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Diversity of factor H-binding protein in *Neisseria meningitidis* carriage isolates

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ABSTRACT

Several meningococcal vaccines under development for prevention of serogroup B disease target the factor H-binding protein (FHbp), an immunogenic lipoprotein expressed on the surface of *Neisseria meningitidis*. Based upon sequence and phylogenetic analyses, FHbp can be classified into 3 protein variants (1, 2 or 3) or 2 subfamilies (A or B). The potential effect of FHbp-containing vaccines on meningococcal carriage is not known. We determined the diversity of FHbp among a population of carriage isolates obtained from Georgia and Maryland high school students in 1998 and 2006–2007. Analysis of the *fHbp* gene sequence from 408 carriage isolates identified 30 different FHbp protein sequences. The majority of carriage isolates harbored FHbp proteins belonging to variant 2/subfamily A. Association between FHbp proteins and genetic lineage was observed among the carriage isolates. However, split decomposition analysis, together with tests of linkage disequilibrium and pairwise homoplasy suggest recombination at *fHbp* contribute to allelic diversity. Of note, the FHbp proteins in serogroup B vaccines under development are either absent or not well represented in this carriage population. The FHbp genetic repertoire observed in carriage isolate populations will be useful in understanding the potential impact of FHbp-containing vaccines on meningococcal carriage.

1. Introduction

Neisseria meningitidis is a major cause of meningitis and meningococcemia world-wide [1]. Based upon the composition of its polysaccharide capsule, most invasive *N. meningitidis* can be classified into 5 major serogroups – A, B, C, W-135 and Y. Current vaccines licensed in the U.S. target serogroups A, C, W-135 and Y polysaccharide capsules. However, serogroup B polysaccharide-based vaccines have not been feasible since the serogroup B capsule resembles glycosylated proteins expressed in human tissue during fetal development [2,3]. Serogroup B vaccine strategies have therefore focused on antigenic meningococcal outer membrane proteins [4].

Factor H binding protein (FHbp) is a 28 kDa, surface-exposed meningococcal lipoprotein that is capable of inducing high levels of bactericidal antibodies [5,6]. Binding of human factor H by FHbp in the bloodstream of infected individuals permits *N. meningitidis* to effectively evade host immune responses by preventing complement mediated bacterial cell lysis [7]. Based on sequence diversity, FHbp proteins are classified into either 3 variants (1–3) or 2 subfamilies (A and B) [5,6]. More recently, a modular protein structure has been described for FHbp whereby a combination of 5 distinct variable segments define 6 unique modular groups (I–VI) [8].

N. meningitidis colonizes the nasopharynx of ~10% of the human population. While serogroup C conjugate vaccines have been shown to reduce meningococcal disease incidence in part

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through indirect effects because of reductions in serogroup C pharyngeal carriage, less is known about the effect of protein-based meningococcal vaccines on pharyngeal carriage [9,10]. The published data are insufficient to draw definitive conclusions about the impact of serogroup B outer membrane protein vaccines on carriage [11–14]. However, high levels of antibody to the surface exposed FHbp may impact carriage and the effect could be substantial given that the presence of FHbp is independent of *N. meningitidis* serogroup.

Currently, three vaccine strategies employing FHbp are in clinical trials for the prevention of serogroup B meningococcal disease. One strategy utilizes a bivalent vaccine comprised of one FHbp protein from each subfamily (protein IDs 45 [variant 3, subfamily A] and 55 [variant 1, subfamily B]). A second multi-component vaccine targets three outer membrane proteins including a variant 1/subfamily B FHbp antigen (protein ID 1) [15–17]. A third strategy utilizes native outer membrane vesicles from a pathogenic serogroup B strain genetically modified to decrease toxicity and overexpress immunogenic proteins including FHbp protein ID 1 [18]. In the present study, the distribution of FHbp genotypes in carriage isolates among US high school students was determined.

2. Methods

2.1. Isolate collection and data sources

This investigation included 408 isolates obtained from 2 previous meningococcal carriage studies performed in the US – 194 from 1998 and 214 from 2006 to 2007. The first study examined the genetic lineage of 194 carriage isolates from 2730 high school students in 2 Georgia counties in 1998 – one county experiencing a high incidence of meningococcal disease, and the other county for comparison [19,20]. The second carriage study yielded 325 meningococcal isolates from 3 surveillance periods during the 2006–2007 school year from 3314 students in 4 high schools each in Baltimore County, Maryland and Douglas County, Georgia [21]. When multiple isolates were obtained from individual students at different time periods, all isolates with different genotypes (defined by multi-locus sequence typing (MLST) and outer membrane protein genotyping of *porA*, *porB* and *fetA*) were selected for FHbp analysis. For students that had multiple isolates with the same genotype, only the first isolate collected was selected for FHbp analysis. Thus, 111 isolates from 106 students in Maryland and 103 isolates from 83 students in Georgia were selected for evaluation of *fHbp* genotypes from the 2006 to 2007 study. Serogroup was determined by either slide agglutination or serogroup specific PCR [22]. Isolates that did not agglutinate and did not amplify specific capsular synthesis genes were considered non-groupable, unencapsulated. Isolates that did not agglutinate but amplified a specific capsular synthesis gene were considered groupable, unencapsulated. There were 77 isolates from the Georgia 1998 collection that were non-groupable by slide agglutination and not available for SGS-PCR. To determine if the carriage isolates in this study represented unique *fHbp* sequences, the neissera.org FHbp database (http://pubmlst.org/neisseria/fHbp/) was searched. In addition, FHbp data from invasive U.S. isolates collected from 2000 to 2007 by the Active Bacterial Core surveillance (ABCs) network were also examined (personal communication, Leonard Mayer) [23,24].

2.2. Genotyping

The Georgia 1998 and Maryland 2006–2007 carriage isolates were characterized by MLST and OMP genotype profile at the University of Pittsburgh as previously described [25,26]. MLST and OMP genotyping were performed on the Georgia 2006–2007 carriage isolates by the Centers for Disease Control and Prevention using the same methods. All *fHbp* genotyping was performed at the University of Pittsburgh by PCR amplification and subsequent sequence analysis. Briefly, 1 μl of genomic DNA prepared by boil preparation was PCR amplified with 1.0 μM CDC5UNI (5′-CTATTCTGVGTATGACTAG-3′) and CDC3UNI (5′-GTCCGAACGGTAAATTATYGT-3′) in a final reaction 50 μl reaction volume containing 1X AmpliTaq Gold PCR Buffer (15 mM Tris–HCl, pH 8.0, 50 mM KCl), 2.5 mM $MgCl_2$, 0.2 mM each deoxynucleoside triphosphate and 1.5 units AmpliTaq Gold DNA polymerase (Applied Biosystems). Cycling conditions were 95 °C for 5 min, followed by 30 cycles of 95 °C for 1 min, 50 °C for 1 min, and 72 °C for 1.5 min with a final extension of 72 °C for 7 min. PCR products were prepared for sequencing by precipitation with 20% polyethylene glycol 8000 (Sigma–Aldrich, St. Louis, MO) in a 2.5 M NaCl solution followed by 2–70% ethanol washes. The cleaned PCR product was resuspended in 30 μl distilled H_2O and 1 μl of this product was sequenced with 0.66 pmol of CDC5UNI, CDC3UNI and each of the following primers: 581F, 5′-CCGCCGAACTCAAAGCAGAT-3′; 310R, 5′-TGTAGGCGAACGACGGCGGA-3′; 205R, 5′-CGCCTG ACCTTGTCGTTCTT-3′; 540F, 5′-AATCGAACATTTGAAATCGC-3′. Sequencing reactions were performed in 10 μl volumes using Big Dye Terminator v3.1 (Applied Biosystems). Sequencing reactions were cleaned by ethanol precipitation and analyzed on an AB3730 sequence detection system (Applied Biosystems). Resulting sequence files were either assembled into contigs using DNAstarSeqMan software v6.1 (DNAstar, Madison WI) and manually queried against the *fHbp* sequence typing database at pubmlst.org/neisseria/fhbp/ to obtain alleles or were uploaded to the Meningococcus Genome Informatics Platform (MGIP) website: mgip.biology.gatech.edu for automatic *fHbp* allele calling [27,28]. In the PubMLST database, unique nucleotide and peptide sequences are arbitrarily assigned allele number in order of discovery [28]. In this manuscript, FHbp protein ID (e.g., FHbp protein ID 25) corresponds to the deduced amino acid sequence peptide ID in the PubMLST database. In addition, FHbp modular groups were assigned according to the PubMLST database. The combination of MLST alleles defined the sequence type (ST). STs were assigned to a clonal complex defined at http://pubmlst.org/neisseria/info/complexes.shtml. The association between FHbp protein sequence and genetic lineage as defined by clonal complex (CC) was determined using the asymmetric Goodman–Kruskal Lambda statistic (λ) [29]. This method calculates the reduction in error for predicting the FHbp protein ID based upon knowledge of the genetic lineage of the isolates.

2.3. Comparative sequence analysis

The software MEGA 4.1 was used to calculate the pairwise distances for *fHbp* gene sequences using the Kimura 2-parameter model [30]. Estimates of linkage disequilibrium (LD) were performed using two measures: r^2 and D' and the program PERMUTE. The correlation coefficients between physical distance and these LD measures for all *fhbp* base pairs and the associated *p* values were calculated using the null hypothesis that in the absence of recombination there should be zero correlation between physical distance and LD measures [31]. The pairwise homoplasy index (PHI) available within SplitsTree 4.11.3 was used to measure the mean incompatibility score of *fHbp* sequences and SplitsTree 4.11.3 was used to generate a summary network of the relatedness of *fHbp* sequences for each population [32]. GARD (*G*enetic *A*lgorithm *R*ecombination *D*etection) was used to detect discordant phylogenetic signals in alignments of *fHbp* sequences and provide an estimate of the number and location of break points [33].