A Conserved Major Pilin subunit in *Neisseria meningitidis*

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INTRODUCTION

The type IV pilus (tfp) of *Neisseria meningitidis* is an important virulence factor, essential for processes such as adhesion and DNA uptake. The pilus is composed of pilin subunits encoded by *pilE*, which polymerise to form a fibre that extends through a pore in the outer membrane to the bacterial surface (Figure 1). Two classes of pili (Class I & Class II) have been described in Neisseria species (Diaz *et al*., 1984). They are distinguished by their reaction with antibody SM1, which fails to recognize Class II pili. The basis of this difference in reactivity has not been defined precisely but is related to differences in the major pilin subunit. It has previously been shown that commensal Neisseria express class II pilin, *N. gonorrhoeae* expresses class I pilin and meningococci harbour either class I or class II pilin genes (Aho *et al*., 1997).

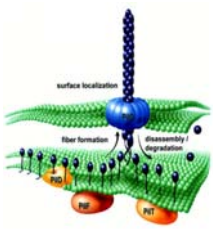


Figure 1: From Wolfgang *et al*., 2000.

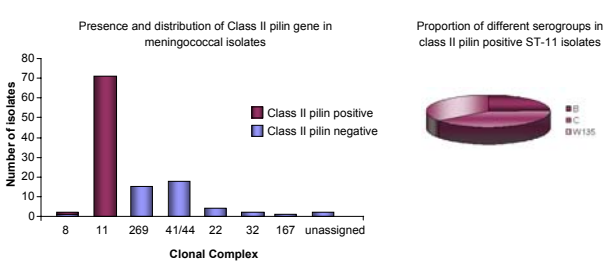
Current data concerning pilus assembly, structure and function in *N. meningitidis* has been assimilated from studies on pilus components of *N. gonorrhoeae*, or strains of *N. meningitidis* expressing class I pilin. The goal of this work is to determine the prevalence and distribution of class II pilin in meningococcal strains and analyse immune responses to class II pilin and investigate its potential as a vaccine antigen.

RESULTS

1. We performed PCR analysis of 115 *N. meningitidis* clinical isolates of different serogroups and clonal complexes from the UK and Spain (1999-2008) using primers specific for the class II *pilE* locus. As shown in Figure 2, we found that:

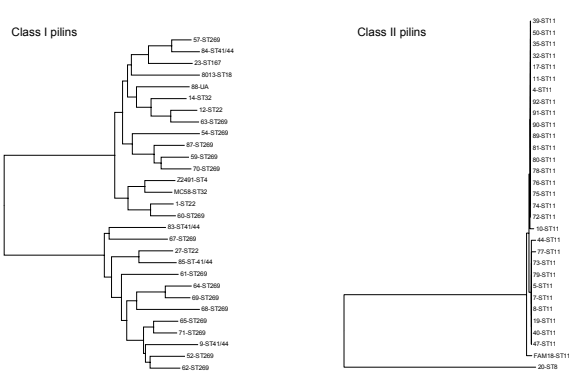
- 72/115 isolates had the gene encoding class II pilin
- 99% of these isolates belonged to the ST-11 clonal complex
- class II pilin was found in serogroups B, C & W135

Figure 2.



2. We amplified, cloned and sequenced the *pilE* gene from 56 meningococcal strains. Full length nucleotide sequence was obtained for 30 class II *pilE* genes and 26 class I *pilE* genes and was used to predict pilin sequence. Phylogenetic analysis was performed using MEGA and Clustal W2 at EBI (www.ebi.ac.uk). We found that:

- as expected, there is significant variation in the sequences of class I pilins
- class I pilins vary even among strains of the same clonal complex
- there is little variation among the class II pilins in ST-11 strains



3. Type four pilins share common features including a conserved N-terminal domain and a variable C-terminal domain. The C-terminus contains two cysteines that border a hypervariable domain (D-region). This region is exposed on the surface of the assembled pilus fibre (Figure 4); the variation arises via gene conversion and is thought to result from exposure to the immune system, providing a mechanism of immune escape.

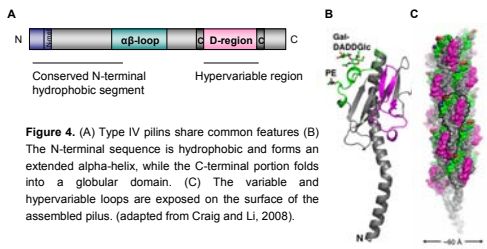


Figure 4. (A) Type IV pilins share common features (B) The N-terminal sequence is hydrophobic and forms an extended alpha-helix, while the C-terminal portion folds into a globular domain. (C) The variable and hypervariable loops are exposed on the surface of the assembled pilus. (adapted from Craig and Li, 2008).

Inspection of the pilin sequences from the strains belonging to the ST-11 clonal complex revealed that, in contrast to previously described class I pilins, the D-region is shorter and is invariant (Figure 5).

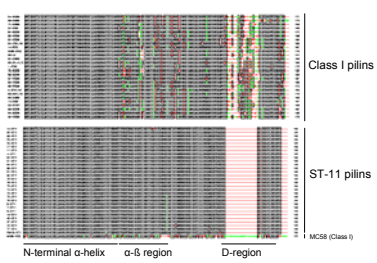
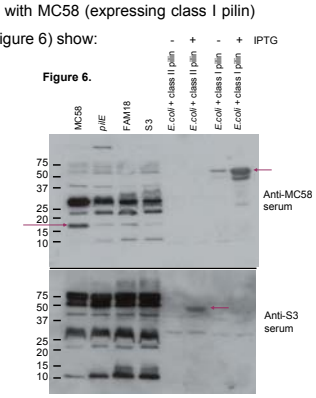


Figure 5. Prettyplot representation of alignment of meningococcal PilE sequences from isolates expressing class I pilin and from isolates belonging to the ST-11 clonal complex expressing class II pilin. Conserved residues within each alignment are black on grey background, similar residues are green and variable residues are red. Gaps are shown by red lines. Class I pilins have variable D-regions that are comprised of 32-35 residues. Class II pilins in ST-11 isolates have a shorter D-region, which is invariant.

4. Pilin subunits from Neisseria are immunogenic and studies have shown that the D-region is immunodominant while the conserved regions are nonimmunogenic (Hansen *et al*., 2007). We therefore investigated the antigenicity of class II pilin from an ST-11 strain. Truncated pilin was expressed as a fusion protein in *E. coli* and western blotting performed using sera from mice infected with MC58 (expressing class I pilin) or S3 (ST-11, class II pilin). Preliminary results (Figure 6) show:

- anti-MC58 serum contains antibodies that recognise class I pilin in Neisseria extracts & class I pilin expressed in *E. coli* (arrows).
- anti-S3 serum contains antibodies that recognise class II pilin expressed in *E.coli* (arrow).

Figure 6.



CONCLUSIONS

We have found that hypervirulent strains of *N. meningitidis* belonging to the ST-11 clonal complex express a highly conserved major pilin subunit protein (class II pilin) which has important differences compared to the previously studied variable pilin (class I pilin) from Neisseria species.

The striking conservation of the pilins we have identified in these isolates indicates that they do not undergo antigenic variation. Class I pilins vary significantly due to antigenic pressure so this is a remarkable difference with potentially important consequences on immune responses to pili.

As pili are surface expressed structures, the conservation of class II pilin in ST-11 strains raises the possibility of including it as an antigen in future multivalent vaccines.

REFERENCES

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