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LETTER TO THE EDITOR

Within-Host Sampling of a Natural Population Shows Signs of Selection on Pde1 during Bacterial Meningitis

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After the *pde1* gene was found to be essential for growth in an experimental meningitis model (1), Cron et al. further showed in a 2011 study that *Streptococcus pneumoniae* mutants with *pde1* (SP2205 in TIGR4; SPD2032 in D39) and its paralogue *pde2* (SP1298 in TIGR4; SPD1153 in D39) knocked out exhibited reduced host cell adherence and attenuated virulence in a mouse model of meningitis (2). Following work confirmed that Pde1 acts as a phosphodiesterase, cleaving c-di-AMP into pApA (3, 4). These signaling molecules are known to have broad effects on the cell (5) and were again shown to affect growth and virulence in a mouse model of pneumonia. In both studies, the authors suggested that these proteins are promising vaccine targets; however, further evidence of their importance in human infection is needed to bolster these claims.

In a recent study of 674 adults with culture-proven pneumococcal meningitis (6), we searched concurrently sampled bacterial genomes from the blood and cerebrospinal fluid (CSF) for adaptation to either niche occurring postinvasion (7). Here we present results of additional analysis performed using this study that support the conclusions of Cron et al. with respect to a natural population.

First, we observed that *pde1* did not appear to be under selection in the sampled population, as the ratio of nonsynonymous to synonymous mutations was neutral (dN/dS = 0.89) and contained variants with a site frequency spectrum similar to that of other genes (Fig. 1a and b; Tajima's $D = -1.69$; $P = 0.94$). However, comparing the variations between samples taken from the same patient during meningitis and given the overall small number of mutations occurring during the rapid progression of disease, *pde1* showed a significant enrichment of mutations ($P < 10^{-10}$). As all these mutations were nonsynonymous, this strongly implies that selection acts on *pde1* during the course of invasive disease.

We computationally predicted (8, 9) the effect of the 19 mutations observed to occur in *pde1* during meningitis and have plotted these along with the predicted functional domains in Fig. 1c. Of these mutations, 14 are predicted to change protein function, without causing a loss of function (LoF). The mutations are not evenly distributed across the gene and are mostly clustered in the DHH family domain or just before it. While this does not allow a singular interpretation of the effect of these variants on gene function, we are able to conclude that selection appears to be operating on *pde1* during meningitis.

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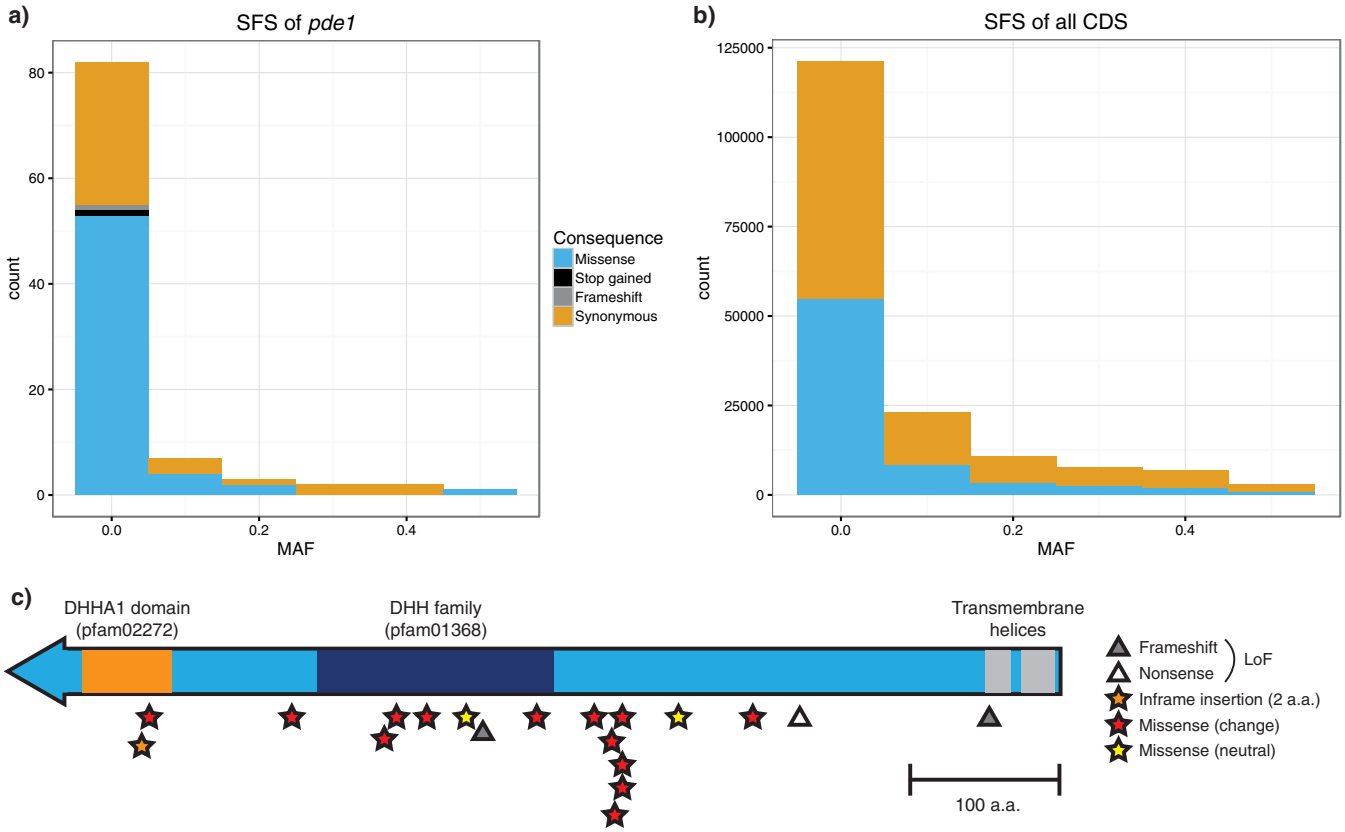

FIG 1 Evidence of selection on *pde1* during meningitis. Panels a and b show the site frequency spectra (SFS; histograms of minor allele frequency) of mutations in just *pde1* and in all coding regions (CDS), respectively. Variants are colored according to the predicted effect. Panel c shows the positions and predicted effects of mutations observed in *pde1* during cases of meningitis and pfam predicted domains. MAF, minor allele frequency; a.a., amino acids.

This corollary from our study therefore strongly supports the conclusion of Cron et al. that *pde1* is essential for virulence and additionally shows variation to be important in specific regions of *pde1* which should be considered in follow-up work. Together, these studies give good evidence that Pde1 might be an important component of a pneumococcal protein vaccine.

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