

Editorial relating to Blue-201605-1042OC.R1

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The article by Nebenzahl-Guimaraes and colleagues “Transmissible *Mycobacterium tuberculosis* strains share genetic markers and immune phenotypes” is a novel look at the whole genome determinants of *Mycobacterium tuberculosis* transmission. Relative to many other bacterial species, *Mycobacterium tuberculosis* has comparatively little sequence diversity (1). This lack of diversity has limited findings from genotype-phenotype association studies that have employed low resolution genotyping techniques.

Existing evidence is suggestive of a *Mycobacterium tuberculosis* lineage specific effect on virulence (2–4), mutation rate (5), immune response (6) and transmissibility (7) although the findings vary by setting and are limited when comparison is made to controls strains that have been passaged in the laboratory.

Many studies have examined the host and environmental factors that influence transmission and second cases of disease (8, 9). It is well documented for instance that smear positive index cases (10, 11), those with cavitation (12, 13) and contacts with human immunodeficiency virus infection give rise to more secondary cases of disease (14). However, the genotypic determinants of *Mycobacterium tuberculosis* transmission remain poorly understood.

Transmission is conventionally deemed to have occurred when a previously skin test negative contact of tuberculosis disease becomes skin test positive after exposure to an index case. It is important to clarify that this study examines the association with second cases of tuberculosis disease and not transmission as it is conventionally defined. Understanding which tuberculosis index cases give rise to a second case of tuberculosis disease is arguably more important than understanding which individuals give rise to an infection that may never cause disease.

The behavioural aspects of transmission such as abandoning treatment, inadequate nutrition and index-contact mixing are difficult to predict. However, the genome of *Mycobacterium tuberculosis* is a relatively fixed entity with a mutation rate of approximately 0.5 single nucleotide polymorphisms per genome per year (15). Therefore, better understanding of the genetic determinants of transmission could enable clinicians and public health professionals to identify individuals at high risk of transmission independent of other factors. If pathogen genetic factors can be shown to influence transmission the possibility of isolating the most transmissible index cases either in their own home or in hospital until culture negative could effectively bottle-neck the emergence of the most transmissible strains.

Using a pre-defined host risk factor criteria “cluster propensity to propagate” (CPP) Nebenzahl-Guimaraes and colleagues selected 100 strains which were deemed to be highly likely to transmit but didn’t and vice versa. The authors identified signals of homoplasmy in

strains that were clustered (associated with a second case of disease) versus strains that were not using the PhyC method.

Implementing PhyC regionally the authors identified five genomic regions that were statistically significant “Targets of Independent Mutations” (TIMS). These TIMS included three genes and two intergenic regions. Four of these TIMs were then confirmed to be associated with clustering in an independent dataset of 143 strains. Twelve single nucleotide polymorphisms identified in TIMs that covered genes *Rv0197* and *espE* were all predicted to adversely affect the respective proteins making an effect on phenotype more likely.

The effect of these polymorphisms on cytokine production was also examined by comparing strains with and without the polymorphisms. Mutations in *espE* significantly decreased IL-10 and TNF- α production in monocytes while mutations in *Rv2813-2814c* increased TNF- α , IL-1 β and IL-10 production. Mutations in *PE-PGRS56* were shown to significantly influence the production of IFN gamma from T-cells while mutations in *Rv2813-2814c* significantly decreased reactive oxygen species production in neutrophils.

This study has a number of strengths; the combination of genotypic association, functional protein prediction and the evaluation of the effect on cytokine production lends weight to the findings. The large and well characterized Netherlands Tuberculosis Register also allowed the authors to select clustered and un-clustered strains from a diverse national dataset. The confirmation of these findings in a separate dataset also helps to limit the possibility of false positive discoveries.

Nebenzahl-Guimaraes and colleagues do also rightly highlight some limitations of the data. Phenotype misclassification of strains into “transmissible” and “non transmissible” groups is one possible bias. This can be influenced by many factors including the recent importation of strains. The length of follow up for each case also impacts on the number of secondary cases likely to be detected. In this study this bias is partly mitigated by the fact that most of the un-clustered cases had resided within the Netherlands for 4 years. The Netherlands is a low HIV setting, however the lack of contact data also limits the conclusions somewhat. Clearly the immune status and demographics of the contacts will influence the incidence of secondary cases of tuberculosis disease and this could account for why some of the un-clustered cases with high bacterial burden and CPP score did not give rise to secondary cases of disease. The lack of isogenic mutant wild type comparisons also limits the conclusions from the cytokine analysis.

These limitations however should not detract from the novelty and aim of the study, namely to attempt to identify genetic determinants of transmission. Ultimately as the authors’ conclude, the study of these markers in prospective household follow up studies will help determine the likely functional consequence of these mutations on transmission. The search for genetic markers of transmission is a cause worth pursuing. If they can be found then the possibility of genome based infection control measures and treatments directed at blocking transmission will be a step closer.

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