6.20

PHYSIOLOGICAL IMPACT OF THE EVOLUTION OF THE *rpoB* MUTATION

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Bacilli within an infected lung cavitary lesion spontaneously evolve mutations that confer resistance and are subsequently selected following antibiotic treatment. During this evolutionary process both drug susceptible and drug resistant bacilli may be present. This mix state of susceptive and resistant bacilli captured at a distinct point in time may change during the course of infection and drug selection. The complexity of the population structure in each sputum sample may thus define the outcome of molecular and phenotypic drug resistance testing which in turn may determine how the patient will be treated. We hypothesize that the *rpoB* mutation will influence the transcriptome of the rifampicin mono-resistant isolate compared to the progenitor rifampicin susceptible isolate.

A sputum sample from an individual patient containing a heterogeneous population of both a rifampicin mono-resistant Beijing Ser531Leu clone and its susceptible progenitor was selected. DNA was extracted and sequenced using the Illumina HiSeq platform and analyzed using an in-house bioinformatic pipeline. RNA was extracted and sequenced using the Illumina platform and analyzed using Chipster, an open source bioinformatic platform.

The small number of variants between the two isolates suggests that the resistant isolate evolved from the susceptible progenitor. Our comparative transcriptomic analysis showed that microevolutionary events within the *rpoB* gene had a considerable influence on transcription. Consequently, the expression of bacilli's stress response, sigma factors, and regulatory genes were down regulated. This in turn led to a down-regulation of expression of a large number of genes, suggesting that the rifampicin resistant mutant has an altered physiology.

6.21

MYCOBACTERIUM TUBERCULOSIS DRUG RESISTANCE MUTATIONS AND UNDERSTANDING OF PK/PD: TREATMENT AND CARE IMPLICATIONS

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Russian Federation has the third-highest burden of multidrug-resistant tuberculosis (MDR-TB) in the world and complicated by high rates of human immunodeficiency virus (HIV) co-infection which leads to mortality and risk for acquired *Mycobacterium tuberculosis* drug resistance. Treatment outcomes may be a consequence of pharmacokinetic/pharmacodynamics (PK/PD) variability.

In Irkutsk, we aimed to describe pharmacokinetic variability, minimum inhibitory concentrations (MICs) for key anti-TB drugs and their molecular correlates of resistance, and to determine if PK/PD variability associates with treatment response.

Consecutive people living with HIV initiating TB treatment at Irkutsk Regional TB Referral Hospital were recruited. After 2 weeks of treatment, medications were directly administered and plasma samples collected at 2 and 6 hours after administration. Drug concentrations were measured using validated liquid chromatography-mass spectrometry assays for peak concentration (C_{max}), the highest value in the dosing interval, and area under the concentration curve from time 0 to 6 hours (AUC_{0-6}). *M. tuberculosis* MIC testing was performed using the MYCOTB Sensititre plate. A drug was classified as active when C_{max} was greater than MIC. PK/PD variability as a predictor of treatment outcome was determined by classification and regression tree (CART).

69 patients with HIV had PK/PD testing. Mean age was 34 years (SD±6.2), 45 (65.2%) were male. Mean CD4 count was 180 (±202) cells/mL. Thirty-six (52.2%) had drug susceptible TB, 10 (14.5%) MDR-TB, 17 (24.6%) pre-extensively drug-resistant (XDR)-TB and 6 (8.7%) with XDR-TB. Based on PK/PD testing, patients were treated with a lower number of active drugs (3.25±1.40) compared to the number presumed to be active when initially prescribed (4.81±0.94), p ≤ 0.001. Fifty patients had treatment outcomes and 16 (32.0%) had treatment failure. In CART analysis, regardless of molecular mutation for drug resistance, having less than 4.5 active drugs as redefined by PK/PD testing, correctly identified 15 of 16 (93%) of patients with treatment failure.

In Irkutsk, PK/PD testing predicted treatment outcome for patients with HIV/TB. Screening for mutations in *M. tuberculosis* resistance determining regions is an important method for constructing initial regimens, but should be followed by PK/PD testing to attain the highest likelihood of drug activity.

6.22

GENOMICS AND LOCAL ADAPTATION OF *MYCOBACTERIUM AVIUM*

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Mycobacterium avium subsp. *hominissuis* (MAH) is a human pathogen that causes *M. avium* complex (MAC) lung disease, which is difficult to cure by current antibiotics treatment. It has been suggested that MAH circulates between the human body and the environment. Despite its clinical significance, the genetic mechanisms underlying local adaptation of this pathogen are unknown due to a lack of population-wide genomic data. To overcome this issue, we evaluated the genetic population structure of MAH using genome-scale data from 36 global strains (including 12 Japanese strains sequenced in this study), and then sought to identify alleles unique to Asian populations by comparative genomic analysis. The population structure analysis was extended to include 652 global strains using the multiple-locus variable-number tandem repeats data set, which revealed that two genetic population groups dominated the Asian isolates.

By analyzing mutual homologous recombination and gene content, we revealed that MAH reproduces sexually and has an unlimited gene repertoire. The results of these analyses predict the presence of a chromosome

exchange mechanism called "distributive conjugative transfer (DCT)," recently discovered in other mycobacterial species (Gray et al., Science. 2016. 354: 347−350). We also identified chromosomal loci where allelic variation was correlated with geographic origin. One noteworthy discovery was that the origin of genes responsible for trehalose biosynthesis differed between Asian and other MAH populations. Furthermore, we observed transmission of alleles encoding "mammalian cell entry proteins" between East Asian populations. Therefore, sexual reproduction, likely via DCT, plays a critical role in local adaptation of MAH.

As a new concept, we present a model for the life cycle of *M. avium*, in which *M. avium* generates progeny with diverse genomes via "mating" in a common environmental pool, prior to infection of human hosts. After infection, the progeny is subjected to natural selection within the host, followed by the re-release of clones with adaptive alleles into the environment. This concept may also be relevant for other mycobacterial species.

6.23

UTILITY OF WHOLE GENOME SEQUENCING (WGS) OF *MYCOBACTERIUM TUBERCULOSIS* COMPLEX ISOLATES IN PRACTISE

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Since 2016, all culture positive *M. tuberculosis* complex isolates have been subjected to WGS to allow comparison with the current laboratory diagnosis in the Netherlands. The utility of WGS was investigated for 1) identification of (sub) species and genotypes; 2) drug susceptibility testing; and 3) investigation on tuberculosis transmission.

The new SNP-IT (SNPs to identify TB) method, developed at the RIVM/the Netherlands and Oxford University/ the UK, traces SNPs shared exclusively by members of each (sub) species, lineage, and sub-lineage. The total number of unique SNPs identified ranged from 23 for *M. bovis* to 6.837 for *M. canettii*. The SNP-IT method was applied to 1.157 routine samples from 2016/2017 in the Netherlands and compared with identification by Reverse Line Blot, PhyResSe, and Coll SNP-barcode. This comparison showed that SNP-IT more accurately identifies all animal (sub) species and is more specific in identifying sub-lineages of lineage 4. A small proportion (n = 176) of lineage 4 isolates could not be identified by SNP-IT due to high similarity to the H37Rv reference genome; these are in the Coll SNP-barcode system identified as lineage 4.5/4.7/4.8.

Phenotypic drug susceptibility testing (MGIT) was compared with the detection of resistance-associated mutations by WGS for first-line antibiotics rifampicin, isoniazid, ethambutol, and pyrazinamide. In total, 1.134 isolates from 2016/2017 in the Netherlands were included. For all drugs, the negative predictive value (NPV) was > 99%. In general, rifampicin and isoniazid had most optimal scores. For rifampicin, the sensitivity was 100%, specificity 99.8%, the positive predictive value 95%, and the NPV 100%. This was 98%, 99.2%, 92.5%, and 99.8%, respectively, for isoniazid. WGS was also able to predict intermediate/low level resistance for rifampicin, isoniazid, and pyrazinamide. A minority of isolates showed discrepancy between MGIT and WGS results; these isolates are re-tested to explain discrepancy results.

Both VNTR typing and WGS were applied to all isolates from 2016. In total, 535 isolates were genotyped, of which 25% (134/535) were clustered by VNTR and 15% (82/535) by WGS. The proportion of identified epi-links among WGS clustered cases (50%) was much higher than among VNTR clustered cases (31%). This study was repeated with isolates from 2016 and 2017 to analyse transmission over two years.

6.24

MINOR GENETIC DETERMINANTS OF SECOND-LINE INJECTION DRUGS RESISTANCE IN *MYCOBACTERIUM TUBERCULOSIS*

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Second-line injection drugs group, which include kanamycin (KAN), capreomycin (CAP), and amikacin (AM), is one of the cornerstones used in the treatment of MDR tuberculosis. Though the main resistance mechanism leading to cross-resistance to all three drugs described in *Mycobacterium tuberculosis* is the alteration of 16S rRNA, other mechanisms also could be found in clinical strains, such as promoter mutations of the *eis* and *whiB7* genes leading to KAN resistance, and TlyA inactivating mutations leading to CAP resistance. In consequence, a noticeable number of resistant strains do not carry any known mutations.

We performed the next-generation sequence analysis of the 5 Beijing and one Haarlem lineage clinical *M. tuberculosis* strains with discordant results of phenotypic resistance to injection drugs, and genetic analysis of *rrs*, *eis*, *tlyA*, and *whiB7* loci. The sequencing data were analyzed using the Galaxy web platform (http://usegalaxy.org). Further bioinformatic analysis of the obtained SNPs was performed with custom Python scripts and public databases ReSeqTB and PolyTB.

We found 2126−2229 SNPs for Beijing lineage and 1606 SNPs for Haarlem lineage isolates compared to the referent H37Rv strain. Upon the exclusion of known mutations associated with resistance, fitness compensation and deep bioinformatic analysis, the list of candidate SNPs, potentially associated with resistance, was shortened to 10−100 for each strain. The novel putative mechanisms of resistance included mutations in elongation factor EF-G, phosphotransferase Aph, hypothetical protein Rv0147, secretion protein EspG2, and aspartate aminotransferase AspC.

The diversity of drug resistance mechanisms reflects the complexity of microevolution of *M. tuberculosis* and impacts the sensitivity of molecular tests. Improvement of our knowledge of drug-resistance mechanisms would facilitate the discovery of new drugs together with the prediction of drugs interactions and promote the development of molecular assays.