AMERICAN SOCIETY FOR MICROBIOLOGY | Infection and Immunity®

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

John A. Lees,[a] Matthijs Brouwer,[b] Arie van der Ende,[c,d] Julian Parkhill,[a] Diederik van de Beek,[b] Stephen D. Bentley[a]

Wellcome Trust Sanger Institute, Hinxton, United Kingdom[a]; Department of Neurology, Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Center, Amsterdam, The Netherlands[b]; Department of Medical Microbiology, Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Center, Amsterdam, The Netherlands[c]; Netherlands Reference Laboratory for Bacterial Meningitis, Academic Medical Center, Amsterdam, The Netherlands[d]

KEYWORDS DHH family protein, bacterial meningitis, *pde1*, pneumococcal pathogenesis, selection

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.

Citation Lees JA, Brouwer M, van der Ende A, Parkhill J, van de Beek D, Bentley SD. 2017. Within-host sampling of a natural population shows signs of selection on Pde1 during bacterial meningitis. Infect Immun 85:e01061-16. https://doi.org/10.1128/IAI.01061-16.

Editor Liise-anne Pirofski, Albert Einstein College of Medicine

Address correspondence to Stephen D. Bentley, sdb@sanger.ac.uk.

Ed. Note: The author of the published article did not feel that a response was necessary.

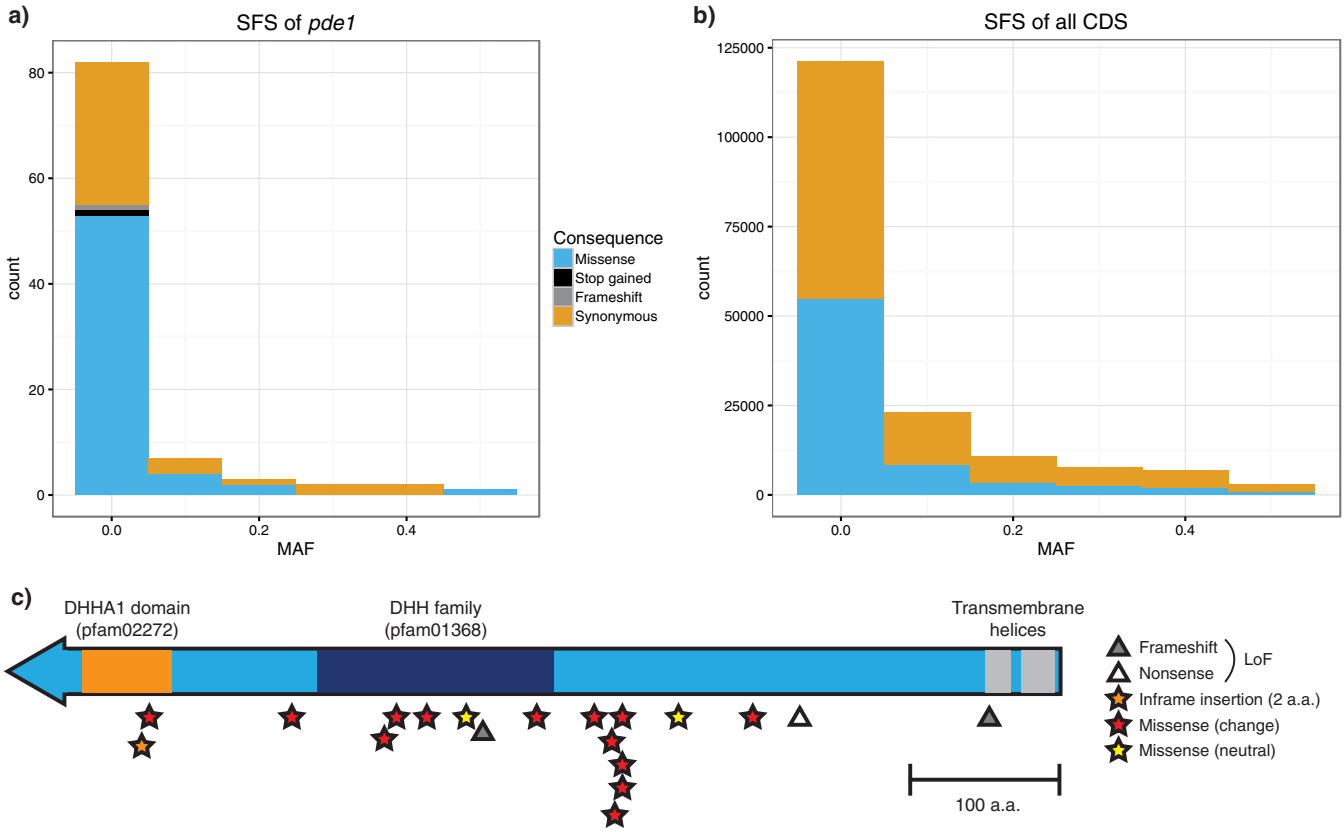

FIG 1 Evidence of selection on *pde1* during meningitis. Panels a and b show the site frequency spectra (SFS; histograms of minor allele frequency) of mutations in just *pde1* and in all coding regions (CDS), respectively. Variants are colored according to the predicted effect. Panel c shows the positions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

REFERENCES

1. Molzen TE, Burghout P, Bootsma HJ, Brandt CT, van der Gaast-de Jongh CE, Eleveld MJ, Verbeek MM, Frimodt-Møller N, Østergaard C, Hermans PWM. 2011. Genome-wide identification of Streptococcus pneumoniae genes essential for bacterial replication during experimental meningitis. Infect Immun 79:288–297. https://doi.org/10.1128/IAI.00631-10.
2. Cron LE, Stol K, Burghout P, van Selm S, Simonetti ER, Bootsma HJ, Hermans PWM. 2011. Two DHH subfamily 1 proteins contribute to pneumococcal virulence and confer protection against pneumococcal disease. Infect Immun 79:3697–3710. https://doi.org/10.1128/IAI.01383-10.
3. Bai Y, Yang J, Eisele LE, Underwood AJ, Koestler BJ, Waters CM, Metzger DW, Bai G. 2013. Two DHH subfamily 1 proteins in Streptococcus pneumoniae possess cyclic di-AMP phosphodiesterase activity and affect bacterial growth and virulence. J Bacteriol 195:5123–5132. https://doi.org/10.1128/JB.00769-13.
4. Kuipers K, Gallay C, Martínek V, Rohde M, Martínková M, van der Beek SL, Jong WS, Venselaar H, Zomer A, Bootsma H, Veening JW, de Jonge MI. 2016. Highly conserved nucleotide phosphatase essential for membrane lipid homeostasis in Streptococcus pneumoniae. Mol Microbiol 101:12–26. https://doi.org/10.1111/mmi.13312.
5. Tamayo R, Pratt JT, Camilli A. 2007. Roles of cyclic diguanylate in the regulation of bacterial pathogenesis. Annu Rev Microbiol 61:131–148. https://doi.org/10.1146/annurev.micro.61.080706.093426.
6. Bijlsma MW, Brouwer MC, Kasanmoentalib ES, Kloek AT, Lucas MJ, Tanck MW, van der Ende A, van de Beek D. 2016. Community-acquired bacterial meningitis in adults in the Netherlands, 2006–14: a prospective cohort study. Lancet Infect Dis 16:339–347. https://doi.org/10.1016/S1473-3099(15)00430-2.
7. Lees JA, Kremer PHC, Manso AS, Croucher NJ, Ferwerda B, Serón MV, Oggioni MR, Parkhill J, Brouwer MC, van der Ende A, van de Beek D, Bentley SD. 2017. Large scale genomic analysis shows no evidence for pathogen adaptation between the blood and cerebrospinal fluid niches during bacterial meningitis. Microb Genom 3:1–12. https://doi.org/10.1099/mgen.0.000103.
8. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM. 2012. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly 6:80–92. https://doi.org/10.4161/fly.19695.
9. Choi Y, Sims GE, Murphy S, Miller JR, Chan AP. 2012. Predicting the functional effect of amino acid substitutions and indels. PLoS One 7:e46688. https://doi.org/10.1371/journal.pone.0046688.