in the analysis.

Socio-demographic and clinical data and ethical consideration

The clinical diagnosis for GBS was confirmed using the criteria defined by the National Institute of Neurological Disorders and Stroke (NINDS) and further classified on the basis of electrophysiological criteria into various subtypes: axonal [acute motor axonal neuropathy (AMAN) and acute motor sensory axonal neuropathy (AMSAN)], demyelinating [acute inflammatory demyelinating polyneuropathy (AIDP)] and unclassified (inexcitable nerves, and equivocal).^{23,24} Disease severity was defined using the Medical Research Council (MRC)-sum-score at entry and disease outcome was assessed using the GBS disability scale (GBS-DS) after six months follow-up. Patients with a MRC sum score < 40 were defined as severely affected and 40–60, mildly affected. At 6 months follow-up, good outcome was defined as able to walk independently (GBS-DS of 1 or 2) and poor outcome as unable to walk independently (GBS-DS of 3, 4, or 5).^{17,18} This study was reviewed and approved by the ethical committee of both the DMCH and icddr,b and written informed consent was obtained from all enrolled participants.

Isolation of genomic DNA

Genomic DNA was extracted from whole blood collected in lithium heparin coated anti-coagulation blood collection tubes. The QIAamp[®] DNA Blood Midi Kit (Qiagen, Hilden, Germany) was used to isolate genomic DNA according to the manufacturer's instructions. DNA samples were dissolved in 1× TE buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0) and stored at –80°C. Before SNP detection, genomic DNA was diluted in Milli-Q water to the working concentration (10 ng/μL) and stored at –20°C.

Determination of *TLR4* (Asp299Gly and Thr399Ile) genotypes

The *TLR4* allelic variants Asp299Gly (rs4986790) and Thr399Ile (rs4986791) were genotyped using a mismatched polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay, as previously described.²⁵ The forward primers were specially designed to introduce a restriction enzyme recognition site. The regions encoding the Asp299Gly and Thr399Ile positions were amplified using published primer sequences and amplification profiles.^{17,25} Primer for *TLR4* Asp299Gly were forward 5'-GATTAGCATACTTAGACTA-CTACCTCCATG-3', reverse 5'-GATCAACTTCTGAAAAA-GCATTCCCAC-3', and for *TLR4* Thr399Ile were forward 5'-GGTTGCTGTTCTCAAAGTGATTTGGGAGAA-3', reverse 5'-ACCTGAA-GACTGGAGAGTGAGTTAAATGCT-3'. The PCR products were subjected to restriction digestion with the respective restriction endonucleases, *NcoI* for +A896G & *HinfI* for +C1196T. The digested fragments were separated on 3% agarose gel and visualized by ethidium bromide staining.

Serological assays

Serological tests were performed on the pretreatment serum of patients with GBS to assess anti-ganglioside antibodies and antecedent *C. jejuni* infection. Enzyme-linked immunosorbent assay (ELISA) was used to detect IgG, IgM and IgA antibodies against *C. jejuni* and IgG against the nerve GM1-ganglioside, as described previously.^{26,27}

Statistical analysis

Hardy–Weinberg equilibrium was assessed for control group using the Chi-square test. Associations of the *TLR4* SNPs with disease susceptibility and clinical features of GBS were assessed by Fisher's exact test and interpreted with odds ratios (OR) and 95% confidence intervals (CI); *P*-values \leq 0.05 were considered significant. All statistical tests were adjusted using the Bonferroni correction to exclude type I errors. Haplotype analysis was performed to assess the associations of haplotypes between two groups using SHEsis software (<http://analysis.bio-x.cn/myAnalysis.php>). Haplotypes with a frequency $<$ 0.03 were ignored in the analysis.²⁸ Statistical analyses were performed using Graph Pad Prism (version 5.01; GraphPad Software, Inc., La Jolla, CA, USA) and SPSS (16.0 version, Chicago, IL, USA).

Results

Study population

The median age, 141 patients with GBS was 28 years (range, 4 to 75-years-old) and 149 healthy controls were

34 years (range, 17 to 75-years-old). Overall, 88% (124/141) of patients with GBS reported antecedent symptoms: diarrhea (58%; 72/124) was the leading antecedent symptom, followed by respiratory tract infections (21%; 26/124), and fever (11%; 14/124). More than two-thirds (70%; 99/141) of patients were severely affected with GBS, 60% (61/101) of cases were classified as the axonal variant and 41% (58/141) were anti-ganglioside (GM1) antibody positive (Table 1).

The *TLR4* Asp299Gly polymorphism is associated with susceptibility to GBS

The associations between the Asp299Gly and Thr399Ile genotypes and the risk of GBS are shown in Table 2. Genotype of *TLR4* Asp299Gly polymorphism was not associated with increased susceptibility to GBS ($P = 0.5387$, OR = 1.26, 95% CI = 0.69–2.31). The Gly299Gly genotype was detected in eight of the 141 (6%) patients with GBS, but not in any of the 149 healthy controls. The minor 299Gly allele was significantly associated with susceptibility to GBS ($P = 0.0137$, OR = 1.97,

Table 1. Demographic and clinical features of the healthy controls and patients with GBS.

Features	Healthy controls <i>n</i> = 149 (%)	Patients with GBS <i>n</i> = 141 (%)
Sex		
Male:female (%)	52:77 (35/52)	103: 38 (73/27)
Age		
Median age, years (range)	34 (17–75)	28 (4–75)
Area of residence		
Rural	–	86 (61)
Urban	–	55 (39)
Antecedent events		
Total	–	124 (88)
Diarrhea	–	72 (58)
Respiratory infection	–	26 (21)
Fever	–	14 (11)
Other	–	15 (12)
Severity based on MRC sum score		
Severely affected (<40)	–	99 (70)
Mildly affected (40–60)	–	42 (30)
Anti-ganglioside antibodies		
GM1-positive	3 (2)	58 (41)
GM1-negative	146 (98)	83 (59)
GBS subtype (<i>n</i> = 101)		
AMAN	–	56 (55)
AMSAN	–	5 (5)
AIDP	–	21 (21)
Unclassified	–	14 (14)

AMAN, acute motor axonal neuropathy; AMSAN, acute motor-sensory axonal neuropathy; AIDP, acute inflammatory demyelinating polyneuropathy.

Table 2. Genotype and allele distributions of the Asp299Gly and Thr399Ile *TLR4* polymorphisms among patients with GBS and healthy controls.

<i>TLR4</i> SNPs	GBS		Healthy controls		<i>P</i> -value	OR (95% CI)
	<i>n</i> = 141	Frequency	<i>n</i> = 149	Frequency		
<i>TLR4</i> genotypes						
Asp299Asp	106	0.752	124	0.832	–	Reference
Asp299Gly	27	0.191	25	0.168	0.5387	1.26 (0.69–2.31)
Gly299Gly	8	0.057	0	0.000	NC	NC
Thr399Thr	111	0.787	123	0.826	–	Reference
Thr399Ile	28	0.199	26	0.174	0.6507	1.19 (0.66–2.16)
Ile399Ile	2	0.014	0	0.0	NC	NC
<i>TLR4</i> alleles						
Asp Allele	239	0.848	273	0.916	–	Reference
Gly Allele	43	0.152	25	0.084	0.0137 ¹	1.97 (1.17–3.31)
Thr Allele	250	0.887	272	0.913	–	Reference
Ile Allele	32	0.113	26	0.087	0.3332	1.34 (0.78–2.31)

Bonferroni-adjusted significance threshold was 0.0167 for genotypes and 0.025 for alleles.

NC, not calculated.

¹Statistically significant

95% CI = 1.17–3.31); this effect remained statistically significant after Bonferroni adjustment ($P = 0.0137/2 = 0.0069$). The genotype ($P = 0.6507$, OR = 1.19, 95% CI = 0.66–2.16) and allele frequencies ($P = 0.3332$, OR = 1.34, 95% CI = 0.78–2.31) of the Thr399Ile polymorphism was homogeneously distributed between patients with GBS and healthy controls (Table 2).

The *TLR4* Asp299Gly polymorphism is associated with the axonal subtype of GBS

The genotype and allele frequencies of the Asp299Gly and Thr399Ile SNPs were compared between subgroups of patients with different clinical subtypes of GBS and healthy controls. The Gly299Gly genotype was present at a higher frequency among patients with the axonal

variant (AMAN) of GBS than the demyelinating variant and healthy controls. Allele disparity was also observed between patients with different subtypes of GBS and healthy controls. The allele frequency of the 299Gly allele in the subgroup of patients with the AMAN subtype was double that of healthy controls and the 299Gly allele was significantly associated with the axonal variant of GBS ($P = 0.0120$, OR = 2.37, 95% CI = 1.26–4.47; Table 3). The genotype and allele frequencies of the *TLR4* Thr399Ile polymorphism were not significantly associated with the electrophysiological subtypes when compared to healthy control. The frequency of the 399Ile allele was higher among patients with the axonal subtype of GBS compared to healthy controls (15% vs. 9%; Table 3), though this difference was not significantly associated ($P = 0.070$, OR = 1.87, 95% CI = 0.97–3.60).

Table 3. Association between the Asp299Gly and Thr399Ile *TLR4* SNPs and GBS subtypes.

<i>TLR4</i> Polymorphism	Subtype			AMAN vs. controls		AIDP versus controls	
	AMAN <i>n</i> = 56 (%)	AIDP <i>n</i> = 21 (%)	Control <i>n</i> = 149 (%)	<i>P</i> -value	OR	<i>P</i> -value	OR
Asp299Asp	40 (72)	17 (81)	124 (83)	–	Reference	–	Reference
Asp299Gly	12 (21)	4 (19)	25 (17)	0.3073 ^a	1.49 (0.69–3.23)	0.959 ^a	1.17 (0.36–3.77)
Gly299Gly	4 (7)	0 (0)	0 (0)	NC	NC	NC	NC
Asp Allele	92 (82)	38 (90)	273 (91)	–	Reference	–	Reference
Gly Allele	20 (18)	4 (10)	25 (9)	0.0120 ^{a1}	2.37 (1.26–4.47)	0.961 ^b	1.15 (0.38–3.48)
Thr399Thr	40 (72)	17 (81)	123 (82)	–	Reference	–	Reference
Thr399Ile	15 (27)	4 (19)	26 (17)	0.1670 ^a	1.77 (0.86–3.68)	0.8998 ^b	1.11 (0.35–3.58)
Ile399Ile	1 (1)	0 (0)	0 (1)	NC	NC	NC	NC
Thr Allele	95 (85)	38 (90)	272 (91)	–	Reference	–	Reference
Ile Allele	17 (15)	4 (10)	26 (9)	0.070 ^a	1.87 (0.97–3.60)	0.905 ^b	1.10 (0.36–3.33)

'a' Fisher's exact test; 'b' Yates correction; ¹statistically significant; OR, odds ratio with 95% confidence interval; NC, not calculated.

No significant association between *TLR4* SNPs and the clinical and serological features of GBS

The patients with GBS were classified into subgroups based on their clinical and serological characteristics. The frequencies of the dominant (reference) allele and minor alleles of the Asp299Gly and Thr399Ile SNPs were not significantly different between *C. jejuni* seropositive and seronegative patients with GBS, anti-GM1-Ab seropositive and seronegative patients, severely affected and mildly affected patients, or patients with good and poor outcomes at 6-months (Table 4).

TLR4 haplotype analysis for patients with GBS and healthy controls

The frequency of the Asp299-Thr399 haplotype was significantly higher among healthy controls than patients with GBS ($P = 0.0451$, OR = 0.63, 95% CI = 0.40–0.99), though this effect was not significant after Bonferroni adjustment. The frequencies of the Gly299-Thr399 (0.073 vs. 0.039) and Gly299-Ile399 (0.080 vs. 0.045) haplotypes were slightly higher among patients with GBS than healthy controls, but these differences were not significant (Table 5).

Discussion

TLR4 recognizes LPS and is an important mediator of the inflammatory response in the first line of host defense.²⁹ The primary aim of this study was to investigate the

association between *TLR4* polymorphisms (Asp299Gly and Thr399Ile) and susceptibility to GBS in the Bangladeshi population. The minor 299Gly allele of the *TLR4* Asp299Gly polymorphism was associated with significantly higher susceptibility to GBS in our population. However, no associations were found between the Asp299Gly and Thr399Ile SNPs and the clinical features of GBS.

Previous studies have assessed immunogenetic risk factors for GBS among Indian and Dutch populations.^{17,18} The homozygous *TLR4* Gly299Gly genotype was significantly associated with susceptibility to GBS in the Indian study.¹⁷ However, while the Dutch cohort included a larger number of patients ($n = 242$), no associations were observed between *TLR4* polymorphisms and susceptibility to GBS.¹⁸ In agreement with the Dutch study, we did not observe any significant difference in the genotype frequencies of the Asp299Gly and Thr399Ile SNPs between the patients with GBS and healthy controls. However, we found the minor 299Gly allele of *TLR4* was associated significantly with an increased risk of GBS. One explanation for these disparities between studies could be variations in host response in terms of varied susceptibility of populations in different regions of the world to pathogenic infections. In addition, polymorphisms in the extracellular domain of *TLR4* may alter the ability of the host to respond to environmental stress.¹⁵ Thus, our findings provide evidence that the minor *TLR4* 299Gly allele might be a potential immunogenetic factor for GBS and may contribute to differences in disease susceptibility to various infections, including *C. jejuni*. Previous findings suggested a heterogeneous pattern of *TLR4* Asp299Gly and

Table 4. Association between the Asp299Gly and Thr399Ile *TLR4* polymorphisms and the clinical characteristics of GBS.

Variable	Reference Allele/risk allele	Allele distribution	Odds Ratio	95% CI	P-value
Disease severity Severe ($n = 99$)/Mild ($n = 42$)	299Asp	168/71	1.025	0.505–2.080	0.945
	299Gly	30/13			
	399Thr	176/74	1.081	0.488–2.395	0.848
	399Ile	22/10			
Outcome at 6 months Poor ($n = 19$)/Good ($n = 111$)	299Asp	31/189	0.773	0.314–1.901	0.575
	299Gly	7/33			
	399Thr	35/195	1.615	0.465–5.615	0.447
	399Ile	3/27			
Anti-GM1-Ab Positive ($n = 58$)/Negative ($n = 83$)	299Asp	95/144	0.691	0.360–1.326	0.423
	299Gly	21/22			
	399Thr	99/151	0.578	0.276–1.211	0.232
	399Ile	17/15			
<i>C. jejuni</i> infection Positive ($n = 86$)/Negative ($n = 55$)	299Asp	144/95	0.841	0.427–1.658	0.617
	299Gly	28/15			
	399Thr	148/102	0.500	0.216–1.157	0.100
	399Ile	24/8			

95% CI, 95% confidence interval; MRC sum score: < 40, severely affected; 40–60, mildly affected.

Table 5. Haplotype frequency for the Asp299Gly and Thr399Ile *TLR4* polymorphisms among patients with GBS and healthy controls.

<i>TLR4</i> haplotype	GBS (frequency)	Control (frequency)	χ^2 -test	Fisher's <i>P</i> -value	OR (95% CI)
A-C (Asp299-Thr399)	229 (0.814)	260 (0.874)	4.018	0.0451 ¹	0.63 (0.40–0.99)
A-T (Asp299-Ile399)	10 (0.034)	13 (0.042)	0.270	0.6033	0.80 (0.34–1.88)
G-C (Gly299-Thr399)	21 (0.073)	12 (0.039)	3.221	0.0728	1.95 (0.93–4.08)
G-T (Gly299-Ile399)	22 (0.080)	13 (0.045)	2.970	0.0849	1.83 (0.91–3.67)

Global results for all four haplotypes: Total number in GBS = 282; total number in healthy controls = 298.

Global $\chi^2 = 6.575$, degrees of freedom = 3, Fisher's *P*-value = 0.0818.

¹Statistically significant, Bonferroni adjusted significance threshold was $(0.05/4) = 0.0125$ for haplotypes.

Thr399Ile polymorphisms (either alone or in combination) among different ethnic groups in Iran.³⁰

Evidences suggest that systemically and locally released cytokines and their contribution in immune-mediated of peripheral nerves damage are important in the pathogenesis of GBS.³¹ The *TLR4* Asp299Gly polymorphism has been shown to result in improper activation of dendritic cells via the CD14–TLR4 complex and enhances the stimulation of activated T cells to release elevated levels of chemokines and proinflammatory cytokines that induce nerve damage.¹⁵ High TLR2 protein expression is observed in inflamed nerve tissues and TLR4 and TLR9 were upregulated during disease progression in a rat model of experimental autoimmune neuritis (EAN).^{32,33} Our findings indicate an association between the *TLR4* 299Gly allele and axonal damage in patients with GBS, as previously reported in an Indian population,¹⁷ but not in a European population.¹⁸ One possible explanation behind these discrepancies could be the higher prevalence of the axonal variant of GBS in patients from Asian countries than Europe.

Several reports have described an association between *TLR4* polymorphisms and an attenuated blunted immune response towards LPS.¹⁵ Individuals with the Asp299Gly polymorphism may exhibit modified cellular responses; changes in the rotation and charge of the ligand (LPS) docking site may affect the interaction between LPS and the TLR4 receptor.¹² Indeed, the Asp299Gly polymorphism was related to an increased risk of gram-negative infections,¹⁶ which may be associated with elevated TNF- α production.¹¹ Elevated production of the proinflammatory cytokine TNF- α may stimulate the inflammatory cells of the innate immune system and enhance the risk of GBS.¹¹ However, the exact mechanism by which *TLR4* SNPs affect the humoral immune response to *C. jejuni* in patients with GBS is unknown. In GBS, *C. jejuni* lipooligosaccharides (LOS) trigger the production of cross-reactive antibodies to peripheral nerve gangliosides. Structural changes in *TLR4* induced by SNPs may reduce the ability of the host to eliminate bacterial components. However, we observed no significant associations between

the *TLR4* polymorphisms and *C. jejuni* infection, ganglioside mimicry or GBS disease severity.

Previous studies of *TLR4* SNPs and co-segregation of *TLR4* genotypes in various populations have reported significant associations between *TLR4* polymorphisms and several inflammatory diseases, including rheumatoid arthritis, malaria, septic shock, and urinary tract infection.^{34–37} However, other studies reported no significant relationships between these SNPs and disease pathogenesis.^{38,39} The *TLR4* SNPs are associated with increased risk of developing septic shock and urinary tract infection.^{36,37} However, polymorphisms in the *TLR4* gene were associated with a reduced prevalence of diabetic neuropathy in type 2 diabetes.⁴⁰

One of the limitations of this study was the moderate sample size, particularly for the genotype analysis of patients with GBS and healthy controls. Furthermore, at the time of enrolment and hospital admission, most patients (~70%) were severely affected (disability scale of 3 to 5) and almost 80% of patients had improved by 6 months follow-up (disability scale of ≤ 2), which may have limited our ability to detect an association between *TLR4* SNPs and clinical outcome.

In conclusion, we explored the contribution of the Asp299Gly and Thr399Ile *TLR4* polymorphisms to the development of GBS in the Bangladeshi population, and found the minor 299Gly allele was associated with increased susceptibility to GBS. However, the genotypes of *TLR4* SNPs appear to have no significant influence on the clinical manifestation of GBS. Additional data on populations with different ethnicities needs to be obtained to fully understand the effect of *TLR4* polymorphisms on the pathogenesis and severity of GBS.

Acknowledgments

This research activity was funded by icddr,b, Dhaka, Bangladesh. icddr,b acknowledges with gratitude the commitment of the Government of Bangladesh to its research efforts, and also gratefully acknowledges the following donors who provide unrestricted support: the Government

of the People's Republic of Bangladesh, Global Affairs Canada (GAC), the Swedish International Development Cooperation Agency (Sida) and the Department for International Development, UK (DFID). We are indebted to the neurologists who referred their patients to us. We thank Smriti Akter for her assistance in transporting biological specimens from hospital to the icddr,b laboratory.

Author Contributions

ZI, IJ, RUA and MMK conceived and designed the study. IJ, ZI, MIR, SH, QDM and BI participated in data acquisition and interpretation of data analysis. IJ and ZI drafted the manuscript, which was critically reviewed by all other authors. All authors read and approved the final manuscript before submission.

Conflict of Interests

Nothing to report.

References

1. Van Den Berg B, Walgaard C, Drenthen J, et al. Guillain-Barré syndrome: pathogenesis, diagnosis, treatment and prognosis. *Nat Rev Neurol* 2014;10:469.
2. Willison HJ, Jacobs BC, Van Doorn PA. Guillain-barre syndrome. *Lancet* 2016;388:717–727.
3. Ang CW, Jacobs BC, Laman JD. The Guillain-Barré syndrome: a true case of molecular mimicry. *Trends Immunol* 2004;25:61–66.
4. Tam CC, Rodrigues LC, Petersen I, et al. Incidence of Guillain-Barré syndrome among patients with *Campylobacter* infection: a general practice research database study. *J Infect Dis* 2006;194:95–97.
5. Myhr K-M, Vågnes K, Marøy T, et al. Interleukin-10 promoter polymorphisms in patients with Guillain-Barré syndrome. *J Neuroimmunol* 2003;139:81–83.
6. Islam Z, Jahan I, Ahammad RU, et al. FAS promoter polymorphisms and serum sFas level are associated with increased risk of nerve damage in Bangladeshi patients with Guillain-Barré syndrome. *PLoS ONE* 2018;13:e0192703.
7. Jahan I, Ahammad RU, Farzana KS, et al. Tumor necrosis factor- α -863C/A polymorphism is associated with Guillain-Barré syndrome in Bangladesh. *J Neuroimmunol* 2017;310:46–50.
8. Medzhitov R, Janeway C Jr. Innate immunity. *N Engl J Med* 2000;343:338–344.
9. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol* 2010;11:373.
10. Medzhitov R. Toll-like receptors and innate immunity. *Nat Rev Immunol* 2001;1:135.
11. Ferwerda B, McCall MB, Verheijen K, et al. Functional consequences of toll-like receptor 4 polymorphisms. *Mol Med* 2008;14:346.
12. Rallabhandi P, Bell J, Boukhvalova MS, et al. Analysis of TLR4 polymorphic variants: new insights into TLR4/MD-2/CD14 stoichiometry, structure, and signaling. *J Immunol* 2006;177:322–332.
13. Hoshino K, Takeuchi O, Kawai T, et al. Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. *J Immunol* 1999;162:3749–3752.
14. Du X, Poltorak A, Silva M, Beutler B. Analysis of Tlr4-mediated LPS signal transduction in macrophages by mutational modification of the receptor. *Blood Cells Mol Dis* 1999;25:328–338.
15. Arbour NC, Lorenz E, Schutte BC, et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 2000;25:187.
16. Agnese DM, Calvano JE, Hahm SJ, et al. Human toll-like receptor 4 mutations but not CD14 polymorphisms are associated with an increased risk of gram-negative infections. *J Infect Dis* 2002;186:1522–1525.
17. Nyati KK, Prasad KN, Verma A, et al. Association of TLR4 Asp299Gly and Thr399Ile polymorphisms with Guillain-Barré syndrome in Northern Indian population. *J Neuroimmunol* 2010;218:116–119.
18. Geleijns K, Jacobs BC, Van Rijs W, et al. Functional polymorphisms in LPS receptors CD14 and TLR4 are not associated with disease susceptibility or *Campylobacter jejuni* infection in Guillain-Barré patients. *J Neuroimmunol* 2004;150:132–138.
19. Mockenhaupt FP, Cramer JP, Hamann L, et al. Toll-like receptor (TLR) polymorphisms in African children: common TLR-4 variants predispose to severe malaria. *Proc Natl Acad Sci USA* 2006;103:177–182.
20. Hang J, Zhou W, Zhang H, et al. TLR4 Asp299Gly and Thr399Ile polymorphisms are very rare in the Chinese population. *J Endotoxin Res* 2004;10:238–240.
21. Kim YS, Hwang YJ, Kim SY, et al. Rarity of TLR4 Asp299Gly and Thr399Ile polymorphisms in the Korean population. *Yonsei Med J* 2008;49:58–62.
22. Islam Z, Sarker S, Jahan I, et al. Capsular genotype and lipooligosaccharide locus class distribution in *Campylobacter jejuni* from young children with diarrhea and asymptomatic carriers in Bangladesh. *Eur J Clin Microbiol Infect Dis* 2018;37:723–728.
23. Asbury AK, Cornblath DR. Assessment of current diagnostic criteria for Guillain-Barré syndrome. *Ann Neurol* 1990;27:S21–S24.
24. Hadden R, Cornblath D, Hughes R, et al. Electrophysiological classification of guillain-barré syndrome: clinical associations and outcome. *Ann Neurol* 1998;44:780–788.

25. Najmi N, Kaur G, Sharma S, Mehra N. Human Toll-like receptor 4 polymorphisms TLR4 Asp299Gly and Thr399Ile influence susceptibility and severity of pulmonary tuberculosis in the Asian Indian population. *HLA* 2010;76:102–109.
26. Islam Z, Gilbert M, Mohammad QD, et al. Guillain-Barré syndrome-related *Campylobacter jejuni* in Bangladesh: ganglioside mimicry and cross-reactive antibodies. *PLoS ONE* 2012;7:e43976.
27. Kuijf ML, Van Doorn PA, Tio-Gillen AP, et al. Diagnostic value of anti-GM1 ganglioside serology and validation of the INCAT-ELISA. *J Neurol Sci* 2005;239:37–44.
28. Yong Y, Lin H. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res* 2005;15:97.
29. Beutler B. Tlr4: central component of the sole mammalian LPS sensor. *Curr Opin Immunol* 2000;12:20–26.
30. Ioana M, Ferwerda B, Farjadian S, et al. High variability of TLR4 gene in different ethnic groups in Iran. *Innate Immun* 2012;18:492–502.
31. Nyati KK, Prasad KN. Role of cytokines and Toll-like receptors in the immunopathogenesis of Guillain-Barré syndrome. *Mediators Inflamm* 2014;2014:758639.
32. Zhang Z-Y, Zhang Z, Schluesener H. Toll-like receptor-2, CD14 and heat-shock protein 70 in inflammatory lesions of rat experimental autoimmune neuritis. *Neuroscience* 2009;159:136–142.
33. Deng Y-N, Zhou W-B. Expression of TLR4 and TLR9 mRNA in Lewis rats with experimental allergic neuritis. *NeuroImmunoModulation* 2007;14:337–343.
34. Pirahmadi S, Zakeri S, Mehrizi AA. Multiple genotypes of the commonly co-segregating Toll-like receptor 4 Asp299Gly and Thr399Ile in Baluchi malaria patients from Iran. *Cell J* 2013;15:182.
35. Kilding R, Akil M, Till S, et al. A biologically important single nucleotide polymorphism within the toll-like receptor-4 gene is not associated with rheumatoid arthritis. *Clin Exp Rheumatol* 2003;21:340–342.
36. Lorenz E, Mira JP, Frees KL, Schwartz DA. Relevance of mutations in the TLR4 receptor in patients with gram-negative septic shock. *Arch Intern Med* 2002;162:1028–1032.
37. Hawn TR, Scholes D, Li SS, et al. Toll-like receptor polymorphisms and susceptibility to urinary tract infections in adult women. *PLoS ONE* 2009;4:e5990.
38. Wu S, Huang W, Wang D, et al. Evaluation of TLR 2, TLR 4, and TOLLIP polymorphisms for their role in tuberculosis susceptibility. *Apmis* 2018;126:501–508.
39. Kroner A, Vogel F, Kolb-Mäurer A, et al. Impact of the Asp299Gly polymorphism in the toll-like receptor 4 (tlr-4) gene on disease course of multiple sclerosis. *J Neuroimmunol* 2005;165:161–165.
40. Illig T, Bongardt F, Schöpfer A, et al. The endotoxin receptor TLR4 polymorphism is not associated with diabetes or components of the metabolic syndrome. *Diabetes* 2003;52:2861–2864.