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PorB2/3 Protein Hybrid in *Neisseria meningitidis*

To the Editor: Class 2 and class 3 porin (PorB) proteins are the major proteins found in the outer membrane of *Neisseria meningitidis* (1); they function as porins, allowing the passage of small molecules through the outer membrane. PorB outer membrane proteins are transmembrane proteins with 8 predicted surface-exposed loops (I–VIII), which vary in length and in amino acid sequences. Several sequence analyses of these proteins have shown 4 regions with a high level of amino acid variability in loops I, V, VI, and VII (variable regions [VRs] 1–4) (2). The extensive antigenic variability of these proteins forms the basis of the *N. meningitidis* serotyping scheme (3,4). These 2 classes of proteins are mutually exclusive, and they are expressed by alternate alleles (*porB2* and *porB3*) at the *porB* locus (1).

All *N. meningitidis* strains received in the Spanish Reference Laboratory for *Neisseria* are routinely serotyped by whole-cell ELISA (5) with a set of monoclonal antibodies (MAbs) provided by the National Institute for Biological Standards and Control (South Mimms, UK) that includes the following serotypes: 1 (MN3C6B), 2a (5D4–5), 2b (MN2C3B), 4 (5DC4-C8G8), 14 (MN5C8C), 15 (8B5–5G9), and 21 (6B11F2B5). Those meningococci that appear as nonserotypeable (NT) are analyzed by sequencing the *porB* gene (6). In the case discussed

here, in the sequencing of a NT strain, the *porB* gene showed an unusual sequence.

This strain, isolated in Spain during 2006, was recovered from the cerebrospinal fluid of a patient with meningococcal disease. The *porB* gene sequence shows VR1–4, which is exclusive of PorB3 protein, and VR2-Eb, VR3–2ab, and VR4-Cc, which are typical of PorB2 (GenBank accession no. EF094023). A comparison of this new sequence with the available *porB* sequences in the *Neisseria.org* database (<http://neisseria.org/nm/typing/porB>) enabled a more detailed analysis of the fragments corresponding to *porB3* and *porB2* found in this sequence. The fragment from nt 1 to 213 was identical to the *porB3*–193 allelic variant (VR1–4, VR2-Aa, VR3–7, VR4–14b), and the second part, with nt 233–972 identical to *porB2*–99 (VR1-Dc, VR2-Eb, VR3–2ab, VR4-Cc). The region of 214–232 nt is identical in the 3 variants. Therefore, this is a true hybrid molecule, which appears to have arisen from recombinational events between *porB2*–99 and *porB3*–193 alleles. In fact, this finding has prompted the inclusion of a new family called *porB2/3* hybrid in the *Neisseria.org* database to facilitate the collection of this type of *porB* sequences.

The most likely origin of the *porB2/3* hybrid (4, Eb, 2ab, Cc) is the acquisition of DNA that encodes a VR1–4 sequence by a meningococcus with a *porB2*–99 allelic variant. It is less likely that DNA encoding the *porB* VR2-Eb, VR3–2ab, and VR4-Cc sequences was acquired by a meningococcus with the *porB3*–193 allelic variant because a longer fragment of DNA would have been transferred.

In spite of the presence of a VR1–4, which should be recognized by the set of MAbs used, this strain appeared as NT. A Western blot assay using MAb type 4 showed a good recognition epitope-MAb. Therefore, the failure of MAbs to identify this strain may have been due to the limited ac-

cessibility of the epitope because of the alteration of the PorB protein, which might be affecting its conformation. Once again, genetic characterization should be a preferred method over phenotypic characterization for typing meningococcal strains. Molecular characterization of NT strains in other laboratories might clarify the true frequency of this event.

Intragenic recombination between porin genes of the same allelic family is likely occurring in nature because mosaic gene structure has been reported in *porB* genes. However, *porB2/3* recombinants have never been previously found in the nature. Given the known ability of meningococci to be transformed by DNA from other strains, it is surprising that occurrence of genuine *porB2/3* hybrids has not yet been documented. There is only a report of naturally occurring gonococci expressing a hybrid *porB1a/porB1b* (7) (PorB1a and PorB1b gonococcus porins, as in meningococci, are encoded by 2 families of diverged alleles of the *porB* gene [8]). Gonococcal strains expressing the recombinant *por* genes appear to be particularly susceptible to the bactericidal effect of human serum (9). A similar situation might happen in *N. meningitidis*, with a selective disadvantage in the invasive process of these hybrid strains, explaining the rarity of naturally occurring hybrids. By contrast, mechanisms like this are frequently used by meningococci to avoid the immune response against ordinary antigens. The balance between advantages and disadvantages at this level would show the true implications of this event.

This finding is relevant regardless of its frequency in nature. This report suggests how frequent the recombination events should occur among the meningococcal population: even theoretical mutually exclusive genes can produce hybrid variants; such knowledge is an important step in the development of future vaccines based on protein formulations.

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References

1. Urwin R. Nucleotide sequencing of antigen genes of *Neisseria meningitidis*. In: Pollard AJ, Maiden MCJ, editors. Meningococcal disease: methods and protocols. Totowa (NJ): Humana Press, Inc.; 2001. p. 157–72.
2. Van der Ley P, Heckels JE, Virji M, Hoogerheut P, Poolman JT. Topology of outer membrane porins in pathogenic *Neisseria* spp. *Infect Immun*. 1991;59:2963–71.
3. Frasch CE, Zollinger WD, Poolman JT. Serotype antigens of *Neisseria meningitidis* and proposed scheme for designation of serotypes. *Rev Infect Dis*. 1985;7:504–10.
4. Sacchi CT, Lemos APS, Whitney AM, Solari CA, Brant ME, Melles CEA, et al. Correlation between serological and sequence analysis of the PorB outer membrane protein in the *Neisseria meningitidis* serotyping scheme. *Clin Diagn Lab Immunol*. 1998;5:348–54.
5. Abdillahi H, Poolman JT. Typing of group-B *Neisseria meningitidis* with monoclonal antibodies in the whole-cell ELISA. *J Med Microbiol*. 1988;26:177–80.
6. Abad R, Alcalá B, Salcedo C, Enríquez R, Uría MJ, Diez P, et al. Sequencing of the *porB* gene: a step toward a true characterization of *Neisseria meningitidis*. *Clin Vaccine Immunol*. 2006;13:1087–91.
7. Cooke SJ, Jolley K, Ison CA, Young H, Heckels JE. Naturally occurring isolates of *Neisseria gonorrhoeae*, which display anomalous serovar properties, express PIA/PIB hybrid porins, deletions in PIB or novel PIA molecules. *FEMS Microbiol Lett*. 1998;162:75–82.
8. Carbonetti NH, Simnad VI, Seifert HS, So M, Sparling PF. Genetics protein I of *Neisseria gonorrhoeae*: construction of hybrid porins. *Proc Natl Acad Sci U S A*. 1988;85:6841–5.
9. Carbonetti N, Simnad V, Elkins C, Sparling PF. Construction of isogenic gonococci with variable porin structure—effects on susceptibility to human serum and antibiotics. *Mol Microbiol*. 1990;4:1009–18.

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West Nile Virus in Birds, Argentina

To the Editor: West Nile virus (WNV), genus *Flavivirus*, family *Flaviviridae* has been rapidly dispersing through the Americas since its introduction in 1999 in New York (1). By 2004, serologic studies detected WNV-specific antibodies in birds and horses from Canada to northern South America (2–4). The first report of WNV activity in the Southern Cone of South America surfaced in April 2006, when 3 horses died in Argentina (5). However, established transmission foci in Argentina are unknown. We report evidence for the introduction and establishment of WNV in Argentina as early as January 2005.

Serum samples from free-ranging birds were collected from 5 locations in Argentina and screened for generic flavivirus antibodies by using a blocking ELISA with monoclonal antibody 6B6C-1 (6). Positive serum specimens were further characterized by plaque-reduction neutralization test (PRNT). We identified the etiologic agent responsible for the previous flavivirus infection by using the following criteria: 80% neutralization of reference virus (WNV NY99-4132 or an Argentinean strain of St. Louis encephalitis virus [SLEV CbaAr4005]) in serum diluted at least 1:40 and 4-fold greater titer compared with the other virus.

Overall, 474 (25.6%) of 1,845 serum specimens from 117 bird species collected from January to June

2006 tested positive when using the blocking ELISA; 30% inhibition was the threshold for a positive test. SLEV infections were confirmed in 105 birds by PRNT; WNV infections were confirmed in 43 birds. Anti-WNV antibody titers ranged from 40 to 2,560 in birds collected as early as January 2005 in Córdoba City and as late as June 2006 in Mar Chiquita (Table). Recent WNV activity was indicated by seroconversion in 3 banded rufous hornero in Córdoba City between January and March 2005. Although 659 (1.5%) of serum samples were positive for SLEV, no WNV infection was detected in free-ranging birds collected in 2004. As early as January 2005, WNV was detected in a seroconversion so we suspect WNV was introduced before 2005 at the end of 2004 in all 5 sampling locations and in a variety of ecosystems: Córdoba, periurban (1.1%, 6/543); Mar Chiquita, thorn forest (5.1%, 16/313); Monte Alto, semidry chaco forest (9.8%, 8/82); Montecristo, cropland (9.5%, 2/21); and San Miguel de Tucumán, periurban yungas foothills (4.9%, 12/227).

In 2006, WNV was isolated from equines in Buenos Aires province (5). WNV transmission to resident birds collected further north in Córdoba, Chaco, and Tucumán provinces was detected in 2005 and 2006. Our data suggest that WNV was introduced into Argentina before 2005 and maintained naturally in enzootic foci where numerous bird species from many families were exposed. Presumably, as in North America, locally abundant passerine birds such as turdids (thrushes) are amplifying hosts. If common species of the *Furnariidae* (a family absent from temperate North America) prove to be competent hosts, they could play an important role in WNV transmission in Argentina because of their frequent exposure to WNV. Twelve (12.5%) of 96 *F. rufus* sampled in 2005 and 2006 tested positive.