ing incidence of adult TB cases in 2012, and was related to the economic crisis in Europe. The best strategy for TB case detection in children is contact investigation allowing early diagnosis, which, in turn, allows the implementation of the prophylactic treatment of TB infection, provides successful treatment outcomes, and prevents death. Current molecular diagnostic methods of *Mycobacterium tuberculosis* (Mbt) usually provide limited information that is often not sufficient for the local outbreak and transmission investigations. Implementation of the modern approaches such as Next generation sequencing technologies in the epidemiological studies of childhood TB has a potential to combine TB diagnosis, drug resistance profiling and epidemiological analysis into one test helping to initiate personalized treatment for every patient timely and correctly.

Case report. A patient, 29 years old, was diagnosed with the 3rd TB episode in her life in 2017. Mbt cultures were obtained and genotyped. Molecular genotyping results showed different spoligo and IS6110 RFLP patterns for all three TB episodes in years 2001, 2011, and 2017.

Epidemiological anamnesis revealed that the first TB episode at the 14 years of age in patient was identified in 2001 during household contact investigation — patient`s uncle was diagnosed with TB in 2001. Uncle had TB relapse in 2006. Genotyping results of the uncle`s both Mbt cultures obtained in 2001 and 2006, and patient`s Mbt culture obtained in 2011 revealed the identical spoligotype (SIT1) and IS*6110* pattern with 17 bands for both patients. These results indicated the high possibility of the transmission in the household contact. However, genotyping results from patient's Mbt culture obtained in 2017 showed different genotype.

Whole genome sequencing (WGS) was used for in-depth characterisation of *M. tuberculosis* isolates associated with matched pairs of TB cases. The obtained results were in accordance to the genotyping and drug resistance. In addition, the obtained data provided additional resolution of the microevolution of Mbt subpopulations.

The addition of WGS to the epidemiological data and social network analysis could improve the confirmation of the epidemiological links and evaluation of the transmission dynamics of TB. Additionally, rapid WGS data can be used to identify molecular evidence for strain-specific phenotypic variability including anti-mycobacterial drug resistance, further providing rapid onset of appropriate treatment.

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MOLECULAR EPIDEMIOLOGY OF TUBERCULOSIS IN LATVIA

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Tuberculosis is still one of the major infectious diseases in Latvia, causing serious health problems. While the incidence of the disease has steadily declined in the country since year 2001, the rates of drug-resistant tuberculosis are among the highest within the European Union. Molecular genotyping of *M. tuberculosis* plays an important role both in clinical studies and in the epidemiological investigations, allowing to describe and characterize pathogen`s population structure. Our previous studies have shown that in Riga and Riga region the majority of *M. tuberculosis* isolates belonged to lineage 4 (Euro-American) and lineage 2 (East-Asia). The family distribution of the isolates comprised 25% Beijing, 27% T, and 25% LAM (Latin-American Mediterranean) isolates, while Haarlem, Ural, and X families were detected in 11, 6, and 3%, respectively. A high proportion of Beijing and LAM isolates is alarming, as these *M. tuberculosis* genotypes have been often associated with remarkable pathogenic features such as drug resistance and increased transmissibility. TB incidence in the Latvian region Latgale seems to be higher than the average, and in-depth studies of *M. tuberculosis* isolates in this region could provide additional resolution for the characterization of the lineages circulating in the country. The Latgale region borders with Lithuania in the South, Belarus in Southeast, and Russian Federation in the East. *M. tuberculosis* isolates in this region were studied by the Spoligotyping and IS*6110* RFLP genotyping methods. In total, 56 (73.7%) samples of 76 bacteriologically confirmed TB cases in the year 2017 were available for molecular analysis. The results showed that 52% of isolates could be classified as common genotypes in Latvia (SIT1, SIT42, SIT50, SIT53, SIT254, SIT262, SIT283, SIT1292), while 48% of isolates belonged to SITs which are rarely found in the country or were unique (SIT45, SIT47, SIT52, SIT65, SIT118, SIT150, SIT278, SIT1175, SIT1451). The most common spoligotype belonged to the T1 lineage (SIT53, 16%) followed by SIT1, SIT47 and SIT254 (9% each). Within all samples studied, 14 isolates (25%) formed 4 different clusters with 3–5 members in each. The epidemiological links were confirmed for nine patients in 3 clusters (SIT47, SIT65, and SIT1292). When the prevalence of different spoligotypes was analysed between different countries, a similarity between particular genotypes in Latvia and neighbouring countries was observed. In-depth analysis of these isolates on the international scale could be very useful in order to investigate the possible transmission dynamics of *M. tuberculosis* strains.

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FUNCTIONAL RELEVANCE OF *MYCOBACTERIUM TUBERCULOSIS* DIVERSITY: FROM GENOTYPES TO IMMUNE RESPONSES AND DISEASE SEVERITY

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The genetic diversity of tuberculosis (TB)-causing bacteria has surprised us over recent years. A growing body of evidence attributes a functional relevance to this diversity, both at the clinical and immune response levels. Investigating the full diversity of *Mycobacterium tuberculosis* in nature is however impossible. We recently moved from the study of limited collections of *M. tuberculosis* to an oriented approach, aimed at covering a representation of *M. tuberculosis* heterogeneity. For this, we studied over 600 TB patients in Porto and over 300 matching *M. tuberculosis* isolates. We show a highly homogeneous phylogenetic structure of *M. tuberculosis*, with nearly all cases belonging to Lineage 4 (L4). Within the L4 clade, the most represented sublineage was LAM. This host-pathogen sympatric distribution was however shak-

en by the presence of HIV or diabetes co-morbidities, which oriented the selection of 2 LAM *M. tuberculosis* isolates from TB patients with no comorbidities, but with different TB severities. Despite their close genetic structure, we are finding a distinctive pattern of cytokine production by human and mouse macrophages infected with either isolate. Interestingly, the high TB severity-associated isolate is a poor inducer of cytokine responses. Mechanistically, we relate this poor induction of cytokine production with a differential capacity of the bacteria in activating the host transcriptional machinery, as well as the inflammasome. Furthermore, a different *in vivo* progression of infection by the selected *M. tuberculosis* isolates was also observed. Most notably, the isolate associated with high TB severity showed higher dissemination patterns from the lung to the liver and spleen than that associated with mild TB. To investigate if the link TB severity-cytokine response was broader, we tested the cytokine response induced in human peripheral blood mononuclear cells by a series of other *M. tuberculosis* isolated from patients with different TB severities. A segregation of isolates was observed, with poor inducers of cytokine responses being generally associated with high TB severity. Collectively, our findings suggest that bacterial-intrinsic properties modulate the intensity of the initial immune response with likely consequences for the severity of TB. Our studies open interesting avenues for TB interventions, namely by raising the importance of considering the pathogen diversity when designing host-directed therapies.

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THE ROLE OF THE IS*6110* IN MICRO- AND MACROEVOLUTION OF *MYCOBACTERIUM TUBERCULOSIS* LINEAGE 2

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Genomes of *Mycobacterium tuberculosis* complex members contain the insertion sequence (IS) *6110* which, due to its high quantitative and positional variability, has become a widely used marker in epidemiological studies. The element plays an important role in microorganism genome plasticity, but still many consequences and causes of transposition have not been fully described. This work studies the transposition mechanism of IS*6110* and its impact on the evolution of *M. tuberculosis* (*Mtb*).

Whole-genome sequencing data of 902 *Mtb* lineage 2 isolates was obtained from NCBI and ENA databases. Phylogenetic sublineages were determined based on SNP analysis (120 samples belonged to the ancient Beijing (17 proto-Beijing, 28 Asia Ancestral 1, 13 Asia Ancestral 2, 38 Asia Ancestral 3), 782 samples belonged to the modern Beijing (10 Asian African 1, 29 Asian African 3, 65 Asian African 2, 43 Pacific RD150, 140 Europe/Russia W148 outbreak, 361 Central Asia) (E. Shitikov et al., SciRep, 2017). ISMapper was used to determine the sites of integration of the IS*6110* (Hawkey et al., BMC Genomics, 2015).

We obtained 17 972 points of insertion, which belonged to 865 independent positions in the H37Rv genome. The mean copy number per genome was 19.92 (from 9 to 25). To describe the evolution of an element in the genome, we arranged our samples in the order corresponding to a phylogenetic tree constructed on the basis of SNPs. We determined the stepwise mechanism of transposition, in which the transition to a new subpopulation is accompanied by a change in the localization of several copies of IS. It is important to note that the localization of the element in the ancestral population does not change, which implies a transposition only by "copy-paste" mechanism. In addition, we defined genes (537 sites (256 genes)) and intergenic regions (328 sites), where the element was integrated. Sixteen genes previously identified as being essential under different experimental conditions were found to contain IS. Further we carried out identification of IS*6110* mediated LSPs which showed the presence of recombination events (deletion) between inversely oriented elements.

In conclusion, we determined the evolution and role of IS*6110* for *Mtb* lineage 2 strains. We identified evolutionary and subpopulation-specific sites of integration which can be used for typing and subsequent research.

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NGS DETERMINATION OF MYCOBACTERIAL TRANSRENAL DNA AS POTENTIAL TOOL OF CLINICAL DIAGNOSTIC

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Sputum is a major object for monitoring TB treatment and diagnostics it is strictly depended from bacterial load. There is a substantial need for less variable and more reliable specimen for the diagnosis of tuberculosis and for treatment monitoring. The objective of this study is to estimation diagnostic power of full genome sequencing (NGS) of soluble mycobacterial transrenal DNA (mtr-DNA) in urine of TB patients and TaqMan tests designed after analysis of metagenomic data.

DNA patient with pulmonary tuberculosis (TB) isolated from 4 ml. of urine by QIAamp Circulating Nucleic Acid Kit. Detection of TB positive samples made by previously developed PCR targeted to 45 base pairs fragment of mycobacterial genome. It was chosen 2 positive PCR samples. It were mapped on the reference genome *M. tuberculosis* (NC_000962.3) by BWA. In total were mapped 16 579 paired reads of the one sample (0.83%) and 1 783 754 (64%) of the second sample respectively. There were also analyzed mapped DNA sequences with more than 4x coverage. The median length of mtr-DNA found as 20 bp.

It was found 156 mtr-DNA fragments repeated in both samples. The median length of DNA fragment was found was 20 bp. Five fragments including part of 16S rRNA gene were chosen for design primer and TaqMan probes for targets from 43 to 60 bp. Length of primers and probes were reduced by Locked Nucleic Acid (LNA) bases. The sensitivity and specificity of the developed tests was determined by known DNA samples from urine. The result obtained did not reveal a significant improvement in the sensitivity of the new tests. PCR-RT cutoff remained approximately 40 cycles, like in previously developed tests.

High specificity and sensitivity of NGS and low of PCR suggest that diagnosis and monitoring of tuberculosis by mtr-DNA should be based on NGS, rather than on PCR.

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