INFECTION AND IMMUNITY, June 1999, p. 2928–2934 Vol. 67, No. 6
0019-9567/99/$04.00+0

Deletion of *porA* by Recombination between Clusters of Repetitive Extragenic Palindromic Sequences in *Neisseria meningitidis*

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Received 19 January 1999/Returned for modification 25 February 1999/Accepted 2 April 1999

PorA is an important component in a vaccine against infection with *Neisseria meningitidis*. However, *porA*-negative meningococci were isolated from patients, thereby potentially limiting the role of PorA-mediated immunity. To analyze the mechanism by which the *porA* deletion occurred, the regions upstream and downstream of *porA* from three meningococcal strains (H44/76, H355, and 860183) were sequenced. The *porA* upstream region in strain 860183 contains a cluster of 22 repetitive palindromic RS3 core sequences (ATTCCC-N_8-GGGAAT) and 10 RS3 core sequences (ATTCCC) in direct orientation. The cluster is flanked by neisserial repeats, so-called Correia elements, and can be subdivided into three repeats of 518 bp followed by a truncated repeat. The *porA* upstream region of the other two strains showed deletions, probably caused by a recombination between RS3 core sequences. The *porA* downstream region of H44/76 and H355 contains the IS*1106* element followed by a cluster of 10 palindromic RS3 core sequences, 4 RS3 core sequences, and 1 other RS3 core sequence (GGGAAT) and is followed by a Correia element. This cluster can be subdivided into four direct repeats of 370 bp. Strain 860183 had two such repeats instead of four. Sequence analysis of the *porA*-negative variants indicated that the deletion of *porA* occurred via a recombination between two copies of the 116-bp region, containing two palindromic RS3 core sequences and a single RS3 core sequence. This region is homologous in the upstream and downstream clusters.

The major outer membrane protein PorA of *Neisseria meningitidis* is of interest, since its antigenic variation is used for the subtyping of meningococci (8, 24, 25). In addition, it is under investigation as a component of experimental vaccines against meningococcal infection (13).

The immunization of mice with outer membrane protein complexes results in bactericidal antibodies mainly directed against PorA (35, 36). Its value as a candidate vaccine is derived from experiments in which monoclonal antibodies directed against subtype-specific epitopes on PorA were effective in bactericidal assays and conferred protection in an animal model. However, PorA is subject to antigenic variation, which is thought to be overcome by including multiple antigenic variants of PorA in the vaccine (43). Already, trials with a hexavalent PorA-based vaccine and vaccines in which PorA is a major component have been performed (15).

PorA is expressed by most of the clinical isolates, but its level of expression varies widely (18, 42). Since the stable expression of this protein in meningococci during disease is a prerequisite for the PorA vaccine to be effective, the genetic mechanism of the variable expression of PorA has to be elucidated. Recently, we reported PorA phase variation at the transcriptional level, mediated by a variable polyguanidine stretch between the −10 and −35 domains of the *porA* promoter (42). In this study we describe a *porA*-negative meningococcus isolated from a patient with meningococcal disease. In addition, *porA*-negative variants were selected in vitro from two of nine different isolates. To elucidate the mechanism involved in the deletion of *porA*, DNA sequences upstream and downstream of this gene were evaluated.

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Sequence analysis of the deletion variants indicated that in these three strains a recombination between regions of homology upstream and downstream of *porA* of 116 bp has occurred. The rise of *porA* deletion variants during a meningococcal infection could possibly be a mechanism to evade the host immune defense. Therefore, the protective efficacy of a vaccine on the basis of PorA may be limited.

MATERIALS AND METHODS

Strains, culture conditions, and chromosomal DNA isolation. From the cerebrospinal fluid (CSF) and blood of the same patient *N. meningitidis* 860183 (C:4:P1.1 [CSF] and C:4:P1.NT [blood]) isolates were collected by the Reference Laboratory for Bacterial Meningitis (RLBM), University of Amsterdam, in 1986. In addition, nine isolates, strains 890456 (B:16:P1.5), 900545 (B:4:P1.15), 900111 (B:15:P1.16), 2996 (B:2b:P1.2), 900181 (B:2b:P1.2), 900619 (B:2b:P1.2), 901569 (C:2a:P1.2), H44/76 (B:15:P1.7,16), and H355 (B:15:P1.15), from the collection of the RLBM were used for in vitro studies. The latter two strains were isolated from patients with meningococcal disease during the epidemic period in Norway in the 1970s and are now used as reference strains.

Bacteria were grown on a GC agar base (Difco Laboratories, Detroit, Mich.) containing 1% Vitox supplement (Oxoid Laboratories, Ltd., Basingstoke, United Kingdom) at 37°C in a humidified atmosphere of 5% CO_2 in air. Chromosomal DNA was prepared as described previously (42). Pellicle growth was performed in 5 ml of tryptic soy broth in 20-ml glass tubes without agitation. The bacteria growing at the surface of the medium were diluted twice a week in fresh medium. Aliquots of the diluted culture were plated on GC agar plates and assessed for the presence of PorA- and *porA*-negative variants by colony immunoblotting and Southern colony hybridization, respectively.

Colony immunoblotting. Colonies were transferred to nitrocellulose filters (0.45-mm pore size; Schleicher and Schuell, Dassel, Germany) and immunologically stained as described before (18).

Detection of *porA* by colony hybridization. Colonies were transferred onto a nylon membrane (Hybond-N; Amersham International plc, Little Chalfont, Buckinghamshire, England) by replica plating. The colonies were lysed and denatured, essentially as described by Sambrook et al. (34). Briefly, filters were placed on GB003 gel-blotting paper (Schleicher and Schuell) saturated with 10% sodium dodecyl sulfate for 5 min, transferred to a second sheet of paper saturated with 0.5 M NaOH–1.5 M NaCl, and incubated for 10 min. The filters were neutralized by incubating them on paper saturated with 1.5 M NaCl–0.5 M Tris (pH 8.0) for 5 min. The filters were then transferred to paper saturated with 2× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA [pH 7.7]) and incubated for 5 min. The filters were allowed to dry at room temperature and

TABLE 1. PCR primers used in this study

Primer	Sequence[a]	Reference
IS1	ATTATTCAGACCGCCGGCAG	30
IS2	CCGATATCAGGATCCG	30
IS41	AATAGGATCCTGCCTGATTATCGGGTATCC	This study
PorA11	CCCGCCTGATGGACACCGCCC	This study
PorA111	ATCAGAATTCCCCGCCTGATGGACACCGCCC	This study
PorA13	TTAATCGGCAAGGAAGAGGG	This study
PorA113	AATAGGATCCTTAATCGGCAAGGAAGAGGG	This study
P21	CTGTACGGCGAAATCAAAGCCGGCCGT	24
P22	TTAGAATTTGTGGCGCAAACCGAC	24
P1-1	GGTCAATTGCGCCTGGATGTTCCTG	This study
P1-2	CGCTGATTTTCGTCCTGATGCGGC	This study
PorA3	CCAAATCCTCGCTCCCCTTAAAGCC	42
PorA5	CCGAGACTGCATCCGGGC	42
PorA107	AATAGAATTCGCGCAGCCTCGTGC	This study
PorA10	GGGATACGGAAGTCCAAG	This study
PorA110	AATAGGATCCGGGATACGGAAGTCCAAG	This study

[a] The restriction sites used for insertion in the cloning vector are underlined.

finally baked at 80°C for 1 h. *porA* was detected by hybridization with a digoxigenin (DIG)-labeled PCR product as described for the Southern hybridization.

Southern hybridization. DNA fragments were electrophoresed on a 0.6% agarose gel and transferred to a nylon membrane (Zeta Probe; Bio-Rad) (34). The *porA*- and IS*1106*-specific probes were made by PCR amplification with primers PorA5 and P22 and IS1 and IS2, respectively. Probes were randomly primed and labeled with DIG (Boehringer, Mannheim, Germany) according to the instructions supplied by the manufacturer. After hybridization the probes were detected with anti-DIG antibodies conjugated to alkaline phosphatase and by staining according to the instructions supplied by Boehringer.

Oligonucleotide synthesis. The oligonucleotides used in this study are shown in Table 1 and Fig. 1. Oligonucleotides were synthesized by Perkin-Elmer Nederland B.V., Gouda, The Netherlands.

Detection of *porA* by PCR. The presence of the *porA* gene was assessed by PCR with primers P21 and P22 as described previously (24). The PCR products were analyzed on agarose (1%) gels with the Tris-acetate-EDTA buffer system (34).

Strategy for determination of the sequences of the regions upstream and downstream of *porA*. Primer PorA11, homologous to a sequence downstream of *porA* and the IS*1106* region, was designed according to the sequence obtained after inverse PCR of the *Eco*RI restriction enzyme fragment that hybridized with the IS*1106*-specific probe as well as with the *porA* gene probe. The *Eco*RI restriction fragment containing the 3′ part of *porA* of the chromosomal DNA from strain H355, H44/76, or 860183 was used as the template in a PCR with primers IS1 and PorA11. After reamplification with IS41 and PorA111 as primers, amplicons were inserted into the *Bam*HI/*Eco*RI-linearized vector pUC18 or pUC19 (Invitrogen Corporation, Carlsbad, Calif.). Subclones were made by exonuclease III digestion according to the company's protocol (Promega) and subsequently sequenced.

The upstream region of *porA* was obtained in a way similar to that aforementioned for the downstream region. PorA13 and PorA10 were designed according to the *porA* upstream sequence obtained after targeted genome walking (42). The *Eco*RI restriction fragment containing the 5′ part of *porA* of the chromosomal DNA from strain H44/76 or H355 was used as the template in a PCR with primers P1-1 and PorA13. For strain 860183 the amplification was initially performed with primers PorA3 and PorA13. To increase the specificity the amplicons were further amplified with primers P1-2 and PorA13. After reamplification with primers PorA113 and PorA107 the amplicons were cloned, subcloned, and sequenced as aforementioned.

The *Eco*RI fragment, hybridizing with PorA13 and PorA11, of the *porA*-negative variants was used as the template in a PCR with primers PorA10 and PorA11. After reamplification with PorA11 and PorA110 as primers, the amplicons were characterized as aforementioned with the *porA* downstream sequences.

Fluorescence-based sequencing and analysis. Subclones were sequenced with the fluorescent dye-labeled universal primer −21M13 in a PCR-based sequence reaction by using *Taq* polymerase (Perkin-Elmer) and the reaction mixture supplied by Amersham according to the instructions of Applied Biosystems Incorporated (Foster City, Calif.). The sequences were analyzed on an automatic sequenator (model 370A; Applied Biosystems Incorporated). Sequences were analyzed with computer programs included in the program package PC/GENE (19a). The sequences were aligned with the CLUSTAL program by the method developed by Higgins and Sharp (17).

Nucleotide sequence accession numbers. The nucleotide sequence data will appear in the EMBL, GenBank, and DDBJ nucleotide sequence databases under accession no. AF117212 (*porA* upstream region of strain H355), AF117213 (*porA* upstream region of strain H44/76), AF117214 (*porA* upstream region of strain 860183), AF117215 (*porA* downstream region of strain H355), AF117216 (*porA* downstream region of strain 860183), AF117217 (*porA* downstream region of strain H44/76), AF117218 (*porA* locus after *porA* deletion of strain H355), AF117219 (*porA* locus after *porA* deletion of strain H44/76), and AF117220 (*porA* locus after *porA* deletion of strain 860183).

RESULTS

Identification of *porA*-negative meningococcal clinical isolates. During the routine characterization of clinical meningococcal isolates in the RLBM we identified a group C nonsubtypeable meningococcus (strain 860183) from a blood culture while the CSF isolate from the same patient appeared to be subtype P1.1. Both isolates had the same serogroup and type and were identical according to their outer membrane profile, except for the presence of PorA. Both isolates were subjected to colony immunoblotting with a PorA-specific antibody and colony hybridization with the *porA*-specific probe. All colonies from the CSF isolate appeared to be *porA* positive and PorA positive. In contrast 4% of colonies from the blood isolate were *porA* positive and PorA negative and 96% were *porA* negative and PorA negative. The occurrence of *porA*-negative and PorA-negative colonies indicated the deletion of *porA*.

Isolation of *porA*-negative variants in vitro. Meningococci reveal phenotypic changes, depending upon growth phase and growth rate (31, 32). The pellicle growth of meningococci has been shown to yield phenotypic variants (31). Two of nine isolates (H44/76 [B:15:P1.7,16] and H355 [B:15:P1.15]) tested yielded *porA*-negative variants after pellicle growth. The proportion of *porA*-negative variants on the culture plates of strain H44/76 was 3% after six cycles of culturing and reculturing of the pellicle. A similar proportion of *porA*-negative variants was obtained with strain H355 after 16 cycles of culturing and reculturing of the pellicle. PorA phase variants, which usually

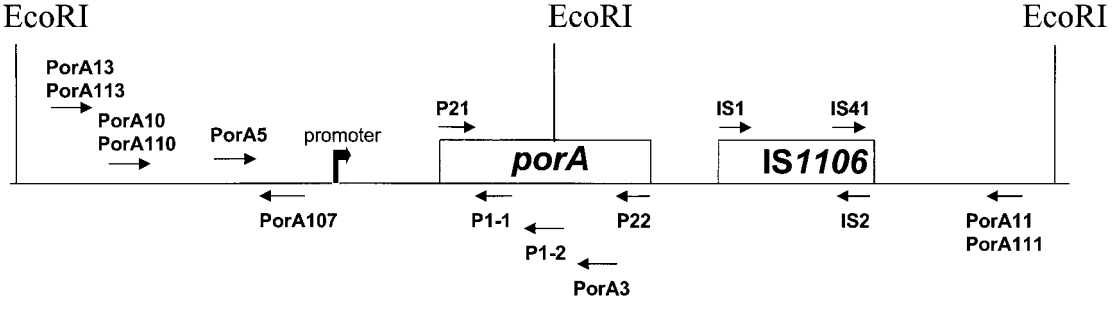


FIG. 1. Schematic representation of the locations of the PCR primers used in this study.

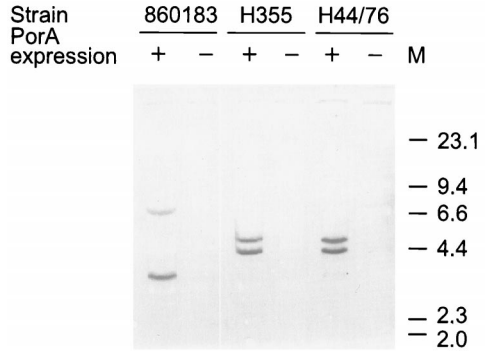


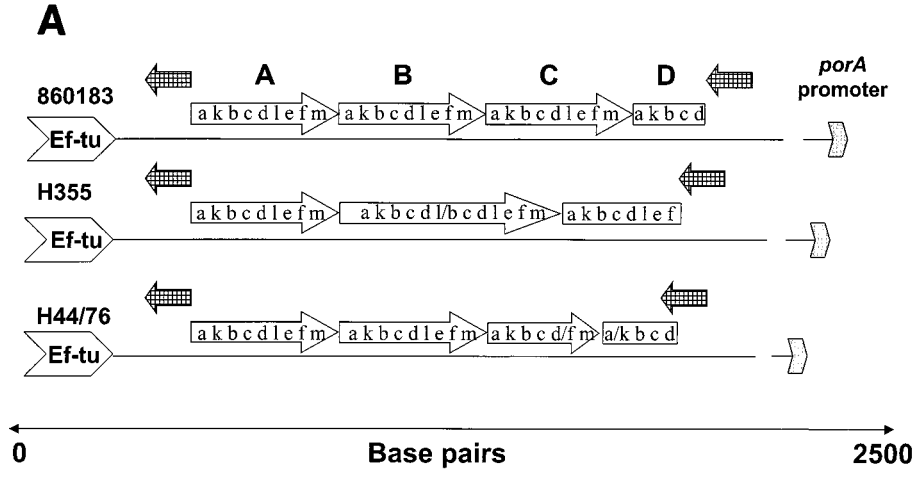
FIG. 2. Southern hybridization analysis of *Eco*RI-digested chromosomal DNA of PorA-positive and PorA-negative variants of *N. meningitidis* H44/76, H355, and 860183. +, PorA-positive variants; −, PorA-negative variants; M, molecular weight markers, in kilobase pairs.

appear with a frequency of 10^{-4} to 10^{-3}, were not observed in these experiments.

Characterization of *porA*-negative variants. Chromosomal DNA digested by *Eco*RI of the *porA*-negative variants and their *porA*-positive counterparts were assessed by Southern hybridization. The *porA* gene has an *Eco*RI restriction site dividing the gene roughly in half. The bands of both restriction fragments were absent in the *porA* deletion variants after hybridization with the *porA* probe, containing the complete *porA* gene, including its promoter (Fig. 1). This means that the deletion extends beyond the size of the probe (1.5 kb) (Fig. 2).

Knight and colleagues (20) have demonstrated that meningococcal isolates can have an IS*1106* element downstream of *porA*. For strains 860183, H355, and H44/76 this was also demonstrated by PCR with primers P21 and IS2 and confirmed by Southern hybridization with the IS*1106*-specific probe (data not shown). In addition, only the smallest *porA Eco*RI restriction fragment reacts with this probe in the Southern hybridization, indicating that this fragment contains the downstream part of *porA*. With the *porA* deletion variants of the three strains the smallest *Eco*RI fragment was absent when assessed with the IS*1106*-specific probe in the Southern hybridization. Together, the Southern hybridization results indicated that with the deletion of *porA* at least 4 kb of the chromosome was lost.

Sequence upstream of *porA*. The sequencing of the *porA* upstream region of each of the three strains revealed a highly repetitive DNA sequence (Fig. 3). No significant open reading frames were found within the 2- to 2.5-kb region. The *porA* upstream sequence is preceded by the 3′-terminal part of the gene coding for the elongation factor Tu (EF-Tu) (Fig. 3A). The *porA* upstream sequence of strain 860183 contains a cluster of 22 palindromic sequences with RS3 (14) core sequences (ATTCCC-N_8-GGGAAT) in an inverted orientation (19, 37)



B

Direct repeat 518 bp DR A, B, C

dRS3a cRS3k
AACCCTGTCG CCGTC**ATTCC** **CGCAAAAGC**G **GGAAT**CTAGG ACGCAGGGTT AAGAAAACCT GCATCCCGTC **ATTCCC**TCAA AAACAGAAAA CAAAATCAG
dRS3b
AAACCTAAAA TCCCGCC**ATT** **CCC**GCAAAAG C**GGGAAT**CCA GTCCGTTCAA TTTCGGTCAT TTCCGATAAA TTCCTGTTGC TTTTCATTTC AG**ATTCCC**A
dRS3c dRS3d
CTTTCGT**GGG** **AAT**GACGGCG GAAGGGTTTT GGTTTTTTCC GATAAATTCT TGAGGCATTG AAATTCCAG**A** **TTCCC**GCCTG CGC**GGGAAT**G CGATTCATA
cRS3l dRS3e
AGTTTCCCGA AATTCCAACA TAACCGAAAC CTGACAGTAA CCGTAGCAAC TGAACCGTC**A** **TTCCC**ACCAC TTTTCGTC**AT** **TCCC**GCGAAA C**GGGAAT**CT
dRS3f
AGAATCTCGG ACTTTCAGAT AATCTTTGAA TATTGCTGTT GTTCTAAGGT CTAG**ATTCCC** GCCTGCGC**GG** **GAAT**GACGAA TCCATCCGCA GGAAACCTG
cRS3m
CACCGCGTC**A** **TTCCC**ACG

FIG. 3. (A) Schematic representation of the structure of the chromosome upstream of *porA* of *N. meningitidis* 860183, H355, and H44/76. Open arrows indicate the 518-bp repeat. The letters in the open arrows refer to the different dRS3 repeats and cRS3 sequences. Hatched arrows indicate the Correia elements (5). (B) Sequences of the different repetitive elements in the *porA* upstream region. The different dRS3 and cRS3 sequences are indicated. Open arrows indicate inverted repeats in the 518-bp repeat. Black arrows indicate the 14-bp direct repeat.

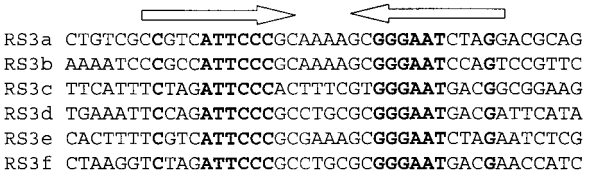


FIG. 4. Homology between the different dRS3 palindromic sequences. The conserved nucleotides are indicated in bold.

and 10 RS3 core sequences (ATTCCC) in the direct orientation. The cluster is flanked by two neisserial repetitive sequences first described by Correia and colleagues (5) in the direct orientation. The pairs of inverted RS3 core sequences are actually inverted repeats of 8 bp; two nucleotides of the inner core sequence are also inverted. In addition, the nucleotides at the fourth position on either side of the inverted repeat are conserved and complementary (Fig. 4). The pairs of inverted RS3 core sequences were termed dRS3 by Morelli et al. (27) to distinguish them from the RS3 sequences originally described by Haas and Meyer (14). In this report we will refer to cases of single RS3 core sequences as cRS3 sequences. The cluster of dRS3 sequences can be subdivided into three repeats of 518 bp, containing six dRS3 sequences (dRS3 a to f) and three cRS3 sequences (cRS3 k to m) in the direct orientation (Fig. 3B). The three 518-bp repeats are followed by another, truncated repeat (Fig. 3A). The dRS3 a sequence in repeat A is part of a larger inverted imperfect repeat of 23 bp. Repeats B to D show minor sequence variations just upstream of dRS3 a, destroying the 23-bp inverted repeat. In addition, a direct repeat of 14 bp (TTTCCGATAAATTC) is found (Fig. 3B).

In comparison to the *porA* upstream region of strain 860183 the *porA* upstream regions of strains H44/76 and H355 show deletions (Fig. 3A). The *porA* upstream region of H355 has a deletion of 280 bp between position cRS3 l of repeat B and dRS3 b of repeat C (Fig. 3A and 5). The *porA* upstream region of strain H44/76 shows two deletions in comparison with strain 860183, i.e., 180 bp between dRS3 d and f of repeat C and 50 bp between dRS3 a and cRS3 k of repeat D, respectively. In strain H355 the fourth repeat (repeat D) is 42 bp shorter than the preceding three repeats and ends with dRS3 f. Repeat D is 160 bp shorter in strains 860183 and H44/76 than in strain H355, presumably by a deletion between dRS3 d and f. The sequence differences between the upstream regions of strain H355 and strain H44/76 are consistent with the results obtained by Southern hybridization. The largest *Eco*RI fragment, containing the *porA* upstream region, of strain H44/76 has a slightly higher electrophoretic mobility than that of strain H355 (Fig. 2). Strains H44/76 and H355 were isolated from patients in Norway during an epidemic in the early 1970s and are from the same clone (4). A comparison with strain 860183 is difficult, because this strain, isolated from a patient in the Netherlands, has a different *Eco*RI restriction enzyme digest pattern than the other two strains.

Sequence downstream of *porA*. The sequences of the region downstream of *porA* in all three strains and strain F207 (20) were essentially similar to each other. However, strain 860183 had only two DR2 repeats (20) downstream of IS*1106* instead of four (Fig. 6). In H44/76 and H355 the sequences downstream of IS*1106* actually also form a cluster comprising 10 dRS3 sequences, four cRS3 sequences (ATTCCC), and one other cRS3 sequence (GGGAAT) and are followed by Correia elements. The orientation of these Correia elements is opposite to that of the Correia elements found upstream of *porA*.

Homology of the *porA* upstream region with different loci in *Neisseria*. The Correia element is found in up to 150 to 200 copies throughout the *Neisseria* genomes, gonococci as well as meningococci (3). In the *porA* locus it is also found downstream of *porA* but in an opposite orientation (Fig. 6) (20).

The complete 518-bp repeat of the *porA* upstream region is not found elsewhere in the *Neisseria* chromosome (either gonococci or meningococci) (12a, 19, 28). However, parts of the repeat show homology with three regions in the *porA* downstream region, dRS3 b to f in the DR1 repeat and dRS3d to f (inverted) and cRS3 l to dRS3 f in the DR2 repeat. Homology is also found in the flanking regions of other genes, mostly coding for outer membrane proteins or other surface structures. These parts are flanked by the RS3 core sequences. Table 2 shows the regions of homology (more than 90% identity) of the 518-bp repeat with other loci in *Neisseria* species.

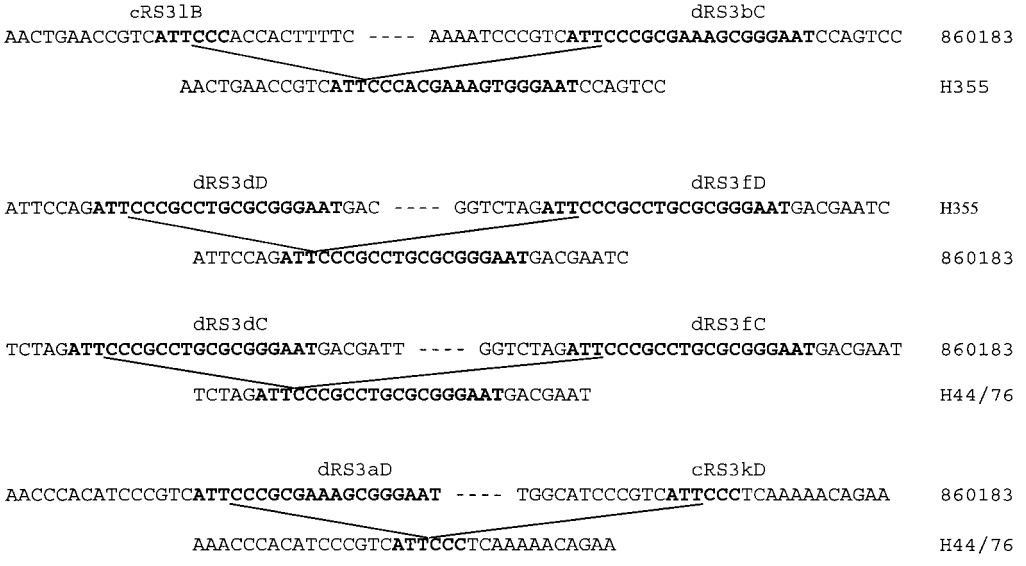


FIG. 5. Putative recombination sites in the *porA* upstream region. The different dRS3 and cRS3 sequences are indicated. The capital letters in the designations refer to the different 518-bp repeats shown in Fig. 3A.

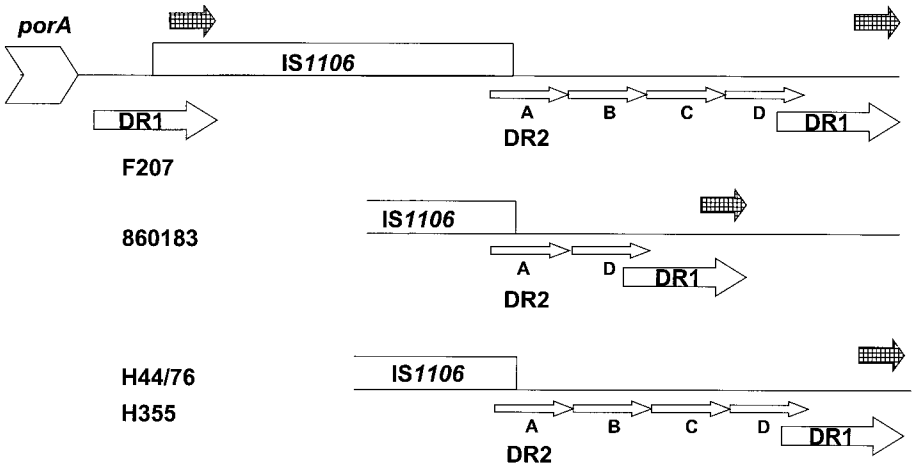


FIG. 6. Schematic representation of the structure of the chromosome downstream of *porA* from *N. meningitidis* F207 (20), H44/76, H355, and 860183. Hatched arrows indicate the Correia elements (5). DR1 and DR2 were originally characterized by Knight et al. (20).

Deletion of *porA* by recombination. To determine which of the three regions with homology upstream and downstream of *porA* is involved in the recombination event that leads to the deletion of *porA*, the *porA* locus in the *porA*-negative variants of the three strains was sequenced. The comparison of these sequences with the sequences of the regions upstream and downstream of *porA* indicates that in all three strains the deletion of *porA* occurred via a recombination between a homologous 116-bp sequence containing cRS3 l to dRS3 f (Fig. 7). It should be noted that the small deletion observed in the *porA* upstream region of strain H355, compared to that of strain 860183, was also observed in the sequence after the deletion of *porA* in strain H355, indicating that this deletion was not caused by PCR amplification.

DISCUSSION

The data presented here show that a patient can have an infection with *porA*-positive and *porA*-negative variants of a meningococcal strain. In addition, *porA*-negative variants can be obtained in vitro. About 4 kb of the chromosome was deleted in the *porA*-negative variants of all three strains which were analyzed in this study. The deletion of *porA* occurred by the recombination between a 116-bp region of homology upstream and downstream of *porA*, containing two dRS3 sequences and one cRS3 sequence. The results of the pellicle growth experiments may give the impression that the deletion occurred with a rather high frequency. However, the pellicle growth experiments do not allow an accurate estimation of the frequency of the *porA* deletion event. Most likely, in these experiments the *porA*-negative variants are accumulated during repetitions of culturing and reculturing of the pellicle.

The RS3 core sequences in the *porA* upstream region form a cluster of as many as 22 dRS3 sequences (two RS3 core sequences in the inverted orientation) and 10 cRS3 sequences (single core sequences in the direct orientation). In addition the cluster is flanked by other neisserial repetitive sequences (5). Downstream of *porA* the DR2 repeats form a cluster of 10 dRS3 sequences and 5 cRS3 sequences, again flanked by a Correia element (opposite to the *porA* upstream Correia elements). Knight and colleagues (20) noticed that the palindromic RS3 core sequences (ATTCCC-N_8-GGGAAT) in the *porA* downstream sequence are similar in structure and distribution to the repetitive extragenic palindromes (REP) of 38 bp, initially identified in *Salmonella typhimurium* and *Escherichia coli* (9, 16, 23). After analysis of the DNA sequences flanking the *opa* genes in *N. meningitidis*, Morelli and coworkers also observed the parallelism between dRS3 sequences and REP sequences (27). In *E. coli* the REP sequences form clusters, which also contain other repeated elements. They were termed bacterial interspersed mosaic elements (12). The neisserial complex dRS3 clusters were termed neisserial interspersed mosaic elements (27). These large clusters of repetitive

TABLE 2. Homology of the 518-bp repeat with different loci in *Neisseria* species

Locus	Organism[a]	Homology with:	Size (bp)	Reference	GenBank accession no.
aroK aroB yafJ	Ng	dRS3 b–dRS3 d	176	3	emb|AJ002783|NGAJ2783
pilS7	Ng	dRS3 a–dRS3 b	126		gb|U58851|NGU58851
pilS7	Ng	dRS3 e–dRS3 f	90		gb|U58851|NGU58851
IS*1106* (DR1)	Nm	dRS3 b–dRS3 f	121	20	emb|Z11857|NMIS1106X
IS*1106* (DR2)	Nm	dRS3 d–dRS3 f (inverted)	71	20	emb|Z11857|NMIS1106X
IS*1106* (DR2)	Nm	cRS3 l–dRS3 f	116	20	emb|Z11857|NMIS1106X
Lactoferrin binding protein B	Nm	dRS3 f–RS3 m	60	21	gb|AF049349|AF049349
15063G epithelial cell invasion protein	Ng	dRS3 d–cRS3 l	84	44	gb|U13708|NGU13708
Outer membrane protein OmC	Ng	dRS3 b–dRS3 c	101	41	gb|L19944|NGOOMC
Pilus biogenesis cluster	Ng	dRS3 b–dRS3 c	109	7	gb|U40596|NGU40596
iga HF13	Nm	dRS3 a–dRS3 b	110	22	emb|X82474|NMIGAF13
iga HF13	Nm	dRS3 f–cRS3 m	60	22	emb|X82474|NMIGAF13

[a] Ng, *Neisseria gonorrhoeae*; Nm, *N. meningitidis*.

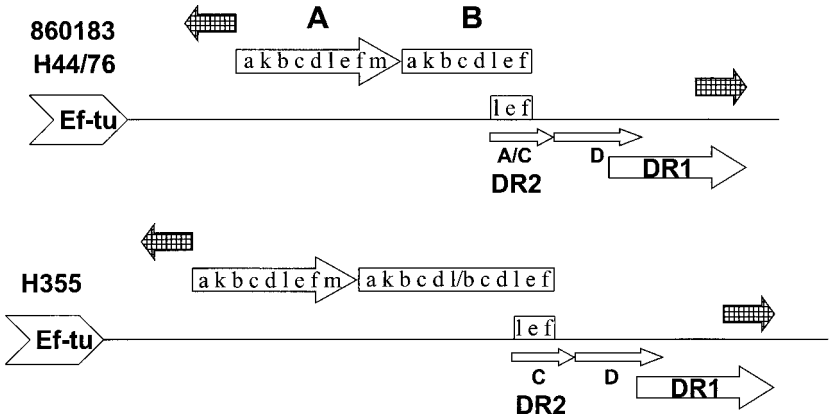


FIG. 7. Schematic representation of the *porA* locus after the deletion of *porA* in *N. meningitidis* H44/76, 860183, and H355. Hatched arrows indicate the Correia elements (5). The different dRS3 and cRS3 sequences are indicated. The capital letters refer to the different 518-bp repeats shown in Fig. 3A.

sequences may give rise to difficulties during bacterial genome sequencing projects. In these projects, genomic fragments are randomly cloned and sequenced. It may be that large repetitive sequences are missed, when the sequences of the different clones are connected during a computerized process.

The comparison of the *porA* upstream sequences of three strains indicated deletions, most likely due to a recombination between RS3 core sequences. The complete 518-bp repeat is only found upstream of *porA*. Partial homology is found with sequences flanking other genes in *Neisseria* strains. Regions showing homology with high identity (>90%) are always flanked by dRS3 sequences, again indicating that these are involved in recombination events. Analysis of the *porA* locus in *porA*-negative variants indicates that the deletion of *porA* occurred by a recombination between a region of 116 bp with homology upstream and downstream of *porA*, possibly between RS3 core sequences. The resemblance between dRS3 sequences and the *E. coli* REP sequence supports this idea. The REP sequence has also been implicated in chromosomal rearrangements (9, 39). The identification of this sequence at the junctions of tandem duplications supports this notion (38). The strains yielding *porA*-negative variants either in a patient or in vitro contained the IS*1106* element distal to *porA*. However, there was variation in the number of DR2 repeats, indicating recombination events leading to the deletion or duplication of some of these repeats. Recombination events in the IS*1106* region were also indicated by the results of Knight et al. (20). They found truncated forms of the IS*1106* region, indicating a recombination between regions within the DR1 repeat. dRS3 sequences were suggested as sequences involved in these recombination events.

The 518-bp repeat contains six dRS3 sequences, and four pairs of these sequences are equally spaced by 76 bp (b-c, c-d, and e-f) to 80 bp (f-a). The two others are also equally spaced by 102 bp (a-b) to 109 bp (d-e). Downstream of *porA* the spacing between the dRS3 sequences is either 75 or 277 bp. This regular structure might indicate that dRS3 sequences are involved in organizing the DNA suprastructure, as proposed for REP sequences of *S. typhimurium* and *E. coli*, as well as in facilitating recombination. It has been shown that the REP sequence binds DNA gyrase (46) and DNA polymerase I (10). The HU protein stimulates the binding of gyrase to these REP sequences (47). These studies with *E. coli* have led to the proposal that REP sequences are involved in the folding of the bacterial nucleoid into independent supercoiled looped domains (11, 39).

PorA is the important component of group B meningococcal protein-based vaccines, since capsule polysaccharides of group B meningococci are poorly immunogenic. Antibodies against PorA are bactericidal and protective in a mouse model (35, 36). However, meningococci avoid the humoral host immune response by antigenic variation within PorA. Point mutations in the VR1 and VR2 regions of the protein (26, 40), and the replacement of epitopes by recombination and small deletions (2, 26) contributes to PorA antigenic variation. In addition, PorA expression is variable by means of the variable *porA* promoter (1, 42). The loss of PorA expression can also be due to the insertion of IS*1301* (1, 29) or to frame shift mutation (1 and our unpublished data). The function of PorA is unknown. It has been reported that PorA-positive as well as PorA-negative meningococci can be cultured from the nasopharynx (6, 45). Our results show that a *porA*-negative variant can also be isolated from the blood of a patient with meningococcal disease. During a preliminary survey, two of a group of 57 nonsubtypeable meningococcal isolates appeared to be *porA* negative (unpublished data). The number of patients infected with *porA*-negative variants is likely to be underestimated. The isolates from patients infected with subtypeable meningococci were not investigated, but they could well contain *porA*-negative variants, since patients can be infected with both *porA*-positive and *porA*-negative variants of a meningococcus. These findings do not rule out the possibility that PorA has an essential function in the pathogenesis of meningococcal disease. In fact, the coexistence of both *porA*-positive and *porA*-negative variants within samples from one patient might indicate that the latter originates from the *porA*-positive variant during the infection. Our results show that this occurs by a recombination between homologous regions of 116 bp upstream and downstream of *porA*. The occurrence of *porA*-negative meningococci in patients together with the ability of PorA phase variation due to promoter variability might be indicative of the limited efficacy of PorA-based vaccines.

ACKNOWLEDGMENTS

The sequencing of *N. meningitidis* MC58 by The Institute for Genomic Research (TIGR [19]) was accomplished with support from TIGR; the sequencing of *Neisseria gonorrhoeae* was accomplished by The Gonococcal Genome Sequencing Project (12a, 33) with support from USPHS/NIH grant AI38399, and the sequencing of *N. meningitidis* Z2491 (serogroup A) was accomplished by The Sanger Centre (28) with support from the Wellcome Trust.

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Editor: D. L. Burns