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Molecular characterization of invasive serogroup B *Neisseria meningitidis* isolates from Spain during 2015–2018: Evolution of the vaccine antigen factor H binding protein (FHbp)

Raquel Abad*, Cristina García-Amil, Carmen Navarro, Elena Martín, Ariadna Martín-Díaz, Julio A Vázquez

National Reference Laboratory for meningococci, National Centre for Microbiology, Instituto de Salud Carlos III, Ctra, Majadahonda-Pozuelo, Km2., 28220 Majadahonda, Madrid, Spain

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SUMMARY

Studies of meningococcal genetic population structure, including the potential associations between surface proteins variants and clonal complexes, are important to understand how new protein MenB vaccines might impact in specific scenarios. With the aim to analyze the diversity of Spanish invasive MenB strains, and genetic variability of the fHbp vaccine antigen, all MenB isolates received at National Reference Laboratory (NRL) from 2015 to 2018 were molecularly characterized.

Material and methods: 108, 103, 87 and 112 invasive MenB strains isolated during 2015–2018, respectively, were received at NRL. The strains were whole genome sequenced, and *porA, fetA*, MLST and *fHbp* variability was analyzed. Potential impact on MenB vaccines coverage was also assessed.

Results: A total of 42, 38 and 3 different FHbp subfamily A, B and A/B hybrid peptides, respectively, were found. FHbp subfamily A peptides were harboured by most of the strains (65.9%), being the most prevalent peptide 45 which was associated with genosubtype 22,14 and cc213. FHbp subfamily B peptides were harboured by 32.4% of the strains, and 6 strains harbouring subfamily A/B hybrid peptides were also found.

The 64.15% of the strains showed FHbp variants "exact-match" or "cross-reactive" to the FHbp variants included in rLP2086 vaccine according to hSBA assays in the rLP2086 clinical development, and 15.85% showed FHbp peptides defined as predictors of FHbp-coverage for 4CMenB vaccine by gMATS.

Conclusions: Due to invasive meningococcal strains temporal variability (eg prevalence of the cc213 increased from 3.6% in 2007 to 33% in 2018) affecting to the presence and distribution of the vaccine antigens, continuous detailed meningococcal surveillance and monitoring of the vaccine antigens is needed to determine the degree and durability of coverage provided by these protein vaccine.

Introduction

Neisseria meningitidis is a major cause of invasive disease worldwide, including both bacterial meningitis and septicemia, but also some other clinical presentations.[1] Serogroups A, C, W, Y and X are responsible for a high proportion of the clinical cases, but serogroup B (MenB) is still the most frequent among invasive isolates in industrialized countries.[2] While there are available polysaccharide conjugate vaccines against serogroups A, C, W and Y, either as monovalent or multivalent formulations,[3] the serogroup B capsular polysaccharide is not an effective candidate as a vaccine antigen.[4] Outer membrane vesicle (OMV) vaccines have been developed with different antigen formulations and have been used to control MenB epidemics associated with single clones.[5] However, the protection provided by OMV vaccines is almost limited to the homologous epidemic meningococcal strain due to the variability of the major antigen, porin A (PorA),[6] and new approaches have been necessary for the development of effective vaccines against serogroup B. In an attempt to solve the problem of antigenic variability of meningococcal proteins for the development of vaccines with broad coverage of MenB iso-

* Corresponding author.
E-mail addresses: rabad@isciii.es (R. Abad), cgamil@isciii.es (C. García-Amil), carnavarro@isciii.es (C. Navarro), elenamartin@isciii.es (E. Martín), jvazquez@isciii.es (J.A. Vázquez).

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lates, an antigen, the factor H binding protein (FHbp), was identified some years ago as a good vaccine candidate.[7,8] FHbp, also called genome-derived *Neisseria* antigen [GNA] 1870 and LP2086, binds human factor H, allowing to the meningococci to resist complement-mediated killing.[9] FHbp has been classified into 3 variants denominated 1, 2 and 3, and alternatively into subfamily A (that corresponds to variants 2 and 3) and B (that corresponds to variant 1) according with the sequence analysis.[2] When FHbp is used as an immunogen, antibodies raised against one subfamily are not cross-protective against the other subfamily. FHbp has shown to be highly variable and, although a broad protection is assumed including variants within the same subfamily, the amplitude of cross-reactivity covering heterologous strains of the same group is still to be elucidated. It is well known that bactericidal activity of anti-fHbp antibodies varies according to the genetic diversity and level of expression of the protein in the different strains.[4]

FHbp has been included in the formulation of two available MenB vaccines: Bexsero® (4CMenB), from GSK, and Trumenba® (rLP2086), from Pfizer. 4CMenB vaccine is composed by three recombinant meningococcal proteins (*Neisseria* heparin binding antigen [NHBA] peptide 2, *Neisseria* adhesin A [NadA] peptide 3.8 and FHbp variant 1 [subfamily B] peptide 1) combined with outer membrane vesicle (OMV) from the MenB strain NZ98/254 expressing the PorA P1.4, as the major antigen,[7] while Trumenba® is including two factor H binding proteins, one from each of the two subfamilies (variant 1 peptide 55 and variant 3 peptide 45).[10] Although some study has documented *N. meningitidis* isolates that do not express FHbp, it is generally accepted that protein is expressed in almost all invasive strains.[9]

FHbp is widely considered as an important vaccine antigen and its contribution on the potential coverage for the 4CMenB vaccine (Bexsero®) has been estimated to range between 53% in USA and Canada,[11,12] 66% in Europe[13] and just 36.3%[14] in Spain. The belonging of an isolate to a clonal complex (cc) has been associated with the ability to produce invasive disease as well as the expression of different phenotypes that may or may not be associated with specific clonal complexes. Studies carried out using Meningococcal Antigen Typing System (MATS), an assay developed to predict strain coverage by 4CMenB, conclude that some clonal complexes can be associated with specific patterns of diversity in FHbp as well as in the other vaccine antigens,[13,14] but did not predict the antigenic profile of a given strain or the likelihood to be killed in the serum bactericidal antibody assay. However, the cc distribution of meningococcal strains within a country could account for variations in antigen coverage.

In Spain, MenB has been predominant since 1997–1998, currently (2018–2019) accounting 153 confirmed cases (36.4%) and an incidence rate of 0.33.[15] Although MenB cases have remain stable from 2013 to 2014, serogroup B causes 61% of cases among children less than 5 years old.[16] In Europe, both protein MenB vaccines are licensed (4MenB in 2013 and rLP2086 in 2017), but only polysaccharide conjugate MenC and MenACYW vaccines are included in the Spanish immunization programme. Continuous detailed meningococcal surveillance and monitoring of vaccine antigens is needed to support national decision-making concerning MenB vaccine strategies.

With the aim to keep track of the *fHbp* evolution in MenB invasive meningococcal strains isolated in Spain and its impact on the MenB vaccines potential coverage, *fHbp* molecular characterization and prevalence was analyzed in invasive MenB isolates received at the Spanish Reference Laboratory for Meningococci (SRLM) from 2015 to 2018. Clonal complex, genosubtype (variable regions 1 and 2 of class 1 protein [PorA VR1 and VR2]) and FetA type (variable region of an iron-regulated outer membrane protein) distribution were also determined.

Material and methods

Neisseria meningitidis *isolates*

In Spain, cases of invasive meningococcal disease (IMD) are compulsory declared, and the SRLM, which performs functions to support the National Health System, receives around 80–85% of the confirmed cases around the country for full characterization. Sample handles by the SRLM is therefore representative of the national situation.

All *N. meningitidis* isolates received in the SRLM are routinely grouped by slide agglutination with specific polyclonal antibodies. All invasive MenB isolates received from 2015 to 2018 (108, 103, 87 and 112 isolates, respectively) were included in this study.

Molecular characterization

Whole genome sequencing is carried out systematically on all *N. meningitidis* invasive cultured isolates received in the SRLM since 2017. In this study, all invasive MenB isolates received from 2015 to 2016 were also retrospectively whole genome sequenced.

DNA from cultured isolates was extracted by using the QIAamp DNA Mini Kit (Quiagen, Hilden, Germany). Multiplexed libraries were created with Nextera XT DNA Library Preparation Kits (Illumina, San Diego, CA, USA) and sequencing was performed on MiSeq or NextSeq Illumina platform (Illumina, San Diego, CA, USA). The short-read sequences obtained were de novo assembled using SPAdes algorithm[17].

The assembled genomes were uploaded to the PubMLST Neisseria database (https://pubmlst.org/neisseria/) and annotated using a gene-by-gene approach through the Bacterial Isolate Genome Sequence Database (BIGSdb) platform as described previously,[18] under the following ids: 38,261, 53,041, 60,422–60,433, 60,447–60,454, 60,456–60,460, 71,806, 71,807, 71,809–71,811, 71,813–71,817, 71,885, 72,234, 72,235, 72,237, 72,243, 72,244, 72,794, 80,309, 80,311, 94,035, 94,036, 94,038, 94,042, 94,043, 94,045, 94,046, 94,180, 98,155–98,199, 98,206–98,404, 98,409–98,507.

Genome assembly data was employed to strain characterization by multilocus sequence typing (MLST),[19] *porA* and *fetA* typing,[20] and to determine *fHbp* allele sequence.

Potential impact on MenB vaccines coverage

FHbp is widely considered as an important vaccine antigen and has been included in the formulation of two licensed MenB vaccines (4CMenB –Bexsero- and rLP2086 –Trumenba-). The FHbp subfamily B peptide 1 is one of the four components included in 4CMenB vaccine; while rLP2086 vaccine is composed of a peptide of each subfamily, FHbp subfamily A peptide 45 and FHbp subfamily B peptide 55. However, FHbp has shown to be highly variable and bactericidal activity of anti-FHbp antibodies varies according to the genetic diversity and the level of the protein expression in the different strains.

Different strategies to know cross-reactivity of the elicited vaccine antibodies with other different FHbp variants have been followed.[21,22] Protective bactericidal response by rLP2086 vaccine, measured by human serum bactericidal antibody assay (hSBA), has been observed against 14 different MenB invasive strains expressing FHbp variants heterologous to the vaccine, so to estimate rLP2086 vaccine potential coverage we considered these FHbp peptides cross-reactive to the vaccine antibodies (Supplementary Table 1). Formulation of 4CMenB vaccine also include other components different to FHbp, so we only estimated FHbp contribution on 4CMenB vaccine potential coverage. Recently, a genetic MATS (gMATS) for predicting 4CMenB vaccine strains coverage, has been defined by associating antigen genotyping and MATS results.[22] For

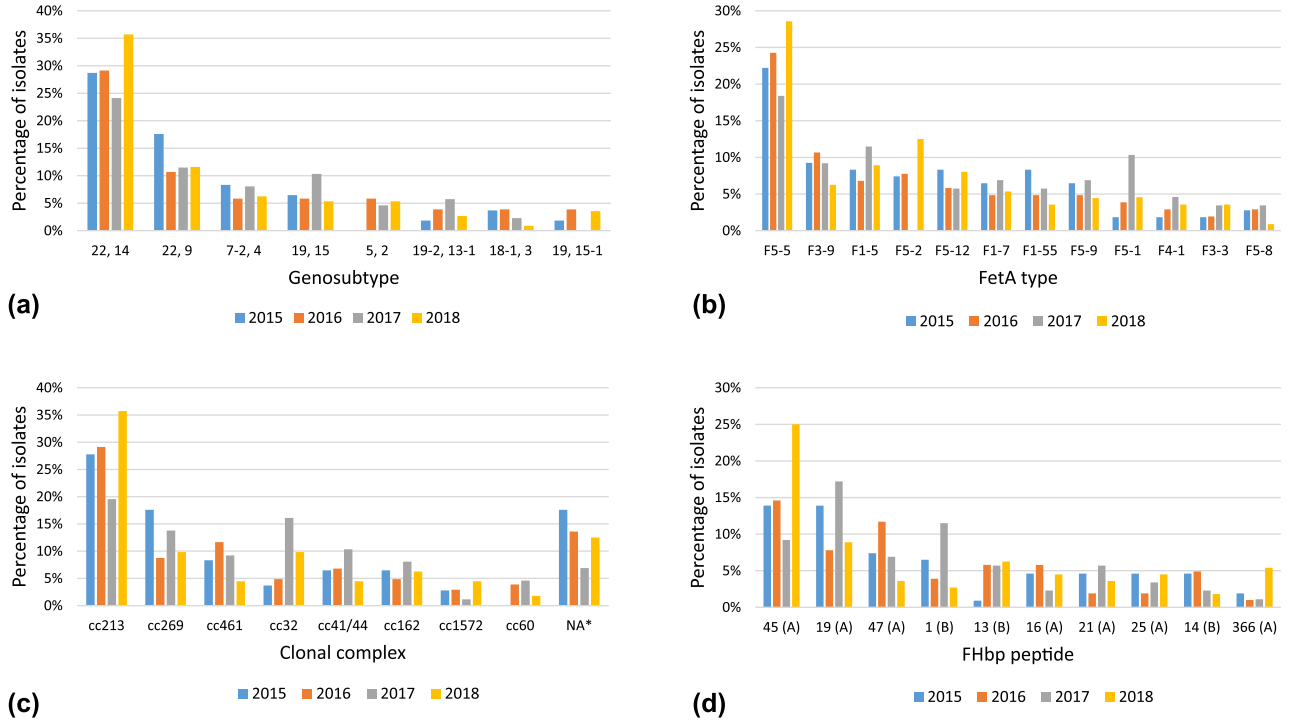

Fig. 1. Invasive MenB isolates received in the Spanish Reference Laboratory for Meningococci (SRLM) from 2015 to 2018, by more representative (presented in ≥ 10 strains) PorA genosubtypes (1a), FetA types (1b), clonal complexes (1c) and FHbp peptides (1d). NA*: Non assigned; (A) FHbp subfamily A; (B) FHbp subfamily (B).

FHbp, peptides for which the percentage of MATS-covered strains was higher than 60% or lower than 40% were considered predictors of coverage or non-coverage, respectively. Peptides not fulfilling these criteria were considered "unpredictable".[22] To estimate FHbp contribution on 4CMenB vaccine potential coverage we considered only FHbp peptides defined as predictors of coverage by gMATS (Supplementary Table 1).

Results

A total of 848 invasive meningococcal disease cases were confirmed in the SRLM between 2015 and 2018 (2015: 165 cases, 2016: 193, 2017: 200 and 2018: 290), of which 523 cases corresponded to MenB cases: 410 (78.39%) MenB isolates, and 113 (21.61%) clinical samples only PCR confirmed and genogrouped. Molecular characterization through whole genome sequencing of the 410 MenB cultured isolates (108 from 2015, 103 from 2016, 87 from 2017 and 112 from 2018) was carry on.

PorA genosubtype, FetA type and clonal complex distribution

Eighty different PorA genosubtypes were identified, of which more than a half (62.5%) were only present in one isolate. The most frequent PorA genosubtypes observed were 22,14 ($n = 122$, 29.76%), 22,9 ($n = 53$, 12.93%), 7–2,4 ($n = 29$, 7.07%) and 19,15 ($n = 28$, 6.83% (Fig. 1a).

The *fetA* gene was not present in 6 strains. Thirty-eight different FetA types were found among the rest of 404 MenB invasive isolates analyzed, of which half appeared in more than one strain. The most frequent FetA type found was F5–5 ($n = 97$, 24.01%), followed by F1–5 ($n = 36$, 8.91%), F3–9 ($n = 36$, 8.91%), F5–2 ($n = 30$, 7.43%) and F5–12 ($n = 29$, 7.18%) (Fig. 1b).

Thirty hundred and fifty-seven isolates (87.07%) belonged to 21 different clonal complexes (cc) and the remaining 53 isolates (12.93%) did not belong to any assigned cc. The most frequent cc observed was cc213 ($n = 117$, 32.77%), followed by cc269 ($n = 51$, 14.29%), cc32 ($n = 34$, 9.52%) and cc461 ($n = 34$, 9.52%) (Fig. 1c).

An important increase of isolates characterized as genosubtype 22,14, FetA type F5–5 and cc213 was observed in 2018 (Fig. 1).

FHbp subfamily and peptide distribution

All 410 *N. meningitidis* isolates were found to contain the *fHbp* gene, however one of them presented an allele (allele 1659) with a frameshift mutation resulting in an internal stop codon.

FHbp subfamily A, B and A/B hybrid peptides were found, in detail, a total of 42 different FHbp subfamily A (15 FHbp variant family 2 and 27 FHbp variant family 3), 38 FHbp subfamily B (FHbp variant family 1) and 3 FHbp A/B hybrid peptides were identified, of which almost half ($n = 37$, 44.6%) were presented only in one isolate.

FHbp subfamily A peptides were harboured by most of the strains ($n = 270$, 65.9%): 120 isolates were harbouring FHbp variant family 2 peptides and 150 isolates were harbouring FHbp variant family 3 peptides. The most common FHbp subfamily A peptide was peptide 45 ($n = 66$), corresponding with rLP2086 vaccine variant A05, which was associated with genosubtype 22,14 and cc213 (all isolates harbouring FHbp peptide 45 showed genosubtype 22,14 and belonged to cc213). Other prevalent FHbp subfamily A peptides found among isolates analyzed were peptide 19 ($n = 48$) and peptide 47 ($n = 30$), mainly associated with cc269 ($n = 26$) and cc461 ($n = 28$), respectively, (Figs. 1d and 2).

FHbp subfamily B peptides were harboured by 133 isolates (32.4%). The most common was peptide 1 (the specific FHbp peptide included in 4CMenB vaccine) which was identified in 24 (5.9%) isolates belonging to different cc (cc32, $n = 16$; cc269, $n = 5$; cc162, cc213 and cc865, $n = 1$ in each) (Fig. 1d) (Fig. 2). FHbp peptide 55, corresponding to the other rLP2086 vaccine variant, B01, was not present in any isolate.

Additionally, 3 different subfamily A/B hybrid peptides were observed: peptide 207 harboured by 2 isolates, peptide 283 har-

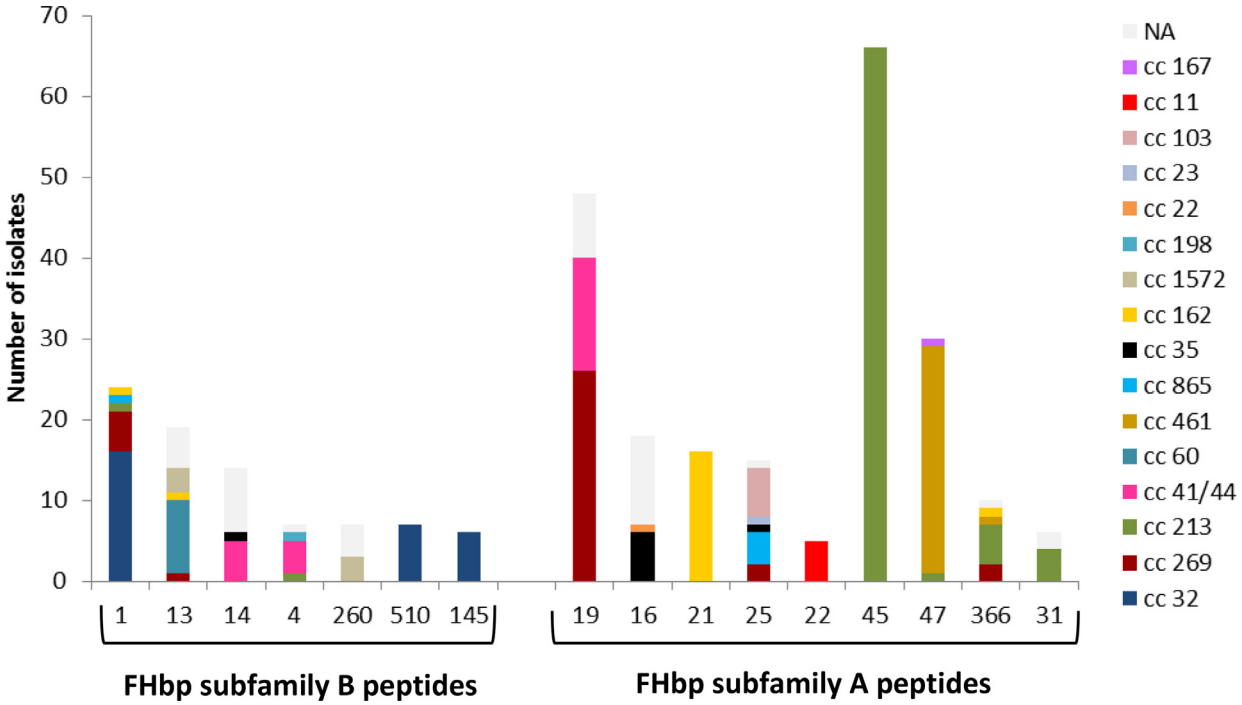

Fig. 2. Distribution of the more representative FHbp peptides (peptides presented in more than 5 strains) by cc in the strains panel studied. NA: Clonal complex non assigened.

boured by 3 isolates, and peptide 1256 harboured by 1 strain. Phylogenetic relationship of the 83 different FHbp peptides found is shown in Fig. 3. FHbp peptide 207 is closer to the subfamily B (84% of similarity with the subfamily B peptide 1) coinciding with the N-terminal region, contrary to FHbp peptide 283 whose N-terminal region corresponds with the subfamily A. FHbp peptide 1256 is a new variant very similar to FHbp 283 peptide (98% of similarity) with only 5 amino acid differences.

The evolution of FHbp subfamilies in Spanish invasive MenB strains during the studied time period is shown in the Fig. 4a. An increase of invasive MenB strains harbouring FHbp subfamily A peptides was observed, representing 63.9% ($n=69$) in 2015 and 73.2% ($n=82$) in 2018. This increasing trend is observed since 2001 when most of the invasive MenB strains harboured FHbp subfamily B (Fig. 4b). Subfamily B FHbp peptides were predominant in a strain panel isolated in Spain from 2001 to 2006 (60%),[23] a decreasing to 50% of this proportion was observed in a study including 300 isolates from 2009 to 2010,[14] reaching a change of the predominant FHbp subfamily in 2015 which was observed in the present study (Fig. 4b).

A higher FHbp subfamily A proportion was observed in all age groups, similar to FHbp subfamily A/subfamily B proportion found in 2015–2018 period of time (65.9% subfamily A vs 32.4% subfamily B). This difference was more noticeable in the 10–14 (76.5% subfamily A vs 23.5% subfamily B) and >64 (84.6% subfamily A vs 15.4% subfamily B) age groups. However, similar FHbp subfamilies distribution was observed in the 5–9 (54.2% subfamily A vs 41.7% subfamily B) and 25–44 (53.6% subfamily A vs 46.4% subfamily B) age groups (Fig. 5).

Potential impact on MenB vaccines coverage

A total of 263 strains (64.15%) showed FHbp variants "exact-match" ($n=66$, 16.10%) or "cross-reactive" ($n=197$, 48.05%) to the FHbp variants included in rLP2086 vaccine (Supplementary Table 1). Based on the genetic information and assuming enough level of expression in all strains, at least 64.15% of the invasive MenB strains would be covered by the rLP2086 vaccine. By age groups, at least the potential coverage ranged from 41.67% (5–9 years age group) to 83.08% (>64 years) (Fig. 6).

On the other hand, a total of 65 strains (15.85%) showed FHbp peptides defined as predictors of FHbp-coverage for 4CMenB by gMATS (24 strains (5.85%) showed the same FHbp peptide included in 4CMenB vaccine, and 41 strains (10%) other different peptides) (Supplementary Table 1). Because 4CMenB is composed for 3 more antigens apart from FHbp, it is not possible to talk about vaccine potential coverage but rather about FHbp contribution on 4CMenB vaccine coverage, which was estimated in at least 15.85%.

Discussion

Real time and continuous IMD molecular surveillance is needed to identify characteristics of circulating meningococci on a national scale, especially the variability and distribution of vaccine antigens. The potential effect of MenB protein-based vaccines depends on the genetic variability (vaccine heterologous peptides need to be cross-reactive to the vaccine antibodies) and the degree of expression of each vaccine antigen (a minimum level of expression is required for bacterial lysis to occur), so monitoring of meningococcal antigenic diversity over time is required to determine the degree and durability of coverage provided by these protein-based vaccines.

A continuous change in the cc distribution of the MenB invasive strains in Spain has been observed from the time period 2001–2007, when cc32 (27%), cc 41/44 (13%), cc269 (10%) and cc11 (8%) were the most prevalent cc.[24] The evolution of the cc over the time shows the emergence of cc213, increasing from 3.6% in 2007[24] to 18% in 2009–2010[14] and currently becoming the most prevalent cc (33%). Cc213 in Spain has been associated with low predicted 4CMenB vaccine strains coverage by MATS (30%)[16] so its incidence should be monitoring carefully. In contrast, cc32 has gone from the most prevalent cc in 2001–2007 representing 27%[24] to 16% in 2009–2010,[14] and currently being only the 10% of the invasive MenB isolates in Spain. A decrease of cc41/44 and cc11 prevalence

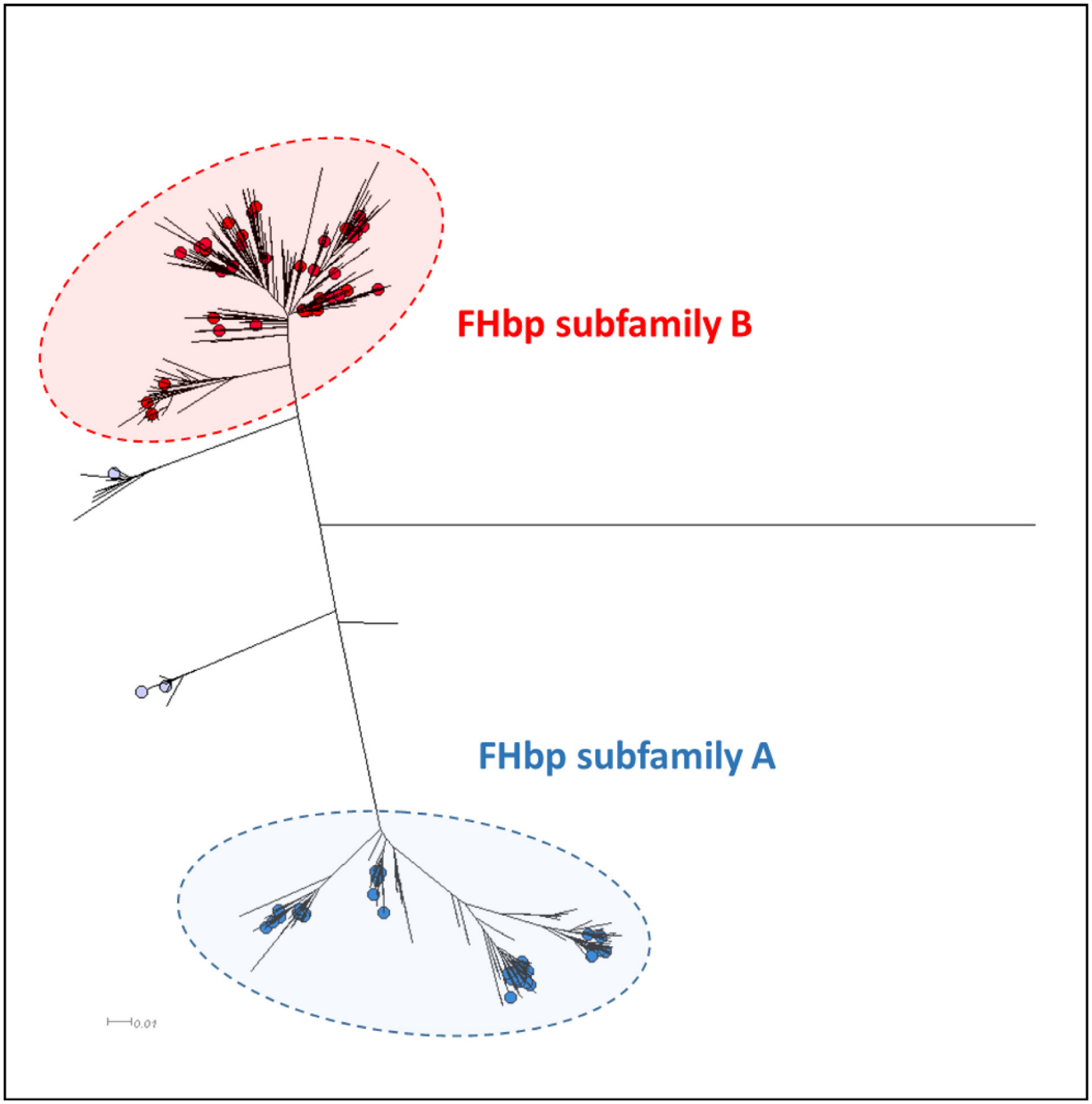

Fig. 3. Phylogenetic relationship of the 1324 different FHbp peptides found in the database (last accessed: 2020–06–12) available in Neisseria Multi Locus Sequence Typing website (https://pubmlst.org/ neisseria/), based on a clustal W alignement and drawn with splitsTree 4. Dots represent the 83 different FHbp peptides found in the total of invasive MenB strains received at SNRL from 2015 to 2018. The scale bar represents phylogenetic distance based on the deduced FHbp peptide sequence.

was also observed, decreasing from 13% and 8% in 2001–2007[24] to 7% and 1%, respectively, in 2009–2010,[14] prevalence which is remained currently.

According with the evolution of the cc, and non-overlapping associations found, changes in the genosubtype and FetA type distribution were also observed. Genosubtype 22,14, mainly associated with cc213, has been increasing from 4% in 2001–2007[24] to 18% in 2009–2010[14] and currently becoming the most prevalent genosubtype (30%). In contrast, genosubtypes 19,15 (mainly associated with cc32), 7–2,4 (mainly associated with cc41/44) and 5–1,10–8 (mainly associated with cc11) has been decreasing in a similar way to the cc decrease observed: from 24%, 10% and 8%, respectively, in 2001–2007,[24] to 12%, 8% and 1% in 2009–2010[26], and being currently 7%, 7% and 1%. Although there were no data on FetA type for 2001–2007, an increase of F5–5 from 2009–2010 (16%) is observed, being currently the most prevalent FetA type (24%). Conversely, FetA types F5–1 and F1–55 showed a decreasing from 11% and 10%, respectively, in 2009–2010[24] to 5% and 6% nowadays.

This population dynamics of the invasive MenB strains in Spain could be relevant for evaluation of the potential MenB proteins vaccines coverage, due to changes in the vaccine antigens distribution. The kept track of the fHbp (an important vaccine antigen presents in both MenB protein-vaccines licensed currently) evolution in MenB invasive meningococcal strains isolated in Spain studied here, showed an important change in the FHbp subfamilies prevalence and distribution. FHbp subfamily B was predominant in the 2001–2006 period, found in 60% of the MenB strains,[23] decreasing to 50% in 2009–2010[14] and to 32% in 2015–2018 (present study). A proportional increase of FHbp subfamily A prevalence was observed, representing 40% in 2001–2006,[23] 50% in 2009–2010[14] and 66% in 2015–2018 (present study).

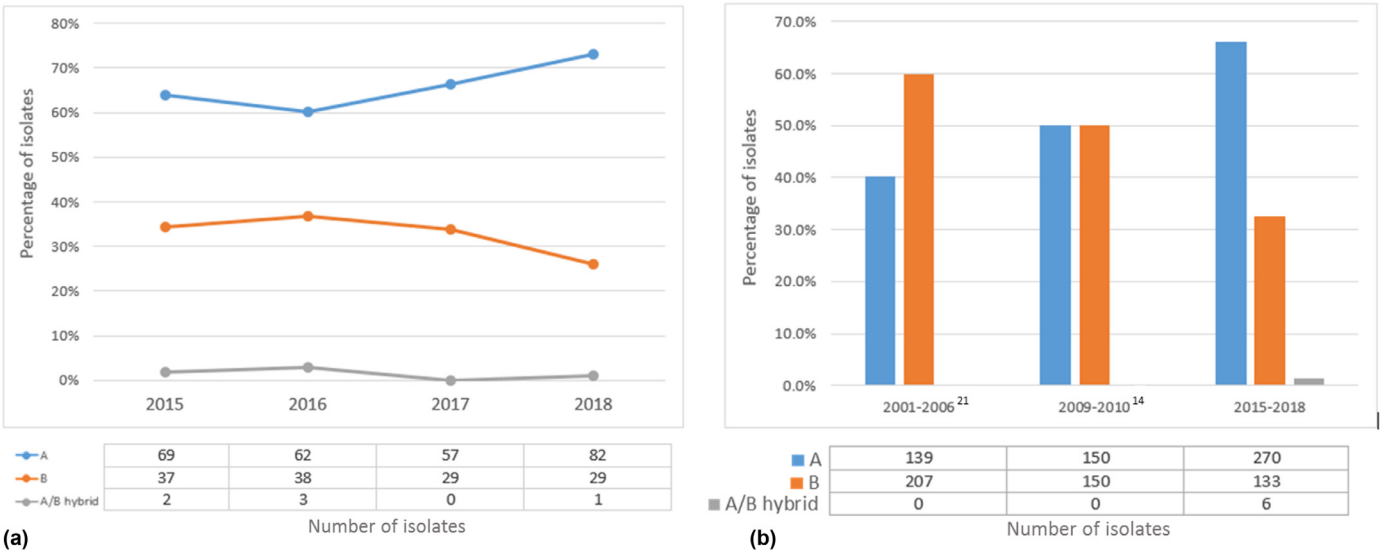

Fig. 4. Distribution of the FHbp subfamilies in MenB invasive meningococcal disease isolates received in the Spanish Reference Laboratory for Meningococci (SRLM) from 2015 to 2018 (4a) and from 2001 to 2018 (4b).

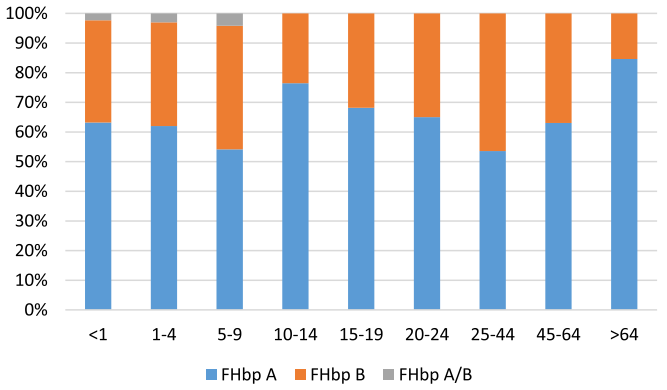

Fig. 5. Distribution of the FHbp subfamilies in MenB invasive meningococcal disease isolates received in the Spanish Reference Laboratory for Meningococci (SRLM) from 2015 to 2018 by age group.

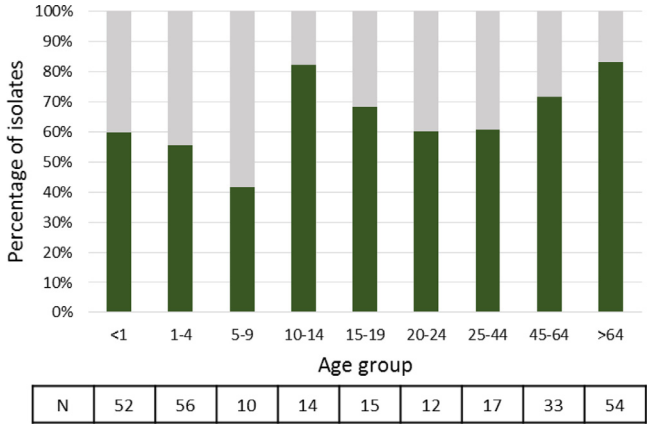

Fig. 6. Minimal rLP2086 potential coverage by age group.

Although allelic variants of fHbp cannot be predicted based on the cc, as it has been previously described,[13, 22] a certain degree of relationship between both variables is observed. If we analyze the comparative evolution of the fHbp subfamilies A and B alleles and the majority clonal complexes we can see that the most significant changes have occurred due to the sharp increase of cc213 (more associated with fHbp subfamily A alleles), and to the significant regression of the cc32 (almost exclusively associated with allele expression of subfamily B). There is also a small, but sustained, increase of cc461 (associated almost exclusively with alleles of subfamily A). Therefore, the dynamics of certain clones in Spain are affecting the proportion of subfamily A and B, and the future evolution of these clones will be relevant for that proportion, and for the potential impact of the vaccines containing this antigen.

Assessing MenB protein-based vaccines by hSBA, (the accepted meningococcal vaccines surrogate of protection) is difficult due to the diversity in the sequence and expression of MenB protein antigens.[4] Different methodologies have been developed to know the MenB protein-based vaccines potential coverage, that is, to know the potential impact on the invasive MenB strains population.

The Meningococcal Antigen Surface Expression (MEASURE) assay, a flow cytometry method, was developed to assess the minimum level of FHbp expression necessary so that a MenB strain is neutralized by antibodies generated by the rLP2086 vaccine.[25] Although the applicability of MEASURE to predict rLP2086 potential coverage to globally invasive MenB strains has been suggested, an analysis based on 1.814 isolates from United Stated and Europe showed that 91% of the MenB isolate set expressed sufficient levels of FHbp to be susceptible to bactericidal killing by rLP2086 vaccine-induced antibodies.[25] MEASURE does not take into account the FHbp sequence diversity (FHbp needs to be expressed in enough quantity but also needs to be recognize by rLP2086 vaccine-induced antibodies). For the purpose of evaluating rLP2086 potential coverage, 14 invasive MenB strains (4 primary and 10 additional), each expressing FHbp variants different from the vaccine antigens, were used for hSBA assays in the clinical development of the rLP2086 vaccine.[21] Since protective bactericidal response by rLP2086 vaccine has been observed against these 14 invasive MenB strains expressing FHbp variants heterologous to the vaccine, it is possible to consider these FHbp peptides variants cross-reactive to the vaccine antibodies. The molecular analysis of the FHbp in Spanish MenB strains population has identified the presence of peptides potentially recognized by rLP2086 induced antibodies in the 64.15% of the strains (16.10% harbouring the exact vaccine FHbp variants, and 48.05% harbouring “cross-reactive” FHbp variants). So, based on the molecular information and assuming enough level of expression, it could be concluded that at least 64.15% of the inva-

sive MenB isolates would be covered by rLP2086 vaccine. However, data should be considered with caution since, on the one hand it has been seen that some strains even expressing sufficient FHbp level were not neutralized by vaccine induced-antibodies in hSBA assays[25] and, on the other hand it is possible that other FHbp peptide variants non tested by hSBA could be recognized by vaccine induced-antibodies. Therefore, both the assumption that all strains expressing these FHbp peptide variants will be vaccine covered and that only these FHbp peptide variants will be recognized by vaccine induced antibodies are probably not entirely valid.

Considering that rLP2086 vaccine is licensed for use in individuals from 10 years old, the potential coverage in this age group (≥10 years) would be at least 73.23% (145 of all 198 isolates from ≥10 years patients would be potentially recognized by vaccine induced-antibodies).

The Meningococcal Antigen Typing System (MATS) was developed to predict strain coverage by 4CMenB vaccine,[26] the other MenB protein-vaccine licensed. MATS combines genotyping for PorA, with three enzyme-linked immunosorbent assays (ELISA) for FHbp, NHBA y NadA, that quantify in meningococcal strains the relative expression and cross-reactivity of antigen variants with vaccine-induced antibodies. Contrary to rLP2086 vaccine which is composed by 2 different FHbp subfamily alleles peptides (FHbp peptides 55 y 45), 4CMenB vaccine in addition to one FHbp subfamily B allele peptide (FHbp peptide 1) is also composed by other three different antigens (NHBA peptide 2, NadA peptide 3.8 and PorA P1.4). Internationally-standardized MATS[27] was shown to provide a conservative predictions of hSBA results from pooled human sera[28, 29] ranging from 66% to 91% in 14 countries.[13, 30] In Spain, 4CMenB vaccine was predicted by MATS to cover the 69% of invasive MenB isolates from 2009 to 2010, and specifically FHbp contribution, either alone or in combination with other vaccine antigens, was estimated in 36%[14]. Recently, a genetic MATS (gMATS) for predicting 4CMenB vaccine strains coverage, has been defined by associating antigen genotyping and MATS results.[22] Predicted strain coverage of 4CMenB by gMATS was assessed for invasive MenB isolates in Spain from 2009 to 2010 in 58%,[22] and FHbp contribution was estimated in at least 28%. Predicted strain coverage by gMATS underestimated MATS results by 10%, it is mainly due to Spain showed a high proportion of “unpredictable” strains (32%).[22] A major difference between gMATS and MATS is the “unpredictable” strains category in gMATS, i.e. strains whose antigen genotype was not possible to associate with either a positive or negative MATS results. Considering only FHbp-specific contribution on predicted strain coverage by gMATS, it was estimated in at least 16% for the analyzed period in this study (2015–2018), while other 16% of the strains showed FHbp alleles “unpredictables” which might be or not recognized by vaccine induced-antibodies. There has been a decrease in the fHbp contribution on the 4CMenB potential coverage (28% in 2009–2010 vs 16% in 2015–2018), however the significance of this finding is unclear as we have not studied the other components of the 4CMenB vaccine, and if this decrease has been or not compensated by the other vaccine antigens would need to be analyzed in future studies.

Finally, a total of 6 strains harbouring 3 different subfamily A/B hybrid peptides were found. Although it is the first time that A/B hybrid peptides were detected in Spain, in the PubMLST database (https://pubmlst.org/neisseria/) appear 17 different A/B hybrid peptides identified in isolates from several countries. The oldest were isolated from UK in 1998 and correspond with 3 invasive serogroup C isolates cc11 harbouring the A/B hybrid FHbp peptide 776, and the most recent is an invasive MenB cc41/44 isolate from Czech Republic in 2017 harbouring the A/B hybrid FHbp peptide 207. It would be important to monitor the frequency of this type of alleles as well as to have information on whether or not they will be recognized by the new MenB protein vaccines.

Although there is a general trend in many countries to a slow increase in the frequency of subfamily A, the higher frequency of subfamily A in this study is surprising, with the important repercussions it can have on the coverage of strains of available vaccines. The increase registered in the last years of cc213 strains in Spain has a positive impact on the potential coverage of Trumenba® and it is possibly associated with less contribution of the FHbp antigen to the coverage by Bexsero®.

Declaration of Competing Interest

RA, CGA, CN, EM and AMT have nothing to disclose. JAV reports personal fees and research grants from GSK, Pfizer, and Sanofi-Pasteur through Instituto de Salud Carlos III.

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This publication made use of the Neisseria Multi Locus Sequence Typing website (https://pubmlst.org/ neisseria/) sited at the University of Oxford (Jolley et al. Wellcome Open Res 2018, 3:124 [version 1; referees: 2 approved]). The development of this site has been funded by the Wellcome Trust and European Union.

This study made use of Genomic and Bioinformatics ISCIII central Units for genomes sequencing and assembly.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.01.030.

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