

# Presynaptic Neuromuscular Transmission Defect in Anti-GQ1b IgG Antibody-Related Disorders

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## Abstract

**Background:** In vitro studies of Miller Fisher syndrome (MFS) have demonstrated anti-GQ1b IgG antibody-mediated presynaptic damage at the neuromuscular junction. Previous studies have provided electrophysiological evidence of presynaptic neuromuscular transmission defect in MFS in anti-GQ1b positive patients, which persisted up to 3 months from initial presentation.

**Methods and results:** In this study, we show that incremental responses were significantly greater in antibody-positive MFS and Bickerstaff's brainstem encephalitis, compared with antibody-negative Guillain-Barre and MFS variants. These responses return to normality by 6 months, in tandem with clinical recovery.

**Conclusions:** The findings have pertinent implications on developing management strategies of these conditions.

## Introduction

Guillain-Barre syndrome (GBS), Miller Fisher syndrome (MFS) and Bickerstaff's brainstem encephalitis (BBE) are immune-mediated disorders with overlapping clinical features [1]. Anti-GQ1b IgG antibody is detected at variable frequencies, up to 95% in MFS, 26% in GBS and 66% in BBE [2].

In vitro studies of MFS have demonstrated anti-GQ1b IgG antibody-mediated presynaptic damage at the neuromuscular junction [3]. This mechanism may be responsible for some of its clinical manifestations.

Repetitive nerve stimulation (RNS) is a validated electrophysiological technique for assessing neuromuscular transmission. Incremental responses to RNS are characteristic of neuromuscular transmission defect at the presynaptic region, as reported in the Lambert-Eaton myasthenic syndrome (LEMS) and botulism [4]. In a previous study, we provided electrophysiological evidence of presynaptic neuromuscular transmission defect in MFS in anti-GQ1b positive patients, which persisted up to 3 months from initial presentation [5,6]. While these findings may represent subclinical phenomena in clinically unaffected muscles, it remains unclear if these effects persist beyond 3 months after clinical presentation. In addition, it remains uncertain if presynaptic neuromuscular transmission defect is present in the related conditions of GBS and BBE. In this study, we present our clinical findings in relation to anti-GQ1b IgG antibody titers for 10 further patients with these conditions.

## Methods

We evaluated 10 consecutive patients presenting with MFS, GBS and BBE in this study with local ethical committee approval previously obtained. GBS patients were diagnosed based on well described clinical and electrophysiological criteria previously published [7,8]. In particular, patients diagnosed as GBS have at least one abnormality (conduction velocity, F latency, distal latency or temporal dispersion) in 2 separate nerves. Table 1 summarizes their clinical data. All patients had a complete neurological examination, as well as routine nerve conduction studies. Anti-GQ1b IgG antibody assays were performed during hospital admission using enzyme-linked immunosorbent assay

(ELISA) methodology. Substrates were obtained from commercial sources (Sigma, St. Louis, USA). 300 ng of GQ1b in 50 ul of methanol was added to microtiter plate wells and evaporated to dryness to coat the wells. The remaining binding sites were blocked with 100 ul of 1% bovine serum albumin in phosphate buffered saline for 4 hours at room temperature. After washing with buffer, 100 ul volumes of sera diluted 1:100 in PBS were added to the wells and incubated overnight at 4 degrees Centigrade. The binding of anti-GQ1b antibodies was measured by applying peroxidase conjugated goat anti-human IgG and developing with hydrogen peroxide and 0.1% O-phenylenediamine in citrate buffer. The reaction was stopped with 1N sulphuric acid after 30 minutes. OD (optical density) readings were made at 492 nm using a Tecan Elisa reader. If the OD reading exceeded the upper limit of normal, we repeated the ELISA test at further dilutions, in order to determine the dilutions at which OD readings were within the linear portion of the curve. We then calculated the titre by using the OD reading taken at the linear portion of the curve, extrapolating it to the expected OD value for serum dilution of 1:100 and multiplying by 1000. Normal values for OD values and antibody titres were obtained from ELISA of sera from 56 normal control subjects previously described [6]. OD values and antibody titres greater than 3 SD above the mean of the controls were considered abnormally elevated. Normal OD values based controls yielded a mean of 0.234 and SD of 0.1. The upper limit of normality (mean + 3SD) was 0.534, which corresponded to an antibody titre of 534 [6].

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Received November 25, 2010; Accepted April 06, 2011; Published April 08, 2011

**Citation:** Lo YL, Seah A, Lim LL, Leoh TH, Dan YF, et al. (2011) Presynaptic Neuromuscular Transmission Defect in Anti-GQ1b IgG Antibody-Related Disorders. J Neurol Neurophysiol 2:111. doi:10.4172/2155-9562.1000111

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Patient	Age	Sex	Clinical	RNS 20 Hz	RNS 50 Hz	Anti-GQ1b IgG titer	CMAP	Imaging	Remarks
<b>Group 1 BBE</b>									
1	31	M	BBE: drowsy, ataxia, bilateral ptosis, weakness & brisk reflexes in upper & lower limbs; power 4/5 in all limbs	1 <sup>st</sup> : +90% (87-93)(1.5) 2 <sup>nd</sup> : +38% (36-40)(1)	1 <sup>st</sup> : +69% (66-71)(1) 2 <sup>nd</sup> : +35% (32-38)(1.5)	2168	11.1	MRI brain: normal	NCS normal; F and H responses normal Had 5 days of immunoglobulin infusion <i>Complete recovery in 3 months</i>
2	60	M	MFS: Diplopia after URTI, complete ophthalmoplegia; areflexia of all limbs	1 <sup>st</sup> : +69% (67-71)(1) 2 <sup>nd</sup> : +26% (24-28)(1)	1 <sup>st</sup> : +72% (70-74)(1) 2 <sup>nd</sup> : +28% (25-31)(1.4)	4855	7.4	MRI brain: normal	NCS; absent H reflexes <i>Complete recovery in 3 months</i>
3	27	M	MFS: complete ophthalmoplegia, ataxia, generalized areflexia	1 <sup>st</sup> : +97% (95-99)(1) 2 <sup>nd</sup> : +38%(36-40)(1.1)	1 <sup>st</sup> : +85% (83-87)(1) 2 <sup>nd</sup> : +41%(39-43)(1.1)	12840	9.1	MRI brain: normal	NCS: absent H reflexes
<b>Group 2 GBS</b>									
4	71	M	GBS: generalized areflexia, ataxia, dysmetria	1 <sup>st</sup> : +34%(32-36)(1)	1 <sup>st</sup> : +33%(31-35)(1)	16	6.7	MRI brain: left basal ganglia hyperintensity	NCS: abnormal F latencies in 4 limbs (upper > 34 ms; lower > 56 ms); H reflexes absent <i>Complete recovery in 3 months</i>
5	51	F	GBS: bilateral lower VII palsy, generalized areflexia	1 <sup>st</sup> : +29%(26-32)(1.3)	1 <sup>st</sup> : +40%(38-42)(1)	3	6.5	CT brain: normal MRI brain: normal	NCS: absent H reflexes & prolonged F latencies in 4 limbs (upper > 36 ms; lower > 60 ms) Clinical deficits resolved in 6 weeks
6	56	F	GBS: lower limb weakness, numbness & generalized areflexia	1 <sup>st</sup> : +9% (8-10)(0.5)	1 <sup>st</sup> : +4% (2-6)(1)	65	8.9	MRI brain: normal MRI lumbosacral spine: hyperintense conus medullaris	NCS: absent H reflexes & prolonged lower limb F latencies (all > 36 ms) Clinical deficits resolved in 2 months
7	57	M	GBS: pharyngeal-cervical-brachial variant	1 <sup>st</sup> : + 10% (8-12)(1.1)	1 <sup>st</sup> : +7% (4-10)(1.5)	1	2.7	MRI brain: normal MRI cervical spine: normal	NCS: prolonged upper limb F latencies ( all > 35 ms) Clinical deficits resolved in 2 months
8	61	M	GBS: bilateral footdrop; lower limb weakness, numbness & generalized areflexia	1 <sup>st</sup> : + 38% (36-40)(1)	1 <sup>st</sup> : +29% (26-32)(1.4)	10	10.8	MRI lumbar spine: mild L3 & L4 spondylosis	NCS: prolonged upper & lower limb F latencies (upper > 36 ms; lower > 56 ms) Clinical deficits resolved in 2 months
9	61	F	MFS variant: ataxia; bilateral VII palsy, reduced lower limb reflexes	1 <sup>st</sup> : +48%(46-50)(1)	1 <sup>st</sup> : +50%(47-63)(2.3)	115	7.5	MRI brain: old right parietal infarct	NCS: absent H reflexes & prolonged F latencies Clinical deficits resolved in 2 weeks
10	53	F	MFS variant: Diplopia; reduced vertical eye movements; absent lower limb reflexes; mild dysmetria	1 <sup>st</sup> : +38% (35-41)(1.5)	1 <sup>st</sup> : +40% (38-42)(1)	7	6.8	MRI brain: hyperintense midbrain signal	NCS: normal <i>Complete recovery in 3 months</i>

M: male; F: female; RNS: repetitive nerve stimulation; URTI: upper respiratory tract infection  
 1<sup>st</sup>: initial RNS study; 2<sup>nd</sup>: repeat RNS study at 6 months; values in brackets indicate range of 5 RNS values (Second bracket denote standard deviation value)  
 Upper limit of normality: 56% for 20 Hz RNS; 52% for 50 Hz RNS  
 CMAP: compound muscle action potential amplitude (mV); refers to amplitude at initial study  
 CMAP & RNS values were measured from the right abductor digiti minimi muscle; abnormal values underlined  
 Upper limit of normal for anti-GQ1b IgG titer: 534

**Table 1:** Summary of clinical, electrophysiological and imaging data.

Patients were classified into Group 1 (anti-GQ1b IgG antibody positive) and Group 2 (anti-GQ1b IgG antibody negative).

Repetitive nerve stimulation (RNS) of the ulnar nerve was performed with right abductor digiti minimi recording. RNS was performed at the following frequencies in random order: 3 Hz at rest, 3 Hz post-exercise, 20 Hz and 50 Hz. Exercise consisted of 20 s of maximal muscle activation. Each RNS was performed at 3-minute intervals. The 3 Hz RNS consisted of 10 stimuli trains. The 20 Hz and 50 Hz RNS consisted of 30 stimuli trains. Surface temperature was maintained between 31 to 32 degrees Centigrade. For 3 Hz RNS, negative peak amplitude percentage decrements were compared between the first and fourth compound muscle action potentials (CMAP). For 20 Hz and

50 Hz RNS, percentage increments between the first CMAP and the CMAP with the largest amplitude was calculated. In view of inherent variability, each RNS value obtained was the average of 5 repeat RNS studies. RNS studies were performed at presentation and at 6 months by an investigator blinded to patients' clinical features. In addition, we studied 20 healthy controls with informed consent using an identical protocol. Ulnar neuropathy was excluded in both controls and patients. Statistical comparisons (Student's t-test and Pearson's correlation) were made with SPSS Version 10.1 for Windows.

## Results

Patient 1 showed features inkeeping with BBE, and also had a change in sensorium [2]. Patients 2 and 3 had the classical triad of MFS.

In contrast, Patients 9 and 10 were collectively termed 'MFS variant', as they showed partial features of MFS (Group 2). Both had incomplete ophthalmoplegia, but not the full classical triad. Incremental responses during RNS in this study were significant for Patients 1, 2 and 3 for both 20 Hz and 50 Hz RNS. All 3 patients had elevated anti-GQ1b IgG. Patients 4, 5, 6, 7 and 8 were classified as GBS (Group 2). These patients had normal antibody levels and none showed significant incremental responses. None of the 10 patients showed decrements beyond those of healthy controls with 3Hz RNS.

With 20 Hz RNS, Group 1 showed significantly greater incremental responses compared to Group 2 (t-test,  $p = 0.0006$ ). With 50 Hz RNS, Group 1 also showed significantly greater incremental responses compared to Group 2 (t-test,  $p = 0.002$ ).

MRI of the brain in all 10 patients were normal, or had incidental unrelated findings. Nerve conduction studies usually suggested proximal conduction abnormalities (prolonged 'F' wave latencies and absent 'H' reflexes). All 10 made uneventful recoveries over a 3-month period.

Repeat studies at 6 months, showed return of increments to within normal limits in all 3 patients.

There was significant correlation of the initial increments (mean of 20 Hz and 50 Hz) and anti-GQ1b IgG antibody titer (Pearson's correlation coefficient  $r = 0.76$ ,  $p = 0.011$ ).

In healthy controls, mean percentage decrement (SD) with 3 Hz RNS was  $-1.7$  ( $-1.9$ ) %. The lower limit of normality at 2SDs below the mean was  $-5.5$ %. Mean percentage increment (SD) with 20 Hz RNS was 28 (14) %. With 50 Hz RNS it was 22 (15) %. At 2SDs above the mean, the upper limits of normality were 56% for 20 Hz RNS and 52% for 50 Hz RNS.

Table 1 summarizes clinical and electrophysiological findings.

Figure 1 shows RNS recordings of Patient 1.

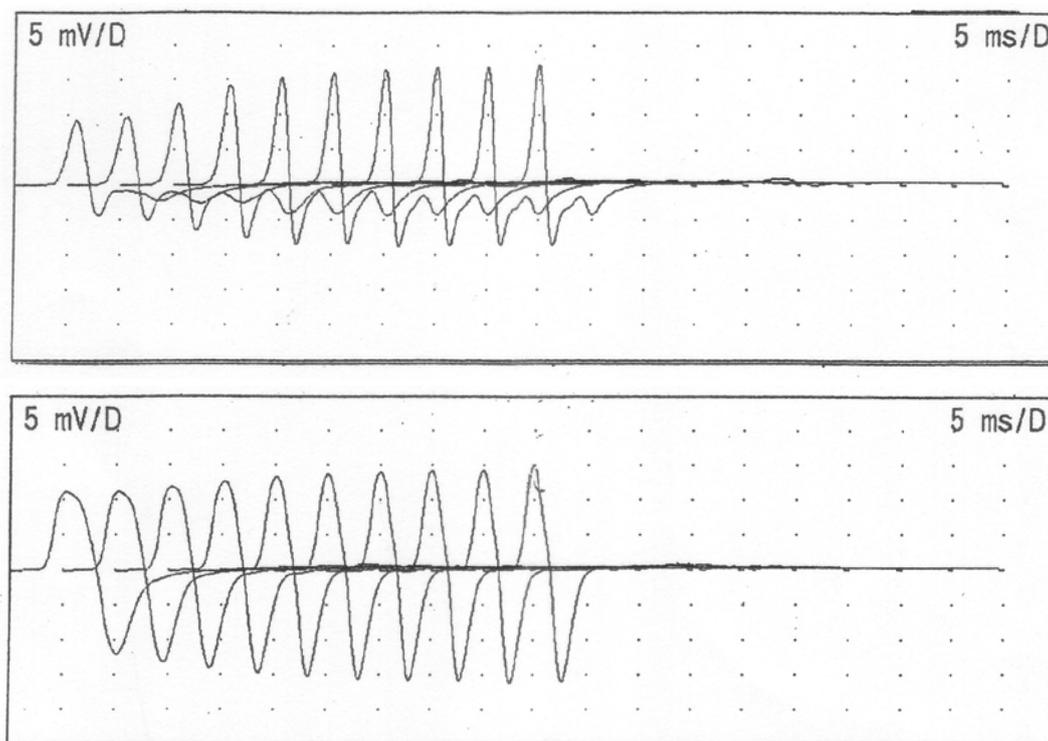
Figure 2 and Figure 3 depict increments plotted against anti-GQ1b IgG titers graphically.

## Discussion

Our findings of significant incremental responses seen in patients with significantly elevated anti-GQ1b IgG antibody levels confirm the previous report of presynaptic neuromuscular transmission defect in the MFS [5]. In addition, significant correlation of increment with anti-GQ1b IgG antibody titer strengthened the role this antibody plays in presynaptic neuromuscular transmission defect. However, this should be interpreted with caution due to the small sample size, and this relation should be further explored in future studies.

In addition to MFS, we have also shown that this phenomenon can occur in BBE. All the 5 patients with GBS did not have elevated antibody titers, or significantly incremental responses. This suggests that anti-GQ1b IgG antibody is closely associated with presynaptic neuromuscular transmission defect, and these 5 cases without electrophysiological evidence of presynaptic dysfunction served as negative controls. Furthermore, the remaining 2 cases classified as 'MFS variant' also did not exhibit elevated antibody titers or significant incremental responses, supporting further the likely role of anti-GQ1b IgG antibody in presynaptic neuromuscular transmission defect.

LEMS is the most widely recognized disorder of presynaptic neuromuscular transmission. A study of 18 patients [4] found that incremental responses were higher in seropositive cases over seronegative cases. The 60% increment found in these cases compared well to our upper limit of increment up to 56% in healthy controls. These

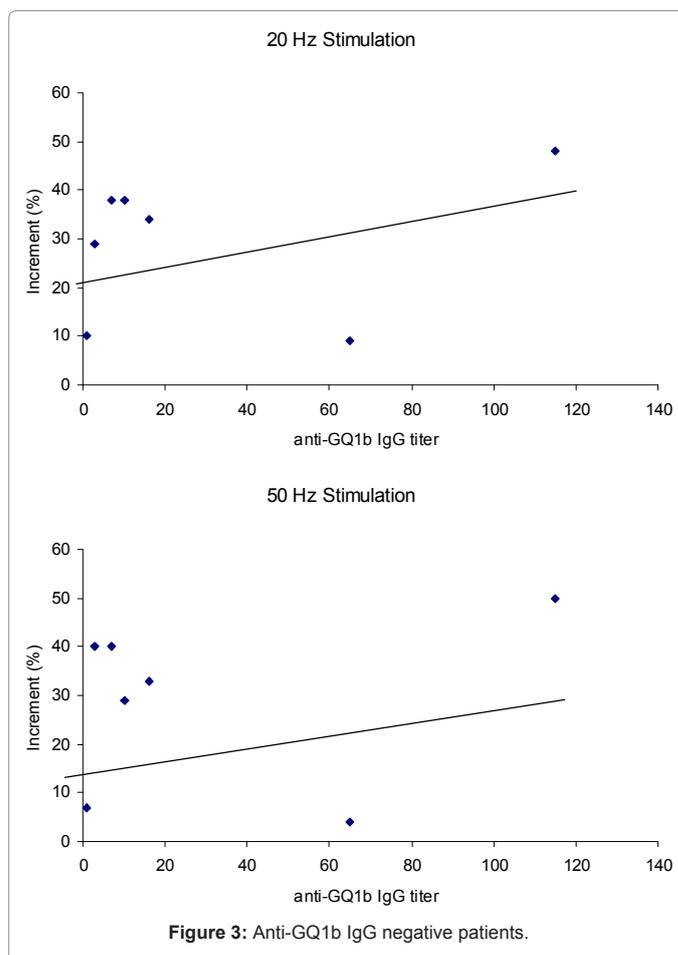
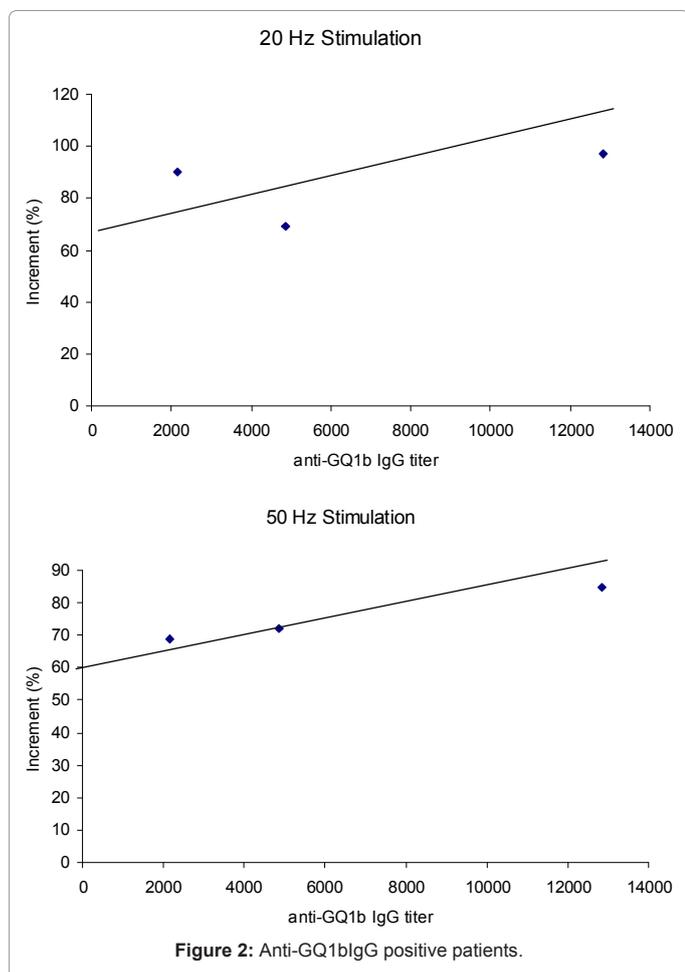


**Figure 1:** RNS recordings of Patient 1. Top trace shows initial increment of +90% at 20 Hz stimulation. The repeat study 6 months later (bottom trace) indicates reduced increment of +38%. Vertical gain and horizontal sweep speeds are depicted in the tracings.

findings can be extended to our study, where significant incremental responses were found only in antibody positive patients. However, only Patient 1 with BBE had mild proximal clinical weakness, whereas the RNS changes observed for Patients 2 and 3 were likely to be subclinical. We were, however, unable to demonstrate definite fatigability or paradoxical potentiation of motor power with exercise in Patient 1, suggesting inherent pathophysiological differences with LEMS. Only Patient 1 (classified as BBE) displayed signs of motor weakness, which improved with intravenous immunoglobulin infusion, and increments subsequently returned to normal. In addition, Patients 2 and 3 did not demonstrate clinical weakness, but increments also normalized at 6 months. It is thus possible that anti-GQ1b IgG antibody may have played a role in BBE, but the effects observed in MFS remained subclinical.

It was previously shown that significant incremental responses in antibody positive MFS patients persisted up to 3 months from disease onset, pointing to relatively permanent nerve terminal structural changes reported in *in vitro* studies [3]. However, the present findings of return of increments to normality at 6 months suggest otherwise, and other underlying mechanisms of nerve terminal injury, apart from anti-GQ1b IgG antibody-mediated effects, may be relevant in the pathogenesis of MFS.

The clinical implications of these findings are pertinent. For BBE, we provide observations to suggest that use of intravenous immunoglobulin may have contributed to clinical and



electrophysiological improvement. Although there is evidence of clinical response to intravenous immunoglobulin administration in BBE [9,10], spontaneous recovery may have occurred. However, correlation of normalization of incremental responses suggesting resolution of presynaptic neuromuscular transmission defect lends additional evidence that neutralization of antibody-mediated action in BBE may have played a role in clinical resolution.

Incremental responses are well defined for small upper limb muscles. Presynaptic neuromuscular transmission defect mediated by anti-GQ1b IgG antibody occurring in a distribution outside the extraocular muscles is supported by a recent study by Liu et al [11], which found that staining with anti-ganglioside antibodies in human limb or axial muscles occurred at the interstitial compartments of the muscle fiber surfaces, suggesting a more widespread location of antibody action. To this end, incremental responses in upper limb muscles may serve as surrogate markers for NM transmission defect in these disorders.

As immunotherapy is the mainstay of treatment in these conditions, complement mediation has to date been recognized as the most downstream pathophysiological event. Hence, utilization of treatment strategies targeting its actions [12,13] would appear to be most pragmatic [14]. As anti-complement therapy can be directed at complement action at the presynaptic level [15], our findings of presynaptic neuromuscular transmission defect may serve as a potential parameter in selection of patients for human therapeutic trials.

Finally, in MFS, use of calpain inhibitors has been proposed as a means to limit anti-GQ1b IgG antibody-mediated longstanding ultrastructural nerve terminal damage [13]. Our findings of reversible abnormal increments in antibody positive MFS patients may thus suggest that some aspects of presynaptic nerve terminal dysfunction may be self-limiting, and further research should be performed to elucidate other mechanisms downstream mediating nerve terminal injury in these patients. The initial important description of anti-GQ1b IgG antibody positivity in MFS alone [16] has thus been extended to involve a spectrum of related disorders, as well as relevant implications on therapy.

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