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Comparison of *Campylobacter jejuni* Isolates Implicated in Guillain-Barré Syndrome and Strains That Cause Enteritis by a DNA Microarray

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We asked whether *Campylobacter jejuni* isolated from patients with Guillain-Barré syndrome (GBS) differ from isolates isolated from patients with uncomplicated gastrointestinal infection using DNA microarray analysis. We found that specific GBS genes or regions were not identified, and microarray analysis confirmed significant genomic heterogeneity among the isolates.

Campylobacter jejuni subsp. *jejuni* (referred to as *C. jejuni* hereafter) is one of the most common causes of bacterial infectious diarrhea and is estimated to cause approximately 2.5 million cases of infectious diarrhea per year in the United States (5). In most cases of *C. jejuni* infection, patients develop acute gastroenteritis and resolve the infection without complications. There is a small subset of patients with *C. jejuni* infection who develop the postinfectious neurologic disorder, Guillian-Barré syndrome (GBS), an acute, immune system-mediated polyneuropathy that leads to ascending paralysis (9, 22). *C. jejuni* has been implicated as one of the leading infectious agents associated with this syndrome (15).

Although not completely understood, the development of GBS after *C. jejuni* infection is thought to be related to molecular mimicry by anti-*Campylobacter* lipooligosaccharide (LOS) antibodies that cross-react with ganglioside epitopes on neural tissue (22). *C. jejuni* express a number of ganglioside-like moieties in the outer LOS core region, including GM_1-like and GD_{1a}-like structures, and some patients with *Campylobacter*-associated GBS develop antibodies to these and other ganglioside-like structures (14). The resulting antibodies are thought to target relevant epitopes in peripheral nerve tissue. causing demyelination and axonal damage (7, 8).

With the availability of the *C. jejuni* sequence (18), genomic and expression analyses using a *C. jejuni* DNA microarray have been made possible (3, 11, 20). In order to better understand the pathogenesis of *C. jejuni* associated with GBS, we compared a collection of isolates from patients with GBS with isolates from patients with uncomplicated gastrointestinal infection using an open reading frame (ORF)-specific *C. jejuni* DNA microarray. The aim of this study was to identify unique differences within the genome that were associated with GBS.

Twelve strains of *C. jejuni* associated with GBS were compared to 12 strains associated with uncomplicated gastrointestinal illness (Table 1). A variety of serotypes are represented in both groups of isolates. Some of the isolates were also previously characterized by multilocus enzyme electrophoresis and represent a number of different electrophoretic types (4, 16).

TABLE 1. Bacterial isolates used in microarray study

Isolate designation[a]	Strain	Country of origin[b]	HS serotype(s)
GBS-associated isolates			
1	HB93-6	P.R. China	2
2	DVL5906	Denmark	2
3	JHU2	USA	4
4	DVL5610	Denmark	4
5	HB93-10	P.R. China	5
6	HB93-13 (ATCC 700297)	P.R. China	19
7	OH4384	Japan	19
8	INP7 (ATCC BAA-528)	Mexico	19
9	HB97-34	P.R. China	37
10	INP16	Mexico	37
11	INP21 (ATCC BAA-530)	Mexico	41
12	INP59 (ATCC BAA-529)	Mexico	41
Enteritis-associated isolates			
14	DVL5384	Denmark	4
15	DVL5529	Denmark	4
16	INP66	Mexico	5
17	D3002	USA	19
18	KB1428	Japan	19
19	INP15	Mexico	19
20	DVL5443	Denmark	37
22	DVL5671	Denmark	41
23	D3007	USA	2
24	81-176	USA	23,36
25	D3027	USA	2
26	DVL5558	Denmark	41
	NCTC 11168	UK	2

[a] Isolate designations used in Fig. 1 and 2.
[b] Abbreviations: P.R. China, People's Republic of China; USA, United States; UK, United Kingdom.

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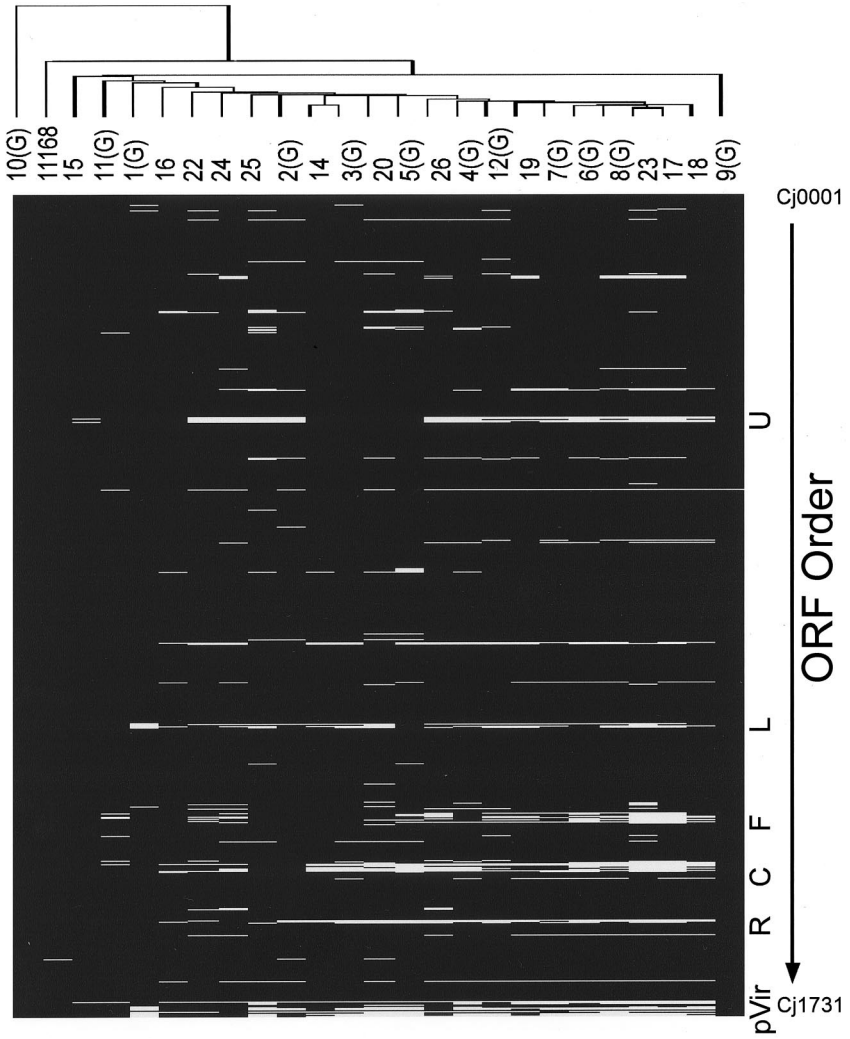

FIG. 1. Comparison of *C. jejuni* isolates to reference strain 11168. Isolates are listed across the top of the schematic by the designations given in Table 1. Strains associated with GBS patients are indicated by (G) after the isolate designation. Conserved genes (black) and divergent or absent genes (white) are indicated. The dendrogram represents a cluster analysis of strains as described in the text. Isolates found within the same node are considered more related than isolates found outside the node. ORF order abbreviations: U, *uxaA*/sugar modification; L, LOS; F, flagella; C, capsule; R, type I restriction enzyme proteins R, M, and S.

DNA microarray analysis was performed blindly, without knowledge of strain designation or clinical outcome. Genomic DNA was prepared using a Wizard Prep kit per the manufacturer's instructions (Promega). All strains were compared to a reference strain composed of DNA from the sequenced strain NCTC 11168 and DNA from the virulence plasmid (pVir) isolated from strain 81-176 (1, 2); the pVir DNA was added to approximate one copy of plasmid per genome. Hybridization was performed using 500 ng of chromosomal DNA from each isolate and the reference array as previously described (11, 19). All strains were hybridized to the microarray in duplicate. After the hybridizations were completed, the microarrays were scanned using an Axon scanner and Genepix 3.0 software (Axon Instruments, Redwood City, Calif.). The data were submitted to the Stanford Microarray Database and retrieved using the following filters: (i) spots with Ch1 pixels > background level plus 1 standard deviation and (ii) values of failed spots are set at 0. The microarray replicates were shown to be reproducible by correlation coefficients and averaged after the data was analyzed and transformed into binary format using a program called Genomotyping Analysis (GACK) (10) (http://cmgm.stanford.edu/falkow/whatwedo/software/software.html). This program calculates an idealized normal distribution curve for each array and assigns a binary value to each data point on the microarray, depending on an estimated probability of a gene being present or absent in a given strain relative to the same probability in reference strain 11168. We designated genes that had an equal probability of being present or absent as present in the analysis. Gene identification used the annotated *C. jejuni* genome available on the Sanger Centre website (http://www.Sanger.ac.uk/Projects/C_jejuni). The microarray data were subjected to hierarchical clustering between arrays with the CLUSTER program, and the results were presented using the TREEVIEW program (19).

Microarray analysis did not identify discrete groups of isolates or any unique features within the genome of the *C. jejuni*

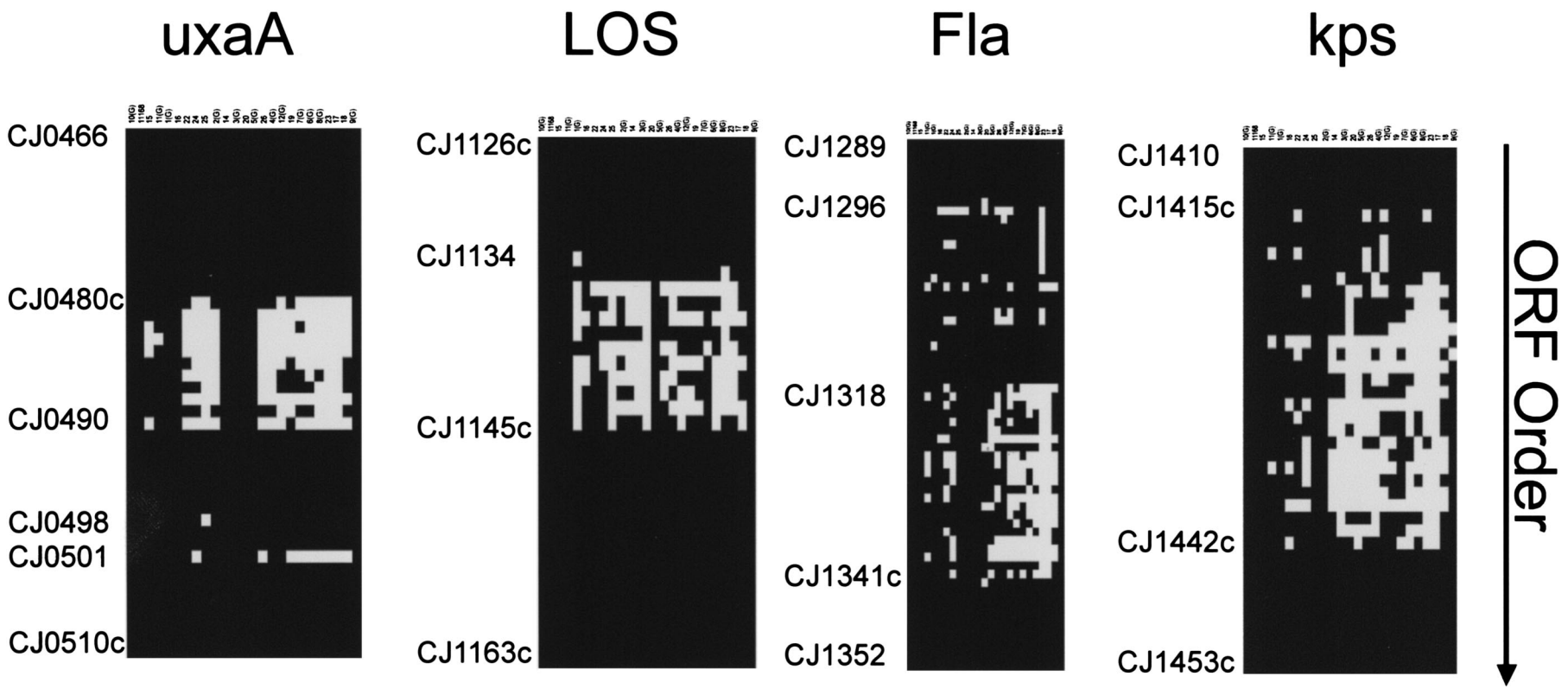

FIG. 2. Detailed view of four major variable regions identified by DNA microarray analysis of *C. jejuni* isolates associated with GBS and enteritis. Isolates are listed across the top of the schematic by the designations given in Table 1. Strains associated with GBS patients are indicated by (G) after the isolate designation. Conserved genes within the genome relative to reference strain 11168 (black) and genes that are divergent or absent from the reference strain (white) are indicated. Fla, flagella; kps, capsule.

isolates associated with GBS. Cluster analysis of the groups of isolates with the same serotype was also performed and failed to segregate isolates associated with GBS from isolates associated with gastroenteritis (Fig. 1). The pVir plasmid was not detected in any of the strains studied. However, pVir has been reported to contain several chromosomal homologues, such as *fliH* and a gene with unknown function that could account for conserved regions within the pVir section of the array (2). Our results suggest that the differences, if any, between strains of *C. jejuni* that were associated with GBS versus those associated only with enteritis were not at the level of the genome. However, we cannot determine from this study whether the lack of hybridization in various regions represents the absence of a particular gene or nucleotide divergence within an existing gene. Additionally, differences due to the presence of genetic elements in either the GBS- or enteritis-related isolates would not necessarily be detected because of the absence of such elements in the genome of the strain used to construct the microarray.

The divergent regions within the *C. jejuni* genome involve well-defined regions (Fig. 2). Compared with strain 11168, GBS- and enteritis-associated strains had 91.4 and 90.9% ORFs in common, respectively. Compared with enteritis-associated strains, GBS-associated isolates had 89% ORFs in common. Approximately 11% of the ORFs contained in strain 11168 were absent in the GBS- and enteritis-associated strains. Many of the genes that were divergent or absent in the GBS- and enteritis-associated strains were in the same region, regardless of how the strains were compared. These regions of the genome include genes involved in production of the capsule, flagella, and LOS. In addition, there were areas of heterogeneity in the *uxaA* locus involved with sugar transport and metabolism of hexuronates leading to the formation of D-2-keto-3-deoxy-D-gluconate and is part of the *exu* regulon (12). There were other less-defined areas of heterogeneity in some strains compared to the genome sequence that were mostly ORFs without an identified function thus far. Variation in type I modification and restriction genes were also observed among the isolates. While the reasons for this variation are unknown, such variation has been observed by our group (Erin Gaynor, personal communication) and we can only speculate that since *C. jejuni* is naturally transformable, it may have evolved multiple mechanisms to avoid incorporating foreign DNA. Taken together, these results suggest that many *C. jejuni* strains undergo extensive surface protein modification. These results are similar to findings obtained from microarray analyses of a series of unrelated strains, as well as in bacteria that were epidemiologically linked (3, 11).

A comparison of *C. jejuni* strains associated with GBS has not previously been studied by DNA microarray analysis. We are also not aware of studies using subtractive hybridization between GBS- and enteritis-related isolates that could reveal additional differences not detected with the microarray approach in the present study. Certain serotypes of *C. jejuni* are known to be overrepresented in cases of campylobacter-induced GBS, such as HS:19 and HS:41, suggesting that strains expressing these serotypes might contain specific virulence factors relevant to GBS (15). Previous molecular typing studies have not, however, identified unique features of GBS-associated strains (6), although the sialyltransferase gene, *cstII*, has been identified as being overrepresented in GBS-related strains of *C. jejuni* (16, 17, 21). On the other hand, there is evidence from a recent study that *C. jejuni* isolates associated with GBS preferentially express GD_{1a}-like structures, regardless of the bacterial serotype (17). Possibly, posttranslational modification of the surface structures might account for the discordant results, suggesting that GBS-associated strains might encode regulatory or modification loci that were not detected by microarray analysis. Such modifications might be an important mechanism in the development of molecular mimicry. Alternatively, differential expression of *Campylobacter* virulence factors might be operative and would be disclosed by expression analysis. Host susceptibility is likely an important factor in the development of GBS (13), and further characterization of the interaction between the host and *C. jejuni* is needed to better understand the pathogenesis of *Campylobacter*-induced GBS.

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