Reviewer's report

Title: Neisseria meningitidis porA, fetA and fHbp gene distribution in Western Australia 2000 to 2011.

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Reviewer: Maurizio Comanducci

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General comments

The paper by Peter Boan et al., “Neisseria meningitidis porA, fetA and fHbp gene distribution in Western Australia 2000 to 2011” reports about genetic characterization of 128 of the 271 meningococcal strains which have been isolated between 2000 and 2011 in Western Australia, and stored at PMH. The focus of the paper is on the sequence variability of genes porA, feA and fHbp, which code for proteins PorA, FetA and fHbp, respectively. PorA is the immunodominant antigen in OMV-based anti-MenB vaccines (Frasch et al., Methods Mol Med. 2001;66:81-107). fHbp is the unique component of the Pfizer anti-MenB vaccine (ref. 6) and one of the multiple components of the Novartis vaccine 4CMenB (ref. 7). FetA is an important outer membrane protein and good vaccine candidate (ref. 10). Some association of PorA and fHbp variants with age and demographics are described in the paper, as well.

The subject of the present paper is quite interesting. The variability of the sub-capsular components of the anti-MenB vaccines is a major problem, because the immunities raised by those antigens are only partially cross-protective against meningococcal strains harbouring different variants of the same antigens. The immunogenicity and related features (efficacy and coverage) of all meningococcal vaccines should be tested by the unique correlate of protection available, which is the Serum Bactericidal Assay (SBA – Gotschlich EC et al., 1969. J Exp Med 129: 1367-1384). Surrogates of SBA have been set up (e.g. MATS), by the producers of the vaccines based on fHbp.

Concerns

1) Due to the fHbp expression level variability, any estimate of efficacy of both fHbp-based Pfizer and Novartis vaccines requires an account on the level of expression of fHbp. The susceptibility to killing of strains harbouring fHbp variants different than the vaccine variants are scarcely predictable by sequence homology. It is crucial to know the fHbp expression level in the target strain, which might be too low. In addition, in the case of the multi-component Novartis vaccine, the relative contribute of other vaccine components should be considered. Therefore, genetic data alone are of poor indicative value to predict any reliable vaccine efficacy. This is actually admitted by the authors as well, in the discussion section (pag 15, lines 15-20).
2) From a Molecular Epidemiology perspective, the lack of all strain genetic characterization (MLST) is a limitation of major impact. Incomplete strain classification, beside impeding the comparison with the worldwide meningococcal epidemiology (also this issue, like the one of point 1, is admitted by the authors, at the end of the discussion section) does not allow to assign strains to any previously described clone, and to fully compare the strain to each other.

3) The lack of genetic characterization (MLST data) of the strains harbouring fHbp variant 2 and PorA P1.22,14-16, which have been postulated as associated with demographics and age, significantly impedes a more complete picture of possible associations. The clonal complexes of those strains could be added to the multivariate regression analysis, in order to evaluate other possible associations. Associations of single outer membrane protein variants with clonal complexes have been clearly demonstrated, and have become a solid reference for the meningococcal scientific community [Urwin R. et al., Infect Immun 2004; 72(10): 5955-62].

Major Compulsory Revisions

1) Adding the MLST data corresponding to all strains. Unfortunately, as specified by the authors at the very end of the discussion, that will cost something, in terms of labour and time constraints. Though, genetic characterization is nowadays expected to be included in high value Molecular Epidemiology papers. In order to do consistent comparisons, amount and quality of data should be comparable worldwide. MLST data are also important to confirm and corroborate the associations described in the paper, by having a more complete picture of all possible associations.

2) Proposing the paper as a careful and detailed analysis of variability of three meningococcal genes, which code for vaccine candidates-components. I would avoid tentative predictions of vaccine efficacy using sequence data, only. Predictions of efficacy should be addressed by functional assays.

Discretionary Revisions

1) All through the paper, I would deal with translated protein sequences, instead of coding genes. It would be simpler, and maybe more interesting.

I agree with the authors it would have been more prudent to use published primers to amplify all genes of interest. Procedures and tools described in the original papers were able to successfully amplify-sequence target genes from any genotype.

2) Page 12, line 2,3 – I suggest to specify how many of the 32 total translated fetA sequences (and of the three fHbp sequences) which were treated as “no results” contained stop codons, and how many were sequences with incomplete matches with the Neisseria database. Sequences with incomplete matches should be submitted to Neisseria database, to be given a new ID number.
3) I think reference #30 would be more appropriate at the end of the period in line 6, page 6. Beside reference #30 in the new location, and in order to give an example of the results expected from (a surrogate of) a functional assay (MATS) I would also add reference Vogel U. et al, Predicted strain coverage of a meningococcal multicomponent vaccine (4CMenB) in Europe: a qualitative and quantitative assessment. Lancet Infect Dis. 2013 May;13(5):416-25

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I do not think any company may gain or lose financially from the publication of this paper.

I have been a Novartis employee until two years ago. Novartis has possibly applied for patents relating to the content of the manuscript.

I can answer no to all other above.