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Identification and Classification of *P1* Variants of *Mycoplasma pneumoniae*

With considerable interest we have read the article by Schwartz et al. (2), who describe a rapid real-time PCR high-resolution melt (HRM) procedure that allows the identification of variants of the *P1* gene of *Mycoplasma pneumoniae*. In that paper, evidence is presented showing that both major and minor variations within the *P1* gene can be detected by this rapid procedure.

One of the *P1* variants described in that paper was found in an *M. pneumoniae* isolate designated isolate 3. In order to characterize the differences between this isolate and other *M. pneumoniae* strains, the part of the *P1* gene that was targeted by HRM analysis was sequenced. On the basis of the sequence data, isolate 3 was suggested to exhibit an "intermediate" genotype between subtypes 1 and 2 at the *P1* locus (2). However, this would be an unexpected finding because we recently reported that subtype 1 and 2 strains can be differentiated on the basis of sequence variation within each RepMP element (i.e., the repetitive DNA elements located in the *P1* gene, as well as elsewhere in the *M. pneumoniae* genome) and that subtype 1 and subtype 2 strains therefore represent evolutionarily diverged strain lineages (3). In addition, each *P1* variant ever reported could be explained by intragenomic homologous DNA recombination events between RepMP elements (3). With this in mind, we performed a BLAST analysis to try to explain the "novel" *P1* sequence of isolate 3 and compare it to the RepMP sequences we previously determined. Interestingly, our analysis showed that the *P1* variant of isolate 3 is identical to a known subtype 2 variant which was previously designated variant 2b by Dumke et al. (1). In this strain, a putative homologous DNA recombination event has transferred sequences from element RepMP2/3-a to the RepMP2/3 element within the *P1* gene, i.e., RepMP2/3-d. Thus, according to the classification scheme of Spuesens et al. (3), isolate 3 can be described as a strain with sequence type 2-*P1*(4-c; 2/3-d[a]d). This classification is in line with the classification of isolate 3 as a subtype 2 strain by PCR-restriction fragment length polymorphism analysis (2). As a consequence, isolate 3 is highly unlikely to have an intermediate genotype between subtypes 1 and 2.

Finally, the HRM procedure presented by Schwartz et al. is a sensitive and promising technique for the detection of, and discrimination between, different genotypes of *M. pneumoniae*. However, extensive sequencing and BLAST procedures should follow the finding of "aberrant" *P1* genotypes in order to classify putatively novel *M. pneumoniae* strains in a correct and standardized fashion. The sequencing and strain classification scheme we previously described (3) may be helpful in these analyses.

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Authors' Reply

We appreciate the comments made by Spuesens et al. concerning our recent report of *P1* variants of *M. pneumoniae* (1). Our assay was designed to rapidly identify type 1 and type 2 strains by HRM technology (2). In addition, our assay has the potential to identify variants within these two groups. One variant (isolate 3) was correctly identified as a "type 2 *P1* variant." However, we did not propose that this isolate represented an evolutionarily divergent strain lineage, as stated by Spuesens et al.

The classification procedure Spuesens et al. have proposed requires 18 different PCR assays, followed by purification, cloning, and sequencing to characterize an isolate (3). Their classification scheme is based on the putative RepMP elements from a single strain (M129). Additionally, a limited number ($n = 8$) of type 2 strains were examined. We are uncertain how Spuesens et al. would classify isolate 3 as "2-*P1* (4c;2/3-d[a]d)" when no sequence data are available on the RepMP4 locus of isolate 3. We are optimistic that, collectively, our rapid typing assay along with genetic analyses such as those proposed by Spuesens et al. may allow for a more comprehensive understanding of *M. pneumoniae* diversity.

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