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RESEARCH ARTICLE

Human leukocyte antigen-*DQB1* polymorphisms and haplotype patterns in Guillain-Barré syndrome

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Abstract

Objective: The etiology of Guillain-Barré syndrome (GBS) remains enigmatic, although genetic and environmental factors are speculated to be associated with this autoimmune condition. We investigated whether polymorphisms and the haplotype structures of the human leukocyte antigen (HLA)-*DQB1* gene relate to the autoimmune response to infection and affect the development of GBS. **Methods**: *HLA-DQB1* polymorphic alleles (*0201, *030x, *0401, *050x, *060x) were determined for 151 Bangladeshi patients with GBS and 151 ethnically matched healthy controls using sequence-specific polymerase chain reaction. Pairwise linkage disequilibrium (LD) and haplotype patterns were analyzed based on D′ statistics and the genotype package in R statistics, respectively. Association studies were conducted using Fisher's exact test and logistic regression analysis. The Bonferroni method was applied to correct for multiple comparisons, whereby the *P*-value was multiplied with the number of comparisons and denoted as *Pc* (*Pc*, *P* corrected). **Results**: No associations were observed between *HLA-DQB1* alleles and susceptibility to disease in the comparison between GBS patients and healthy subjects. Haplotype 9 (*DQB1**0303-*0601) tended to be less frequent among patients with GBS than healthy controls ($P = 0.006$, OR = 0.49, 95% CI = 0.30–0.82; $Pc = 0.06$). Haplotype 5 (*DQB1**0501-*0602) and the *DQB1**0201 alleles were more frequent in the *Campylobacter jejuni*-triggered axonal variant of GBS ($P = 0.024$, OR = 4.06, 95% CI = 1.25–13.18; $Pc = 0.24$) and demyelinating subtype ($P = 0.027$, OR = 2.68, 95% CI = 1.17–6.17; $Pc = 0.35$), though these associations were not significant after Bonferroni correction. **Interpretation**: This study indicates that *HLA-DQB1* polymorphisms are not associated with susceptibility to GBS. In addition, these genetic markers did not influence the clinical features or serological subgroup in patients with *C. jejuni*-triggered axonal variant of GBS.

Introduction

Guillain-Barré syndrome (GBS) is a postinfectious immune-mediated neuropathy that includes the symptoms of flaccid paralysis. Molecular mimicry between the outer core structures of *Campylobacter jejuni* and host nerve gangliosides is one apparent cause of GBS, and instigates a tissue-damaging autoimmune response that determines disease presentation.[1–5] However, the exact mechanisms that lead to induction of nerve fiber demyelination and axonal damage after antecedent *C. jejuni* infection remain to be elucidated. Several subtypes of GBS have been associated with specific *Campylobacter* strains, though a single strain can lead to different subtypes of GBS and only a small percentage (1 in 1000–5000 cases) of patients with *C. jejuni* enteritis develops GBS.[6,7] Thus, molecular mimicry is not the only pathogenic mechanism underlying *C. jejuni*-triggered GBS.[4]

Host genetic factors may play a role by modifying regulatory elements that influence GBS susceptibility and disease pathogenesis. In particular, genetic polymorphisms and the resulting haplotype variations may play an important role in the pathogenesis of GBS.

The human leukocyte antigen (HLA) gene complex is extensively polymorphic. The *HLA-DQB1* gene, the major stimulus of the DQ antigen, is the most polymorphic HLA variant[8–10] and also exhibits the most dense linkage disequilibrium (LD).[11] *HLA-DQB1* allele variations and haplotype patterns may affect the recognition of self and nonself antigens and have been implicated in the pathology of a number of autoimmune diseases.[12] As one of the most polymorphic regions in the HLA gene complex, *HLA-DQB1* has been a focus of inquiry to investigate the genetic and pathophysiological basis of GBS and the associated immune-mediated tissue damage.[13]

Several case-control studies have investigated whether there is an association between HLA class I or II antigens and GBS susceptibility and subgroups.[14–18] Most of these studies did not find any association or observed weak associations with regard to disease susceptibility to GBS. For example, the *DQB1**060x alleles were significantly associated with increased risk of developing GBS in the Indian population, but no association was found in the Dutch population.[14,15] One study reported an increased frequency of *DQB1**03 alleles among *C. jejuni*-infected patients with GBS compared to *C. jejuni*-negative patients, though other studies did not find any association with recent *C. jejuni* infection.[16,17] In our view, these differences could be the consequence of limited sample sizes, as well as geographical variations and differences in GBS subtype.

In this study, we used one of the largest cohorts of GBS patients from low/middle-income countries (LMIC) to evaluate the association of *HLA-DQB1* polymorphisms with GBS disease susceptibility and the clinical features and serological subgroups of GBS. HLA allele distributions vary between patients with different subtypes of GBS.[18] Therefore, considering the varied regional distribution of HLA alleles and high endemicity and severity of GBS in Bangladesh, we also investigated the association between *HLA-DQB1* polymorphic alleles and haplotype patterns with GBS among patients and healthy controls in Bangladesh.

Materials and Methods

Study population

A total of 151 patients with GBS (102 males and 49 females; median age, 29 years [interquartile range, 17–42 years]) diagnosed with GBS at Dhaka Medical College and Hospital (DMCH) using the National Institute of Neurological Disorders and Stroke (NINDS) criteria were enrolled in this study.[19] Patients with GBS were matched with 151 genetically unrelated healthy individuals (77 males and 74 females; median age, 35 years [interquartile range 28–40 years]) without any history of neurological disorders, serious comorbidities (infection, stroke, myocardial infarction, major surgery, etc.), or chronic medical illnesses, with no specific predilection for race, religion, or socioeconomic status during control selection. Written informed consent was obtained from all participants before data collection, clinical examination, and specimen collection. This study was approved by the Institutional Review Board (IRB) and ethics committees of the icddr,b, and Dhaka Medical College and Hospital, Dhaka, Bangladesh.

Peripheral blood and clinical data were collected at entry before treatment for all enrolled patients. The majority of patients with GBS (130/151, 86%) had a history of a preceding illness, either diarrhea (71/130, 55%) or respiratory infection (24/130, 18%) or another preceding illness (35/130, 27%). Electrophysiological studies were performed for 104/151 (69%) patients with GBS; subtype was classified as the axonal type (59/151 [57%]: 55, AMAN and 4, AMSAN); the demyelinating type (27/151, [26%]; AIDP), or unclassified GBS with inexcitable nerves or equivocal findings (18/104 [17%]).[20–22] The severity of disease was assessed at study entry using the medical research council (MRC) sum score at nadir (maximum muscle weakness).[23] Patients with a MRC sum score at nadir of <40 were considered severely affected and between 40 and 60, mildly affected.[24] Disease outcome was measured using the GBS disability score after 6 months follow-up.[25] Antibodies against the *C. jejuni* and antibodies against GM1, GD1a, and GQ1b gangliosides were measured serologically using enzyme-linked immunosorbent assays (ELISAs).[26,27]

Genomic DNA isolation

Whole blood was collected from all 302 participants into lithium heparin anticoagulant-coated blood collection tubes for genomic DNA isolation. The QIAamp® DNA Blood Midi Kit (100; Qiagen, Hilden, Germany) was used to isolate genomic DNA according to the manufacturer's instructions. The eluted DNA samples were dissolved in 1 × TE-buffer (10 mmol/L Tris-Cl, pH 8.0, 1 mmol/L EDTA) and stored at −80°C. DNA samples were diluted in Milli-Q water to a final concentration of 10 ng/μL and stored at −20°C until genotyping.

HLA typing

Sequence-specific PCR (PCR-SSP) was performed for *HLA-DQB1* typing using previously published primer sequences and reaction conditions.[28] A primer pair was added to each PCR reaction as an internal positive control to amplify the third intron of the *DRB1* genes.[29]

Statistical analysis

The associations between the *HLA-DQB1* alleles and susceptibility to GBS and the clinical or serological features of GBS were assessed using Fisher's exact test with Yates' continuity correction and logistic regression analysis. Allele frequencies were reported as *P*-values, odds ratios (ORs), and 95% confidence intervals (CIs). *P*-values less than 0.05 were considered statistically significant. *HLA-DQB1* allelic frequency was estimated by simple counting and the data were processed using Microsoft Excel 2010 (Microsoft, Redmond, WA), GraphPad Prism (version 5.01, GraphPad software, Inc., La Jolla, CA), and SPSS (16.0 version, Chicago, IL). Pairwise LD was analyzed based on D′ statistics for each of the 13 *HLA-DQB1* loci assessed. Haplotype structures and frequencies were estimated from genotypic data and their associations with GBS susceptibility and the clinical and serological subgroups were assessed using logistic regression analysis. Individual alleles with an allele frequency >10% and haplotype frequency >4% within the population were included in the association studies. The Bonferroni method was conducted to correct for multiple comparisons, whereby the *P*-value was multiplied with the number of comparisons and denoted as *Pc* (*Pc*, *P* corrected).

Results

Influence of HLA-DQB1 polymorphisms and haplotype patterns on GBS susceptibility

The influence of 13 *HLA-DQB1* polymorphic loci on susceptibility to GBS was assessed by comparing patients and healthy controls. No alleles were significantly associated with GBS disease susceptibility (Table 1). However, a trend toward a lower frequency in the *DQB1**0601 allele was observed in patients with GBS, but this was not significant when corrections for multiple comparisons were made ($P = 0.045$, OR = 0.60, 95% CI = 0.38–0.96; $Pc = 0.58$; Table 1).

In haplotype analysis, a total of 136 different profiles were observed among the two[13] possible combinatorial patterns for the 13 *HLA-DQB1* polymorphic loci. Eighty-eight and 90 profiles were observed among the patients with GBS and healthy controls, respectively (Fig. 1). Forty-two profiles were common to both groups, with 46 profiles unique to patients and 44 unique to healthy controls (Fig. 1). Of the 136 haplotype patterns, 10 haplotypes (haplotype 1–10) were predominant (frequency > 4%); these 10 haplotypes represented 64% of total predicted haplotype variation. Haplotype 9 tended to be associated with GBS (*DQB1**0303-*0601, $P = 0.006$, OR = 0.49, 95% CI = 0.30–0.82; $Pc = 0.06$; Table 2); no other haplotypes were significantly associated with GBS. Pairwise LD analysis based on D′ statistics indicated significant LD between patients and healthy controls for the *0201-*0302, *0301-*0303, *0301-*0601, *0502-*0503, and *0604-*0605 *HLA-DQB1* alleles after correction (Fig. 2).

Table 1. Frequency distribution of *HLA-DQB1* polymorphisms in patients with GBS and healthy controls.

Allele	GBS $n = 151$ (%)	HC $n = 151$ (%)	*P*-value	Odds ratio (95% CI)
DQB1*0201	56 (37)	48 (32)	0.397	1.26 (0.78–2.03)
DQB1*0301/4	35 (23)	37 (25)	0.893	0.92 (0.55–1.58)
DQB1*0302	63 (42)	70 (46)	0.487	0.83 (0.53–1.30)
DQB1*0303	64 (42)	78 (52)	0.134	0.69 (0.44–1.08)
DQB1*0401	39 (26)	27 (18)	0.125	1.60 (0.92–2.78)
DQB1*0501	31 (21)	36 (24)	0.580	0.83 (0.48–1.42)
DQB1*0502	12 (8)	21 (14)	0.139	0.53 (0.25–1.12)
DQB1*0503	20 (13)	17 (11)	0.726	1.20 (0.60–2.40)
DQB1*0601	51(34)	69 (46)	0.045[a]	0.60 (0.38–0.96)
DQB1*0602	87 (58)	81 (54)	0.562	1.17 (0.75–1.85)
DQB1*0603/8	7 (5)	6 (4)	1.00	1.17 (0.39–3.58)
DQB1*0604	3 (2)	4 (3)	1.00	0.74 (0.16–3.39)
DQB1*0605	4 (3)	5 (3)	1.0	0.79 (0.21–3.02)
DQB1*03	114 (75)	122 (81)	0.330	0.73 (0.42–1.27)
DQB1*05	64 (42)	72 (48)	0.418	0.80 (0.51–1.27)
DQB1*06	111 (74)	117 (77)	0.5	0.80 (0.48–1.36)

GBS, Guillain-Barré syndrome; HC, healthy controls; 95% CI, 95% confidence interval; [a]*Pc* = 0.58 (*Pc*, *P* corrected).

Association of HLA-DQB1 polymorphisms with the clinical features and serological subtypes of GBS

Next, we performed subgroup analysis based on the subtype of GBS and *C. jejuni* seropositivity (Tables 3 and 4). The *DQB1**0201 alleles were significantly more frequent among patients with the demyelinating subtype compared to healthy controls, but this trend was not significant when corrected for multiple comparisons ($P = 0.027$, OR = 2.68, 95% CI = 1.17–6.17; $Pc = 0.35$; Table 3). The *DQB1**0601 alleles were significantly less frequent among patients with the axonal subtype of GBS compared to healthy controls, but significance was

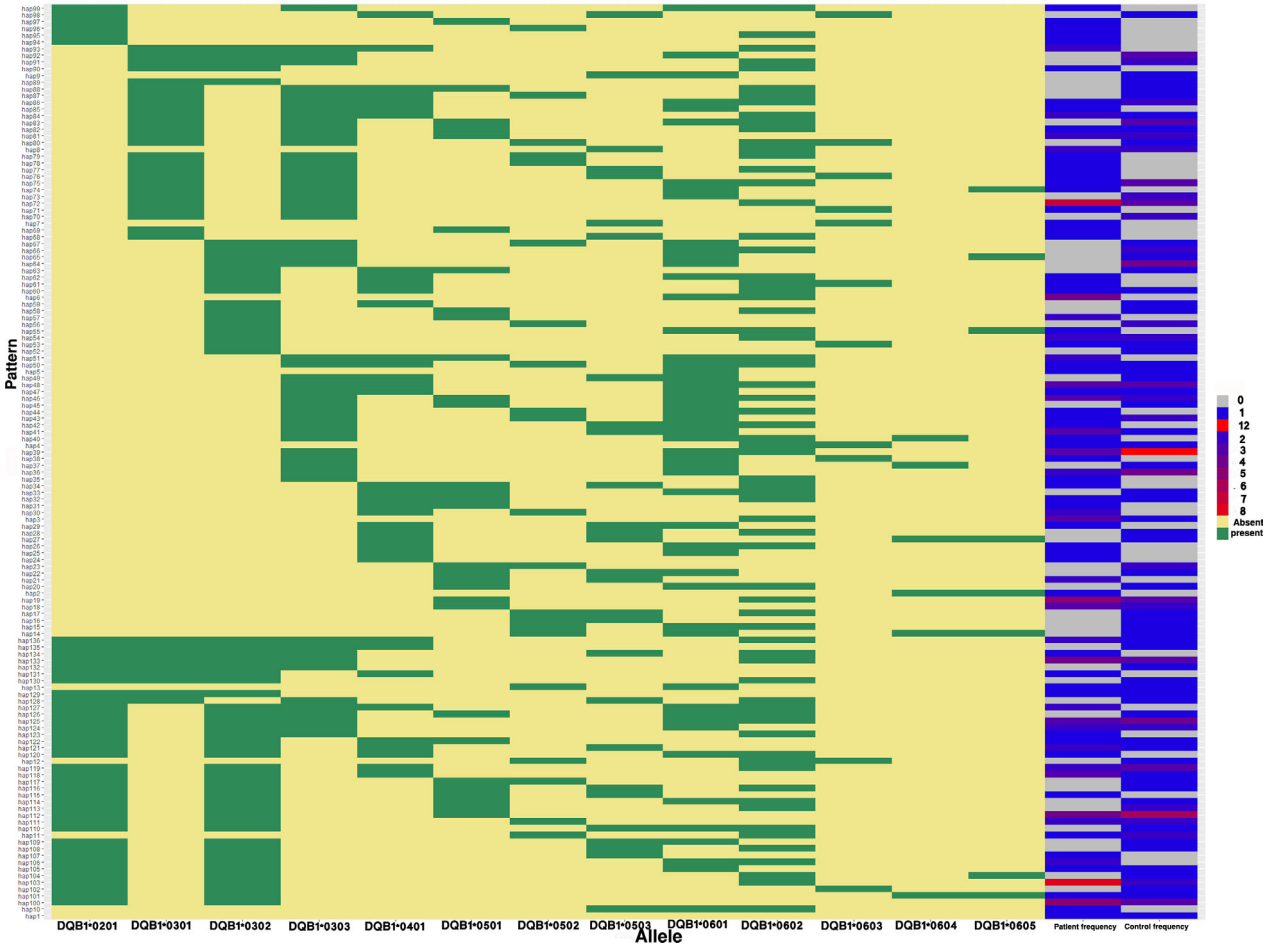

Figure 1. *HLA-DQB1* allelic profiles of patients with GBS and healthy controls. The 136 patterns for the 13 *HLA-DQB1* alleles are presented on the right. Green indicates the presence and yellow indicates the absence of specific alleles for the 13 *HLA-DQB1* loci. The frequencies of the patterns among patients with GBS and healthy controls are presented as color gradients with the frequencies shown on the left.

Table 2. Logistic regression-derived odd ratios for the associations of predominant haplotype (1–10) with GBS and GM1 autoantibodies.

		GBS vs. healthy controls		Anti-GM1-Ab (positive vs. negative)	
Haplotype No.	*HLA-DQB1* alleles	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)
1	*0303 -*0601 -*0602	0.140	0.64 (0.36–1.16)	0.184	0.58 (0.26–1.30)
2	*0301 -*0303 -*0602	1.00	1.0 (0.53–1.87)	0.581	1.23 (0.59–2.59)
3	*0201 -*0302 -*0602	0.529	1.22 (0.66–2.26)	0.247	0.60 (0.26–1.42)
4	*0201 -*0302 -*0501	0.105	0.44 (0.16–1.19)	0.265	0.43 (0.10–1.90)
5	*0501 -*0602	0.265	0.65 (0.31–1.38)	0.881	1.07 (0.44–2.60)
6	*0201 -*0302	0.538	1.16 (0.72–1.89)	0.498	0.81 (0.44–1.49)
7	*0201 -*0302 -*0303 -*0601 -*0602	1.00	1.0 (0.28–3.52)	0.984	2.32–07 (0.00–Inf)
8	*0201 -*0301 -*0302 -*0303 -*0602	0.363	1.79 (0.51–6.23)	0.596	1.44 (0.37–5.60)
9	*0303 -*0601	0.006[a]	0.49 (0.30–0.82)	0.029[b]	0.47 (0.24–0.93)
10	*0303 -*0401 -*0601-*0602	0.430	1.53 (0.53–4.41)	0.467	0.57 (0.12–2.59)

OR, Odds ratio; 95% CI, 95% confidence interval; Anti-GM1-Ab, anti-GM1 antibody seropositive or seronegative; [a]*Pc* = 0.06 (*Pc*, *P* corrected); [b]*Pc* = 0.29 (*Pc*, *P* corrected).

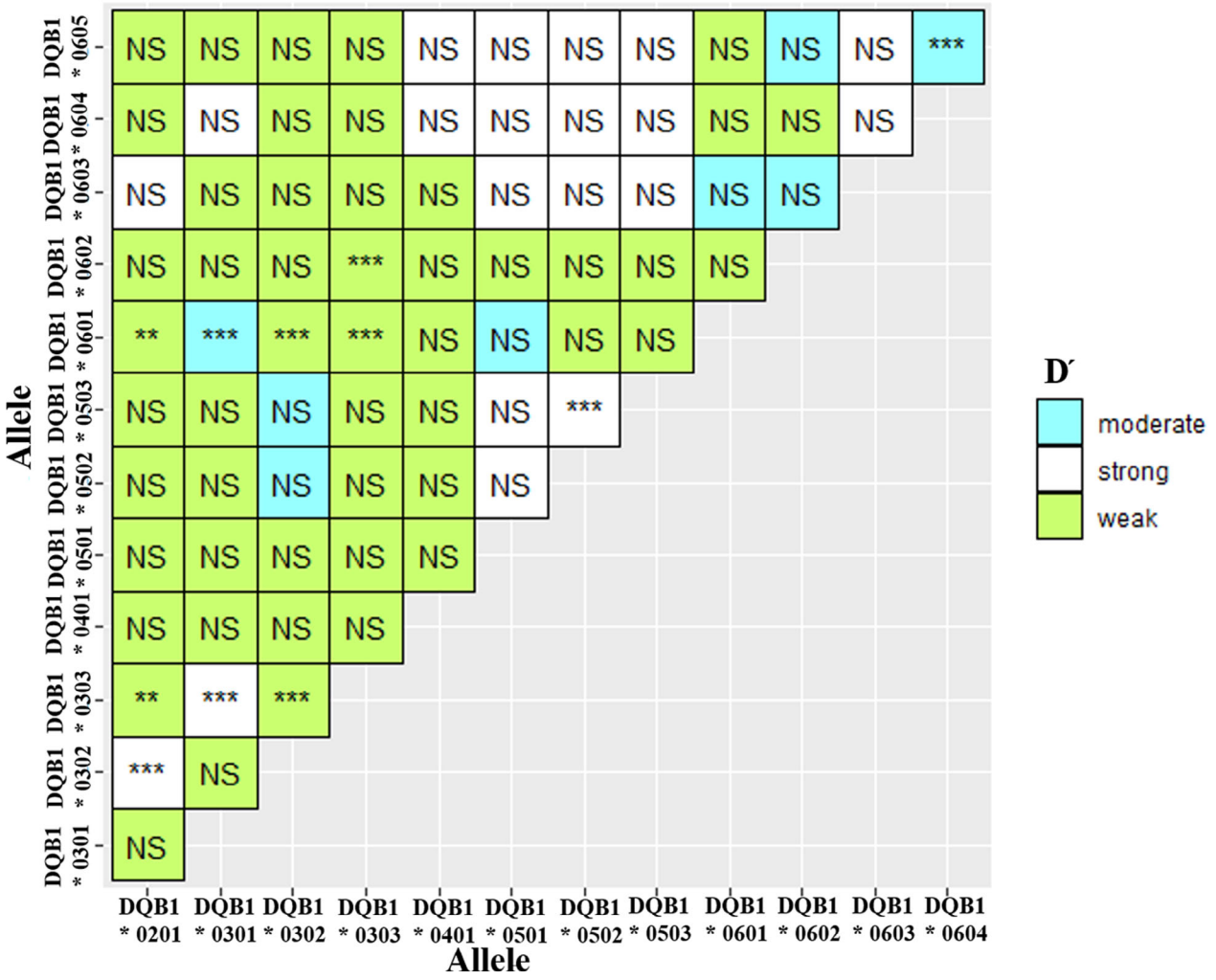

Figure 2. Pairwise linkage disequilibrium (LD) among the 13 *HLA-DQB1* loci based on D′ statistics. D′ > 0.75 indicated strong LD with white shade, D′ 0.5–0.74 indicated moderate LD with cyan shade, and D′ < 0.49 indicated weak LD with green shade. *P*-value overwrite above the respective LD where ***<0.005, **<0.05, *<0.01, Not significant > 0.1.

lost after correcting for multiple comparisons (P = 0.029, OR = 0.48, 95% CI = 0.25–0.92; Pc = 0.37; Table 3). Haplotype 5 (*0501-*0602) was significantly more prevalent in *C. jejuni* seropositive patients with the axonal variant compared to *C. jejuni* seropositive or seronegative patients with demyelinating subtype or unclassified GBS; but, this trend was not significant after Bonferroni correction (P = 0.024, OR = 4.06, 95% CI = 1.25–13.18; Pc = 0.24; Table S1). The *DQB1*0401* alleles were less frequent in *C. jejuni* seropositive patients with the axonal subtype than the *C. jejuni* seropositive or seronegative patients with other subtypes of GBS, but significance was lost after correcting for multiple comparisons (P = 0.045, OR = 0.39, 95% CI = 0.16–0.97; Pc = 0.58; Table S1).

Association of HLA-DQB1 polymorphisms and haplotype variations with autoantibodies in patients with GBS

The distribution of *HLA-DQB1* polymorphisms among antiganglioside antibody (Ab) seropositive patients with GBS is presented in Table S2. Overall, 48% (73/151) of patients with GBS were antiganglioside-Abseropositive: 38% (58/151) were anti-GM1-Abpositive, 15% (23/151) were anti-GD1a-Abpositive, and 9% (14/151) were anti-GQ1b-Abseropositive (Table S2). Among the anti-GM1-Abpositive patients, the frequency of the *DQB1*0601* allele was significantly lower in seropositive patients compared to seronegative patients, but this was not significant when the *P*-values were corrected for the number of

Table 3. Distribution of *HLA-DQB1* polymorphic alleles in patients with the axonal and demyelinating subtypes of GBS and healthy controls.

Allele	Axonal subtype *n* = 59 (%)	Demyelinating subtype *n* = 27 (%)	Healthy controls *n* = 151 (%)	Axonal vs. HC		Demyelinating vs. HC	
				P-value	Odds ratio (95% CI)	*P*-value	Odds ratio (95% CI)
DQB1*0201	21 (36)	15 (55)	48 (32)	0.626	1.19 (0.63–2.23)	0.027[a]	2.68 (1.17–6.17)
DQB1*0301/4	14 (24)	6 (22)	37 (24)	1.00	0.96 (0.47–1.94)	1.00	0.88 (0.33–2.34)
DQB1*0302	23 (40)	15 (55)	70 (46)	0.357	0.74 (0.40–1.37)	0.409	1.45 (0.63–3.30)
DQB1*0303	25 (42)	11 (41)	78 (52)	0.282	0.69 (0.38–1.26)	0.403	0.64 (0.28–1.48)
DQB1*0401	11 (19)	8 (30)	27 (18)	1.00	1.05 (0.48–2.29)	0.190	1.93 (0.77–4.88)
DQB1*0501	17 (29)	4 (15)	36 (23)	0.482	1.29 (0.66–2.54)	0.334	0.56 (0.18–1.71)
DQB1*0502	3 (5)	1 (4)	21 (14)	0.091	0.33 (0.09–1.16)	0.206	0.24 (0.03–1.85)
DQB1*0503	6 (10)	4 (15)	17 (11)	1.00	0.89 (0.33–2.39)	0.745	1.37 (0.42–4.44)
DQB1*0601	17 (29)	12 (44)	69 (46)	0.029[b]	0.48 (0.25–0.92)	1.00	0.95 (0.42–2.17)
DQB1*0602	37 (63)	17 (63)	81 (54)	0.279	1.45 (0.78–2.70)	0.407	1.47 (0.63–3.42)
DQB1*0603/8	2 (4)	0 (0)	6 (4)	1.00	0.85 (0.17–4.33)	nc	–
DQB1*0604	0 (0)	1 (4)	4 (3)	nc	–	1.00	1.41 (0.15–13.15)
DQB1*0605	0 (0)	2 (7)	5 (3)	nc	–	0.597	2.34 (0.43–12.7)

HC, healthy controls; 95% CI, 95% confidence interval; nc, not calculated; [a]*Pc* = 0.35 (*Pc*, *P* corrected); [b]*Pc* = 0.37 (*Pc*, *P* corrected).

alleles ($P = 0.022$, OR = 0.42, 95% CI = 0.20–0.88; *Pc* = 0.28; Table 5). Moreover, haplotype 9 (DQB1*0303-*0601) was less common among anti-GM1-Abseropositive patients than seronegative patients, but this trend was not significant after correction ($P = 0.029$, OR = 0.47, 95% CI = 0.24–0.93; *Pc* = 0.29; Table 2).

Association of HLA-DQB1 polymorphisms with severity and disease outcome in GBS

The patients with GBS were classified as severely affected (74%) or mildly affected (26%) based on MRC sum score. The *DQB1*0303 alleles were significantly more frequent among severely affected patients than mildly affected patients with GBS, but this significance was lost after correcting for multiple comparisons ($P = 0.025$, OR, 2.49; 95% CI, 1.13–5.48; *Pc* = 0.32; Table 6). However, no significant associations were observed between GBS disease severity and the 10 most common haplotype patterns. Furthermore, no significant associations were evident between the candidate alleles or haplotype patterns and disease outcome at 6 months of follow-up.

Discussion

This study investigated the association between *DQB1* alleles and haplotype patterns and GBS susceptibility in Bangladesh. Associations between HLA complex genes and human autoimmune diseases have been described; however, studies of HLA typing among populations with different genetic backgrounds have reported inconclusive associations with GBS.[14,15,17,18,30,31] In this study, we observed no association between *DQB1* alleles or haplotype patterns and disease susceptibility to GBS; the *DQB1* alleles and haplotype patterns had no influence on the clinical and serological subgroups of GBS in Bangladesh after the *P*-values were corrected.

GBS is a heterogeneous disorder with respect to severity, prognosis, and clinical features.[24] In this study, the *DQB1*0303 alleles were significantly associated with the severe form of GBS before correcting for multiple comparisons, implying that *HLA-DQB1* polymorphisms may possibly influence disease severity and the extent of the inflammatory response at the peripheral nerves. Though a Dutch study reported no association between *HLA-DQB1* alleles and disease severity, the *HLA-DRB1*01 allele was associated with the need for mechanical ventilation in patients with GBS.[14]

The associations of individual *HLA-DQB1* polymorphic alleles with GBS have been studied; however, haplotype studies were not performed.[14–16] In this study, we found individual *DQB1* alleles or haplotype were not associated with the development of GBS. However, haplotype 9 (*HLA-DQB1*0601-*0303) was less frequent among patients with GBS in Bangladesh compared to healthy controls and LD analysis also indicated their association among *DQB1* *0601 and*0303 alleles. Moreover, no significant LD was observed between the alleles of the 10 most common haplotype. This implies that the presence of both alleles (*HLA-DQB1*0601-*0303) may exert a reciprocal effect toward the development of GBS in the Bangladeshi population.

The *DQB1*03 allele is significantly associated with *C. jejuni* infection.[16] However, our study revealed a relatively lower frequency of the *DQB1*0303 and *0601 alleles and a slightly higher frequency of the *0502 alleles in *C. jejuni* seropositive patients compared to healthy controls. This discrepancy may be due to local evolutionary

Table 4. Distribution of *HLA-DQB1* polymorphic alleles in healthy controls and *C. jejuni* seropositive and *C. jejuni* seronegative patients with GBS.

					Axonal type			Demyelinating type		
	Healthy controls	Cj-positive patients	*C. jejuni* seropositive $n = 95$ HC vs. Cj (+)		*C. jejuni*					
Allele	$n = 151$ (%)	$n = 95$ (%)	*P*-value	Odds ratio (95% CI)	$n = 59$ (57%)	sero+ $n = 47$ (80%)	sero– $n = 12$ (20%)	$n = 27$ (26%)	sero+ $n = 12$ (44%)	sero– $n = 15$ (56%)
DQB1*0201	48 (32)	35 (37)	0.489	1.25 (0.73–2.15)	21 (36)	17	4	15 (55)	6	9
DQB1*0301/4	37 (24)	18 (19)	0.348	0.73 (0.38–1.36)	14 (24)	9	5	6 (22)	2	4
DQB1*0302	70 (46)	38 (40)	0.357	0.77 (0.46–1.30)	23 (40)	16	7	15 (55)	6	9
DQB1*0303	78 (52)	37 (39)	0.066	0.60 (0.35–1.00)	25 (42)	20	5	11 (41)	4	7
DQB1*0401	27 (18)	22 (23)	0.329	1.38 (0.74–2.61)	11 (19)	7	4	8 (30)	3	5
DQB1*0501	36 (23)	22 (23)	1.00	0.96 (0.53–1.76)	17 (29)	14	3	4 (15)	1	3
DQB1*0502	21 (14)	5 (5)	0.034[a]	0.34 (0.13–0.95)	3 (5)	3	0	1 (4)	0	1
DQB1*0503	17 (11)	14 (15)	0.436	1.36 (0.64–2.91)	6 (10)	5	1	4 (15)	3	1
DQB1*0601	69 (46)	30 (32)	0.033[b]	0.55 (0.33–0.94)	17 (29)	15	2	12 (44)	5	7
DQB1*0602	81 (54)	58 (61)	0.291	1.35 (0.80–2.28)	37 (63)	29	8	17 (63)	8	9
DQB1*0603/8	6 (4)	4 (4)	1.00	1.06 (0.29–3.87)	2 (4)	2	0	0 (0)	0	0
DQB1*0604	4 (3)	2 (2)	1.00	0.79 (0.14–4.40)	0 (0)	0	0	1 (4)	1	0
DQB1*0605	5 (3)	2 (2)	0.710	0.63 (0.12–3.30)	0 (0)	0	0	2 (7)	1	1

Cj, Campylobacter jejuni; sero +, *C. jejuni* seropositive; sero –, *C. jejuni* seronegative; HC, healthy control; 95% CI, 95% confidence interval; [a]*Pc* = 0.44 (*Pc*, *P* corrected); [b]*Pc* = 0.42 (*Pc*, *P* corrected).

Table 5. Distribution of *HLA-DQB1**060x polymorphisms within anti-GM1 antibody seropositive and seronegative patients with GBS.

	Presence of anti-GM1 antibody			
Allele	Positive $n = 58$ (%)	Negative $n = 93$ (%)	*P*-value	Odds ratio (95% CI)
DQB1*0601	13 (22)	38 (41)	0.022[a]	0.42 (0.20–0.88)
DQB1*0602	37 (64)	51 (55)	0.311	1.45 (0.74–2.85)
DQB1*0603/8	0 (0)	7 (8)	nc	–
DQB1*0604	1 (2)	1 (1)	1.00	1.61 (0.10–26.32)
DQB1*0605	1 (2)	3 (3)	0.6	0.53 (0.05–5.18)

95% CI, 95% confidence interval; nc, not calculated; [a]*Pc* = 0.28 (*Pc*, *P* corrected).

Table 6. Distribution of *HLA-DQB1* allele frequency among patients with different severities of GBS.

Allele	Mildly affected $n = 40$ (%)	Severely affected $n = 111$ (%)	*P*-value	Odds ratio (95% CI)
DQB1*0201	13 (33)	42 (38)	0.572	0.79 (0.36–1.69)
DQB1*0301/4	9 (23)	26 (23)	1.00	0.94 (0.40–2.24)
DQB1*0302	18 (45)	45 (41)	0.709	1.2 (0.57–2.48)
DQB1*0303	11 (28)	54 (49)	0.025[a]	2.49 (1.13–5.48)
DQB1*0401	10 (25)	29 (26)	1.00	0.94 (0.41–2.16)
DQB1*0501	11 (28)	20 (18)	0.253	1.72 (0.74–4.02)
DQB1*0502	2 (5)	11 (10)	0.515	0.47 (0.10–2.25)
DQB1*0503	3 (8)	17 (15)	0.281	0.44 (0.12–1.62)
DQB1*0601	10 (25)	40 (36)	0.243	0.59 (0.26–1.34)
DQB1*0602	24 (60)	64 (58)	0.853	1.10 (0.52–2.30)
DQB1*0603/8	2 (5)	5 (5)	1.00	1.12 (0.21–5.99)
DQB1*0604	2 (5)	1 (1)	0.171	5.78 (0.51–65.67)
DQB1*0605	1 (3)	3 (3)	1.00	0.92 (0.09–9.13)

Mildly affected at nadir, MRC sum score ≥ 40; severely affected at nadir, MRC sum score < 40; 95% CI, 95% confidence interval; [a]*Pc* = 0.32 (*Pc*, *P* corrected).

pressure among infectious agents in different ethnic populations. A previous study also indicated the contribution of *HLA-DQB1**030x alleles to regional variation in GBS.[31] Further analysis revealed haplotype 5 (*0501-*0602) was more frequent in the *C. jejuni*-associated axonal variant of GBS compared to other subtypes of GBS. This observation may be one factor explaining the higher prevalence of the axonal subtype of GBS in Bangladesh compared to other regions of the world. Furthermore, this also may explain how human ancestry and race modify *C. jejuni* strain's interaction with an individual's immune system to trigger different subtypes of GBS.[20] In our Bangladeshi population, a higher frequency of the *DQB1**0201 alleles was observed in the demyelinating variant of GBS. However, it is important to confirm and compare our results with studies of other ethnic populations from different regions of the world where the demyelinating variant of GBS predominates.

Campylobacter jejuni-triggered GBS is frequently associated with anti-GM1 antibodies, and GM1 acts as a target pathogenic antigen that triggers the axonal variant of

GBS.[27,32] HLA class II genes are recognized by CD4+ Th cells and are known to influence antibody responses by activating B cells.[33] A previous study observed no association between HLA alleles and the presence of anti-GM1 antibodies. However, the *HLA-DRB1**0803 and *HLA-DQA1**0301 alleles were more frequent in Japanese[34] and Chinese[30] anti-GM1 antibody-positive patients with GBS, respectively, while no significant association was observed between the *HLA-DRB1* and *HLA-DQB1* alleles and anti-GM1 antibody positivity in Dutch patients with GBS.[14] We did not observe a significant association between *HLA-DQB1* alleles and anti-GM1 antibody positivity in Bangladeshi GBS patients.

HLA-DQB1 alleles have diverse effects on susceptibility to autoimmune diseases. A stronger association between the *DQB1**06 alleles and disease susceptibility and a lower frequency of the *DQB1**03 alleles were observed in multiple sclerosis.[35] Similar studies on *HLA-DQB1* polymorphisms showed a higher risk of type I diabetes among individuals with the *DQB1**0201/*0302 alleles, whereas the *DQB1**0301, *DQB1**0601,**DQB1**0602, *DQB1**0603, and *DQB1**05 alleles protect against the development of type I diabetes.[36] Furthermore, the *DQB1**04 alleles confer susceptibility to rheumatoid arthritis, whereas the *DQB1**06 alleles protect against the development of rheumatoid arthritis.[37]

This study has several limitations. Even though we used one of the largest GBS cohorts from developing countries, the sample size was relatively small for investigation of a large number of haplotypes in GBS patients. Here, we only explored the association of *HLA-DQB1* alleles with disease susceptibility and subgroups, without considering other HLA alleles that are also important in GBS pathogenesis.

In conclusion, *HLA-DQB1* gene polymorphisms and haplotype were not associated with susceptibility to GBS in the Bangladeshi population. However, the importance of *HLA-DQB1* polymorphisms in the pathogenesis of GBS still remains unclear. Extensive analysis of a larger cohort of patients (e.g. from the IGOS study)[25] from various ethnic backgrounds is required to confirm our findings on *HLA-DQB1* alleles and haplotype and the development and progression of GBS.

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Author Contributions

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

Conflict of Interest

The authors do not have any conflict of interest to report.

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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. Association studies of axonal subtype patients with antiganglioside antibodies, HLA-DQB1 alleles, haplotype, and LOS**.**

Table S2. Distribution of HLA-DQB1 alleles in antiganglioside antibody-seropositive patients with GBS and in healthy controls.

