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Prevalence of factor H Binding Protein sub-variants among *Neisseria meningitidis* in China

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ABSTRACT

Objective: To study the prevalence of the *fHbp* genes in *Neisseria meningitidis* (*N. meningitidis*) isolates for further evaluation and development of serogroup B meningococcal vaccines in China.
Methods: A panel of 1012 *N. meningitidis* strains was selected from the national culture collection from 1956 to 2016, according to the years of isolation, locations, and strain sources. These were tested by FHbp variant typing. Multi-locus sequence typing (MLST) was performed on 822 of these samples, including 242 strains from clinical strains and 580 carrier-derived strains. Analysis based on sequence types, serogroups, and FHbp variations were used to summarize the prevalence and characteristics of *N. meningitidis*.
Results: There were 8 serogroups of *N. meningitidis* as well as a collection of nongroupable strains in this study. 1008 of 1012 *N. meningitidis* strains tested were positive for the *fHbp* gene. Serogroup A *N. meningitidis* (MenA) strains belonging to ST-1 and ST-5 clonal complexes harbored genes only encoding variant 1 (v1) FHbp. All MenW strains encoded v2 FHbp. 61.9% of clinical MenB strains were positive for v2 FHbp *vs.* 32.1% that were positive for v1. Among *fHbp*-positive carrier-derived MenB strains, v2 FHbp accounted for 90.8%. 79.7% of clinical MenC strains were positive for v1 FHbp and 20.3% were positive for v2 FHbp. Among carrier-derived MenC strains, v2 FHbp predominated. The number of major serogroups of *N. meningitidis* analyzed by MLST was 822, and the encoded FHbp showed CC- or ST-specific characteristics.
Conclusion: fHbp genes were detected in almost all *N. meningitidis* strains in this study. Therefore, it is possible that a vaccine against MenB or meningococci irrespective of serogroups, which includes FHbp, could be developed. Meningococcal vaccine development for China is a complex issue and these findings warrant further attention with respect to vaccine development.

1. Introduction

Meningococcal disease is usually characterized by meningitis and septicemia, and is a devastating infectious disease with a high case fatality rate caused by *Neisseria meningitidis* (*N. meningitidis*). According to the biochemical characteristics of capsular polysaccharide, *N. meningitidis* can be divided into 12 serogroups, of which serogroup A (MenA), B (MenB), C (MenC), W (MenW), X (MenX) and Y (MenY) cause the majority of invasive cases worldwide [1–3].

One of the most cost-effective strategies for prevention and control of meningococcal disease is through immunization. Based on the meningococcal capsular polysaccharide, polysaccharide vaccines and polysaccharide-protein conjugate vaccines are available for prevention of meningococcal infection caused by meningococcal A, C, W and Y organisms [4]. However, as a result of similarity to human neural cell adhesion molecule [5], the MenB capsular polysaccharide is poorly immunogenic and has the potential to cause autoimmune disease [6]. Hence, MenB capsular polysaccharide is not suitable as a vaccine component. Therefore,

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a noncapsular vaccine approach should be considered as an alternative choice for MenB vaccine development.

An outer membrane protein vaccine was the earliest attempt to use antigenic proteins to prevent MenB infection [7,8]. Utilization of OMV (outer membrane vesicles) vaccines based on PorA against MenB meningococcal disease have been investigated since the 1970 s [7,9]. There is a significant limitation concerning these vaccines since they only afford significant protection if the meningococci express the same type of PorA antigen as the vaccine component. Furthermore, they do not confer broad protection against the large number of MenB strains containing diverse PorA globally [9–11]. Therefore, these vaccines are more suitable for the control of local outbreaks [12–14], though next-generation OMV vaccines that are under development have greatly expanded strain coverage [15].

Through genome mining (also referred to as reverse vaccinology), five surface-exposed proteins were selected as components of the recombinant MenB vaccine, Bexsero®, made by GlaxoSmithKline [16–18]. In contrast, Pfizer, who discovered meningococcal antigens using traditional proteomics, developed a bivalent recombinant MenB vaccine called Trumenba® [19]. The common component of these two vaccines is factor H Binding Protein (FHbp). Both of these vaccines have been licenced in certain countries throughout the world [20,21].

FHbp, also referred to as GNA1870 (Genome-Derived Neisseria Antigen 1870) or LP2086 (lipoprotein LP2086) [16,17], is located on the surface of *N. meningitidis*. FHbp is known for its ability to specifically bind to human complement regulatory protein factor H and can inhibit the alternative complement pathway [22,23] thus improving survival of *N. meningitidis* in human blood. According to amino acid sequences, FHbp can be classified into 3 variant groups (variant 1 - variant 3) [16]. Newly discovered nucleotide sequences and peptide sequences can be deposited in the PubMLST database (http://pubmlst.org/neisseria/fHbp/) hosted by the University of Oxford, where they will be arbitrarily assigned numbers [24]. Corresponding sub-variants can then be determined according to the peptide ID prefixed with the variant group number and separated by a full stop [24]. For example, in sub-variant v1.5, 5 is the peptide ID of this FHbp protein sequence, and 1 means this sub-variant belongs to variant group 1.

Bexsero® (4CMenB) is composed of FHbp (v1.1), NadA, NHBA, and MeNZB OMV. Trumenba® (rLP2086) is a bivalent recombinant FHbp vaccine containing v1.55 and v3.45 with lipid-modified FHbps [25]. Both of the vaccines have been shown to have a broad coverage of circulating MenB strains worldwide [20,26–28]. As the outer membrane proteins are not MenB-specific, both recombinant vaccines have been shown to provide cross-protection against *N. meningitidis* against other serogroups apart from MenB [29–31].

China has experienced several epidemics of meningococcal disease caused by MenA and MenC. Introduction of polysaccharide vaccines against MenA and MenC in the national routine immunization program has significantly reduced the disease incidence [32]. However, meningococcal disease caused by MenW and B has increased in the past 10 years [3,32,33]. Previously, no vaccine was available for prevention of meningococcal infections caused by MenB, and the MenACWY vaccine has not yet been used routinely amongst high risk populations in China.

The efficacy of 4CMenB and rLP2086 has been broadly reviewed and recognized in many countries around the world [26,29,31]. However, it remains to be explored whether such vaccines can work well in China. With increasing public health concern associated with meningococcal disease caused by non-MenA and non-MenC strains, it is important to understand the molecular epidemiology of *N. meningitidis* in order to develop a protein-based vaccine against MenB and other serogroups. In this study, a total of 1012 *N. meningitidis* strains collected over the last 60 years in China were analyzed to identify the polymorphism of FHbp among *N. meningitidis* belonging to different serogroups and clonal complexes. It will be conducive to the development of the MenB vaccine in China while further evaluating effects of any potential vaccine.

2. Materials and methods

2.1. Neisseria meningitidis isolates

A total of approximately 3000 *N. meningitidis* isolates have been collected over the last 60 years in China through the National Active, Laboratory-based Surveillance Network which has branches throughout the 31 provinces/major cities/autonomous regions nationwide. These have been stored in the laboratory of the National Institute for Communicable Disease Control and Prevention (ICDC), Chinese CDC. According to the isolation years, locations and strain sources, we selected 1012 samples from this database (Table 1) including 242 isolates from cases with invasive meningococcal disease (by culture of blood or cerebrospinal fluid) and 770 from healthy carriers (by culture of nasopharyngeal swabs). Half of the strains investigated by Zhou et al. [34] were also included in this study. All strains were stored at −80 °C in skimmed milk.

2.2. Bacterial growth and DNA preparation

N. meningitidis strains were cultured on blood culture agar at 37 °C in a 5% CO_2 atmosphere for 18–22 h. All the isolates were reconfirmed by means of Gram-staining and biochemical tests (API-NH, BioMerieux, Marcy L'Etoile, France). Serogrouping was conducted using slide agglutination (Remel, Lenexa, KS, USA) and a PCR approach where primers and conditions were described by Bennett and Zhu [35,36]. The pure cultures of *N. meningitidis* were eluted with saline water, and the supernatant was discarded after centrifugation. Extraction of Genomic DNA was performed via Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions.

2.3. Amplification and sequencing of fHbp genes

A region of approximately 1500 bp was amplified, including the complete encoding sequence of the *fHbp* gene, the 5′ flanking sequence, and part of the downstream nearby gene. Two pairs of primers were used to conduct the amplification: Primer set 1: *gna1870*-F:5′-TGACCTGCCTCATTGATGC-3′, *gna1871*-Ralt: 5′-ATGC CGATACGCAGTCC[G/C]GTAAAC-3′; Primer set 2: *gna1869*-2F: 5′-G AAGAAATCGTCGAAGGCATCAAAC-3′, *gna1870*-R: 5′- CGGTAAAT TATCGTGTTCGGACGGC-3′ [37,38]. The second pair of primers were only used if the first pair failed to amplify the *fHbp* gene.

The PCR was conducted in a total mixture volume of 40 μL containing the following reagents: 4.0 μL of 10 × PCR buffer, 3.2 μL of highly pure dNTPs (2.5 mM for each type of dNTP), 0.32 μL of *EasyTaq*® DNA polymerase (5U/100 μL, TransGen), 26.48 μL of double-distilled water, 2.0 μL of each primer (10 μM) and 2.0 μL of genomic DNA template. The PCR conditions and procedures were as follows; 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 63 °C for 30 s, and 72 °C for 1 min; the final extension was then performed at 72 °C for 5 min. The amplified products were electrophoretically resolved in 1.5% TBE agarose gels stained with Gold View and were visualized under UV light. The sequencing procedure was completed by Tianyi Huiyuan Company, and the sequencing primers were the same as the amplification primers.

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