Sequence Variation in the porA Gene of a Clone of Neisseria meningitidis during Epidemic Spread

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The ET-15 clone within the electrophoretic type (ET)-37 complex of Neisseria meningitidis was first detected in Canada in 1986 and has since been associated with outbreaks of meningococcal disease in many parts of the world. While the majority of the strains of the ET-37 complex are serosubtype P1.5,2, serosubtype determination of ET-15 strains may often be incomplete, with either only one or none of the two variable regions (VRs) of the serosubtype PorA outer membrane protein reacting with monoclonal antibodies. DNA sequence analysis of the porA gene from ET-15 strains with one or both unidentified serosubtype determinants was undertaken to identify the genetic basis of the lack of reaction with the monoclonal antibodies. Fourteen different porA alleles were identified among 38 ET-15 strains from various geographic origins. The sequences corresponding to subtypes P1.5a,10d, P1.5,2, P1.5,10d, P1.5a,10k, and P1.5a,10a were identified in 18, 11, 2, 2, and 1 isolate, respectively. Of the remaining four strains, which all were nonserosubtypeable, two had a stop codon within the VR1 and the VR2, respectively, while in the other two the porA gene was interrupted by the insertion element, IS1301. Of the strains with P1.5,2 sequence, one had a stop codon between the VR1 and VR2, one had a four-amino-acid deletion outside the VR2, and another showed no expression of PorA on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Our results reveal that numerous genetic events have occurred in the porA gene of the ET-15 clone in the short time of its epidemic spread. The magnitude of microevolutionary mechanisms available in meningococci and the remarkable genetic flexibility of these bacteria need to be considered in relation to PorA vaccine development.

Neisseria meningitidis expresses different porins in its outer membrane which may be characterized serologically. Meningococci possess either a class 2 or 3 protein which defines the serotype and a class 1 protein which defines the serosubtype (14). Sequencing of the porA gene, which encodes the class 1 or PorA protein, revealed that antigenic variation occurs within two variable regions (VRs) of the porin, referred to as VR1 and VR2. These are located on loops 1 and 4, respectively, and are responsible for the generation of the two serosubtype specificities (5, 22–25, 31).

In the early 1990s, outbreaks of serogroup C meningococcal disease occurred in Canada. Multilocus enzyme electrophoresis, which has been used extensively for genotyping of N. meningitidis, revealed that the strains responsible for these outbreaks belonged to the electrophoretic type (ET)-37 complex but were characterized by a rare allele at the fumarase locus (4). This new variant, designated ET-15, was subsequently associated with outbreaks of meningococcal disease in parts of the United States, Israel, the Czech Republic, Iceland, Finland, Norway, England, Germany, and Australia (8, 18–20, 28, 34–35). Pulsed-field gel electrophoresis and Southern hybridization analysis of ET-15 strains confirmed a common origin of the strains causing these outbreaks and demonstrated that, in addition to the change in the fumarase locus, large genetic rearrangements had occurred within the ET-37 complex in association with the appearance of this new clone (19).

The predominant serotype-serosubtype combination associated with strains of the ET-37 complex is 2a:P1.5,2 (8), and most ET-15 strains had the phenotype C:2a:P1.5,2. The monoclonal antibody-based typing scheme currently used is, however, not comprehensive, and many isolates can be only partially typed. This is especially true for strains of ET-15 which may present an incomplete serosubtype or are fully nonserosubtypeable (NST). Thus, the phenotypes C:2a:P1.5, C:2a:P1.2, and C:2a:NST have been frequently encountered in ET-15 strains.

We report here the results of sequence analysis of the porA gene in 38 meningococcal ET-15 strains from diverse geographical origins that had an incomplete serosubtype or were NST. This study was undertaken in order to (i) establish the VR1 and VR2 occurring in NST and partially typed strains, (ii) determine the level of expression and specificity of the PorA proteins in selected strains, and (iii) define the extent of genetic variability of the porA gene in strains of a single clone during epidemic spread. The results revealed a high degree of porA gene variability among meningococci of the ET-15 clone which had occurred in about a decade, thus reinforcing the inadequacy of the current serological typing reagents. Many different genetic mechanisms were involved in generating this diversity, further illustrating the evolutionary potential of the meningococcal genome.

MATERIALS AND METHODS

Bacterial isolates. The 38 strains examined were identified as belonging to the ET-37 complex by their allelic variation at 14 enzyme loci (8). In addition, all of them presented the allele 2 at the fumarase locus, identified in Canada as a marker for the ET-15 variant (4). Of these isolates, 23 were selected on the basis of their incomplete serosubtype among 72 ET-15 strains previously analyzed for genome organization (19). This ET-15 collection was supplemented with a further 10 isolates from Australia and 6 strains from Norway that also presented incomplete serotypes. The serological characteristics of the 38 ET-15 strains...
on dot blots (36) were as follows: C2aP1.5 (23 strains), CNTiP1.5 (1 strain), C2aP1.2 (5 strains), C2aNST (8 strains), and B2aP1.2 (1 strain). The 38 strains spanned the years 1988 to 1999 and, with the exception of strain II050775, 2a (5 strains), C:2a:NST (8 strains), and B2aP1.2 (1 strain). The 38 strains were further analyzed in immunoblots (36) for their reactions with monoclonal antibodies specific for the P1.5 (MN22A9.19), P1.2 (3-1-P1.2), and P1.10 (56C5/MN20.F417) epitopes, as well as for the common PorA epitope, P1.C (9-1-P1C). The monoclonal antibodies were kindly provided by W.D. Zollerling and J.T. Poulman.

### RESULTS

#### PCR and sequence analysis

PCR with primers 730 and 733 resulted in the amplification of a product of ca. 1,100 bp for all but two strains. The exceptions were the patient isolate 91297 from Canada and the carrier isolate from Iceland II050775, which both gave a product of ca. 1,900 bp.

The PCR product obtained for the 38 strains was sequenced, and a segment of the porA gene starting seven codons from the signal peptide was compared for all strains. A total of 14 alleles of the porA gene were identified. Each allele was assigned a roman number (Table 1), starting with the subtype commonly associated with strains of the ET-37 complex, P1.5,2. Tables 2 and 3 depict the sequence results obtained for VR1 and VR2, respectively. Five different VR1s and five different VR2s were identified. The deduced amino acid sequences of the corresponding VRs were compared to those previously published (10, 32; Maiden et al., VRD) using the designations of Suken et al. (32). The VR1 and VR2 designations were based on the translated nucleotide sequence and were assigned regardless of the monoclonal antibody interpretation.

#### Table 1. Characteristics of the 38 ET-15 N. meningitidis isolates

| porA allele | Strain no. | Source | Yr | Phenotype | Subtype |
|-------------|------------|--------|----|-----------|---------|
| I           | 95N477**   | Australia | 1995 | B2aP1.2 | P1.5,2  |
| I           | 50447*     | Australia | 1996 | C2aP1.5 | P1.5,2  |
| I           | 57463      | Australia | 1996 | C2aP1.5 | P1.5,2  |
| I           | 88080*     | Canada   | 1988 | C2aP1.5 | P1.5,2  |
| I           | 89486*     | Canada   | 1989 | C2aNST  | P1.5,2  |
| I           | 343/95*    | Czech Republic | 1993 | C2aP1.2 | P1.5,2  |
| I           | 58         | Finland  | 1994 | C2aP1.5 | P1.5,2  |
| I           | M929*      | Israel   | 1993 | C2aP1.2 | P1.5,2  |
| II          | M837*      | Israel   | 1992 | C2aP1.2 | P1.5,2  |
| II          | 15/98*     | Norway   | 1998 | C2aNST  | P1.5,2  |
| IV          | 84/96*     | Norway   | 1996 | C2aNSTP1.5,10d |
| IV          | 114/96*    | Norway   | 1996 | C2aNSTP1.5,10d |
| V           | 961M1966   | Australia | 1996 | C2aNSTP1.5,10a |
| VI          | 94N266*    | Australia | 1994 | C2aNSTP1.5,10a |
| VII         | 98N079     | Australia | 1998 | C2aNSTP1.5,10a |
| VIII        | 94N369*    | Australia | 1994 | C2aNSTP1.5,10a |
| VIII        | 95N668     | Australia | 1995 | C2aNSTP1.5,10a |
| VIII        | 97N221     | Australia | 1997 | C2aNSTP1.5,10a |
| VIII        | 97N236     | Australia | 1997 | C2aNSTP1.5,10a |
| VIII        | 97W004     | Australia | 1997 | C2aNSTP1.5,10a |
| VIII        | 98N005     | Australia | 1998 | C2aNSTP1.5,10a |
| VIII        | 98N101     | Australia | 1998 | C2aNSTP1.5,10a |
| VIII        | 98N097     | Australia | 1998 | C2aNSTP1.5,10a |
| VIII        | 98N161     | Australia | 1998 | C2aNSTP1.5,10a |
| VIII        | 99N001     | Australia | 1999 | C2aNSTP1.5,10a |
| VIII        | 934286     | England   | 1993 | C2aNSTP1.5,10a |
| VIII        | 31268      | England   | 1995 | C2aNSTP1.5,10a |
| VIII        | 17         | Finland   | 1992 | C2aNSTP1.5,10a |
| VIII        | 11/94*     | Norway    | 1994 | C2aNSTP1.5,10a |
| VIII        | 121/95     | Norway    | 1995 | C2aNSTP1.5,10a |
| VIII        | 36/97      | Norway    | 1997 | C2aNSTP1.5,10a |
| IX          | 25103*     | England   | 1995 | C2aNSTP1.5,10a |
| IX          | 85/96      | Norway    | 1996 | C2aNSTP1.5,10a |
| X           | 311340*    | England   | 1995 | C2aNSTP1.5,10a |
| XI          | 67*        | Finland   | 1994 | C2aNSTP1.5,10a |
| XII         | M702/91*   | Iceland   | 1991 | C2aNSTP1.5,10a |
| XIII        | 91297*     | Canada    | 1991 | C2aNSTP1.5,10a |
| XIV         | II050775*  | Iceland   | 1993 | C2aNSTP1.5,10a |

* NT, nonerythrocyteable.  
\( ^{b} \) Subtype = VR loop names, as defined by sequence analysis.  
\( ^{c} \) *, analyzed by SDS-PAGE and immunoblot.

#### Table 2. Nucleotide sequences encoding the VR1 region of the PorA gene in ET-15 N. meningitidis strains

| Representative strain | VR1 DNA sequence | VR1(s) identified in allele no. | Epitope variant |
|-----------------------|------------------|---------------------------------|-----------------|
| 58                    | CGG CCT CAA AAT ATT CAA ... CCT CAG GTT ACT AGG GCG AAA | 1-IV, X, XI | P1.5,2 |
| L194                  | CGG CCT CAA AAT ATT CAA ... CCT CAG GTT ACT AGG GCG AAA | V-IX | P1.5,2 |
| M702/91               | CGG CCT CAA AAT ATT CAA ... CCT TAG | XII | Stop codon |
| 91297                 | CGG CCT CAA AAT ATT CAA ... CCT CAG ACT AGG IS130l (reverse orientation) | XIII | IS130l detected |
| II050775              | CGG CCT CAA AAT ATT CAA ... CCT CAG ACT AGG IS130l (forward orientation) | XIV | IS130l detected |

* Strains representative of the corresponding VR1 nucleotide sequence; other strains with the same epitope are listed in Table 1.  
\( ^{a} \) A period indicates a gap inserted for the purpose of alignment. The underlined segment indicates the insertion recognition site for IS1301, and the boldface letters refer to the mutation A-G.
Of the 14 porA alleles, allele VIII was the most common and was found in 16 of the 38 isolates. It corresponded to subtype P1.5a,10d, as defined by the VRs sequences (Table 1). Figure 1, based on the class 1 porin topology model described by van der Ley et al. (33), shows the loop structure of the molecule with the peptide sequence deduced from the sequence of allele VIII. The sites where the other alleles differed from this sequence are indicated.

Two other strains, 94N266 and 98N079, also with the deduced subtype P1.5a,10d, represented two distinct porA alleles (alleles VI and VII), differing from allele VIII by single base mutations at the same site in the periplasmic region between loops V and VI. These mutations resulted in a G\rightarrow D substitution (allele VI) and a G\rightarrow A substitution (allele VII). Subtype P1.5a,10k (allele IX) was found in two strains, and subtype P1.5a,10a (allele V) was found in one strain, 96M1966. This last strain had, in addition to a different VR2, an E\rightarrow A mutation at the top of loop VII.

The second most common subtype, found in 11 of the 38 strains, was P1.5,2. Eight of these strains had the same porA sequence (allele I), whereas alleles II, III, and X were represented by one strain each. Allele II presented a mutation in the transmembrane region between loops V and VI; allele III had a four-amino-acid deletion in loop IV, just outside of VR2 (shaded box in Fig. 1), and allele X had a stop codon between VR1 and VR2.

Of the 23 isolates with VR2 belonging to the P1.10 sequence family, 21 had the same 10 single base substitutions outside the two VRs (see Fig. 1) in comparison with strains having a VR2 of the P1.2 family. Of these 10 base differences, 3 resulted in amino acid substitutions in loop II, in loop IV, and in the transmembrane region between loops III and IV, respectively. The remaining two strains with VR2 in the P1.10 family, 84/96 and 114/96 (both allele IV and corresponding to subtype P1.5,10d), were found to have all but the first 2 of the 10 substitutions outside the VRs.

The remaining four strains each presented a distinct allele (alleles XI to XIV). Strains M702/91 and 67 had a stop codon

### Table 3. Nucleotide sequences encoding the VR2 region of the PorA in ET-15 N. meningitidis strains

| Representative strain | VR2 DNA sequence | VR2(s) identified in allele no. | Epitope variant |
|-----------------------|------------------|-------------------------------|-----------------|
| 57463                 | CAT TTT GTT CAG CAG ACT | I–III, X, XII P1.2          |
| 96M1966               | CAT TTT GTT CAG AAT AAG | V–VIII P1.10a                |
| 311268                | CAT TTT GTT CAG AAT AAG | IV P1.10d                    |
| 85/96                 | CAT TTT GTT CAG AAT AAT | IX P1.10k                    |
| 67                    | CAT TTT GTT CAG TAG    | XI                            |

\[a\] Strains representative of the corresponding VR2 nucleotide sequence; other strains with the same epitope are listed in Table 1.

\[b\] A period indicates a gap inserted for the purpose of alignment. The mutation between P1.10d and P1.10k is marked in boldface.

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![Figure 1](http://cvi.asm.org/on April 29, 2019 by guest)
interrupting the VR1 and the VR2, respectively, while the porA of 91297 (allele XIII) and II050775 (allele XIV) was interrupted by a single copy of the insertion sequence IS1301 in VR1 (15, 16). The insertion site was identical, but the orientation of IS1301 differed for these two isolates (Table 2). The target consensus for insertion of IS1301, 5'-AYTAG-3' (16), was found within the porA gene of these strains, generated by an A→G mutation in VR1 (Table 2), and was accompanied by a TA duplication.

**GenBank accession numbers.** The following nucleotide sequences were submitted to GenBank: 343/93, allele I (AF173031); M837, allele II (AF173020); 15/98, allele III (AF173024); 84/96, allele IV (AF173025); 96M1966, allele V (AF173030); 94N266, allele VI (AF173026); 98N079, allele VII (AF173028); 94N369, allele VIII (AF173027); 251033, allele IX (AF173029); 311340, allele X (AF173022); 67, allele XI (AF173023); M702/91, allele XII (AF173021); 91297, allele XIII (AF174359); and II050775, allele XIV (AF174360).

**SDS-PAGE and whole-cell immunoblot analysis.** The P1.5a subtype differs from P1.5 by an extra glutamine (Q) residue on the top of the loop I of the PorA. The monoclonal antibody directed against P1.5 recognizes both epitopes on dot blot but reacts more strongly and at a lower concentration with P1.5a subtype strains than with P1.5 strains (E. Wedege, unpublished data). This was illustrated by the fact that all of our strains with the P1.5a sequence reacted with the P1.5 monoclonal antibody on dot blot, while those with the P1.5 sequence had more variable reactions. None of the variants of the P1.10 family, identified by sequencing of the VR2 in this study, reacted with the P1.10 monoclonal antibody on dot blot, although it has been reported that P1.10a may be detected at a high antibody concentration (32). To analyze the cause of some discrepancies between the antibody reaction on dot blot and the sequence data, a selection of the strains were further studied by SDS-PAGE and immunoblotting.

**DISCUSSION**

Genetic characterization of the porA gene of *N. meningitidis* has been utilized extensively as a research tool (3, 10, 12, 22, 23, 31) but, as yet, has not been undertaken routinely for subtype identification. The use of monoclonal antibodies remains the most common technique for assigning serotypes and serosubtypes (1, 13, 27). However, there have been increasing reports of the failure of the existing panel of typing reagents to detect both VRs, and many strains are reported as NST (3, 10, 32). In this study we examined a collection of 38 strains, all of which had previously been identified as belonging to a single newly generated clone of the ET-37 complex (4, 8). This new variant, ET-15, was first identified in 1986 after an extensive outbreak of meningococcal disease in Canada (4) and has later been associated with outbreaks and clusters of meningococcal disease in many different countries (4, 8, 18, 20, 28, 34–35). The ET-15 strains examined here were isolated between 1988 and 1999 in eight countries and were selected on the basis of their phenotypic traits.

Given that the class I outer membrane protein of *N. meningitidis* is an important vaccine target, it is necessary to have a full complement of information regarding the genetic events which can and does arise within this gene during the epidemic spread of a clone. In the ET-15 strains the lack of reaction of the serosubtyping monoclonal antibodies was due to factors other than genetic variation in the sequenced region of the porA gene in three strains: 50447, which should have reacted with P1.2; 89486, which did not express a PorA, although the gene was present; and 84/96, which should have reacted with P1.5.
tions outside the two VRs, in addition to the two subtype-specific VRs. Another recombinational event starting within loop III was probably responsible for the P1.5,10d subtype. Allele VIII dominance among the 38 strains reflects, in part, the fact that a large proportion of Australian strains was included in the analysis and that all Australian strains with allele VIII might have a common origin. Both allele I and allele VIII were found in several countries. Our study does not permit determination of whether the generation of allele VIII has occurred independently several times during the spread of ET-15 or whether after the recombinational event generating allele VIII, ET-15 with allele I and with allele VIII, respectively, has had an intercontinental spread.

Single base mutations from one of the two common subtypes, P1.5,2 and P1.5a,10d, may have generated seven of these alleles, inclusive of three mutations to stop codons in different parts of the gene, resulting in no PorA expression. Three mutations occurred in the transmembrane region (allele II) or in the periplasmic region (alleles VI and VII) of PorA. These regions are not surface exposed, and thus these mutations are unlikely to provide protection against eventual bactericidal antibodies during the pathogenic process. It is noteworthy, however, that all three mutations are located within a fragment of the PorA molecule that has been shown to be one of the immunodominant T-cell epitopes recognized in humans (37). The other variants resulted from duplication of a codon, duplication of a codon triplet, deletion of four codons, and two cases of insertion of IS1301. In addition, one strain with allele I did not express a PorA, but the reason for the lack of expression has not been elucidated. Mutation in the promoter region of the porA gene has previously been shown to result in a total loss of expression (3).

Insertional inactivation of the porA gene by IS1301 has been described previously (3, 26). IS1301 was found at the same location within the VR1 in two ET-15 strains in this study, resulting in no PorA production and a phenotype of C:2a:NST. The orientation of IS1301 differed, however, between these two strains. The ability of IS1301 to insert with different orientations has been described previously: orientation of IS1301 varied among the 12 insertion sites in the chromosome of N. meningitidis B1940 (16). The target consensus for insertion of IS1301 is 5'-AYTAG-3', and this target site was found within the porA gene of our strains, with a A→G mutation being a prerequisite for the fulfillment of the insertion site criteria (15, 16). In all cases of inactivation of porA genes by IS1301 reported so far, the strains involved were serogroup C with either serotype 2a (26) or subtype P1.5,2 (3), phenotypes that are frequently associated with strains of the ET-37 complex. Although the multilocus genotypes of these specific strains have not been analyzed, they were recently isolated in Canada and England during increased meningococcal disease associated with ET-15 (4, 8). The two isolates identified here were from Canada and Iceland and represented two different genetic events, as evidenced by the reverse orientation of the insertion element. We found IS1301 inactivation of the porA gene in 2 of the 38 ET-15 isolates; in a recent study, 1 of 10 strains from Quebec with the P1.5,2 subtype, also probably ET-15, presented IS1301 (3). Thus, one could surmise that inactivation of the porA gene by IS1301 is not an infrequent event in the ET-15 clone.

The porA gene has been targeted as a diagnostic marker and has been extensively utilized in PCR strategies (9, 26, 29). In spite of the numerous diagnostic porA gene PCRs that have been undertaken in our laboratory and others, IS1301 has not been detected in clinical samples analyzed by porA PCRs other than those reported by Newcombe et al. (26). Thus, inactivation of the porA gene of meningococcal strains by IS1301 is unique to ET-15.

The distribution of IS1301 in the genome of meningococcal strains belonging to various genetic lineages was recently studied and shown to rarely occur in clones associated with epidemic disease (17). Especially, only 3 of 103 (3%) of serogroup C strains of the ET-37 complex possessed a copy of IS1301 anywhere in their genome (17), and two of these, one from Australia and one from Norway, were ET-15. Whether the insertion site for these two strains was also the porA gene has not been determined, but it is unlikely since the strains were subtypeable.

The range of VR families identified in the ET-15 strains was limited. For the VR2, the prototype subtype 2 was found, as well as several variants of the subtype 10 family: 10a, 10d, and 10k. The VR1 family was less diverse, with only subtypes 5 and 5a being represented. Although the porA gene has a mosaic structure (11) resulting from frequent recombinational events (30), within the period of 13 years since the origin of ET-15 only related VRs have been seen in association with the clone. Thus, genetic exchange has been restricted to a few closely related porA genes, perhaps because of structural constraints from other antigens in the outer membrane. Numerous genetic events other than recombination have been responsible for the allelic variation of the porA gene in these 38 strains. They have led to either minor modification of the epitopes, resulting in the failure of the monoclonal antibodies to adequately detect the VRs or the total loss of expression of the PorA protein. Deletion of a single amino acid is sufficient to hinder the recognition of the epitopes by the monoclonal antibodies (10, 12, 22, 31, 32). Other genes of ET-15 also are subject to high rate of genetic changes. Microevolution among strains of ET-15 isolated in the Czech Republic since 1993 was newly evidenced by study of the polymorphism in staD, pilA, and pilD genes (21).

Previously, the uniformity of the porA genes of serogroup C strains isolated from various locations was explained by the fact that these strains were of limited genetic diversity and recombination with other meningococci occurred infrequently (10). Our results reveal, however, the generation of a considerable degree of porA diversity among a selection of 38 strains belonging to the ET-15 clone of the ET-37 complex, all of which must have occurred since the emergence of this new clone after 1986. The porA gene is an important epidemiological marker, and comprehensive analysis of this gene can provide valuable data, especially during an outbreak of meningococcal disease. With the development of wild-type outer membrane vesicle vaccines (6) and polyvalent PorA vaccines (7), full characterization is also crucial if the PorA antigen is to be a useful vaccine target.

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REFERENCES

1. Abdillahi, H., and J. T. Poolman. 1987. Whole-cell ELISA for typing Neisseria meningitidis with monoclonal antibodies. FEMS Microbiol. Lett. 48:367–371.
2. Altschul, S. F., W. Gish, E. W. Myers, and D. J. Lipman. 1990. Basic local assignment search tool. J. Mol. Biol. 215:404–410.
3. Arhin, F. F., F. Moreau, J. W. Coulton, and E. L. Mills. 1998. Sequencing of 
porA from clinical isolates of Neisseria meningitidis defines a subtyping 
scheme and its genetic regulation. Can. J. Microbiol. 44:56–63.

4. Ashton, F. E., J. A. Ryan, A. Borczyk, D. A. Caugant, L. Mancino, and D. 
Huang. 1991. Emergence of a virulent clone of Neisseria meningitidis sero-
type 2a that is associated with meningococcal group C disease in Canada. 
J. Clin. Microbiol. 29:2480–2493.

5. Barlow, A. K., J. E. Heckels, and I. N. Clarke. 1989. The class 1 outer 
membrane protein of Neisseria meningitidis: gene sequence and structural 
and immunological similarities to gonococcal porins. Mol. Microbiol. 3:131– 
139.

6. Bjune, G., E. A. Hobly, J. K. Gronnesby, O. Arnesen, J. H. Fredriksen, A. 
Halstensen, E. Holten, A.-K. Lindbakh, H. Nokleby, E. Rosenqvist, L. K. 
Solberg, O. Cluss, J. Eng, L. O. Frosholm, A. Lystad, L. S. Bakkeiteig, and B. 
Hareide. 1991. Effect of outer membrane vesicle vaccine against group B 
meningococcal disease in Norway. Lancet 338:1093–1096.

7. Cartwright, K., R. Morris, H. Rumke, A. Fox, R. Borrow, N. Begg, P. Rich-
mond, and J. Poolman. 1999. Immunogenicity and reactivity in UK infants of a novel 
meningococcal vesicle vaccine containing multiple class 1 (PorA) outer membrane proteins. Vaccine 17:2612–2619.

8. Caugant, D. A. 1998. Population genetics and molecular epidemiology of 
Neisseria meningitidis. APMIS 106:505–525.

9. Caugant, D. A., E. A. Hobly, L. O. Frosholm, and P. Brandtzaeg. 1996. 
Polymerase chain reaction for case ascertainment of meningococcal menin-
gitis: application to the cerebrospinal fluids collected in the course of the 
Norwegian meningococcal serogroup B protection trial. Scand. J. Infect. Dis. 
28:149–153.

10. Feavers, I. M., A. J. Fox, S. Gray, D. M. Jones, and M. C. Maiden. 1996. 
Antigenic diversity of meningococcal outer membrane protein PorA has 
implications for epidemiological analysis and vaccine design. Clin. Diag. 
Lab. Immunol. 3:444–450.

11. Feavers, I. M., A. B. Heath, J. A. Bygraves, and M. C. J. Maiden. 1992. 
Role of horizontal genetic exchange in the antigenic variation of the class 1 
outer membrane protein of Neisseria meningitidis. Mol. Microbiol. 6:489–495.

12. Feavers, I. M., M. C. J. Maiden, and S. T. Wernet. 1991. Proteomic 
analyses of meningococcal serosubtyping antibodies. p. 314–315. In J. S. Evans, S. E. 
McGuinness, B. T., A. K. Barlow, I. N. Clarke, J. E. Farley, A. Anilionis, J. T. 
Poolman, and J. E. Heckels. 1998. Sequencing of porA defines the epitopes responsible for serosubtype specificity. J. Exp. Med. 171:1371–1382.

13. McGuinness, B. T., I. N. Clarke, P. R. Lambden, A. K. Barlow, J. T. Pool-
man, D. M. Jones, and J. E. Heckels. 1991. Point mutation in meningococcal 
porA gene associated with increased endemic disease. Lancet 337:514–17.

14. Newcombe, J. K. Cartwright, S. Dyer, and I. McFadden. 1996. Naturally 
occuring insertional inactivation of the porA gene of Neisseria meningitidis by 
integration of IS1301. Mol. Microbiol. 30:453–454.

15. Poolman, J. T., and H. Abdillahi. 1988. Outer membrane protein serosub-
typing of Neisseria meningitidis. Eur. J. Clin. Microbiol. 7:291–292.

16. Ringuelet, L., M. Lorange, A. Ryan, and F. Ashton. 1995. Meningococcal 
infestions in the Province of Quebec, Canada, during the period 1991 to 
1992. J. Clin. Microbiol. 33:53–57.

17. Saunders, N. B., W. D. Zollinger, and V. B. Rao. 1993. A rapid and sensitive 
PCR strategy employed for amplification and sequencing of porA from a single colony-forming unit of Neisseria meningitidis. Gene 137:153–162.

18. Maynard Smith, J. 1992. Analyzing the mosaic structure of genes. J. Mol.
Evol. 34:126–129.

19. Suker, J., I. M. Feavers, M. Achtman, G. Morelli, J.-F. Wang, and M. C. J. 
Maiden. 1994. The porA gene in serogroup A meningococci: evolutionary 
stability and mechanism of genetic variation. Mol. Microbiol. 12:253–265.

20. Suker, J., I. M. Feavers, and M. C. J. Maiden. 1996. Monoclonal antibody 
reactivity of patients with meningococcal disease in comparison with 
clinical reference strains of Neisseria meningitidis. J. Clin. Microbiol. 34:727–736.

21. van der Ley, P., J. E. Heckels, M. Virji, P. Hoogerhout, and J. T. Poolman. 
1991. Topology of outer membrane porins in pathogenic Neisseria spp. 
J. Clin. Microbiol. 29:263–270.

22. Wiertz, E. J. H. J., J. A. M. van Gaans-van den Brink, H. Gausepohl, A. 
Prochnicka-Chalifour, P. Hoogerhout, and J. T. Poolman. 1992. Identification 
of T cell epitopes occurring in a meningococcal class 1 outer membrane 
protein using overlapping peptides assembled with simultaneous multiple 
peptide synthesis. J. Exp. Med. 176:79–88.