Published in final edited form as:

Int J Med Microbiol. 2009 February ; 299(2): 133–139. doi:10.1016/j.ijmm.2008.06.010.

A common gene pool for the *Neisseria* FetA antigen

Julia S. Bennett^{a,*}, Emily A. L. Thompson^a, Paula Kriz^b, Keith A. Jolley^a, and Martin C. J. Maiden^a

^aThe Peter Medawar Building for Pathogen Research and Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3SY, UK

^bNational Reference Laboratory for Meningococcal Infections, National Institute of Public Health, Prague, Czech Republic

Abstract

Meningococcal FetA is an iron-regulated, immunogenic outer membrane protein and vaccine component. The most diverse region of this protein is a previously defined variable region (VR) that has been shown to be immunodominant. In this analysis, a total of 275 *Neisseria lactamica* isolates, collected during studies of nasopharyngeal bacterial carriage in infants were examined for the presence of a *fetA* gene. The *fetA* VR nucleotide sequence was determined for 217 of these isolates, with *fetA* apparently absent from 58 isolates, the majority of which belonged to the ST-624 clonal complex. The VR in *N. lactamica* was compared to the same region in *Neisseria meningitidis*, *Neisseria gonorrhoeae* and a number of other commensal *Neisseria*. Identical *fetA* variable region sequences were identified among commensal and pathogenic *Neisseria*, suggesting a common gene pool, differing from other antigens in this respect. Carriage of commensal *Neisseria* species, such as *N. lactamica*, that express FetA may be involved in the development of natural immunity to meningococcal disease.

Keywords

FetA; *Neisseria meningitidis*; commensal *Neisseria*; gene pool

Introduction

Neisseria meningitidis is a major cause of meningitis and septicaemia. Although vaccines based on serogroups A, C, Y and W135 are effective, a comprehensive vaccine is not yet available, and the development of a vaccine against serogroup B meningococci is hampered by the similarity of its capsule to host antigens and its poor immunological reactivity (Finne et al., 1987). Serogroup B meningococci are responsible for a large burden of disease in North America and Europe, causing over 85% of meningococcal disease in England and Wales since the introduction of a serogroup C conjugate vaccine (Gray et al., 2006) and so the development of an effective vaccine based on these organisms is a priority for many public health authorities.

Approaches to designing vaccines that protect against serogroup B meningococcal disease have focused on immunogenic non-capsular outer membrane proteins. Some vaccine formulations include the immunodominant outer membrane protein FetA (Wedegge et al.,

*Corresponding author. Mailing address: The Peter Medawar Building for Pathogen Research and Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3SY, UK. Telephone: +44 (0)1865 281537, Fax: +44 (0)1865 281275, julia.bennett@zoo.ox.ac.uk.

1998; Vipond et al., 2005), an iron regulated, TonB-dependent enterobactin receptor (Pettersson et al., 1990; Ala'Aldeen et al., 1994; Beucher & Sparling, 1995). A recent topology model of FetA, also known as FrpB, predicts a 22-stranded β -barrel with 11 surface exposed loops and an N-terminal plug domain that closes the barrel (Kortekaas et al., 2007). Loop 5 of this model corresponds to a variable region (VR) previously shown to contain epitopes for mouse monoclonal antibodies (van der Ley et al., 1996) and currently used in high resolution molecular typing of meningococci (Jolley et al., 2007). Although FetA is known to be diverse in meningococci, with allelic variants generated by both point mutation and horizontal genetic exchange (Thompson et al., 2003), research suggests that it could be a valuable component of vaccines that combine variant sequences from both FetA and PorA (Urwin et al., 2004).

Acquisition of natural immunity to meningococcal disease in childhood may follow nasopharyngeal colonization by non-pathogenic species of *Neisseria* such as *N. lactamica* that express immunologically cross-reactive surface antigens shared with the meningococcus (Gold et al., 1978; Griffiss et al., 1987). *N. lactamica* expresses FetA (Sanchez et al., 2006) and the *N. lactamica* isolate (020-06) that has been completely sequenced at The Wellcome Trust Sanger Institute (http://www.sanger.ac.uk/Projects/N_lactamica/) has a FetA antigen with around 90% sequence identity to meningococcal strains Z2491 (Parkhill et al., 2000), MC58 (Tettelin et al., 2000) and FAM18 (Bentley et al., 2007), and is of similar size. Vaccines based on *N. lactamica* whole cells, outer membrane proteins, or outer membrane vesicles have been proposed (Griffiss et al., 1991; Oliver et al., 2002) and a vaccine based on *N. lactamica* outer membrane vesicles is being developed, with FetA as a component (Finney et al., 2007). However, the antigenic determinants in commensal *Neisseria* that induce cross-reactive immune responses to meningococcal infection, have not been defined (Tang et al., 1999), and the variability of FetA in commensal *Neisseria* and its relationship to meningococcal FetA has not been documented.

In this study, 275 *N. lactamica* isolates, collected from infants and a small number of siblings and parents during studies of nasopharyngeal bacterial carriage, were examined to assess the diversity of the FetA VR in this species. These isolates, collected in the UK, had previously been characterized by multilocus sequence typing (MLST) (Maiden et al., 1998; Bennett et al., 2005) with 72 distinct sequence types (STs) identified. The FetA VR sequences from *N. lactamica* were compared to the VR sequences from other non-pathogenic and pathogenic *Neisseria* to investigate relatedness and the potential for this commensal *Neisseria* antigen contributing to meningococcal immunity.

Materials and methods

Isolates

Details of the 275 *N. lactamica* isolates, collected in Oxfordshire, UK, are available from the *Neisseria* MLST database: <http://pubmlst.org/neisseria/> (Jolley et al., 2004). The genetic diversity of these *N. lactamica* isolates was similar to that seen in *N. lactamica* from other locations and likely to be representative of the global diversity. There is no evidence that these isolates form a separate population and the collection contained representatives from five of six clonal complexes currently defined for *N. lactamica* (Bennett, 2006). Nucleotide sequences of the *fetA* VR from these isolates were compared to sequences determined for a study of antigenic diversity in meningococcal FetA (Thompson, et al., 2003). These sequences were obtained from the 107 meningococcal isolates used to validate MLST (Maiden, et al., 1998), and assembled to represent the global diversity of meningococci in the mid to latter part of the twentieth century. Also included were *fetA* VR nucleotide sequences from a collection of meningococci obtained in the Czech Republic and characterized using MLST, which comprised 339 carried meningococci isolated between

1992 and 1997 (Jolley et al., 2002; Yazdankhah et al., 2004; Jolley et al., 2005; Bennett et al., 2007) and 53 disease related meningococci, collected during 1993. Gene sequences from nine other meningococcal isolates were obtained from GenBank with the following accession numbers: AL162753; AE002548; U55377; U55378; U67310; U67311; U67312; U67313 and U67314.

Nucleotide sequences of the *fetA* VR were also determined for a collection of 33 non-pathogenic members of the genus *Neisseria* which had undergone microbiological and biochemical characterization (Barrett & Sneath, 1994). The collection included six *Neisseria cinerea*; one *Neisseria flava*; three *Neisseria flavescens*; 10 *N. lactamica*; six *Neisseria mucosa*; five *Neisseria polysaccharea* and two *Neisseria subflava*. A total of 11 *fetA* VR nucleotide sequences were available for *Neisseria gonorrhoeae*, including nine obtained from isolates provided by Prof. C. A. Hart, University of Liverpool, UK, and two from GenBank with accession numbers U13980 and AF115385. The VR nucleotide and peptide sequences used are available from the *Neisseria* FetA database: <http://neisseria.org/nm/typing/feta/>.

Nucleotide sequence determination

Nucleotide sequence determination of the FetA VR was undertaken as described previously (Thompson, et al., 2003). Briefly, sequence templates were generated using PCRs with primers 12 and 4 (van der Ley, et al., 1996), and occasionally A14 (Carson et al., 1999), and then purified by precipitation with polyethylene glycol and sodium chloride (Embley, 1991). The termination products were generated by cycle sequencing using combinations of the primers S1, S8, S12, S13, S15 (Thompson, et al., 2003), and BigDye terminators (Applied Biosystems). The products were then separated with either a Prism 3700 DNA analyser or a Prism 377 DNA analyser (Applied Biosystems). The sequence of each strand was determined at least once, and the resultant DNA sequences were assembled using the STADEN suite of computer programs (Staden, 1996).

Analysis of sequence data

Nucleotide sequences were manually aligned and translated using the SeqLab program, part of the GCG Wisconsin package, version 10.3 (Womble, 2000). Alignments were based on amino acid sequence similarity, and codon integrity was maintained. The sequence data was imported into MEGA 3.1 (Kumar et al., 2004) to construct Neighbour-joining trees from amino acid p-distances. The VR nucleotide sequences from *N. lactamica* were compared with those from *N. meningitidis*, using DnaSP 4.0 (Rozas et al., 2003) to calculate shared polymorphisms and fixed differences, and Arlequin 2.0 (Schneider et al., 2000) to compute pairwise F_{ST} . F_{ST} values indicate levels of gene flow between populations, with a value of one indicating no gene flow (100% differentiated populations) and zero indicating free genetic exchange (no population differentiation).

Results and discussion

Nucleotide sequences of the *fetA* VR were determined for 217 *N. lactamica* isolates collected in Oxfordshire, with *fetA* apparently absent from 58 isolates. The *fetA* gene was considered absent if it could not be amplified by the PCR employing combinations of the *fetA* PCR and sequencing primers which are complimentary to conserved DNA sequences within *Neisseria*. Particular VRs were associated with MLST STs, and the majority of the isolates that lacked *fetA* belonged to the ST-624 clonal complex, with *fetA* apparently absent from all isolates belonging to this complex. Lack of *fetA* has also been reported among meningococcal isolates (Claus et al., 2007; Marsh et al., 2007), with deletions mediated by repeat arrays flanking the gene. Deletion in the meningococcus, however, is considered to be

a rare occurrence. The majority of *N. lactamica* isolates used in this study were obtained from UK population studies and it is not known whether *fetA* deletion is common in other isolate collections. Isolates of *N. lactamica* belonging to the ST-624 clonal complex have also been collected in Germany and Malawi (<http://pubmlst.org/neisseria/>), although it has not been established whether *fetA* is absent from these related isolates.

Among the Oxfordshire *N. lactamica* isolates, 28 unique *fetA* VR nucleotide sequences, corresponding to 28 unique peptide sequences, were determined. These were grouped on the basis of peptide sequence similarity and sequence length into five VR families, as previously defined for the meningococcus and designated F1-F5 (Thompson, et al., 2003). A total of 14 VR sequences were identified as family F1 variants, two as family F2, five as family F3, three as family F4 and four as family F5. Of the 28 variants, 17 were present in isolates obtained from single individuals. The most common variant was F1-25, found among *N. lactamica* isolates with seven different STs obtained from 10 unrelated individuals.

Neighbour-joining trees, one for each of the five VR families, were constructed from FetA amino acid sequences from all eight species analysed (Figure 1). The phylogenies produced show that sequences from named species could be more closely related to sequences from other *Neisseria*, than to their own defined species and that identical peptide sequences were distributed among many neisseriae. There was little evidence for distinct, species specific clusters and examples of both commensals and pathogens were present within each family. An interrogation of the FetA database (<http://neisseria.org/nm/typing/feta/>) provided other examples of VR sharing between species. For example, the *fetA* VR sequence from FAM18, a representative of the ST-11 complex and a disease causing serogroup C strain (Bentley, et al., 2007), is the same as the *fetA* VR from *N. lactamica* isolates obtained from an infant between the age of 10 weeks and six months. The majority of amino acid sequences were encoded by single nucleotide sequences, apart from five family F5 variants (5-1, 5-6, 5-8, 5-12, 5-19), which differed by one to two nucleotides. The sharing of identical nucleotide sequences among species could be a consequence of either shared ancestry, which seems unlikely for a gene sequence under positive selection, or recent interspecies genetic exchange.

VR nucleotide sequences from *N. lactamica* and *N. meningitidis* were compared to determine gene flow and genetic similarity (Table 1). The nucleotide sequences from the other *Neisseria* were not included in this analysis as there were too few examples from each species in the dataset. The frequency of polymorphisms shared between *N. lactamica* and *N. meningitidis* indicated a high level of genetic similarity. There were no fixed differences between the nucleotide sequences in any of the five VR families examined, and F_{ST} values of zero indicated high gene flow between *N. lactamica* and *N. meningitidis*, with sequences not statistically genetically different ($p > 0.05$).

The high levels of diversity among the VRs, the sharing of identical nucleotide sequences, the sharing of polymorphisms, the absence of fixed differences and a F_{ST} value of zero between *N. lactamica* and *N. meningitidis*, indicate a common gene pool for the *fetA* VR, with frequent genetic exchange among neisseriae at this locus. A common gene pool has also been described for *tbpB* (Linz et al., 2000), although it differs from *fetA* in that imports of DNA from commensal species into meningococci are from a distinct *tbpB* family and reduce variant fitness (Zhu et al., 2001). Horizontal genetic exchange must be sufficiently rare among the *Neisseria* to maintain species as distinct biological entities (Maiden et al., 1996) and not all antigens have common gene pools. The *N. lactamica* porin for example, is distinct from the porins of *N. meningitidis* and *N. gonorrhoeae* (Derrick et al., 1999) (Bennett et al., in press), and some meningococcal antigens, such as TspA (Oldfield et al., 2007) and NadA (Comanducci et al., 2002), may not be expressed by *N. lactamica*.

Commensal *Neisseria* expressing immunologically cross-reactive surface antigens shared with meningococci are vaccine candidates, although at present only *N. lactamica* and its antigens have been studied intensively as components in vaccines against meningococcal disease (Oliver, et al., 2002; Gorringe et al., 2005; Sardinas et al., 2006). As *N. lactamica* is acapsular (Griffiss, et al., 1987) and does not possess the immunodominant antigen PorA (Derrick, et al., 1999), other antigens would be necessary to induce anti-meningococcal immunity. Antigens from *N. lactamica* and other commensal species, that have a high degree of sequence similarity to *N. meningitidis*, such as the FetA VR, are likely to be the most effective.

It has been proposed that carriage of commensal *Neisseria*, such as *N. lactamica* contributes to the development of natural immunity to meningococcal disease (Gold, et al., 1978). Expression of outer membrane proteins such as FetA could be involved in this immunity. This should be taken into consideration when designing vaccines that include these cross-reactive antigens, as it has been suggested that their use could impede the acquisition of natural immunity to meningococcal disease by preventing colonization by commensals (Sanchez et al., 2002). However, the effect of vaccination on commensals related to *N. meningitidis* has yet to be established in humans.

Acknowledgments

Julia Bennett was funded by the Meningitis Research Foundation. Emily Thompson was funded by a Biotechnology and Biological Sciences Research Council CASE studentship awarded to the University of Oxford and the National Institute for Biological Standards and Control. Paula Kriz was supported by grant No. 1A8688-3/05 of the Internal Grant Agency of Ministry of Health of the Czech Republic. Martin Maiden is a Wellcome Trust Senior Research Fellow and Keith Jolley is funded by The Wellcome Trust.

The *N. lactamica* samples were obtained from studies carried out by the Oxford Vaccine Group supported by the Wellcome Trust, reference number: 056886/2/994/Z. We would like to thank the staff of the Oxford Vaccine Group for supporting the collection of samples and the families and children who participated in the studies. The authors are grateful to Tony Hart for providing the gonococcal samples.

References

- Ala' Aldeen D, Davies HA, Borriello SP. Vaccine potential of meningococcal FrpB: studies on surface exposure and functional attributes of common epitopes. *Vaccine*. 1994; 12:535–541. [PubMed: 7518627]
- Barrett SJ, Sneath PHA. A numerical phenotypic taxonomic study of the genus *Neisseria*. *Microbiology*. 1994; 140:2867–2891. [PubMed: 8000550]
- Bennett, JS. The Relationship of *Neisseria lactamica* to the Pathogenic *Neisseria*: Implications for Vaccine Development [D.Phil thesis]. University of Oxford; Oxford: 2006.
- Bennett JS, Griffiths DT, McCarthy ND, Sleeman KL, Jolley KA, Crook DW, Maiden MC. Genetic diversity and carriage dynamics of *Neisseria lactamica* in infants. *Infect. Immun*. 2005; 73:2424–32. [PubMed: 15784588]
- Bennett JS, Jolley KA, Sparling PF, Saunders NJ, Hart CA, Feavers IM, Maiden MC. Species Status of *Neisseria gonorrhoeae*: Evolutionary and Epidemiological Inferences from MLST. *BMC Biology*. 2007; 5:35. [PubMed: 17825091]
- Bentley SD, Vernikos GS, Snyder LA, Churcher C, Arrowsmith C, Chillingworth T, Cronin A, Davis PH, Holroyd NE, Jagels K, Maddison M, Moule S, Rabinowitsch E, Sharp S, Unwin L, Whitehead S, Quail MA, Achtman M, Barrell B, Saunders NJ, Parkhill J. Meningococcal Genetic Variation Mechanisms Viewed through Comparative Analysis of Serogroup C Strain FAM18. *PLoS Genetics*. 2007; 3:e23. [PubMed: 17305430]
- Beucher M, Sparling PF. Cloning, sequencing, and characterization of the gene encoding FrpB, a major iron-regulated, outer membrane protein of *Neisseria gonorrhoeae*. *J. Bacteriol*. 1995; 177:2041–2049. [PubMed: 7721696]

- Carson SDB, Klebba PE, Newton SMC, Sparling PF. Ferric enterobactin binding and utilisation by *Neisseria gonorrhoeae*. J. Bacteriol. 1999; 181:2895–2901. [PubMed: 10217784]
- Claus H, Elias J, Meinhardt C, Frosch M, Vogel U. Deletion of the meningococcal fetA gene used for antigen sequence typing of invasive and commensal isolates from Germany: frequencies and mechanisms. J Clin Microbiol. 2007; 45:2960–4. [PubMed: 17626167]
- Comanducci M, Bambini S, Brunelli B, Adu-Bobie J, Arico B, Capecchi B, Giuliani MM, Massignani V, Santini L, Savino S, Granoff DM, Caugant DA, Pizza M, Rappuoli R, Mora M. NadA, a novel vaccine candidate of *Neisseria meningitidis*. J. Exp. Med. 2002; 195:1445–54. [PubMed: 12045242]
- Derrick JP, Urwin R, Suker J, Feavers IM, Maiden MCJ. Structural and evolutionary inference from molecular variation in *Neisseria* porins. Infect. Immun. 1999; 67:2406–2413. [PubMed: 10225902]
- Embley TM. The linear PCR reaction: a simple and robust method for sequencing amplified rRNA genes. Lett. Appl. Microbiol. 1991; 13:171–174. [PubMed: 1370053]
- Finne J, Bitter Suermann D, Goridis C, Finne U. An IgG monoclonal antibody to group B meningococci cross-reacts with developmentally regulated polysialic acid units of glycoproteins in neural and extraneural tissues. J. Immunol. 1987; 138:4402–7. [PubMed: 3108388]
- Finney M, Vaughan T, Taylor S, Hudson MJ, Pratt C, Wheeler JX, Vipond C, Feavers I, Jones C, Findlow J, Borrow R, Gorringe A. Characterization of the Key Antigenic Components and Pre-Clinical Immune Responses to a Meningococcal Disease Vaccine Based on *Neisseria lactamica* Outer Membrane Vesicles. Human Vaccines. 2008; 4:23–30. [PubMed: 17921703]
- Gold R, Goldschneider I, Lepow ML, Draper TF, Randolph M. Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in infants and children. J. Infect. Dis. 1978; 137:112–121. [PubMed: 415097]
- Gorringe A, Halliwell D, Matheson M, Reddin K, Finney M, Hudson M. The development of a meningococcal disease vaccine based on *Neisseria lactamica* outer membrane vesicles. Vaccine. 2005; 23:2210–3. [PubMed: 15755597]
- Gray SJ, Trotter CL, Ramsay ME, Guiver M, Fox AJ, Borrow R, Mallard RH, Kaczmarski EB. Epidemiology of meningococcal disease in England and Wales 1993/94 to 2003/04: contribution and experiences of the Meningococcal Reference Unit. J. Med. Microbiol. 2006; 55:887–96. [PubMed: 16772416]
- Griffiss JM, Brandt B, Jarvis GA. Natural immunity to *Neisseria meningitidis*. In: Vedros, NA., editor. Evolution of meningococcal disease. Vol. II. CRC Press Inc.; Boca Raton, FL: 1987. p. 99–119.
- Griffiss JM, Yamasaki R, Estabrook M, Kim JJ. Meningococcal molecular mimicry and the search for an ideal vaccine. Trans. R. Soc. Trop. Med Hyg. 1991; 85(Suppl 1):32–36. [PubMed: 1725072]
- Jolley KA, Brehony C, Maiden MC. Molecular typing of meningococci: recommendations for target choice and nomenclature. FEMS Microbiol. Rev. 2007; 31:89–96. [PubMed: 17168996]
- Jolley KA, Chan MS, Maiden MC. mlstdbNet - distributed multi-locus sequence typing (MLST) databases. BMC Bioinformatics. 2004; 5:86. [PubMed: 15230973]
- Jolley KA, Kalmusova J, Feil EJ, Gupta S, Musilek M, Kriz P, Maiden MC. Carried Meningococci in the Czech Republic: a Diverse Recombining Population. J. Clin. Microbiol. 2002; 40:3549–3550.
- Jolley KA, Wilson DJ, Kriz P, McVean G, Maiden MC. The influence of mutation, recombination, population history, and selection on patterns of genetic diversity in *Neisseria meningitidis*. Mol. Biol. Evol. 2005; 22:562–9. [PubMed: 15537808]
- Kortekaas J, Pettersson A, van der Biezen J, Weynants VE, van der Ley P, Poolman J, Bos MP, Tommassen J. Shielding of immunogenic domains in *Neisseria meningitidis* FrpB (FetA) by the major variable region. Vaccine. 2007; 25:72–84. [PubMed: 16914236]
- Kumar S, Tamura K, Nei M. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Briefings in Bioinformatics. 2004; 5:150–63. [PubMed: 15260895]
- Linz B, Schenker M, Zhu P, Achtman M. Frequent interspecific genetic exchange between commensal *neisseriae* and *Neisseria meningitidis*. Mol. Microbiol. 2000; 36:1049–1058. [PubMed: 10844690]
- Maiden MCJ, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, Zhang Q, Zhou J, Zurth K, Caugant DA, Feavers IM, Achtman M, Spratt BG. Multilocus sequence typing: a portable

- approach to the identification of clones within populations of pathogenic microorganisms. *Proc. Natl. Acad. Sci. USA*. 1998; 95:3140–3145. [PubMed: 9501229]
- Maiden MCJ, Malorny B, Achtman M. A global gene pool in the *neisseriae*. *Mol. Microbiol.* 1996; 21:1297–1298. [PubMed: 8898397]
- Marsh JW, O'Leary MM, Shutt KA, Harrison LH. Deletion of *fetA* gene sequences in serogroup B and C *Neisseria meningitidis* isolates. *J. Clin. Microbiol.* 2007; 45:1333–5. [PubMed: 17287336]
- Oldfield NJ, Bland SJ, Taraktsoglou M, Dos Ramos FJ, Robinson K, Wooldridge KG, Ala' Aldeen DA. T-cell stimulating protein A (TspA) of *Neisseria meningitidis* is required for optimal adhesion to human cells. *Cell. Microbiol.* 2007; 9:463–78. [PubMed: 16965515]
- Oliver KJ, Reddin KM, Bracegirdle P, Hudson MJ, Borrow R, Feavers IM, Robinson A, Cartwright K, Gorringe AR. *Neisseria lactamica* protects against experimental meningococcal infection. *Infect. Immun.* 2002; 70:3621–6. [PubMed: 12065503]
- Parkhill J, Achtman M, James KD, Bentley SD, Churcher C, Klee SR, Morelli G, Basham D, Brown D, Chillingworth T, Davies RM, Davis P, Devlin K, Feltwell T, Hamlin N, Holroyd S, Jagels K, Leather S, Moule S, Mungall K, Quail MA, Rajandream MA, Rutherford KM, Simmonds M, Skelton J, Whitehead S, Spratt BG, Barrell BG. Complete DNA sequence of a serogroup A strain of *Neisseria meningitidis* Z2491. *Nature*. 2000; 404:502–506. [PubMed: 10761919]
- Pettersson A, Kuipers B, Pelzer M, Verhagen E, Tiesjema RH, Tommassen J, Poolman JT. Monoclonal antibodies against the 70-kilodalton iron-regulated protein of *Neisseria meningitidis* are bactericidal and strain specific. *Infect. Immun.* 1990; 58:3036–3041. [PubMed: 1696939]
- Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*. 2003; 19:2496–7. [PubMed: 14668244]
- Sanchez S, Abel A, Arenas J, Criado MT, Ferreira CM. Cross-linking analysis of antigenic outer membrane protein complexes of *Neisseria meningitidis*. *Res. Microbiol.* 2006; 157:136–42. [PubMed: 16135405]
- Sanchez S, Troncoso G, Criado MT, Ferreira C. In vitro induction of memory-driven responses against *Neisseria meningitidis* by priming with *Neisseria lactamica*. *Vaccine*. 2002; 20:2957–63. [PubMed: 12126908]
- Sardinas G, Reddin K, Pajon R, Gorringe A. Outer membrane vesicles of *Neisseria lactamica* as a potential mucosal adjuvant. *Vaccine*. 2006; 24:206–14. [PubMed: 16115701]
- Schneider, S.; Roessli, D.; Excoffier, L. Arlequin version 2.000: a software for population genetic data analysis. University of Geneva; Geneva: 2000.
- Staden R. The Staden sequence analysis package. *Mol. Biotechnol.* 1996; 5:233–241. [PubMed: 8837029]
- Tang C, Moxon R, Levine MM. For discussion: live attenuated vaccines for group B meningococcus. *Vaccine*. 1999; 17:114–7. [PubMed: 9987144]
- Tettelin H, Saunders NJ, Heidelberg J, Jeffries AC, Nelson KE, Eisen JA, Ketchum KA, Hood DW, Peden JF, Dodson RJ, Nelson WC, Gwinn ML, DeBoy R, Peterson JD, Hickey EK, Haft DH, Salzberg SL, White O, Fleischmann RD, Dougherty BA, Mason T, Ciecko A, Parksey DS, Blair E, Cittone H, Clark EB, Cotton MD, Utterback TR, Khouri H, Qin H, Vamathevan J, Gill J, Scarlato V, Masignani V, Pizza M, Grandi G, Sun L, Smith HO, Fraser CM, Moxon ER, Rappuoli R, Venter JC. Complete genome sequence of *Neisseria meningitidis* serogroup B strain MC58. *Science*. 2000; 287:1809–1815. [PubMed: 10710307]
- Thompson EAL, Feavers IM, Maiden MCJ. Antigenic diversity of meningococcal enterobactin receptor FetA, a vaccine component. *Microbiology*. 2003; 149:1849–58. [PubMed: 12855736]
- Urwin R, Russell JE, Thompson EA, Holmes EC, Feavers IM, Maiden MC. Distribution of Surface Protein Variants among Hyperinvasive Meningococci: Implications for Vaccine Design. *Infect. Immun.* 2004; 72:5955–62. [PubMed: 15385499]
- van der Ley P, van der Biezen J, Suttmuller R, Hoogerhout P, Poolman JT. Sequence variability of FrpB, a major iron-regulated outer-membrane protein in the pathogenic *neisseriae*. *Microbiology*. 1996; 142:3269–3274. [PubMed: 8969523]
- Vipond C, Wheeler JX, Jones C, Feavers IM, Suker J. Characterization of the protein content of a meningococcal outer membrane vesicle vaccine by polyacrylamide gel electrophoresis and mass spectrometry. *Human Vaccines*. 2005; 1:80–4. [PubMed: 17038831]

- Wedeg E, Høiby EA, Rosenqvist E, Bjune G. Immune responses against major outer membrane antigens of *Neisseria meningitidis* in vaccinees and controls who contracted meningococcal disease during the Norwegian serogroup B protection trial. *Infect. Immun.* 1998; 66:3223–31. [PubMed: 9632589]
- Womble DD. GCG: The Wisconsin Package of sequence analysis programs. *Methods Mol. Biol.* 2000; 132:3–22. [PubMed: 10547828]
- Yazdankhah SP, Kriz P, Tzanakaki G, Kremastinou J, Kalmusova J, Musilek M, Alvestad T, Jolley KA, Wilson DJ, McCarthy ND, Caugant DA, Maiden MC. Distribution of Serogroups and Genotypes among Disease-Associated and Carried Isolates of *Neisseria meningitidis* from the Czech Republic, Greece, and Norway. *J. Clin. Microbiol.* 2004; 42:5146–53. [PubMed: 15528708]
- Zhu P, van der Ende A, Falush D, Brieske N, Morelli G, Linz B, Popovic T, Schuurman IG, Adegbola RA, Zurth K, Gagneux S, Platonov AE, Riou JY, Caugant DA, Nicolas P, Achtman M. Fit genotypes and escape variants of subgroup III *Neisseria meningitidis* during three pandemics of epidemic meningitis. *Proc. Natl. Acad. Sci. USA.* 2001; 98:5234–9. [PubMed: 11287631]

Figure 1. Neighbour-joining trees showing the relationships among five families of FetA VR sequences from *Neisseria*.
Model: Amino acid p-distance.

Table 1
Genetic variation of *fetA* VR families from *N. lactamica* and *N. meningitidis*

	<i>fetA</i> family				
	F1	F2	F3	F4	F5
No. of <i>N. lactamica</i> isolates	88	6	49	42	43
No. of <i>N. meningitidis</i> isolates	224	2	153	30	99
No. of <i>N. lactamica</i> nucleotide variants	17	2	5	3	5
No. of <i>N. meningitidis</i> nucleotide variants	22	2	9	6	19
Length of aligned nucleotide sequences	96	78	117	120	126
Total no. of polymorphic sites	58	6	52	38	67
Total no. of polymorphisms	79	7	62	42	77
F_{st} within family *	0.00	0.00	0.00	0.00	0.00
Shared polymorphisms	59	0	50	16	55
Fixed differences	0	0	0	0	0

* $p > 0.05$, indicating that the populations were not significantly genetically different