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Long-Term Persistence of a Discotheque-Associated Invasive *Neisseria meningitidis* Group C Strain as Proven by Pulsed-Field Gel Electrophoresis and *porA* Gene Sequencing

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A cluster of a *Neisseria meningitidis* serogroup C strain causing invasive disease was investigated. Five out of seven cases were associated with a particular discotheque. The strains were indistinguishable, as revealed by pulsed-field gel electrophoresis and sequencing of variable regions of the *porA* gene, but caused strikingly different clinical presentations during 5 months.

Neisseria meningitidis causes both endemic and epidemic disease (8, 12, 13, 18). Although the vast majority of the estimated 2,600 annual cases of meningococcal disease in the United States are sporadic, the frequency of serogroup C meningococcal clusters has increased in, for example, military camps and universities (6–8). During recent years, however, meningococcal serogroup C spread has also been associated with discotheques, hence the designation "Disco Fever." A limited outbreak that involved six individuals who attended a dance club in Argentina was reported (4). A similar epidemic involving 10 young adults who visited a nightclub in Australia was described (9).

Characterization of meningococcal strains isolated during outbreaks is crucial in understanding an epidemic. Isolates may change their phenotype by, for example, capsular switching, justifying approaches other than serogroup typing when disease-causing strains are traced (19). In addition to the standard pulsed-field gel electrophoresis (PFGE) (15, 19), multilocus enzyme electrophoresis (13), ribosomal DNA restriction profiles (21), and PCR analysis followed by restriction fragment length polymorphism analysis of the *porA* gene have, among other methods, been used for characterization of *N. meningitidis* serogroup C (5, 14).

Blood culturing was performed using aerobic flasks (BacT/Alert; Organon Teknika, Durham, N.C.). Cerebrospinal fluid (CSF) was cultured on Columbia blood agar (Difco, Detroit, Mich.) and chocolate agar, and enrichment culturing was performed with brain heart infusion medium including factors V and X (Difco). Serogrouping was carried out by coagglutination (11), and all isolates were serotyped and serosubtyped with monoclonal antibodies for outer-membrane protein (1). PFGE was done using a contour-clamped homogeneous electric field 2 apparatus (Bio-Rad Laboratories, Richmond, Calif.). For *porA* gene sequencing, chromosomal DNAs were directly isolated from bacterial suspensions using Dynabeads DNA DIRECT system I (Dynal, Oslo, Norway). The *porA* gene was amplified by PCR, and variable region 1 (VR1), VR2, and VR3 were labeled with a BigDye Terminator Cycle Sequencing kit followed by sequencing with an ABI PRISM 310 genetic analyzer (Perkin-Elmer, Foster City, Calif.). To assign VR sequences to families (2), deduced amino acid sequences of the VRs were aligned with sequences available in the *N. meningitidis* PorA VR database (http://mlst.zoo.ox.ac.uk/porA-vr/), where the VR family designation is based on the scheme of Suker et al. (17).

Three patients (Table 1; patients D, E, and F) with *N. meningitidis* serogroup C disease were admitted to a local hospital on three subsequent days. The first patient (patient D) was a 25-year-old male who fell ill with fever, petechiae, cutaneous bleedings, and hypotension. The patient developed a fulminant septicemia and a fatal disseminated intravascular coagulation within 5 h. The following day, a 21-year-old female attended the hospital due to a swollen knee joint (Table 1; patient E). Septic arthritis was suspected, and *N. meningitidis* group C was isolated from the joint fluid. The third patient, a 21-year-old male with an artificial eye (enucleation performed due to an uveal tumor at the age of 3 years), suffered from conjunctivitis and displayed symptoms of meningitis (Table 1; patient F). None of these patients knew each other or listed close friends when answering the question of social contacts. However, they had all visited the same discotheque in Malmö on the same night (Fig. 1). The strains from all three patients were phenotypically identical (C2aP1.nst). Genosubtyping showed the same nucleotide sequences in VR1, VR2, and VR3 of the *porA* gene, namely, those of genosubtypes 5a, 10d, and 36b (Table 1). Healthy individuals who had had contact with patients D, E, and F were either checked with pharyngeal swab cultures or directly prescribed ciprofloxacin. One strain of *N. meningitidis* with full identity with the invasive isolates was detected among the healthy contacts (Table 1).

All invasive *N. meningitidis* group C strains ($n = 11$) collected in the surveillance area (Skåne, Sweden; population, 1.1 million people) during 1992 were analyzed. Four strains isolated from patients A, B, C, and G displayed the same PFGE patterns and VRs in their *porA* genes as the strains associated with the discotheque (Table 1). Patient C was a 24-year-old male who also had visited the same discotheque 3 weeks before patients D, E, and F (Fig. 1). He was admitted with septicemia and menigitis complicated by convulsions and required respirator therapy for 6 days. Patient G, a 21-year-old male who had visited the discotheque in July, fell ill in early September. A few days before his admission to the hospital, he had gotten a splinter in his right ankle, accompanied by local tenderness and arthritis in his right knee joint. Surprisingly, meningococci with full identity with the cluster strain were isolated from his knee

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TABLE 1. Summary of cases, contacts with healthy individuals, and unrelated cases

Patient, healthy contact, or unrelated patient	Age (yr)	Gender[a]	Date of presentation (day/mo/yr)	Time (days) between discotheque visit and disease	Relationship to patient(s)	Clinical presentation	Site(s) yielding positive culture(s)	Serogroup	Serotype: serosubtype	PFGE pattern	*porA* gene subtype[b]		
											VR1	VR2	VR3
Patients													
A	9	M	30/04/1992			Sore throat, septis	Blood	C	2a:nst	I	5a	10d	36b
B	2	F	11/05/1992			Septis	Blood	C	2a:nst	I	5a	10d	36b
C	24	M	28/06/1992	≈5		Septis, meningitis	Blood	C	2a:nst	I	5a	10d	36b
D	25	M	23/07/1992	5		Septis[c]	Blood, CSF	C	2a:nst	I	5a	10d	36b
E	21	F	24/07/1992	6		Arthritis	Knee joint	C	2a:nst	I	5a	10d	36b
F	21	M	25/07/1992	7		Conjunctivitis, meningitis	Blood, CSF, pharynx, socket	C	2a:nst	I	5a	10d	36b
G	21	M	09/09/1992	≈65		Arthritis, fever	Knee joint, pharynx	C	2a:nst	I	5a	10d	36b
Healthy contacts													
1	25	M	26/07/1992		Boyfriend of patient E		Pharynx	C	2a:nst	I	5a	10d	36b
2	16	M	26/07/1992		Brother of patient D		Pharynx	C	15:P1.6	II	18	25b	38a
3	24	M	26/07/1992		Friend of patient D		Pharynx	C	4:nst	III	5a	10a	36b
4–10	1–52		26–28/07/1992		Contact with patients D, E, and F		—[d]						
Unrelated patients													
1	1	F	30/07/1992				CSF	C	2b:nst	IV	5	2b	36b
2	17	F	18/12/1992				CSF	C	15:P1.15	V	19	15a	36
3	24	M	30/12/1992				Blood	C	nt:P1.7,9	VI	7	9	35a
4	37	F	30/12/1992				CSF	C	2a:nst	VII	5	2	36b

[a] Abbreviations: F, female; M, male.

[b] The amino acid sequences of VR1 to VR3 (with subtypes in parentheses) were as follows: VR1, PLQNIQPQVTKR (5), PLQNIQQPQVTKR (5a), AQAANGGASGQVKVTKVTKA (7), PPSKGQTGNKVTKG (18), and PPSKSQPQVKVTKA (19); VR2, HFVQQTPKSQPTLVP (2), HFVQQPPKSQPTLVP (2b), YVDEQSKYHA (9), HFVQNKQNQPPTLVP (10a), HFVQNKQNKQNQPPTLVP (10d), HYTRQNNTDVFVP (15a), and TYTEGSSGVFTPVP (25b); and VR3, LLGSGSDQ (35a), LLGSTSDE (36), LLGSGSDE (36b), and LLGRIGEDDE (38a).

[c] Fatal case.

[d] No positive pharyngeal specimens.

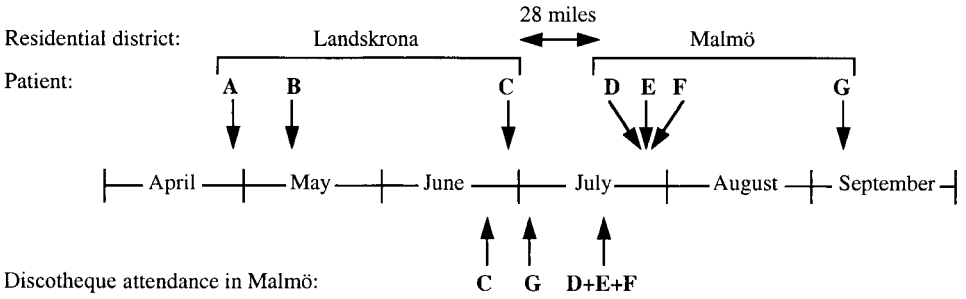

FIG. 1. Chronology of occurrences of meningococcal disease and discotheque attendance. Upper arrows indicate dates when patients fell ill, and lower arrows indicate dates when patients C to F attended the discotheque. The distance between the two residential districts is shown.

joint. Two additional patients carrying the cluster strain were detected in our study; patient A was a 9-year-old boy (Table 1) who initially presented with a sore throat, followed by a fulminant septicemia. Due to necrosis in a finger, amputation of the two distal phalanges had to be performed. Suspected cerebral damage resulting in a considerably delayed puberty was observed after 4 years. Patient B was a 2-year-old girl suffering from septicemia without any manifest sequelae. No epidemiological connection was found either between patients A, B, and C (except for living in the same city [Landskrona, Sweden; population, 37,000]) or between the different disco attendees. The genetic diversity within the group C meningococci in the surveillance area is illustrated by the characterization of the four unrelated cases during 1992 (Table 1).

A relationship between alcohol consumption, tobacco smoke exposure, male gender, and meningococcal disease is statistically proven (4, 7). These hallmarks are, however, less prominent during sporadic serogroup C meningococcal disease (13, 16). Except for environmental factors, it is presently obscure why patients are differently affected by the same strain. Various disease manifestations from fulminant septicemia to less severe disease, including primary arthritis (20) and conjunctivitis (3), were observed in our study (Table 1). The meningeal tropism and interactions with most human cell types by *N. meningitidis* (10) suggests that a patient's phenotype as well as acquired specific and nonspecific immunity plays a crucial role in the clinical outcome. In conclusion, we found that *porA* gene sequencing (genosubtyping) together with PFGE provided meaningful information during a meningococcal outbreak with the cluster strain lasting 5 months.

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