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CONCISE COMMUNICATION

Campylobacter jejuni O:19 serotype-associated Guillain–Barré syndrome in a child: the first case reported from Greece

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We present a case of Guillain–Barré syndrome (GBS) following *Campylobacter jejuni* HS serotype O:19 infection in a child. Antibodies against *C. jejuni* and autoantibodies to the peripheral nerve gangliosides GM1 were positive, a pattern correlating well with the existence of an inflammatory neuropathy like GBS. The patient shared the HLA-B35 and HLA-DR8 antigens, which have been found to be increased in GBS patients with previous *C. jejuni* infection. As this is the first diagnosed *C. jejuni*-associated GBS case reported from Greece, further clinical and epidemiologic investigations are warranted.

Keywords Guillain–Barré syndrome, *Campylobacter jejuni*, *Campylobacter jejuni* O:19 serotype

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Campylobacter jejuni is one of the most commonly recognized causes of bacterial gastroenteritis. The organism is transmitted via contaminated food and water, and colonizes the intestinal tract of many animals, including poultry, cattle and swine, without causing any illness [1]. Campylobacter enteritis is an acute diarrheal disease with clinical manifestations like those of other acute bacterial gut infections, and a definitive diagnosis can be made only by detecting the organism in the feces [2]. The disease is self-limiting, lasting for 3–5 days. The rare extraintestinal infections include bacteremia, hepatitis, cholecystitis, pancreatitis, abortion and perinatal infection, hemolytic–uremic syndrome, nephritis, proctitis, peritonitis, myocarditis, and splenic rupture [3].

Postinfectious complications are triggered by immunopathologic mechanisms and include reactive arthritis, Reiter's syndrome [4,5], and the autoimmune-mediated disorders of the peripheral nervous system, such as Guillain–Barré syndrome (GBS) and Miller–Fisher syndrome [6,7]. The last two may arise as the result of production of antibodies to *C. jejuni* lipopolysaccharides, which cross-react with gangliosides or other structures present in peripheral nerves [8].

Several heat-stable (HS) O-serotypes (Penner serotypes) of *C. jejuni* have been isolated from GBS patients, and include O:1, O:2, O:4, O:5, O:10, O:16, O:19, O:23, O:37, O:41, O:44, and O:64 [9]. According to clinical and epidemiologic reports from the USA, Japan, China and Mexico, serotype O:19—a serotype causing mild gastroenteritis—seems to be over-represented in *C. jejuni*-associated GBS patients [9]. Although the above-mentioned GBS-associated serotypes are distributed worldwide [9], very few data are available from Europe about the incidence of *C. jejuni*-associated GBS and the serotypes involved.

We present a case of GBS following *C. jejuni* HS serotype O:19 infection in a child. As far as we know, this is the first case reported from Greece.

An 11-year-old-male was admitted to the First Department of Pediatrics of Athens University, 'Aghia Sophia' Children's Hospital, because of muscle weakness of acute onset. The patient lives

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in a small town in central Greece. Family history, pregnancy, delivery as well as motor and mental development were normal. Ten days prior to admission, the patient had a cough that lasted for 3–4 days. Six days prior to admission, he complained of intense abdominal pain that lasted for 1 day. He was examined in the local hospital, but no firm diagnosis was established. Three days prior to admission, he complained of muscle pains involving mainly the calf muscles. The pain increased in intensity and, during the last day prior to admission, the patient was unable to walk.

On admission, his mental functions and his cranial nerves were normal. He was unable to walk and had a muscle weakness (grade 3 in the 0–5 scale) more prominent in the proximal muscles of the lower limbs. A milder weakness (grade 4) was found in the anterior tibial muscles and in the distal muscles of his arms. Tendon reflexes could be produced with great difficulty and were absent in the Achilles tendon bilaterally. Sensory examination was normal. The rest of the clinical examination was also normal.

Laboratory investigations were as follows: hemoglobin 13 g/dL, hematocrit 38%, white cell count 9000 μl, polymorphs 63%, lymphocytes 30%, and platelets 406 000/mL; creatin phosphokinase (CPK), aldolase, serum glutamate oxalacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), γ-glutamyl transpeptidase (GT) alkaline phosphatase, urea, creatinine and serum electrolytes were normal. Cerebrospinal fluid (CSF) (on admission) yielded the following findings: 4 leukocytes/mL, total protein 40 mg/dL, and glucose 55 mg/dL. A second CSF sample (on the 10th day of hospitalization) showed the following picture: 15 leukocytes/mL, total protein 138 mg/dL, and glucose 65 mg/dL.

In view of the probable diagnosis of GBS, the patient received from the first day of hospitalization a course of intravenous immunoglobulin at a dosage of 2 g/kg of body weight over a period of 2 days. The patient remained weak and unable to walk for 5 days. During that period, cervical rigidity was also noticed. After that period, a steady improvement was seen. From the 11th day after his admission, in view of the positive stool cultures for *C. jejuni* (see below), he received oral erythromycin 1500 mg daily. On his 15th day of hospitalization, he had only mild weakness (grade 4) of the proximal muscles of the lower limbs, and minimal weakness (grade 5–) of the muscles of his hands. He was discharged on the 17th day after admission. When re-examined 1 month later, he had no muscle weakness and his tendon reflexes were brisk.

Special laboratory investigations for differential diagnosis included antibodies to cytomegalovirus (CMV), *Mycoplasma pneumoniae*, Epstein–Barr virus (EBV) and rubella virus; results for all of these were negative. Because of the clinical signs and the history of a preceding gastrointestinal infection, on the day of admission a stool specimen was sent for bacteriologic and virologic (poliovirus) examination to the hospital laboratory, as well as to the National Reference Laboratory for *Campylobacter* Serotyping of the Medical School of Athens, in order to be processed with enrichment culture isolation techniques for *Campylobacter*. The sample was plated on Skirrow's medium and inoculated in Preston enrichment broth. Cultures were incubated at 42 °C for 48 h in a micro-aerophilic atmosphere (Biomérieux, Marcy l'Etoile, France), and then Preston broth was subcultured on Skirrow's medium and incubated for 48 h at 42 °C. Primary culture was negative for *Campylobacter*, but the subculture of Preston broth yielded a confluent growth of typical colonies for *Campylobacter* which were oxidase and catalase positive. The strain was identified as *C. jejuni* subsp. *jejuni* through microscopic examination of fuchsin-stained culture smears (S-shaped and curved rods), positive hippurate hydrolysis, and differentiation with the commercial system API-Campy (Biomérieux). Antimicrobial susceptibility was tested by MIC determination with E test (AB Biodisk, Solna, Sweden). The strain was susceptible to erythromycin, nalidixic acid, ciprofloxacin, ampicillin, amoxicillin–clavulanic acid, and gentamicin. Serotyping by the Penner method using a commercial serotyping kit (Denka Seiken, Tokyo, Japan) characterized the strain as serotype O:19. Stool cultures requested on days 10 and 19 after admission were negative for *Campylobacter*.

Three serum samples at about 7-day intervals were requested for determination of antibodies against *C. jejuni* and autoantibodies to the peripheral nerve gangliosides. Antibodies to *C. jejuni* were determined by the indirect immunofluorescent assay using as antigen methanol-fixed whole bacteria of the patient's isolate (*C. jejuni* O:19). Five pools, each consisting of 50 sera of healthy blood donors, were used as controls. Autoantibodies to peripheral nerve gangliosides, including monosialoganglioside (GM1), IgG and IgM, sulfatides,

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disialoganglioside (GD1a) and asialoganglioside (ASGM1), were determined by the enzyme-linked immunosorbent assay (ELISA), using methanol-fixed antigen-coated 96-well microElisa plates and peroxidase-conjugated secondary anti-IgG and IgM goat antibodies [10].

On day 4 after admission, antibodies to *C. jejuni* were positive in a serum dilution (titer) of 1 : 64, while the control sera had titers of up to 1 : 8. On days 10 and 19, the antibody titers were still positive at 1 : 128. Anti-GM1-IgG antibodies were positive in the first serum sample (day 4) in significant titers (1 : 16 000, with negative controls 1 : 400) and shifted to negative (1 : 400) on days 10 and 19 after admission. All other anti-ganglioside antibodies were negative. The seropositivity pattern to peripheral nerve gangliosides correlated well with the existence of an inflammatory neuropathy, particularly GBS.

The importance of host factors, such as HLA antigens, in the pathogenesis of *C. jejuni*-associated GBS has been pointed out. In some research studies, however, subgrouping of GBS patients according to *C. jejuni* infection revealed a tendency for an increased frequency of HLA-DRB1*0803 [11], or an increased frequency of HLA-B54 and –CW1 [12], HLA-DQB1*03 [13] and HLA-B35 [14]. Thus, HLA haplotypes of our patient were determined in order to reveal any matches with the worldwide data.

HLA class I and II phenotypes and genotypes were established by serologic and molecular techniques, respectively. HLA class I (A, B, C) and II (DR, DQ) specificities were determined by serologic (NIH standard technique) and molecular techniques at the genomic level, for all the specificities included in the HLA Nomenclature of 1998 [15] (Elpha-Biotest, High Resolution, Germany; Pel. Freeze, Medium Resolution, USA; Inno-Lipa, Murex, Belgium).

Our patient shared the HLA-B35 and HLA-DR8 antigens that have been found in increased prevalence in GBS patients with previous *C. jejuni* infection, both in Japanese groups of patients [11,14].

The neurologic signs of a peripheral neuropathy, the history of a preceding gastrointestinal infection, the isolation of *C. jejuni* serotype O:19 from the patient's stool, the positive anti-GM1-IgG antibodies and typical immunogenetic profile are typical of a *C. jejuni*-associated GBS. So far, there have been no reports about the incidence of such cases in Greece. As far as we know, *C. jejuni* Penner serotype O:19 is one of the most commonly encountered in GBS in the USA and Japan [9]. In a *C. jejuni* serotyping study of strains from infected children in Athens, serotype O:19 accounted for 1.6% of all serotypes (unpublished observations), a rate commensurate with those reported from the USA and Japan [9]. However, in a British study, none of four *C. jejuni* isolates from GBS cases were O:19 [16].

The clinical entity 'GBS' can be divided into three forms: the acute inflammatory demyelinating polyneuropathy (AIDP), which has as its main characteristic feature the macrophage-mediated demyelination of the peripheral nerves; the axonal pattern—with axonal involvement—consisting of the acute motor axonal neuropathy (AMAN), and the rare acute motor-sensory axonal neuropathy (AMSAN); and Miller–Fisher syndrome, characterized by acute onset of ataxia (unsteadiness of gait, areflexia (loss of reflexes) and ophthalmoplegia (an inability to move the eyes) [17]. The differentiation between AIDP and AMAN is based on the different clinical, electrophysiologic and serologic manifestations. In the present case, the electrophysiologic manifestations could not be evaluated, as an electromyogram (EMG) had not been requested because of the mild clinical course of the disease and the quick recovery. This mild clinical course, in association with the presence of anti-GM1-IgG antibodies only, is more compatible with the less severe form of AIDP [9]. GBS following *C. jejuni* infection may be more severe than GBS following other inciting events [18]. However, the quick recovery of the patient in the present case may be due to the early onset of treatment with intravenous immunoglobulin.

Concerning the bacteriologic diagnosis, the role of enrichment broth (Preston broth) was remarkable in the isolation of *Campylobacter* from the stools of the patient. The use of enrichment culture in the investigation of patients with acute *Campylobacter* diarrhea has been contested, but it may be beneficial in instances where small numbers of organisms may be expected [19]. There is a lag time of 1–3 weeks between campylobacter infection and the onset of GBS. Since the median period of excretion of *Campylobacter* from stool samples is only 16 days [20], at the onset of GBS bacteria are either absent from stool, or present in very low numbers, and enrichment cultures enhance the possibility of detecting them.

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The over-representation of certain HLA alleles in GBS patients, especially in those with previous *C. jejuni* infection, has been estimated in population studies, in different ethnic groups. For the Greek population, there are no data currently available, but our ongoing association study seems to reveal a tendency for an increased frequency of HLA-B35, HLA-B44, HLA-DR11 and HLA-DR13 [21] in the GBS group as a whole. Thus, no inclusive results or comments can be elicited, before an association HLA study is completed, but the fact that our patient has, at the same time, two of the five HLA antigens that have been associated with *C. jejuni*-positive GBS patients, even with different immunogenetic backgrounds, supports the 'immunogenic' character of these antigens in the pathogenesis of GBS.

In many countries, special care is given to the elimination of *Campylobacter* from its natural reservoirs, especially poultry, in order to decrease the incidence of enteritis, as well as the incidence of the sequelae of infection. GBS is the most severe of them, in terms of clinical outcome and costs of treatment. As this is the first case reported from Greece, further clinical and epidemiologic investigations are warranted.

REFERENCES

1. Centers for Disease Control. *Campylobacter* isolates in the United States 1982–86. *MMWR* 1988; SS-2: 1.
2. Popovic-Uroic T, Gmajnicki B, Kalenic S, Vodopija I. Clinical comparison of *Campylobacter jejuni* and *C. coli* diarrhea. *Lancet* 1988; i: 176–7.
3. Skirrow M, Blaser M. Clinical aspects of campylobacter infection. In: Nachamkin I, Blaser M, eds. *Campylobacter*. Washington, DC: ASM Press, 2000: 69–88.
4. Peterson MC. Rheumatic manifestations of *Campylobacter jejuni* and *C. fetus* infection in adults. *Scand J Rheumatol* 1994; 23: 167–70.
5. Schaad UB. Reactive arthritis associated with campylobacter enteritis. *Pediatr Infect Dis* 1982; 1: 328–32.
6. Constant OC, Bentley CC, Denman AM, Lehane JR, Larson HE. The Guillain–Barré syndrome following campylobacter enteritis with recovery after plasmapheresis. *J Infect* 1983; 6: 89–91.
7. Molnar GK, Mertsilo J, Gerkko M. Guillain–Barré syndrome associated with campylobacter infection. *BMJ* 1982; 285: 652.
8. Speed B, Kaldor J, Cavanogh P. Guillain–Barré syndrome associated with *Campylobacter jejuni* enteritis. *J Infect* 1984; 8: 85–6.
9. Nachamkin I, Allos BM, Ho TW. *Campylobacter jejuni* infection and the association with Guillain–Barré syndrome. In: Nachamkin I, Blaser M, eds. *Campylobacter*. Washington, DC: ASM Press, 2000: 155–75.
10. Sadiq SA, Thomas FP, Kilidireas K *et al.* The spectrum of neurologic disease associated with anti-GM1 antibodies. *Neurology* 1990; 40: 1067–72.
11. Ma JJ, Nishimura M, Mine H *et al.* HLA and T-cell receptor gene polymorphisms in Guillain–Barre syndrome. *Neurology* 1998; 51: 379–84.
12. Koga M, Yuki N, Kashiwase K, Tadokoro K, Juji T, Hirata K. Guillain–Barre and Fisher's syndromes subsequent to *Campylobacter jejuni* enteritis are associated with HLA-B54 and CW1 independent of anti-ganglioside antibodies. *J Neuroimmunol* 1998; 88: 62–6.
13. Rees JH, Vaugham RW, Kondeatis E, Hughes RAC. HLA class II alleles in Guillain–Barre syndrome and Miller Fisher syndrome and their associations with preceding *Campylobacter jejuni* infection. *J Neuroimmunol* 1995; 38: 53–7.
14. Yuki N, Sato S, Tsuji S, Ohsawa T, Miyatake T. Frequent presence of antiGQ1b antibody in Fisher's syndrome. *Neurology* 1993; 43: 414–7.
15. Bodmer JG, Marsh SG, Albert ED *et al.* Nomenclature for factors of the HLA System, 1998. *Hum Immunol* 1999; 60: 361–95.
16. Rees JH, Soudain SE, Gregson NA, Hughes RAC. *Campylobacter jejuni* infection and Guillain–Barré syndrome. *N Engl J Med* 1995; 333: 1374–9.
17. Ho TN, McKhann GM, Griffin JW. Human autoimmune neuropathies. *Annu Rev Neurosci* 1998; 21: 187–226.
18. Vriesendorp FJ, Mishu B, Blaser M, Kosk CL. Serum antibodies to GM1 peripheral nerve myelin and *Campylobacter jejuni* in patients with Guillain–Barré syndrome and controls: correlation and prognosis. *Ann Neurol* 1993; 34: 130–5.
19. Nachamkin I. Microbiologic approaches for studying *Campylobacter* in patients with Guillain–Barré syndrome. *J Infect Dis* 1997; 176(suppl 2): S106–114.
20. Svedhem A, Kaijser B. *Campylobacter fetus* subspecies *jejuni*: a common cause of diarrhea in Sweden. *J Infect Dis* 1980; 142: 353–9.
21. Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor–recipient matching in cadaveric transplantations. *Tissue Antigens* 1992; 39: 225–35.

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