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Evolution to serum resistance through phase variable opa genes

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Background

Neisseria gonorrhoeae, the causative agent of gonorrhea, has evolved to evade killing by complement. The degree of complement resistance varies among clinical isolates of N. gonorrhoeae. While some genetic contributors to serum resistance, such as variant alleles of the major porin PorB, have been identified, our understanding of the genetic basis of complement resistance in N. gonorrhoeae is incomplete. For example, N. gonorrhoeae strains that express opacity associated proteins (Opa) are more resistant to complement, but the difficulty of reliably genotyping the repetitive and phase-variable opa loci has limited our understanding of how Opa proteins contribute to complement resistance.

Aim/Methods

A panel of 204 diverse clinical N. gonorrhoeae strains was tested for complement resistance (survival in 10% normal human serum). Using conditional genome-wide association studies (GWAS), we evaluated genetic

contributors to serum resistance in this panel. To further probe evolutionary pathways to complement resistance, we performed in vitro evolution in normal human serum for nine N. gonorrhoeae strains of diverse genetic backgrounds. We then analyzed the genomes and transcriptomic profiles of resistant strains, including long-read genome sequencing to resolve phase-variable loci, to determine the mechanisms of complement resistance that emerged.

Results
GWAS revealed PorB as the main contributor to complement resistance, as expected. However, in vitro evolution experiments found that phase variation and increased expression levels of specific opa genes are associated with complement resistance. Passaging serum resistant strains in vitro without serum pressure resulted in a partial reversal of serum resistance in most evolved strains, further supporting the hypothesis that phase variation underlies these rapid phenotypic changes. We also observed that evolved complement resistance varied in different batches of pooled human serum, suggesting that some Opa proteins confer resistance via a mechanism that is specific to different antibody repertoires present in the sera.

Conclusions
We confirmed that PorB is a main genetic contributor to serum resistance in our large, diverse collection of N. gonorrhoeae strains. This work also highlights the contribution of opa genes to serum resistance, demonstrating the importance of examining phase-variable loci and other regions that are not resolvable by standard whole-genome sequencing pipelines.