Implications of Differential Age Distribution of Disease-Associated Meningococcal Lineages for Vaccine Development

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New vaccines targeting meningococci expressing serogroup B polysaccharide have been developed, with some being licensed in Europe. Coverage depends on the distribution of disease-associated genotypes, which may vary by age. It is well established that a small number of hyperinvasive lineages account for most disease, and these lineages are associated with particular antigens, including vaccine candidates. A collection of 4,048 representative meningococcal disease isolates from 18 European countries, collected over a 3-year period, were characterized by multilocus sequence typing (MLST). Age data were available for 3,147 isolates. The proportions of hyperinvasive lineages, identified as particular clonal complexes (ccs) by MLST, differed among age groups. Subjects <1 year of age experienced lower risk of sequence type 11 (ST-11) cc, ST-32 cc, and ST-269 cc disease and higher risk of disease due to unassigned STs, 1- to 4-year-olds experienced lower risk of ST-11 cc and ST-32 cc disease, 5- to 14-year-olds were less likely to experience ST-11 cc and ST-269 cc disease, and ≥25-year-olds were more likely to experience disease due to less common ccs and unassigned STs. Younger and older subjects were vulnerable to a more diverse set of genotypes, indicating the more clonal nature of genotypes affecting adolescents and young adults. Knowledge of temporal and spatial diversity and the dynamics of meningococcal populations is essential for disease control by vaccines, as coverage is lineage specific. The nonrandom age distribution of hyperinvasive lineages has consequences for the design and implementation of vaccines, as different variants, or perhaps targets, may be required for different age groups.

Neisseria meningitidis, the meningococcus, is a pathogen of global significance that causes sporadic cases and periodic epidemics and pandemics of meningitis and septicemia. The disease is associated with high mortality rates and severe sequelae in many patients who recover. Disease rates vary with age, with the highest rates for children and young adults (1–3). However, the meningococcus is usually carried asymptomatically in the nasopharynx of approximately 10% of the human population (4–7). Carriage rates in the population also vary with age, being lowest among infants and young children and rising to a peak among adolescents and young adults (8–10).

Twelve immunochemically distinct meningococcal polysaccharide capsules have been described (12), corresponding to meningococcal serogroups, but most disease is caused by meningococci expressing serogroups A, B, C, Y, W, and X (13). Worldwide invasive meningococcal serogroup distributions vary with region (3, 13); serogroups A, W, and X predominate in Africa, particularly in the “meningitis belt” region (14), whereas most disease in Western Europe is associated with meningococci expressing serogroup B and C capsules. Serogroups B and C also predominate in North and South America and in high-income countries such as New Zealand and Australia (3, 13, 15, 16). Serogroup Y disease has emerged recently as a public health concern in the United States and Canada (17, 18) and more recently in Europe (3, 11, 19, 20). The emergence of serogroup Y–associated lineages in disease and carriage populations underlines the dynamic nature of meningococcal lineages and the importance of understanding their epidemiology and evolutionary history.
MATERIALS AND METHODS

European meningococcal disease isolates. Bacterial samples were obtained from European reference laboratories over 3 years, i.e., 2000 to 2002. A structured sampling program was undertaken to ensure a representative sample; laboratories that received ≤80 disease isolates per year submitted all isolates, and laboratories that processed >80 disease isolates per year sent every third isolate, with the exception of the England and Wales Meningococcal Reference Unit, which sent every tenth isolate, as the unit received >1,000 samples per year. A total of 4,183 samples were received and processed by the European Meningococcal MLST Centre (EMMC) (25), and multilocus sequence typing (MLST) was completed for 4,048 of the samples.

MLST and sequence assembly. MLST was performed as described previously (26, 27). Separation of the labeled extension products was carried out on a 3700 or 3730 capillary DNA analyzer (Applied Biosystems). Assembly and editing of MLST sequence data generated were carried out using STARS (http://sourceforge.net/projects/stars) and Staden software, with Pregap 4 version 1.3 and Gap version 4.7 (28). For each isolate, sequences for each of the seven loci were assigned allele numbers according to age group, with the lowest diversities found in the 25- to 24-year-old children. The diversity of genotypes in a setting of endemicity represents a challenge for vaccine development and implementation. Detailed characterization of disease and carriage isolates, including the age distribution of disease-associated lineages, is essential for the improvement of disease prevention and control strategies.

RESULTS

There has been a decline in meningococcal disease incidence in Europe since 1999, decreasing from 1.9 cases per 100,000 individuals in 1999 to 0.73 cases per 100,000 individuals in 2010 (21). This decline is due in part to implementation of the meningococcal C conjugate (MCC) vaccine in a number of European countries. However, this cannot account for reductions in serogroup B disease incidence, which may be attributable to natural fluctuations. With new protein-based substitute serogroup B vaccines such as Bexsero (22) being licensed in Europe, it is important to monitor changes in the meningococcal population, particularly as herd immunity effects for this type of noncapsular vaccine are largely unknown. Outer membrane protein-based vaccines have been implemented previously in Europe, but that was in response to single-clone outbreaks (23, 24). The diversity of genotypes in a setting of endemicity represents a challenge for vaccine development and implementation. Detailed characterization of disease and carriage isolates, including the age distribution of disease-associated lineages, is essential for the improvement of disease prevention and control strategies.

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present in this vaccine has been carried out in recent years, in collections of disease-associated serogroup B-expressing organisms (34–36). Based on the meningococcal antigen typing system (MATS) assay, there is predicted coverage of 78% of disease-associated serogroup B-expressing meningococci in Europe (36). As Bexsero antigen data are not available for this collection, an estimate based on previous studies (34) showing strong associations of antigen and cc would give a baseline coverage of almost 50% for this data set regardless of serogroup (with ST-41/44 cc, ST-32 cc, and ST-8 cc serving as proxies for NHBA-2 and P1.4, fHbp-1.1, and NadA-3.8, respectively). Representative, well-sampled, isolate collections such as the EU MeningNet collection provide insights into the dynamics of the population biology of the meningococcus and facilitate the planning and implementation of interventions, including immunization programs. Since serogroups and other vaccine antigens are known to be associated with cc, the differences we observed in the distribution of cc with age concur with, and extend, previous work in both Europe and North and South America. Studies have indicated a higher prevalence of non-BC serogroups in older age groups, with serogroup B disease being proportionally the greatest among subjects <1 year of age (15, 16, 19, 21, 37–39). The present analysis also indicated that the diver-

![FIG 1 Age distribution of disease isolates in the European meningococcal disease collection.](image)

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Age distribution among the most common clonal complexes in the European meningococcal disease collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonal complex</td>
<td>No. (%) of isolates per age group</td>
</tr>
<tr>
<td></td>
<td>&lt;1 yr</td>
</tr>
<tr>
<td>ST-41/44</td>
<td>143 (14)</td>
</tr>
<tr>
<td>ST-11</td>
<td>49 (5)</td>
</tr>
<tr>
<td>ST-32</td>
<td>61 (9)</td>
</tr>
<tr>
<td>ST-8</td>
<td>21 (8)</td>
</tr>
<tr>
<td>ST-269</td>
<td>20 (8)</td>
</tr>
<tr>
<td>Others*</td>
<td>88 (14)</td>
</tr>
<tr>
<td>Unassigned</td>
<td>46 (17)</td>
</tr>
<tr>
<td>Total</td>
<td>428</td>
</tr>
</tbody>
</table>


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The relatively lower prevalence of ST-11 cc and ST-269 cc among individuals <14 years of age was consistent with findings in Canada, where there was an association of ST-269 cc in 11- to 40-year-olds and many fewer cases among <1-year-olds (40). Also, a study of meningococcal disease in Poland over 10 years demonstrated a significantly higher frequency of ST-11 cc-associated disease among individuals >5 years of age (41). As with ST-11 cc and ST-269 cc, the serogroup B-associated ST-32 cc was less likely to affect subjects <4 years old. These findings are consistent with the presence of the meningococcal disease-associated (MDA) phage, a candidate virulence factor associated with adolescent disease, in these lineages (42).

The nonrandom variation in age distributions of meningococcal lineages has several consequences. It demonstrates that the different ccs have different phenotypes in terms of disease association and probably carriage. Genotypes that are thought to be comparatively less invasive, along with those not assigned to a cc, were more likely to affect the very young or relatively old (<1 or >65 years of age), which may be due to these cohorts being vulnerable to higher rates of disease caused by less-invasive meningococci, perhaps as a consequence of poorer immune responses. Niche competition with commensal organisms such as Neisseria lactamica, which has its highest rates of carriage in 1- to 2-year-olds, may have an influence on meningococcal carriage and thus potentially disease (43, 44). Immunologically mature individuals such as older adolescents and young adults may more easily clear less-virulent strains but then be more susceptible to more-virulent strains such as ST-11 cc, which may have shorter durations of carriage. Some of these differences may be due to behavioral factors or potential virulence factors such as the MDA phage, which may affect the expression of certain genes. These differences in age associations may have consequences for the design and implementation of vaccines, as different variants or perhaps targets may be required for different age groups. Like other well-studied vaccine candidate antigens, such as PorA and FetA, those included in newly developed vaccines such as Bexsero have associations with clonal complexes (34, 35). Therefore, it is expected that they will also have different age distributions, which may have consequences for vaccine implementation.

Given the diversity of the meningococcal population, a relatively small number of genotypes are associated with disease. In Europe, five ccs (ST-41/44, ST-11, ST-32, ST-8, and ST-269 ccs) accounted for 77% of the disease isolates. These hyperinvasive lineages are a subset of those observed globally and represent a minority of carried meningococci (45–47). The prevalence of particular hyperinvasive lineages in carriage changes over time, and this is reflected in the rates of disease that they cause. For instance, in the past decade previously rare serogroup Y-associated lineages increased in prevalence, in both disease and carriage, in Europe (3, 11, 19, 20, 48–51). It is therefore necessary to maintain surveillance to identify changes in the distribution of types, including the emergence of new clones and possible capsule-switching events in
the face of immunization campaigns. This should include monitoring changes in genotype distributions, in disease and carriage, according to age group. Initiatives such as the Meningitis Research Foundation Meningococcus Genome Library are valuable resources that will allow such analyses using the latest molecular epidemiological tools. In the absence of serogroup B conjugate polysaccharide vaccines, the control of meningococcal disease will rely on the implementation of protein-based vaccines, the coverage of which will vary as changes occur in the circulating meningococcal populations over time. Any implementation of new vaccines, such as Bexsero, that target proteins that are derived from particular serogroup B meningococci but may be shared by strains belonging to other serogroups, will therefore require intensive epidemiological surveillance.

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REFERENCES


