# Campylobacter gene polymorphism as a determinant of clinical features of Guillain–Barré syndrome

M. Koga, MD, PhD; M. Takahashi, DVM, PhD; M. Masuda, MD, PhD; K. Hirata, MD, PhD; and N. Yuki, MD, PhD

Abstract—Background: Ganglioside epitopes on Campylobacter jejuni are hypothesized as the key to the development and characterization of Guillain-Barré syndrome (GBS), but a comprehensive theory has yet to be established. A C jejuni gene, cst-II, involved in the biosynthesis of ganglioside-like lipo-oligosaccharide, shows a polymorphism (Asn/Thr51) that affects ganglioside epitopes. Objective: To examine the hypothesis that this polymorphism determines autoantibody reactivity, and thereby neurologic presentations in GBS. Methods: C jejuni isolates were collected from 105 GBS (including its variants) and 65 uncomplicated enteritis patients. The authors examined the frequency of cst-II and polymorphism (Asn/Thr51) in connection with the bacterial ganglioside epitopes, autoantibody reactivities against GM1, GD1a, and GQ1b, and patients' neurologic findings. Results: Neuropathic strains more frequently had cst-II, in particular cst-II (Thr51), than did enteritic ones (85% vs 52%; p < 0.001). Strains with *cst-II* (Asn51) regularly expressed the GQ1b epitope (83%), whereas those with cst-II (Thr51) had the GM1 (92%) and GD1a (91%) epitopes. The presence of these bacterial epitopes in neuropathy patients corresponded to autoantibody reactivity. Patients infected with C jejuni (Asn51) more often were positive for anti-GQ1b IgG (56% vs 8%; p < 0.001) and had ophthalmoparesis (64% vs 13%; p < 0.001) and ataxia (42% vs 11%; p = 0.001). Patients who had C jejuni (Thr51) more frequently were positive for anti-GM1 (88% vs 35%; p < 0.001) and anti-GD1a IgG (52% vs 24%; p = 0.006) and had limb weakness (98% vs 71%; p < 0.001). *Conclusions:* The genetic polymorphism of *C jejuni* determines autoantibody reactivity as well as the clinical presentation of Guillain-Barré syndrome (GBS), possibly through modification of the host-mimicking molecule. The GBS paradigm is the first to explain the detailed pathogenesis of a postinfectious, autoimmune-mediated, molecular mimicry-triggering disorder.

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Guillain–Barré syndrome (GBS) is characterized by limb weakness and loss of tendon reflexes, but has a variety of other neurologic presentations.<sup>1</sup> Anti-GM1 and anti-GD1a IgG antibodies are associated with axonal GBS,<sup>2</sup> whereas anti-GQ1b IgG antibody is specific to patients with Fisher syndrome (FS) or GBS with ophthalmoplegia.<sup>3</sup> Campylobacter jejuni is the most frequent antecedent infectious agent in GBS.<sup>4</sup> The critical factor that causes the development of neuropathy after *C jejuni* infection is un-

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known, but the bacterial lipo-oligosaccharide (LOS) is a candidate because its terminal sugar regions mimic the sugar residues of the gangliosides GM1, GD1a, and GQ1b.<sup>5-7</sup> The development of axonal GBS model after inoculation of rabbits with the GM1-like LOS has provided conclusive evidence for the hypothesis that the ganglioside-mimicry of *C jejuni* LOS is a cause of GBS.<sup>8</sup> However, determinant factor of anti-ganglioside antibody specificity and neurologic presentation in GBS remains unclear.

Determination of the complete genome sequence of *C jejuni* NCTC 11168 showed that many LOS biosynthesis genes are encoded in a large cluster.<sup>9</sup> A subsequent study identified the genes involved in the

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Address correspondence and reprint requests to Dr. M. Koga, Department of Neurology, Dokkyo University School of Medicine, Kitakobayashi 880, Mibu, Shimotsuga, Tochigi 321-0293, Japan; e-mail: kogamrk@dokkyomed.ac.jp

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From the Departments of Neurology (Drs. Koga, Hirata, and Yuki) and Microbiology (Dr. Masuda), Dokkyo University School of Medicine, Tochigi; and Department of Microbiology (Dr. Takahashi), Tokyo Metropolitan Institute of Public Health, Japan.

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transfer of galactose, N-acetylgalactosamine, and sialic acid to the LOS outer core.<sup>10</sup> Because ganglioside classification is based on the sialylation type (see figure E-1 on the *Neurology* Web site at www. neurology.org),<sup>11</sup> sialyltransferase-encoding genes may be associated with the variation in the ganglioside epitope on LOS. It was reported that cst-II, a gene encoding sialyltransferase,<sup>10,12</sup> was present in all eight strains with GQ1b-like LOS and proposed that its presence is associated with various ganglioside-like LOSs, although *cst-II* frequency did not differ between the GBS/FS and uncomplicated enteritis strains studied.<sup>13</sup> In contrast, based on tests of 28 GBS isolates, it was reported that the cst-II gene is more often present in GBS isolates than in enteritis isolates.<sup>14</sup> Whether the presence of this gene is a risk factor for developing neuropathy after *C jejuni* enteritis has yet to be proved.

The ganglioside-like structure of the *C jejuni* LOS is, in part, determined by the multiple mechanisms the bacterium uses to turn on or off a gene or to modulate the substrate specificities of its glycosyltransferases, as well as by different gene contents.<sup>15</sup> Interestingly, variation in the nucleotide sequence of cst-II might affect enzymatic activity; Cst-II (Thr51) has only  $\alpha$ -2,3-sialyltransferase activity (monofunctional), whereas Cst-II (Asn51) has both  $\alpha$ -2,3- and  $\alpha$ -2,8-sialyltransferase activities (bifunctional).<sup>15</sup> Because both  $\alpha$ -2,3- and  $\alpha$ -2,8-sialyltransferase activities are required for the biosynthesis of GQ1b mimics such as GT1a- or GD1c-like LOS,6,7 cst-II polymorphism is assumed to affect autoantibody reactivities through change in the ganglioside epitope on the LOS outer core, resulting in the diverse neurologic features shown by patients with GBS. In this study we used 105 C jejuni isolates from patients with GBS or a clinical variant and compared cst-II gene frequency in the neuropathic and enteritic strains. We also examined the hypothesis that the genetic polymorphism of the bacterium produces the differences in the clinical manifestation of GBS.

Methods. Bacterial strains and patients. Since 1990, we have received more than 8,000 requests from Japanese physicians to test serum anti-ganglioside antibodies from patients presenting with various neurologic disorders. On receipt of serum samples from patients with GBS or a clinical variant, we request the primary physicians to do a stool culture and to send the patient's stool specimen to the Tokyo Metropolitan Institute of Public Health for C jejuni isolation. From 1990 to 2002, 81 C jejuni strains were isolated successfully by one of the authors (M.T.), and 24 strains were obtained from hospitals. These were used in this study (n = 105: GBS, 80; FS, 20; Bickerstaff brainstem encephalitis,<sup>16</sup> 3; acute ophthalmoparesis,<sup>17</sup> 1; acute oropharyngeal palsy,<sup>18</sup> 1). Most of the strains used were included in our previous study.<sup>19</sup> Two, OH4384 and OH4382, were obtained from sibling GBS patients<sup>6,20</sup>; the others were from sporadic cases with geographically equal distributions. A questionnaire was filled in by the primary physician as to whether diagnostic criteria for GBS or a clinical variant were fulfilled. We also reviewed the patients' medical records to ascertain diagnoses and neurologic findings. GBS, FS, Bickerstaff brainstem encephalitis, and acute ophthalmoparesis diagnoses were all based on clinical criteria.<sup>21,22</sup> ELISAs were used to test for the presence of IgG antibodies to GM1, GD1a, and GQ1b, as reported elsewhere.<sup>23</sup> Sixty-five strains that had been isolated from patients with uncomplicated enteritis and

collected throughout Japan were the controls. Anti-ganglioside antibodies were not tested in the enteritis patients because previous studies showed that such patients did not have the autoantibodies. $^{24,25}$ 

Detection of ganglioside epitopes on LOS. Crude LOS fractions were prepared from the isolates, as described elsewhere,<sup>26</sup> with minor modifications.<sup>27</sup> We first performed sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and silver staining to ensure LOS had been extracted then used western blotting with the cholera toxin B-subunit (a ligand for GM1oligosaccharide structure) to examine whether the GM1 epitope was present on it. A 5 µL portion of the bacterial lysate was added to each well, after which the samples were separated on 15% tricine-SDS-polyacrylamide gels (SPU-15S series, Atto Corporation, Tokyo, Japan).<sup>28</sup> After electrophoresis, the LOS samples were blotted on polyvinylidene difluoride membranes (Atto Corporation), and the membranes incubated at 4 °C for 2 hours with the peroxidase-conjugated cholera toxin B-subunit (List Biologic Laboratories, Campbell, CA) diluted 1:2,000 in phosphate-buffered saline containing 0.5% casein. Binding was made visible with 4-chloro-1-naphtol (Konica Immunostaining HRP-1000, Konica, Tokyo, Japan).

Ganglioside-like LOS also was investigated by thin-layer chromatography (TLC) with immunostaining because this method decreases the volume of reagent needed in the immunostaining step. The reagents used are the monoclonal antibodies (GB1 [anti-GD1a], GB2 [anti-GM1], and FS1 [anti-GQ1b]),<sup>8,27</sup> and sera from patients with GBS (S6960 [anti-GM1] and S5174 [anti-GD1a]) or FS (S7577 [anti-GQ1b]). A 10  $\mu$ L portion of each bacterial lysate was spotted on a precoated Silica Gel 60 TLC plate (Merck, Darmstadt, Germany), developed with an *n*-propanol-water-25% ammonia solution (6:5:1, by volume), after which the plates were immunostained as reported elsewhere.<sup>23</sup> The TLC analysis was done by one of the authors (M.K.).

PCR and DNA sequencing. A single bacterial colony was suspended in 300 µL of sterile deionized distilled water and boiled for 10 minutes. After centrifugation at 15,000 rpm for 1 minute, the supernatant was used as the template in the PCR amplification. Oligonucleotide primers were designed based on the reported Cjejuni cst-II sequence (GenBank accession no. AF400048): forward directional primer, 17F (5'-TTTCTGGAAATGGACCAAGTTT-3'), and reverse, 220R (5'-CGGTCTCATATTCTTGATTTTGG-3'). These primers amplify the 204 bp fragment of the cst-II gene carrying the 51st codon. Amplification reactions were run in a total volume of 50  $\mu L$  with 20 pmol of each primer, 10  $\mu L$  of template, and 2.5 units of Taq DNA polymerase (TaKaRa Ex Taq, Takara Bio Inc., Shiga, Japan), 40 nmol dNTPs, and buffer (2 mM Mg<sup>2+</sup>), as recommended by the manufacturer. After a denaturation step of 5 minutes at 95 °C, the amplification mixture underwent 30 cycles of amplification, each cycle consisting of 10 seconds at 94 °C, 10 seconds at 55 °C, and 30 seconds at 72 °C, in a GeneAmp PCR System 9700 (PE Biosystems, Foster City, CA). PCR products were purified with a QIAquick PCR Purification Kit (Qiagen K.K., Tokyo, Japan). Nucleotide sequences of PCR products were determined with an ABI BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) and an ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

Statistical analysis. Frequency differences between the groups were compared by means of Fisher's exact test using SPSS 12.0J software (SPSS Inc., Chicago, IL). A difference was considered significant when the two-sided p value was less than 0.05.

**Results.** cst-II Gene frequency and polymorphism (Thr/ Asn51). PCR and direct nucleotide sequence analyses showed that the cst-II gene more often was present in neuropathic than enteritic strains (table 1). Although the 51st codon of cst-II is reported to be AAT (corresponding to Asn) or ACC (Thr),<sup>15</sup> some strains in our study had AAC (Asn). This indicates that a single base change (A to C or C to A) which may occur during infection can alter the substrate specificity of Cst-II (monofunctional or bifunctional). cst-II (Thr51) was more common in the neuropathic (50%) than enteritic (25%) strains (p = 0.001), whereas cst-II (Asn51) did not differ (34% vs 26%; p = 0.31). Also, 9 of 16

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**Table 1** Comparison of genetic and phenotypic properties of neuropathic and enteritic Campylobacter jejuni strains

	Neuropathic	Enteritic	p Value	
	strains, n = $105$	strains, n = 65		
Gene				
cst-II	89 (85)	33 (51)	< 0.001	
cst-III	9 (9)	15 (23)	0.012	
${ m GM1}$ epitope*				
Cholera toxin	62 (59)	25(38)	0.01	
GB2	63 (60)	25(38)	0.007	
S6960	60 (57)	22(34)	0.004	
GD1a epitope*				
GB1	51 (49)	13 (20)	< 0.001	
S5174	49 (47)	12 (18)	< 0.001	
GQ1b epitope*				
FS1	22 (21) 13 (20)		1.0	
S7577	32 (30) 17 (26)			

Values are n (%).

\* The reagents used are the monoclonal antibodies (GB1 [anti-GD1a], GB2 [anti-GM1], and FS1 [anti-GQ1b]), and sera from patients with Guillain-Barré syndrome (S6960 [anti-GM1] and S5174 [anti-GD1a]) or Fisher syndrome (S7577 [anti-GQ1b]). Cholera toxin (B-subunit) is a ligand for GM1-oligosaccharide structure.

neuropathic strains without *cst-II* had the *cst-III* gene,<sup>12</sup> and this gene was significantly less frequent in the neuropathic strains compared to the enteritic ones (see table 1).

Ganglioside-like LOS. Although we used two or three reagents in the detection of each ganglioside epitope, the overall results were identical, except for some discrepancies probably due to differences in the sensitivities of the reagents (see table 1). Figure E-2 shows the ganglioside epitope detection in representative strains. Neuropathic strains more commonly expressed GM1 and GD1a epitopes than did enteritic strains. In contrast, the frequency of the GQ1b epitope did not differ between them. Immunostaining results for the patients' sera showed that the GM1 and GD1a epitopes commonly were present in the same neuropathic strains (n = 48; 46%), whereas the GQ1b epitope was present isolatedly (n = 27; 26%). GQ1b and GM1



Figure. The ganglioside-like lipo-oligosaccharide of Campylobacter jejuni and anti-ganglioside autoantibody reactivity in patients with Guillain–Barré syndrome or a variant. Frequencies of positive IgG antibodies against GM1 (left), GD1a (middle), and GQ1b (right) are compared between patients whose isolates carry ganglioside epitope on lipo-oligosaccharide (LOS) (pale bars) or not (dark bars). \*p = 0.04; \*\*p < 0.001.

epitopes coexisted in five strains. Seventeen (16%) of the strains had none of the ganglioside epitopes examined.

cst-II Polymorphism (Thr/Asn51) was closely related to the ganglioside epitopes on LOS in both the neuropathic and enteritic strains. Asn51 strains more often expressed GQ1b epitope than did the others (table 2). In contrast, most Thr51 strains carried GM1- and GD1a-like LOS. Because the 53rd codon of *cst-II* is reported to affect  $\alpha$ -2,3and α-2,8-sialyltransferase activities in an Asn51 strain,<sup>15</sup> we analyzed its association with ganglioside-like LOS. Of the seven strains with cst-II (Asn51/Ile53) thought to have relatively low  $\alpha$ -2,3- and  $\alpha$ -2,8-sialyltransferase activities,<sup>15</sup> four had both GQ1b and GM1 epitopes, three had none. In contrast, all 22 strains (Asn51/Ser53 and Asn51/ Gly53) thought to have high  $\alpha$ -2,8-sialyltransferase activity had only the GQ1b epitope. Four of seven strains (Asn51/ Tyr53) also had only the GQ1b epitope. The other three had neither epitope.

Serologic and neurologic features. Detailed neurologic information was available for 97 neuropathy patients, and pretreatment serum samples were obtained within 4 weeks of neuropathic onset for 94. Serum anti-ganglioside autoantibody reactivities overall corresponded to the ganglioside epitopes on the LOSs of the isolates (figure): GM1 epitope on LOS and anti-GM1 IgG (p value < 0.001);

Table 2 Association of bacterial properties with cst-II content and polymorphism in neuropathic Campylobacter jejuni strains

	cst-II			cst-II (Asn51)			cst-II (Thr51)		
	Present	Absent	p Value	Present	Absent	p Value	Present	Absent	p Value
Lipo-oligosaccharide	n = 89	n = 16		n = 36	n = 69		n = 53	n = 52	
GM1 epitope*	53 (60)	7(44)	0.28	4 (11)	56 (81)	< 0.001	49 (92)	11(21)	< 0.001
GD1a epitope*	48 (54)	1 (6)	< 0.001	0	49 (71)	< 0.001	48 (91)	1 (2)	< 0.001
GQ1b epitope*	31(35)	1 (6)	0.04	30 (83)	2(3)	< 0.001	1(2)	31 (60)	< 0.001

Values are n (%).

\* Judged by results of thin-layer chromatography with immunostaining of patients' sera (S6960 [anti-GM1], S5174 [anti-GD1a], and S7577 [anti-GQ1b]) having high anti-ganglioside antibody titer.

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Table 3 Association of patients' clinical features with cst-II content and polymorphism of their Campylobacter jejuni isolates

	cst-II			cst-II (Asn51)			cst-II (Thr51)		
	Present	Absent	p Value	Present	Absent	p Value	Present	Absent	p Value
IgG antibody to	n = 80	n = 14		n = 32	n = 62)		n = 48	n = 46	
GM1	49 (61)	9 (64)	1.0	7(22)	51 (82)	< 0.001	42 (88)	16 (35)	< 0.001
GD1a	32 (40)	4 (29)	0.56	7(22)	29 (47)	0.03	25(52)	11 (24)	0.006
GQ1b	20 (25)	3 (21)	1.0	18 (56)	5 (8)	< 0.001	2(4)	21 (46)	< 0.001
Neurological sign	n = 81	n = 16		n = 33	n = 64		n = 48	n = 49	
Ophthalmoparesis	25(31)	4(25)	0.77	21 (64)	8 (13)	< 0.001	4 (8)	25(51)	< 0.001
Facial palsy	14(17)	1 (6)	0.45	11 (33)	4 (6)	0.001	3 (6)	12 (24)	0.02
Bulbar palsy	17(21)	0	0.07	13 (39)	4 (6)	< 0.001	4 (8)	13(27)	0.03
Limb weakness	69 (85)	13 (81)	0.71	22(67)	60 (94)	0.001	47 (98)	35(71)	< 0.001
Ataxia	17(21)	4 (25)	0.74	14 (42)	7(11)	0.001	3 (6)	18 (37)	< 0.001
Sensory disturbance	26 (32)	3 (19)	0.38	9 (27)	20 (31)	0.82	17(35)	12 (24)	0.27
Diagnosis	n = 89	n = 16		n = 36	n = 69		n = 53	n = 52)	
Guillain–Barré syndrome	68 (76)	12(75)	1.0	17(47)	63 (91)	< 0.001	51 (96)	29 (56)	< 0.001
Fisher syndrome	16 (18)	4 (25)	0.50	14 (39)	6 (9)	< 0.001	2(4)	18 (35)	< 0.001

Values are n (%).

GD1a epitope and anti-GD1a IgG (p value = 0.04); and GQ1b epitope and anti-GQ1b IgG (p < 0.001).

As expected, *cst-II* polymorphism was closely related to serum anti-ganglioside autoantibody reactivities, and therefore to neurologic features, whereas gene content was not (table 3). Patients from whom C jejuni (Asn51) had been isolated more often had serum anti-GQ1b IgG autoantibody, cranial nerve palsies, and ataxia. In contrast, Cjejuni (Thr51) was associated with anti-GM1 and anti-GD1a IgG antibodies and limb weakness. The diagnosis was GBS for most patients with C jejuni (Thr51), whereas it was GBS for 47% and FS for 39% of patients with C jejuni (Asn51). Conversely, compared to the enteritis patients, FS patients more often had been infected by C jejuni (Asn51) (70% vs 26%; p value = 0.001), whereas GBS patients had been by C jejuni (Thr51) (64% vs 25%; p value < 0.001). Variation in the 53rd codon was not associated with any type of autoantibody or neurologic feature in spite of its association with ganglioside-like LOS (data not shown).

Discussion. The pathogenesis of many postinfectious disorders is still unknown, no autoantigens or virulence factors having been identified. For example, why some group A streptococci can cause acute rheumatic fever and others acute glomerulonephritis is unclear.<sup>29</sup> In contrast, we are the first to show the detailed molecular mechanism of GBS after *C jejuni* enteritis, based on our and others findings that the genetic polymorphism of the bacterium alters the substrate specificity of the LOS biosynthesis enzyme<sup>15</sup> and that autoantibody reactivity determines the clinical presentation of GBS.<sup>30</sup> These findings suggest that the genetic polymorphism of antecedent agents determines autoantibody reactivities and clinical manifestations through change to the host-mimicking molecule in some post-infectious disorders. We believe that GBS is the first paradigm to explain the detailed pathogenesis of a postinfectious, autoimmune-mediated, molecular mimicrytriggering disorder.

We confirmed results of a previous report that *cst-II* more frequently exists in GBS than in enteritis isolates, indicative that its presence is a risk factor for developing GBS.<sup>14</sup> However, considerable numbers of strains from patients with uncomplicated enteritis also had *cst-II* gene, indicating that *cst-II* gene is necessary but not adequate for initiating autoimmune response, although cst-II genotype is important in determining antibody reactivity when autoimmune response is triggered. Certain other genes (e.g., cst-III<sup>12</sup> or an unidentified gene) might produce the enzyme protein instead, subsequently sialylating LOS. Three genes, cst-I, -II, and -III, are reported to encode sialyltransferase protein,<sup>12,15</sup> but cst-I was lacking in some strains with sialylated LOS and therefore is unlikely to be responsible for LOS sialylation.<sup>10</sup> Because Cst-III appears to have only  $\alpha$ -2,3-sialyltransferase activity (monofunction),<sup>15</sup> *cst-II* content must be essential for  $\alpha$ -2.8sialyltransferase activity and thereby biosynthesis of the GQ1b epitope.<sup>13,15</sup>

Most of the previous studies have failed to find a specific *C jejuni* genotype for GBS and FS.<sup>31-35</sup> It was recently reported that the class A LOS biosynthesis locus was over-represented in GBS-associated as compared to enteritis strains, whereas all four of the FS-related strains belonged to class B.<sup>36</sup> The authors suspected that the frequent expression of a GM1 epitope in class A and a GQ1b epitope in class B strains is responsible for the development of GBS and FS. Their findings, however, do not provide the answers as to which difference leads to diverse

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ganglioside-mimics (GM1 and GQ1b) in spite of there being almost the same class A and B gene profiles.<sup>15</sup> In contrast, our data clearly indicate that both presence and polymorphism of bacterial *cst-II* have a major role in the type of ganglioside-like structure on LOS, thereby determining autoantibody reactivity and the pattern of neurologic presentation. Phase variation owing to a homopolymeric G-tract in cst-II or other LOS synthesis genes also may be related to what ganglioside epitopes are present.<sup>10</sup> Along with those of a previous study,<sup>15</sup> however, our findings suggest that mainly it is variation in the cst-II 51st codon and supplemental variation in the 53rd codon that determine which ganglioside epitopes are present on LOS, but only the former was related to the autoantibody reactivity in and neurologic features of neuropathy patients. Interestingly, Haemophilus influenzae, a pathogen recently suggested to cause GBS and FS,  $^{\rm 27,37}$  also has sialy lated LOS.  $^{\rm 38}$ Three genes (lic3A, siaA, lsgB) have been cloned for that sialylation enzyme.<sup>39,40</sup> Whether the polymorphism of *H* influenzae genes also is related to autoantibody reactivity and consequently to the neurologic features of GBS and FS requires investigation.

*C jejuni* strains which had been isolated from anti-GQ1b antibody-positive patients often carried GQ1b epitope on the LOS.<sup>41</sup> The specificity of antiganglioside antibody induced by immunization of *C jejuni* LOS overall corresponds to ganglioside epitopes on the LOS in rabbits.<sup>42</sup> In this study, we showed that the target ganglioside (GM1, GD1a, or GQ1b) for serum autoantibody corresponds to the LOS-mimicking gangliosides of isolates from individual patients. However, other gangliosides such as GM1b and GalNAc-GD1a could be target antigens for autoantibodies in GBS<sup>30</sup> and further investigation is necessary to explain the variety of the clinical manifestation of GBS in more detail.

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# **Neuro** Images

# Isolated insular infarction eliminates contralateral cold, cold pain, and pinprick perception

Frank Birklein, MD, PhD; Roman Rolke, MD; and Wibke Müller-Forell, MD, PhD, Mainz, Germany

Functional imaging suggests that the insular cortex is important for pain processing.<sup>1</sup> We performed quantitative sensory testing (QST) in a 64-year-old man with acute left insular infarct. At day 5, diffusion-weighted MRI (figure) and QST (warm, cold, heat, and cold pain; tactile, vibratory, pinprick, and pressure pain) were performed. Contralateral to the infarct, the patient did not feel cold ( $\Delta$ 32 °C), cold pain ( $\Delta$ 32 °C), or pinprick pain (>630 mN). The other sensory modalities were normal. There were no other neurologic disturbances apart from a mild receptive aphasia. This case illustrates that isolated insular infarction indeed leads to sensory disturbances,<sup>2</sup> which might even be modality specific.

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Address correspondence and reprint requests to Dr. F. Birklein, Department of Neurology, University of Mainz, Langenbeckstrasse 1, D-55101 Mainz, Germany; e-mail: birklein@neurologie.klinik.uni-mainz.de



Figure. Diffusion-weighted MR image shows the acute ischemic infarct, which is restricted to the middle/posterior left insular cortex.



## Isolated insular infarction eliminates contralateral cold, cold pain, and pinprick perception Frank Birklein, Roman Rolke and Wibke Müller-Forell *Neurology* 2005;65;1381 DOI 10.1212/01.wnl.0000181351.82772.b3

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