The genetic basis of Pseudomonas aeruginosa pathoadaptation to the airways of cystic fibrosis patients

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Objectives: Advances in genome sequencing have made it feasible to sequence multiple genomes of the same lineage of bacterial pathogens as they evolve in their human hosts. Nonetheless, only little is known about how evolution processes compare between genotypic different lineages of the same pathogenic species when they evolve within their human hosts.

Methods: Here, we analyze the genomes of 474 isolates of P. aeruginosa sampled from 34 young Danish CF patients. Our phylogenetic analysis reveals the patients to be infected by 53 different clone types of P. aeruginosa, and for 36 of the clone types we sequenced multiple longitudinal isolates, enabling us to decipher the within-host evolutionary history of each of these lineages.

Results: We found the 36 lineages to exhibit mutational convergence in 56 pathoadaptive genes (genes mutated in >5 clone types), in which the host environment imposed a selection for mutations. The pathoadaptive genes were related to motility, antibiotic resistance, remodeling of regulatory networks, biofilm formation, and extracellular virulence factors. Furthermore, our results show that mutation of downstream transcriptional regulators was contingent upon the mutation of upstream regulators in the same regulatory network.

Conclusion: In conclusion, we have identified adaptive trajectories generic to P. aeruginosa in the CF environment, and elucidated how early mutations predict later evolutionary events. Knowledge of pathoadaptive mutations and evolutionary contingency may help prediction of bacterial persistence and development of future therapeutic targets against the infection.

In vitro non-mucoid to mucoid phenotypic switching in clinical isolates of Pseudomonas aeruginosa is induced by mannitol

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Objectives: Nebulised mannitol is a novel intervention that promotes airway clearance. It is therefore important to assess the effects (if any) on altering baseline CF physiology with mannitol on co-habitating bacterial pathogens. The aim was therefore to examine in vitro the effect of mannitol on Pseudomonas aeruginosa [PA] and other CF bacteria to switch from a non-mucoid to a mucoid phenotype.

Methods: 64 CF clinical isolates were examined, i.e. PA (n=51), Burkholderia cenocepacia (n=6), P. putida (n=4), Stenotrophomonas maltophilia (n=2) and Pseudomonas sp. (n=1). In selecting these, any with a previous history of mucoidy were excluded and none of the isolates examined had ever demonstrated mucoidy. All isolates were pre-screened for mucoidy and any which were mucoid were excluded. Isolates were cultured on Nutrient Agar [NA] and on NA + 4% [w/v] mannitol for 48h at 37°C. Following this, they were examined for the presence of a mucoid phenotype.

Results: 5/51 (9.8%) PA organisms exhibited a switch in phenotype from non-mucoid to mucoid, whereas none of the other organisms examined showed any switch in phenotype. This mucoidy was lost when those switching PA organisms were re-cultured onto nutrient agar without mannitol and mucoidy was re-instated, when subsequently recultured back onto medium containing 4% mannitol. Such switching phenomenon was not observed when repeated using sodium chloride. To date, all studies have been in vitro and we are uncertain as to what happens in the in vivo scenario.

Conclusion: Such switching to the mucoid state in PA could be important for bacterial virulence, antibiotic penetration/treatment, as well as microbiological reporting.

Sequence-function analysis of clinical LasR variants of Pseudomonas aeruginosa

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Chronic respiratory infection with P. aeruginosa is a significant cause of morbidity in people with cystic fibrosis. P. aeruginosa establishes chronic lung infection in part through genotypic and phenotypic adaptations. One of these may be inactivating mutation of the lasR gene, encoding the lasR transcriptional regulator. A LasR mutant is often an inherent mucoid strain. However, a recent study [1] demonstrated that mucoidy is induced by mannitol. In selecting these, any with a previous history of mucoidy were excluded and none of the isolates examined had ever demonstrated mucoidy. All isolates were pre-screened for mucoidy and any which were mucoid were excluded. Isolates were cultured on Nutrient Agar [NA] and on NA + 4% [w/v] mannitol for 48h at 37°C. Following this, they were examined for the presence of a mucoid phenotype.

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Use of genome sequencing to study population diversification and transmission of a Pseudomonas aeruginosa epidemic strain

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Since its emergence in the mid 1990s, we have studied the transmissible Liverpool Epidemic Strain (LES), a CF-adapted clone that is widespread amongst CF patients in the UK and has been reported in North America. We have analysed populations of the LES from the spuata of multiple chronically infected adult CF patients. Following the establishment of a chronic infection in the lungs of CF patients, populations of P. aeruginosa LES adapt and diversify due to mutation. Hence, multiple single strain isolates from the same patient sample can exhibit diversity in phenotypes such as quorum sensing, mucoidy and antibiotic resistance.

Using phenotypic analysis and whole genome sequencing we have compared LES populations between patients and followed population changes over time, including during periods of exacerbation. Our data suggest that populations are highly variable between patients and dynamic within patients over time periods of several months. In addition, we have analysed archived, historical isolates and isolates from multiple geographical locations to determine the distribution of specific mutations identified using genome sequencing. These studies give us insights into transmission pathways within and between CF units.

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