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## Novel Nonstructural Protein 4 Genetic Group in Rotavirus of Porcine Origin

**To the Editor:** Infection with group A rotavirus is the main cause of acute gastroenteritis in infants and young children worldwide and in young animals of many species, including piglets. In recent years, several epidemiologic studies designed to monitor the appearance of novel or atypical rotavirus antigenic types have provided evidence for the increasing antigenic diversity of group A rotaviruses (*1*–*3*). In addition to the 2 rotavirus classification systems, VP7 (G) and VP4 (P) genes, the virus can also be classified on the basis of the nonstructural glycoprotein 4 (NSP4)–encoding gene. Sequence analyses of the NSP4 gene indicated the presence of at least 5 distinct genetic groups among human and animal rotaviruses, termed A to E (*1*,*4*,*5*). Among human rotaviruses, the diversity of NSP4 genes has been restricted mainly to genetic groups A and B; only a few human strains possess genetic group C. Conversely, all 5 NSP4 genetic groups (A–E) have been identified in rotaviruses of animal origins. To our knowledge, porcine rotaviruses (PoRVs) have been reported to belong only to NSP4 genetic group B (*1*).

During an epidemiologic survey of PoRV from June 2000 through July 2001, a total of 175 fecal specimens were collected from diarrheic piglets from 6 different farms in Chiang Mai Province, Thailand. Of these, 39 (22.3%) specimens were positive for group A rotavirus (*6*). A novel and unusual PoRV CMP034 strain was isolated from a 7-week-old piglet during this survey. Molecular genetic characterization showed that the CMP034 strain carried a novel P[27] genotype with a new lineage of G2-like rotavirus genotype (*7*). We performed a molecular analysis of the NSP4 gene of this strain in comparison with those of other NSP4 gene sequences available in the GenBank database.

The full-length of NSP4 gene was amplified by NSP4–1a and NSP4–2b primer pairs (*8*). The PCR amplicon was sequenced in both directions by using the BigDye Terminator Cycle Sequencing kit (PerkinElmer-Applied Biosystems, Inc., Foster City, CA, USA) on an automated sequencer (ABI 3100; PerkinElmer-Applied Biosystems, Inc.). The sequence of CMP034 was compared with those of reference strains available in the National Center for Biotechnology Information GenBank database by using BLAST (www.ncbi.nlm.nih.gov/blast). The NSP4 nucleotide sequence of the CMP034 strain was deposited in GenBank under accession no. DQ534017.

The complete NSP4 nucleotide sequence of PoRV CMP034 strain was 750 bp and contained a single long open reading frame coding for a protein of 175 aa. Comparative analysis of the CMP034 NSP4 sequence with those of the 5 representative established genetic groups (A–E) showed the highest sequence identity, at 92.6% nt and 96.9% aa levels, with 1 PoRV strain, P21–5 (*9*). However, CMP034 and P21–5 shared a low degree of sequence identity with other NSP4 genetic groups. The NSP4 sequence identities of the CMPO34 and P21-5 strains ranged from 74% to 78% nt and 75%–79% aa levels with those of genetic group A; 77%–86% nt and 79%–86% aa levels with genetic group B; 69%–73% nt and 75%–78% aa levels with genetic group C; 62%–65% nt and 55%–60% aa levels with genetic group D; and only 43%–50% nt and 29%–33% aa levels with genetic group E. The phylogenetic tree confirmed that PoRV strains CMP034 and P21–5 were located exclusively in a separated branch, which was distantly related to the other 5 known NSP4 genetic groups (Figure). However, a bootstrap support for the separation of

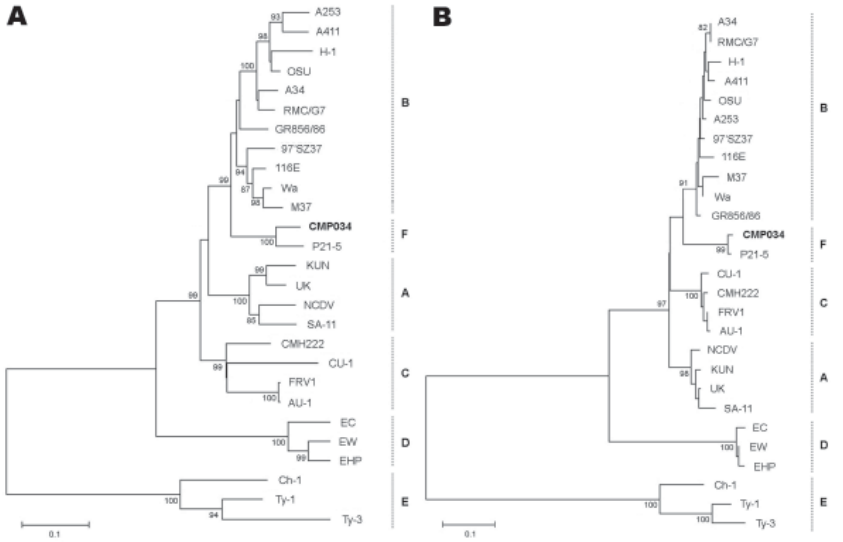


Figure. Phylogenetic analyses of the NSP4 nucleotide (A) and amino acid (B) sequences displaying the relationships between porcine rotavirus strain CMP034 (shown in **boldface**), P21–5, and other 5 known NSP4 genetic groups. Bootstrap values are shown at the branch nodes. Branch length for a 10% nucleotide difference is indicated at the bottom.

the gene into a separate lineage is very strong with nucleotide sequencing but weak by amino acid analysis in this phylogentic tree. Our finding indicates that PoRV strains CMP034 and P21–5 are likely a novel NSP4 genetic group and, therefore, tentatively proposed as a NSP4 genetic group F.

On the basis of the accumulated evidence of transmission of rotaviruses between pigs and other animal species, including humans, pigs are regarded as 1 potential reservoir for the emergence of unusual or novel strains of rotaviruses (*6*,*7*). In our study, the virus carried a novel NSP4 genetic group that has been isolated from a diarrheic piglet in Thailand. The NSP4 sequence analysis of our CMP034 strain revealed a PoRV strain closely related genetically to the NSP4 gene sequence of PoRV strain P21–5 isolated in Slovenia (*9*). PoRV strains CMP034 and P21–5 shared the same VP4 genotype as P[27] with over 90% aa sequence identity. The only difference observed between the 2 strains was that CMP034 belonged to the G2-like genotype whereas P21–5 belonged to G1 genotype. The relatedness between NSP4 sequences of strains CMP034 and P21–5 was confirmed by phylogenetic analysis, which showed that both CMP034 and P21–5 clustered closely together in a branch separated from those of other 5 NSP4 genetic groups. This finding suggests that NSP4 of PoRV strain CMP034 and P21–5 may have derived from the same ancestor. The isolation of 2 strains of rotaviruses with a close genetic relatedness of NSP4 gene from Thailand and Slovenia, 2 countries that are located in different continents, may indicate that this novel NSP4 genetic group has already been introduced into PoRVs worldwide. To verify this hypothesis, extensive epidemiologic surveillance of rotavirus in pigs may need to be conducted in several other regions of the world.

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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 accessibility 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*]). Gonoccocal 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.