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2-hour Oral Session

Control of phase variable epigenetic virulence regulation in pneumococci

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Objectives: We have identified a novel phase variable epigenetic control mechanism in *Streptococcus pneumoniae* with an impact on gene expression and important phenotypes such as virulence in experimental models of infection (Manso et al., 2014). This genetic system is based on the rapid inversion of the specificity determinants of the type I restriction modification (RM) system SpnD39III which results in a change in methylation of the bacterial genome. Similarly organised RM loci are present in multiple other species. The objective of this work was to investigate the contribution of the CreX recombinase encoded by this locus to the phase variable rearrangement of epigenetic control.

Methods: we have developed and validated an allele quantification tool based on a PCR, restriction and GeneScan analysis. Gene expression of *creX* was evaluated by RNAseq and *creX* mutant strains tested for recombination efficiency.

Results: The recombination within the SpnD39III locus reverses the orientation of a *creX* gene encoded in the locus. RNAseq data show that the *creX* gene is expressed two fold more when co-directional to the *hsdRMS* genes due to read-through at the end of the operon. Deletion of the *creX* gene resulted in an approximately two fold decrease in recombination on the large inverted repeats and a greater than four fold decrease on the shorter repeats of the locus.

Conclusion: Our data on the recombination of the SpnD39III locus in *S. pneumoniae* indicate a direct but not exclusive involvement of *creX* in the recombination of the *hsdS* genes responsible for the epigenetic control of pneumococcal virulence.

Manso AS, MH Chai, JM Atack, L Furi, M De Ste Croix, R Haigh, C Trappetti, AD Ogunniyi, LK Shewell, M Boitano, TA Clark, J Korlach, M Blades, E Mirkes, AN Gorban, JC Paton, MP Jennings, MR Oggioni. 2014. A random six-phase switch regulates pneumococcal virulence via global epigenetic changes. Nature Communications. 5:5055 doi: 10.1038/ncomms6055