

## Can *Campylobacter coli* induce Guillain-Barré syndrome?

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*Campylobacter jejuni* enteritis is the most frequently identified infection preceding the Guillain-Barré syndrome (GBS) and neural damage is thought to be induced through molecular mimicry between *C. jejuni* lipo-oligosaccharide

(LOS) and human gangliosides [1]. It has been questioned whether or not other *Campylobacter* species, including *C. curvus*, *C. upsaliensis* and *C. coli*, could be similarly involved [2–4]. This is relevant because it would imply that bacterial factors considered important in the aetiology of GBS crossed species barriers. Two prior reports have appeared where *C. coli* was putatively associated with a case of GBS [2, 3].

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We here present two female patients with GBS, one from the Netherlands (patient GB50) and one from France (patient 664H2004). From a faecal specimen obtained for both patients, a *C. coli* strain was isolated. On the basis of surface protein profiling, the strains were unequivocally demonstrated to belong to the species *C. coli* (results not shown). The strains were encoded GB50 and 664H2004, respectively, and stored at  $-80^{\circ}\text{C}$ . For patient GB50, a serum sample obtained at the acute GBS phase was available. This sample was also stored at  $-80^{\circ}\text{C}$ .

Strains were grown on Mueller-Hinton agar at  $37^{\circ}\text{C}$  for 48 h, after which DNA was extracted, as described by Pitcher et al. [5]. Amplified fragment length polymorphism (AFLP) analysis was performed, as described by Duim et al. [6]. In brief, 1  $\mu\text{g}$  of genomic DNA was digested with the *HindIII-HhaI* restriction enzyme combination and site-specific adaptors were ligated to the restriction fragments. Primers complementary to the adaptor and restriction site sequence were used in pre-selective and selective polymerase chain reaction (PCR) amplifications. The amplified and fluorescently labelled fragments were loaded on an ABI Prism 377 automated sequencer. GeneScan version 3.1 (Applied Biosystems) was used for data collection, and the AFLP profiles were imported, using the CrvConv filter, in BioNumerics 4.61 (Applied Maths, Belgium) for normalisation and further analysis. The obtained AFLP profiles were included in an in-house AFLP reference frame, containing

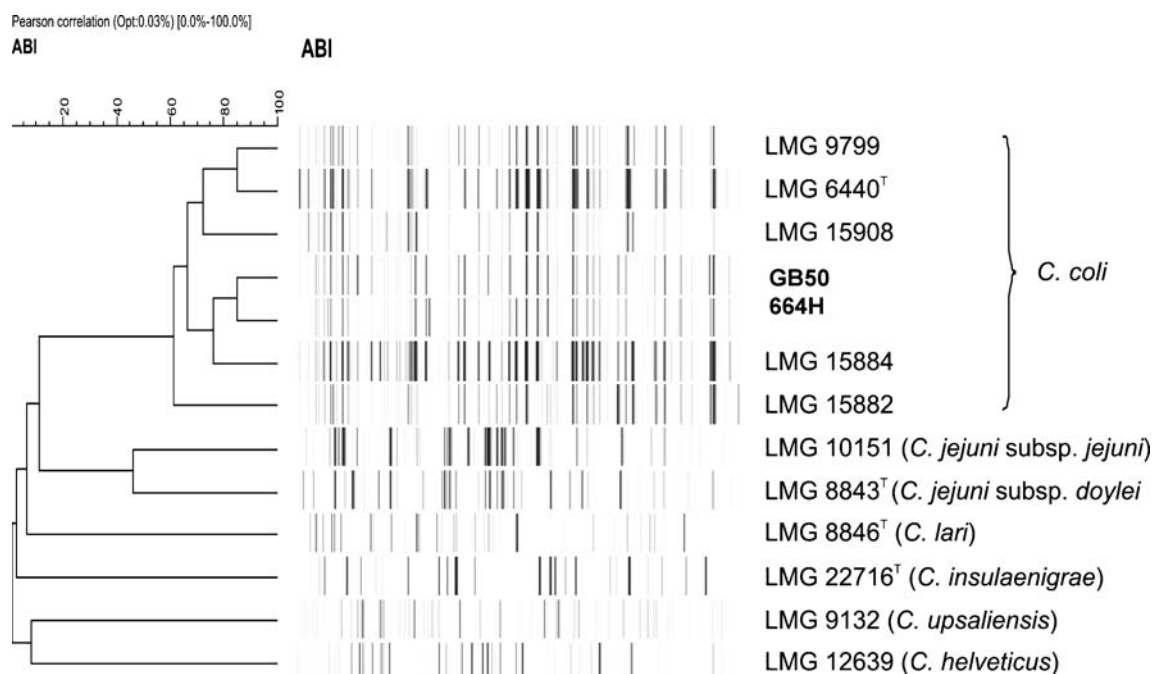
profiles from all known *Campylobacter* species. Similarity between the normalised fingerprints was determined by the Pearson product moment correlation coefficient and a UPGMA dendrogram was constructed. The profiles from both isolates clearly formed a cluster together with *C. coli* reference strains (Fig. 1).

Using LOS gene cluster-specific PCR tests, the 664H2004 strain was demonstrated to harbour a B-type gene cluster [7]. For GB50, neither the *cst2* nor the *cgtA* gene was shown to be present. This convincingly demonstrates that this strain does not harbour the neuropathy-associated LOS gene cluster types A, B or C. Using primers based on the *C. coli* RM2228 LOS gene cluster, we amplified the LOS locus of GB50, but we could not amplify the LOS locus of 664H2004. Complete sequencing of the novel GB50 LOS gene cluster revealed 12 open reading frames (ORFs) that included five putative glycosyltransferases and a few ORFs seemingly unrelated to LOS biosynthesis (Table 1).

The LOS of *C. coli* strain GB50 did not bind cholera toxin, hence, the presence of a GM1-like ganglioside mimic could be excluded. The acute phase pre-treatment serum from patient GB50 showed a high level of IgG activity to the LOS from the GB50 strain (Table 2). This activity was significantly higher than in the serum from ten healthy blood donors. In addition, in the serum from patient GB50, IgM activity was found for this LOS, although it was less than the IgG activity. Some of the healthy blood donors also showed this elevated level of IgM. Probing the LOS with six specific monoclonal anti-ganglioside antibodies

(DG-1, DG-2, TBG-3, EG-7, EG-3 and EG-1) did not reveal any reactivity. These monoclonal antibodies were raised by immunisation with *C. jejuni* LOS and bound to various (combinations of) gangliosides (Table 2). Interestingly, regular mass spectrometry analysis on O-deacylated *C. coli* LOS [1] did not reveal a structural overlap between this LOS and the previously determined *C. jejuni* LOS structures. It appeared that the GB50 LOS did not contain sialic acid based on MS/MS analysis. The LOS from the 664H2004 strain was shown to contain di-NeuAc based on MS/MS analysis.

The serum from patient GB50 was tested in a standardised enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to the gangliosides GM1, GM2, GD1a, GD1b, GD3 and GQ1b [8]. Serum was positive for IgG, IgM and IgA antibodies to the gangliosides GM1 and, to a lesser extent, to GD1b (Table 2). These gangliosides share the terminal Gal( $\beta$ 1–3)GalNAc to which these antibodies are probably directed. Accordingly, the serum from this patient contained high IgG, IgM and IgA activity to the non-ganglioside glycolipid asialo-GM1, which has the same terminal disaccharide. Such a structure, however, was not identified in the LOS of the *C. coli* isolate from this patient. This suggests that this patient might have been co-infected with another organism expressing a GM1 mimic, possibly a *C. jejuni* strain, responsible for a non-GBS-related induction of a cross-reactive antibody response to GM1. In inhibition ELISA experiments, anti-GM1 IgG activity in 1:400 dilutions of serum from GB50 was not reduced by LOS from this strain in concentrations of up to



**Fig. 1** Amplified fragment length polymorphism (AFLP) analysis of the two *Campylobacter coli* strains isolated from patients GB50 and 664H2004. Note that, despite their diverse geographic origins, these strains cluster, but clearly fall within the *C. coli* cluster

**Table 1** Information on the lipo-oligosaccharide (LOS) gene cluster for strain GB50 (GenBank accession number EU374214)

Source sequence		1...11261	
Nucleotide position	Gene orientation	Gene annotation	Putative gene function
1–108	+	waaC	Heptosyltransferase I
101–988	+	htrB	Lipid A biosynthesis acyltransferase
985–2538	+	orf3	Putative glycosyltransferase
2535–3587	+	orf4	Putative glycosyltransferase
3584–4783	–	orf5	Putative glycosyltransferase
4911–6215	+	orf6	Putative CDP-glycerol:polyglycerophosphatase
6212–7246	+	orf7	Hypothetical protein
7316–8335	+	orf8	Transposase-like insertion element
8214–8924	+	orf9	Hypothetical protein
8917–9837	+	orf10	Putative DNA methyltransferase
10021–10437	+	orf11	Putative glycerol-3-phosphate cytidyltransferase
10456–11261	–	waaV	Putative glycosyltransferase

200 µg/ml, demonstrating that these antibodies do not cross-react with the LOS from the *C. coli* isolate from the patient. In control studies, this antibody activity was reduced to a level of less than 5% by pre-incubation with LOS from a *C. jejuni* isolate GB2 from another GBS patient, in which mimicry with GM1 was previously demonstrated by MS [9].

Overall, we have comprehensively characterised two *C. coli* strains isolated from GBS patients with a variety of state of the art technologies. One strain (664H2004) harboured disialylated LOS, but, unfortunately, no serum was available to measure either anti-ganglioside or anti-LOS antibodies in the GBS patient herself. The other strain

from the Dutch patient (GB50) had non-sialylated LOS, which showed no binding to cholera toxin or to monoclonals specific for several gangliosides. However, the serum from patient GB50 from which *C. jejuni* GB50 was isolated did contain antibodies to the LOS from this strain, indicating that this infection led to a specific immune response to *C. coli* strain GB50. The role of this immune response in the pathogenesis of GBS is currently unknown, although other examples of GBS patients infected with non-ganglioside mimicking strains of *C. jejuni* have been described [10]. The serum from GB50, however, also contained antibodies to GM1 and GD1a, which do not

**Table 2** Characterisation of *Campylobacter coli* strains isolated from two patients with Guillain-Barré syndrome (GBS)

	GB50	664H2004
Genotyping strains		
LOS biosynthesis cluster	Non-typable (no sialic acid incorporating enzymes encoded)	Class B (does contain Cst2)
Structure of LOS (MS/MS analysis)		
Presence sialic acids	–	Di-NeuAc
Binding studies with LOS		
Cholera toxin B-subunit	–	–
Anti-ganglioside mAbs <sup>1</sup>	–	–
IgG in serum patient GB50	++ <sup>2</sup>	–
IgM in serum patient GB50	+ <sup>2</sup>	–
Serum anti-ganglioside antibodies <sup>3</sup>		
IgG	GM1 (6400), GD1b (400)	n.a.
IgM	GM1 (800), GD1b (200)	n.a.
IgA	GM1 (200), GD1b (100)	n.a.
Cross-reactivity serum antibodies		
Anti-GM1 IgG in serum GB50 <sup>4</sup>	–	–

n.a.; not available

<sup>1</sup> Monoclonal antibodies DG-1 (to GM1), DG-2 (to GM1/GD1b/GA1), TBG-3 (to GD1a), EG-7 (to GD1b), EG-3 (to GQ1b) and EG-1 (to GT1a/GQ1b)

<sup>2</sup> Significantly higher than antibody activity to this LOS in serum from ten healthy blood donors

<sup>3</sup> Serum IgG, IgM and IgA tested to GM1, GM2, GD1a, GD1b, GD3 and GQ1b

<sup>4</sup> The cross-reactivity of 1:400 diluted serum anti-GM1 IgG antibodies from patient GB50 to LOS from *C. coli* GB50 and 664H2004 were determined by inhibition enzyme-linked immunosorbent assay (ELISA)

cross-react with LOS from GB50, as expected, although they do cross-react with LOS from a *C. jejuni* with a known GM1 mimic. These serological findings in patient GB50 strongly suggest that this patient was infected with at least two micro-organisms, one of which induced the anti-ganglioside antibodies leading to GBS (possibly a *C. jejuni* strain), and *C. coli*, which also induced an immune response, but probably did not trigger GBS.

In summary, we could not produce any evidence that molecular mimicry plays a role in the pathogenesis of GBS that is preceded by a *C. coli* infection. It is still not clear whether *C. coli* isolated from the faeces of GBS patients can play a role in the development of GBS. Additional GBS patients and their *C. coli* strains need to be reported and characterised in detail in order to better understand the pathogenesis of GBS in these patients.

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