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PCR-Restriction Fragment Length Polymorphism Analysis of *Campylobacter jejuni* Genes Involved in Lipooligosaccharide Biosynthesis Identifies Putative Molecular Markers for Guillain-Barré Syndrome▽

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Molecular mimicry of *Campylobacter jejuni* lipooligosaccharides (LOS) by gangliosides in peripheral nerve tissue probably triggers the Guillain-Barré syndrome due to the induction of cross-reactive antibodies. PCR-restriction fragment length polymorphism analysis of *C. jejuni* genes involved in the biosynthesis of LOS demonstrated that specific genes were associated with the expression of ganglioside mimics and the development of neuropathy.

Campylobacter jejuni probably triggers the Guillain-Barré syndrome (GBS) through molecular mimicry between lipooligosaccharides (LOS) in the bacterial cell wall and gangliosides in human peripheral nerve tissue (1). Various ganglioside-mimicking structures have been identified in the LOS fraction of the *C. jejuni* cell wall (9). This variation in LOS structure is the result of differences in the presence of LOS biosynthesis genes and of DNA sequence polymorphism within these genes (4). Based on the differences in gene content observed so far, eight different classes of the LOS biosynthesis gene locus can be identified (6, 10). However, only strains with a class A, B, or C LOS locus express ganglioside mimics (3). Previously, we demonstrated that class A and B LOS biosynthesis gene loci are associated with GBS and its variant, the Miller Fisher syndrome (MFS), and with the expression of ganglioside mimics (5). In search of other and/or more specific markers for GBS/MFS or the expression of ganglioside mimics, we describe a study in which the presence and heterogeneity of individual genes within the class A, B, and C LOS loci were studied by a comparative PCR-restriction fragment length polymorphism (RFLP) analysis of neuropathy-associated and control *C. jejuni* strains.

The *C. jejuni* strains used in this study have been described before and represent a genetically heterogeneous population (see Table 2) (5, 11). The presence of GM1-like, GQ1b-like, or "any" ganglioside mimics in the LOS of the strains has also been determined previously by mass spectrometry analysis or immunological methodologies (2, 3, 6). GD3-like or GD1c-like LOS structures were considered to be GQ1b-like mimics (6). Only strains with a class A, B, or C LOS locus express ganglioside mimics. Therefore, specific PCR tests were developed for the individual genes within the class A, B, and C LOS loci (Table 1). When necessary, primer sequences were selected for both class C and class A/B genes to cover intrinsic sequence variabilities as effectively as possible. PCR assays were performed using a Biomed thermal cycler (model 60; Theres, Germany) with a program consisting of 40 cycles of the following cycling protocol: 1 min at 94°C, 1 min at 55°C, 1 min at 72°C. For some amplifications, timing needed to be adapted. For RFLP analysis, PCR products were subjected to overnight incubation at 37°C with the enzymes AluI, DdeI, HindIII, and DraI (Boehringer-Mannheim) in separate reactions. Length determination of the PCR and the RFLP products was performed by agarose gel electrophoresis (1 to 3%, depending on the fragment size). Single band differences led to the introduction of a novel type. The differential presence of the genes was further confirmed by hybridization studies. PCR fragments were labeled with an ECL chemiluminescence kit (Amersham Pharmacia Biotech, Freiburg, Germany) according to the instructions of the manufacturer and hybridized to spot blots containing 200 ng of DNA from the various strains. In short, after 2 h of prehybridization, 500 ng of each PCR product was labeled and hybridized overnight at 42°C. After they were washed, blots were incubated for 1 min in 20 ml of detection reagent. Films were developed after 1-, 5-, and 30-min exposures. Statistical analysis was performed with Instat (version 2.05a; GraphPad Software, San Diego, CA). A P value of <0.05 was considered significant.

The results of the PCR-RFLP and confirmatory hybridization analyses are summarized in Table 2. In 17% of all positive hybridization signals (the percentage varied per gene), we observed a negative corresponding PCR. In these cases, we considered the gene to be present because sequence heterogeneity at the primer site may result in a negative PCR. For strains

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TABLE 1. Survey of *Campylobacter jejuni* NCTC 11168 and HS:19 LOS biosynthesis genes, including primers for amplification of the respective genes

Code[a]	Gene[b]	Positions[c]	Size (bp)	Proposed function	Primer sequences (5′–3′)[d]
Cj1133, ORF1	*waaC*	126680–127708 <1–360	1,029	Heptosyl transferase I	TAATGAAAATAGCAATTGTTCGT GATACAAAAATCACTTTTATCGA
Cj1134, ORF2	*htrB*	127698–128585 350–1237	888 888	Lipid A biosynthesis acyltransferase	ACGCGTATAGAAGAAGAAGATAT AGATTCATTTGCATCCTTGTA
Cj1135, ORF3		128582–130129 1234–2490	1,548 1,257	Two-domain glucosyltransferase	ATGAATCTAAAGCAAATAAGTGCTATTATC TACATTGTATATGGGGATTACTACACCTAC ATGAATCTAAAACAAATAAGCGTTATTATC (class A/B) CGATAATCATCTTTGTTTAAAATTTCTTCG
Cj1136, ORF4		130133–131305 2786–3955	1,173 1,170	β-1,3-Galactosyltransferase	ATGAAAAAAGTAGGTGTAGTAATCC TCAATGATAGATAAAATCATTTTGCAAGT ATGAAGAAAATAGGTGTAGTTATACC (class A/B) TTAAATATTTTTTATTTTTTGCC
ORF5	*cgtA*	4025–5068	1,044	β-1,4-*N*-Acetylgalactosaminyltransferase	ATGCTATTTCAATCATACTTTGTG TTAAAACAATGTTAAGAATATTTTTTTAG
Cj1137c, ORF14		131289–132284	996	Putative glycosyltransferase	CAACTTTGCAAAATGATTTTATCTATCATT ATGCAAATACAACAAAACAATTC
Cj1138, ORF15		132342–133511	1,170	Putative glycosyltransferase	AACATAGAAGGTAATAATCCTTATG AGGGTTTTAATAGTTGCAATTTCTC
Cj1139c, ORF6	*wlaN*, *cgtB*	133500–134411 5054–5959	912 906	β-1,3-Galactosyltransferase	ATGAAATTTTTAATATCTTTACGGAATTAA ATGAGTCAAATTTCCATCATACTACCAACT ATGTTTAAAATTTCAATCATCTTACC (class A/B) TATTTATTTTAGCTAACAATGTAACC
Cj1140, ORF7	*cst-II*, *cst-III*	134466–135350 6048–6923	885 876	α-2,3- or α-2,3/α2,8-sialyltransferase	ATGAGTATGAATATTAATGCTTTGG TTATCTATTTTTATTTGCATATTTTTC ATGAAAAAAGTTATTATTGCTGGAAATG (class A/B) TTATTTTCCTTTGAAATAATGCTTTATTC
Cj1141, ORF8	*neuB1*	135331–136362 6924–7964	1,032 1,041	Sialic acid synthetase	ATGCAAATAAAAATAGATAAATTAACTAT TCATTCAAAATCATCCCATGTCTTTGCACT ATGAAAGAAATAAAAATACAAAATATAATC (class A/B) CGCAAAATCCTCATAGCTTAACTGAGTATC
Cj1142, ORF9	*neuC1*	136359–137474 8021–9079	1,116 1,059	*N*-Acetylglucosamine-6P 2-epimerase	ATGAAAAAAATAGTTTTTGTTAGCGGAACT TCATTTTTTATCCATGAATATTTTTTGCTT GTGAAAAAAATCCTTTTTATAACAGG (class A/B) GTGTGTTAAGTTTAAAAAATTCTCCGC
ORF10		9076–9741	666	CMP-sialic acid synthetase	ATGAGCTTAGCAATAATCCCTGCTC (class A/B) TTATTTTTTCCATATCTGTTCAGCC
ORF11		9729–10562	834	Sialate-*O*-acetyltransferase	ATGGAAAAATAACCTTAAAATGC (class A/B) AAATAGATTAAAAATTTTTTTTGATTTTAG
Cj1143, ORF5/10	*neuA1*	137471–139081	1,611	β-1,4-*N*-Acetylgalactosaminyltransferase, CMP-sialic acid synthetase	ATGACTTTGTTTTATAAAATTATAGC TTATAAGAAGCTTATATTATTAACAC
Cj1144c, ORF16		139076–139669	594	Hypothetical protein	GAGTTAATAATATAAGCTTCTTTATAA GGATCTGGAGTAGCAGCGTTTAGTGA
Cj1145c, ORF17		139605–139922	318	Hypothetical protein	TAAACATATCATATCAAGATCTACCCATAC TTGAATTAAAAGATGCAAATGAAATTGTTC
Cj1146c, ORF12	*waaV*	139906–140730 10554–11366	822 813	Putative glycosyltransferase	ATGCCACAACTTTCTATCATAATCCCGC CTAATTTCTTTGCTTCATCAACCCTTC ATGCCACAACTTTCTATCATAATCCCGC (class A/B) TTTTAATCTATTTTTCACCCCTGCTTC
Cj1148, ORF13	*waaF*	140789–141748 11347–>11474	960	Heptosyltransferase II	ATGAAAATTTTTATACATCTTCCCACCTGGT AGATCATAGATGAGAGTTTTTAAGTAAATT

[a] The codes represented in the form Cjxxxx correspond to the gene numbering of strain NCTC 11168. The codes representing open reading frame x (ORFx) correspond to the gene numbering by Gilbert et al. (4).

[b] Gene nomenclature as found in the literature.

[c] Nucleotide positions are based on strain NCTC 11168 (GenBank accession numbers AL139077 and AL139078) and/or on strain OH4384 (GenBank accession number AF130984).

[d] Primer sequences are based either on strain NCTC 11168 or on strain OH4384 (indicated by the addition of "class A/B" following the forward primer sequence). For some genes two primer sets were used.

with a class A, B, or C LOS locus, the gene content, as determined by PCR and hybridization analyses, was largely in accordance with the expected gene content based on the type of LOS locus. However, there were some discrepancies. *orf6* could not be detected in 8 out of 34 (24%) strains with a class A, B, or C LOS locus, although its presence was expected based on the type of LOS locus. A possible explanation may be a failure to detect *orf6* due to extensive sequence heterogeneity within *orf6*, as reflected by the large number of different RFLP types (Table 2). On the other hand, it is also possible that *orf6* is really absent in these strains. In five strains with a LOS class other than A, B, or C, one or more genes considered to be unique for class A, B, or C strains gave a positive PCR and hybridization signal. Further analysis is needed to determine whether these positive signals were caused by the actual presence of the target genes in the LOS locus or by the presence of

TABLE 2. Characteristics of *C. jejuni* strains and results of the PCR-RFLP and hybridization analyses for the LOS biosynthesis locus

Strain[a]	Serotype(s)[b]	LOS class	Ganglioside epitope[c]			Gene[d]																		
			Any	GM1	GQ1b	1	2	3	4	5	14	15	6	7ab	7c	8	9	5/10	10	11	16	17	12	13
GB2	UT	A	+	+	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
GB3	19	A	+	+	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
GB11	2	A	+	+	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
GB16	13, 66	A	+	−	+	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
GB18	19	A	+	+	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
GB19	4, 50	A	+	−	+	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
GB21	13, 65	A	+	+	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
GB22	13, 64	A	+	+	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
GB23	4, 13, 43	A	+	−	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
GB26/27	1, 44	A	+	−	−	+	+	+	+	+	−	−	−	+	−	+	+	−	+	+	−	−	+	+
GB28	19, 38	A	+	+	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
OH4382	19	A	+	−	+	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
GB5	4, 64	B	−	−	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
MF6	4, 64	B	+	−	+	+	+	+	+	+	−	−	−	+	−	+	+	−	+	+	−	−	+	+
MF7	35	B	+	−	+	+	+	+	+	+	−	−	−	+	−	+	+	−	+	+	−	−	+	+
MF8	23, 36	B	+	−	+	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
GB17	4, 13, 64	B	+	−	+	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
MF20	2	B	+	−	+	+	+	+	+	+	−	−	−	+	−	+	+	−	+	+	−	−	+	+
GB25	2	B	+	−	+	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
GB1	1	C	−	−	−	+	+	+	+	+	+	+	+	−	+	+	+	+	−	−	+	+	+	+
GB13/14	2	C	+	+	−	+	+	+	+	+	+	+	+	−	+	+	+	+	−	−	+	+	+	+
GB15	5, 34	F	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
GB24	31	K	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+
GB4	37	P	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+
HS:4	4	A	+	+	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
HS:10	10	A	+	−	+	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
HS:19	19	A	+	+	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
HS:23	23	B	−	−	−	+	+	+	+	+	−	−	−	+	−	+	+	−	+	+	−	−	+	+
HS:35	35	B	−	−	−	+	+	+	+	+	−	−	−	+	−	+	+	−	+	+	−	−	+	+
HS:36	36	B	+	−	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+	+	−	−	+	+
HS:1	1	C	+	−	−	+	+	+	+	+	+	+	+	−	+	+	+	+	−	−	+	+	+	+
HS:2	2	C	+	−	−	+	+	+	+	+	+	+	+	−	+	+	+	+	−	−	+	+	+	+
11168	2	C	+	+	−	+	+	+	+	+	+	+	+	−	+	+	+	+	−	−	+	+	+	+
HS:64	64	D	−	−	−	+	+	+	−	−	−	−	+	−	−	+	+	−	−	−	−	−	+	+
HS:3	3	H	−	−	−	+	+	+	−	−	−	−	+	−	−	+	+	−	−	−	−	−	+	+
E98-652	2	B	+	+	+	+	+	−	−	+	−	−	−	+	−	+	+	−	+	+	−	−	+	+
E98-1033	10	B	+	+	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+	−	−	−	+	+
E98-682	1	C	+	+	−	+	+	+	+	+	+	+	+	−	+	+	+	+	−	−	+	+	+	+
E98-1087	1, 10, 44	C	+	+	−	+	+	+	+	+	+	+	−	−	+	−	+	−	−	−	+	+	+	+
E98-706	31, 63	D	−	−	−	+	+	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	+
E98-623	6, 57	E	−	−	−	+	+	−	−	+	−	−	−	+	−	+	+	−	+	−	−	−	+	+
E98-624	22, 57	E	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+
No. of RFLP types[e]						17	17	1–2	1–1	1	1	1	3–10	8	1	2–9	1–8	1	7	1	1	2	1–6	9

[a] Neuropathy-associated strains: GBxx and OH4382, GBS-associated isolates; MFxx, MFS-associated isolates. Enteritis-associated strains: Exx, isolates from enteritis patients; HS:xx, Penner (HS) type strains; 11168, NCTC 11168 genome strain.

[b] Penner (HS) serotypes; UT, untypeable.

[c] +, present, −, absent. The presence of ganglioside epitopes was determined previously by immunological methods or, when available, mass spectrometry (2, 3, 6).

[d] Gene numbers are open reading frame (ORF) numbers (as shown in Table 1). +, detected by PCR/hybridization analysis; −, not detected by PCR/hybridization analysis.

[e] Data represent the number of RFLP types detected per gene. In cases in which the PCR analysis was performed with two primer sets, two numbers are displayed; the first is the number of RFLP types based on NCTC 11168 primer set PCR, and the second is the number of RFLP types based on HS:19 (OH4384) primer set PCR.

the gene (or a homologue) elsewhere in the genome. Indications for both forms of LOS cluster heterogeneity were documented previously by Parker et al. (10).

Table 3 shows the putative association of the various LOS biosynthesis genes with neuropathy. *orf11*, encoding a sialate-*O*-acetyltransferase, was detected significantly more frequently in neuropathy-associated strains and particularly in strains associated with ophthalmoplegia (GBS and MFS) than in controls (Table 3). *orf10*, located next to *orf11* and encoding a CMP-sialic acid synthetase, also occurred more frequently in strains associated with ophthalmoplegia than in controls, but the difference was not statistically significant in the total group of neuropathy-associated strains (Table 3). Because both *orf10* and *orf11* are unique for classes A and B LOS loci, these findings are in accordance with our previous observations that the class A and B LOS loci are associated with neuropathy. We also demonstrated previously that *orf10*, but not *orf11*, is necessary for the biosynthesis of ganglioside mimics and the induction of antiganglioside antibodies in mice (5). Very recently, it was demonstrated that the product of *orf11* has sialate-*O*-acetyltransferase activity and catalyzes the transfer of *O*-acetyl groups onto oligosaccharide-bound sialic acid (7). However, whether or not this sialate-*O*-acetyltransferase plays a functional role in the pathogenesis of GBS remains to be determined.

We also investigated the correlation between the presence of individual LOS biosynthesis genes and the expression of ganglioside-like structures. Three genes were associated with the occurrence of a GQ1b-like epitope in the bacterial LOS, *orf7ab* (*cst-II*), *orf10*, and *orf11* (Table 4). Thus, the previously

TABLE 3. Association of gene presence with disease potency as determined for GBS/MFS-associated strains versus controls[a]

Genomic region	No. (%) of strains associated with:			No. (%) of controls (n = 18)	P value[b]
	GBS-O (n = 17)	GBS + O/MFS (n = 7)	GBS/MFS (n = 24)		
ORF3	16 (94)	7 (100)	23 (96)	15 (83)	NS
ORF4	14 (82)	7 (100)	21 (88)	14 (78)	NS
ORF5	15 (88)	7 (100)	22 (92)	17 (94)	NS
ORF14	2 (12)	0 (0)	2 (8)	5 (28)	NS
ORF15	2 (12)	0 (0)	2 (8)	5 (28)	NS
ORF7a	12 (71)	7 (100)	19 (79)	10 (56)	NS/NS (0.06)/ NS
ORF7b	2 (12)	0 (0)	2 (8)	5 (28)	NS
ORF8	14 (82)	7 (100)	21 (88)	15 (83)	NS
ORF9	14 (82)	7 (100)	21 (88)	16 (89)	NS
ORF5/10	2 (12)	0 (0)	2 (8)	4 (22)	NS
ORF10	12 (71)	7 (100)	19 (79)	9 (50)	NS/0.027/NS (0.1)
ORF11	12 (71)	7 (100)	19 (79)	7 (39)	0.09/0.008/ 0.011
ORF16	2 (12)	0 (0)	2 (8)	5 (28)	NS
ORF17	2 (12)	0 (0)	2 (8)	5 (28)	NS

[a] GBS-O, GBS without ophthalmoplegia; GBS + O/MFS, GBS with ophthalmoplegia or MFS; GBS/MFS, all neuropathies. *orf1, orf2, orf12*, and *orf13* were detected in all strains and therefore were not relevant for statistical analysis. *orf6* was not subjected to statistical analysis because its detection with PCR/hybridization techniques was not reliable, probably due to cross-hybridization with gene homologues and sequence heterogeneity. ORF, open reading frame.

[b] GBS-O, GBS + O/MFS, and GBS/MFS versus controls were tested, respectively. Only p values of ≤ 0.1 are given. NS, not significant.

described association between the *cst-II* gene and the expression of a GQ1b-like epitope was confirmed in the present study (8, 11). Furthermore, we found that all genes that are unique for classes A and B or class A, B, and C LOS loci were significantly associated with the expression of a ganglioside mimic (Table 4), which is concordant with the observation that only the class A, B, and C LOS loci contain the genes that are necessary for the biosynthesis of ganglioside mimics.

Variation in gene content is only one of the five known mechanisms that allow *C. jejuni* to vary the structure of its LOS (4). Other mechanisms, which include phase variation due to homopolymeric tract polymorphism and single or multiple mutations, may be detected by RFLP analysis of the LOS biosynthesis genes. This approach enables the screening of large groups of strains for polymorphism in multiple genes. The number of RFLP types that were found in the current study varied strongly per gene (Table 2). Interestingly, most genes in the class C strains were very homogeneous, with only one or two different RFLP types. This is in contrast with the large number of RFLP types that were observed for many genes in the class A and B strains. It is possible that the class C locus has evolved more recently than the less homogeneous class A and B loci (3). Considerable heterogeneity with more than six RFLP types per gene was detected in *orf1* (*waaC*), *orf2*, *orf6* (*cgtB*), *orf7ab* (*cst-II*), *orf9* (*neuC*), *orf10* (*neuA*), *orf12*, and *orf13* (*waaF*). These genes are involved in different steps of LOS biosynthesis, including the biosynthesis and transfer of sialic acid, a crucial component of gangliosides (Table 1). Such variation on the genetic level may imply functional differences in the products of these genes, leading to qualitative or quantitative variation in expression of ganglioside mimics. It has been demonstrated previously that specific polymorphism in the *cst-II* gene determines whether the LOS contains mono-NeuAc (the "GM1-like") or di-NeuAc (the "GQ1b-like") ganglioside (4). However, in this study, we did not detect an association between the *cst-II* RFLP type and the mono- or disialylated LOS (results not shown). This may be due to the limited sensitivity of RFLP analysis compared with that of DNA sequence analysis. For the other genes, we did not find either a specific RFLP type that was associated with neuropathy or the expression of ganglioside mimics (results not shown). It may, therefore, be necessary to perform elaborate DNA sequence analysis to determine the putative role of point mutations in the expression of ganglioside mimics and pathogenesis of post-*Campylobacter* infection neuropathy.

In conclusion, PCR-RFLP analysis demonstrated considerable variation in gene content and overall sequence heterogeneity in the *C. jejuni* LOS biosynthesis locus. We confirmed

TABLE 4. Association of gene presence with the expression of ganglioside mimics on the LOS[a]

Genomic region	Expression of ganglioside mimics								
	GM1-like epitope		P value	GQ1b-like epitope		P value	Any ganglioside epitope		P value
	+ (n = 15)	− (n = 27)		+ (n = 11)	− (n = 31)		+ (n = 30)	− (n = 12)	
ORF3	14	24	NS	10	28	NS	29	9	NS (0.06)
ORF4	14	19	NS	10	23	NS	29	4	<0.001
ORF5	15	22	NS	11	26	NS	30	7	<0.001
ORF14	4	3	NS	0	7	NS	6	1	NS
ORF15	4	3	NS	0	7	NS	6	1	NS
ORF7a	11	18	NS	11	18	0.009	24	5	0.026
ORF7b	4	3	NS	0	7	NS	6	1	NS
ORF8	14	22	NS	11	25	NS	29	7	0.005
ORF9	15	22	NS	11	26	NS	30	7	<0.001
ORF5/10	3	3	NS	0	6	NS	5	1	NS
ORF10	11	17	NS	11	17	0.008	24	4	0.009
ORF11	10	16	NS	11	15	0.003	23	3	0.004
ORF16	4	3	NS	0	7	NS	6	1	NS
ORF17	4	3	NS	0	7	NS	6	1	NS

[a] Only P values of <0.1 are given. P values of <0.05 are considered significant. NS, not significant. *orf1, orf2, orf12*, and *orf13* were detected in all strains and therefore were not relevant for statistical analysis. *orf6* was not subjected to statistical analysis because its detection with PCR/hybridization techniques was not reliable, probably due to cross-hybridization with gene homologues and sequence heterogeneity. ORF, open reading frame. Values are number of strains with presence (+) or absence (−) of indicated ganglioside mimics on LOS.

and extended previous observations that specific LOS biosynthesis genes are associated with neuropathy and the biosynthesis of ganglioside mimics. RFLP analysis did not demonstrate sequence heterogeneity within genes that was associated with neuropathy or the expression of ganglioside mimics. This technique may not be suitable to detect such polymorphism due to its limited sensitivity.

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