

# Phase variation of NadA, PorA and Other Surface Proteins During Meningococcal Carriage

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## INTRODUCTION

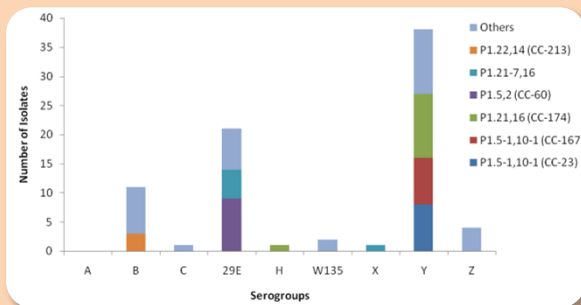
Many surface antigens of meningococci are subject to phase variation or high frequency, reversible switches in expression. Phase variation is mediated by hypervariable tandem DNA repeat tracts present within the promoters or reading frames of specific genes. Phase variation of PorA is driven by a polyC repeat tract in the core promoter, NadA by a 5'TAAA repeat tract upstream of the core promoter and Hpu/HmbR by polyG repeat tracts in the reading frame.

The aim of our study was to characterize how often phase variation events occur during longitudinal carriage of meningococci.

Meningococcal isolates, representative of persistence for 4 to 24 weeks, serum and saliva samples were obtained from first year University of Nottingham students starting in November 2008. Twenty colonies were picked after minimal *in vitro* passage from each carrier at each timepoint. Repeat tracts of PorA, NadA, Hpu and HmbR were analysed by either PCR and automated sequencing or PCR using fluorescently-labelled primers and sizing of PCR products by gene scan.

## RESULTS

Carriage rates rose from 48% at the first time point to 62% after 24 weeks. Greater than 50% of the isolates belonged to four PorA types: P1.5-1,10-1, P1.21,16, P1.5,2 and P1.21-7,16. These dominant PorA types were associated with serogroups Y and 29E and five clonal complexes. A high proportion of isolates (43.8%) possessed the Y capsule (Figure 1).



**Figure 1:** Serogroups, PorA types and major clonal complexes of carriage isolates obtained in November 2008. Note that serogroup Y isolates with a P1.5-1,10-1 serosubtype were split into two clones – CC-23, F4-1 and CC-167, F1-3).

Carriage was maintained for at least 6 months in 79% of volunteers (from a total of 29). Molecular typing showed that the strains carried by 61% of these carriers remained unchanged.

Mutations in the repeat tracts associated with phase variable expression of PorA, NadA, HpuA and HmbR were easily discernible (Figure 2).

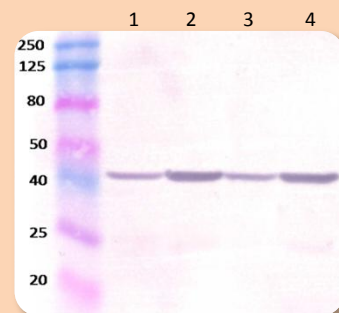
PorA type	Volunteer	Week 0	Week 4	Week 12	Week 24
P1.21,16	V43	12 / 12 / 13	-	11 / 12 / 12	-
	V51	12 / 12 / 12	12 / 12 / 12	11 / 12 / 13	11 / 12 / 13
	V52	12 / 12 / 13	11 / 12 / 13	12 / 12 / 11	-
	V54	13 / 14 / 14	15 / 14 / 13	15 / 12 / 11	-
	V58	11 / 12 / 13	12 / 12 / 13	-	11 / 12 / 12
	V59	12 / 13 / 13	12 / 12 / 13	12 / 12 / 12	12 / 12 / 13
	V88	11 / 11 / 9	11 / 9 / 10	11 / 9 / 10	11 / 9 / 9
	V138	12 / 12 / 13	12 / 12 / 13	12 / 12 / 13	-
	V114	11 / 9 / 10	12 / 9 / 10	12 / 9 / 10	-
P1.5,2	V134	11 / 10 / 10	12 / 10 / 10	12 / 10 / 10	-
	V185	12 / 10 / 10	12 / 10 / 10	-	12 / 10 / 10

**Figure 2:** Changes in repeat tract length of PorA, NadA, HpuA and HmbR during longitudinal carriage (24 weeks).

## CONCLUSION

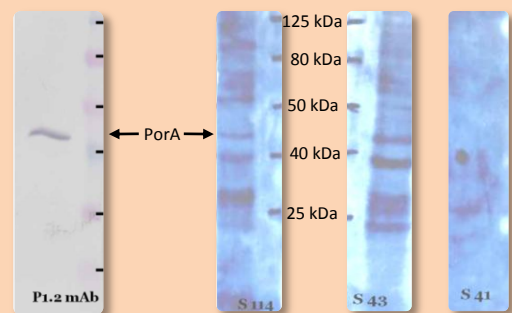
- Frequent changes in the number of repeat units were observed, indicative of switches in gene expression.
- Meningococcal isolates were all derived from mucosal surfaces suggesting that mucosal immune responses may be the major selective force driving phase variable changes in gene expression.
- Further studies of the mucosal and humoral responses in this cohort will highlight:
  - + the potential importance of phase variable antigens as vaccine targets; and
  - + the likelihood of phase variation-mediated immune evasion.

Changes in the repeat tracts of *porA* genes from two volunteers (V114 and V134, see Figure 2) resulted in increases in expression of the PorA protein (Figure 3).



**Figure 3:** Western blot of P1.2 mAb against whole cell lysates of meningococcal isolates obtained from volunteers V114 and V134. Lanes 1 and 2 are V114 isolates obtained at 0 and 12 weeks respectively while lanes 3 and 4 are V134 isolates obtained at 0 and 12 weeks respectively.  $1 \times 10^9$  cells were used to prepare each lysate.

Changes in expression patterns could be due to a change in antibody levels. The presence of antibodies reactive with PorA, and other outer membrane proteins, in sera from carriers was confirmed with western blot analyses (Figure 4).



**Figure 4:** Western blots of P1.2 mAb and human sera against outer membrane prep of meningococcal isolate from volunteer V119 (P1.5,2). S114, S43 and S41 are human sera samples from V114 (P1.5,2 carrier), V43 (P1.21,16 carrier) and V41 (non-carrier) respectively.