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INTRODUCTION

Distinguishing the cause of tuberculosis (TB) recurrence is crucial for tracing transmission chains and guiding timely treatment. TB reinfection can be mistaken for a relapse or treatment failure due to a changed drug resistance pattern after initially successful treatment. TB reinfection could likely occur in the household, within the community, or even in the hospital ward during treatment. The actual rate of TB reinfection in Lithuania has never been reported, and the reasons for TB recurrence remain poorly understood.

OBJECTIVE

The aim of this study was to assess the rate of true TB relapses *versus* reinfection among patients with TB recurrence in Lithuania using whole-genome sequencing (WGS).

METHODS

The study included 62 *Mycobacterium tuberculosis* isolates, recovered from as many TB patients who had at least one reported TB relapse (20/29; 69%) or treatment failure (9/29; 31%) episode and were treated in Vilnius University Hospital Santaros Klinikos between 2016 and 2023. WGS was performed with Illumina NovaSeq 6000 sequencer in 2x150 bp paired-end mode. The multiple sequence alignment of alleles was constructed using the MTBseq v1.0.3 bioinformatic pipeline, setting the distance at 5. To differentiate between relapse and reinfection, a cut-off of 5 single nucleotide polymorphisms was used. Subsequently, Randomized Axelerated Maximum Likelihood (RAxML) v.8.2.12 was used to produce a robust bootstrap-supported phylogenetic tree. The resulting phylogenetic tree was visualized and annotated using the online interface Interactive Tree of Life (iTOL).

RESULTS

The most prevalent genotype was Beijing (2.2.1) (30/62; 48.4%), followed by LAM (4.3.3) (15/62; 24.2%), mainly T (4.8 and 4.7) (10/62; 16.1% and 1/62; 1.6%, respectively), Ural (4.2.1) (5/62; 8.1%), and H37Rv-like (4.9) (1/62; 1.6%). The majority (12/20; 60%) of all reported relapses were caused by true relapse, while reinfections accounted for 40% (8/20), (**Figure 1**). Of 9 patients reported as treatment failures, 4 were reinfections, 1 was both reinfection and treatment failure and only 4 were true treatment failures. Among 13 reinfected patients, 6 were reinfected with different genotype (**Figure 1**).

CONCLUSIONS

Only 55% (16/29) of patients were correctly differentiated as relapse or treatment failure by clinicians. A high rate of misclassified reinfections as relapse or treatment failure due to limited diagnostics poses a challenge to TB control efforts. Thus, the implementation of WGS may improve timely diagnosis and TB control in Lithuania.

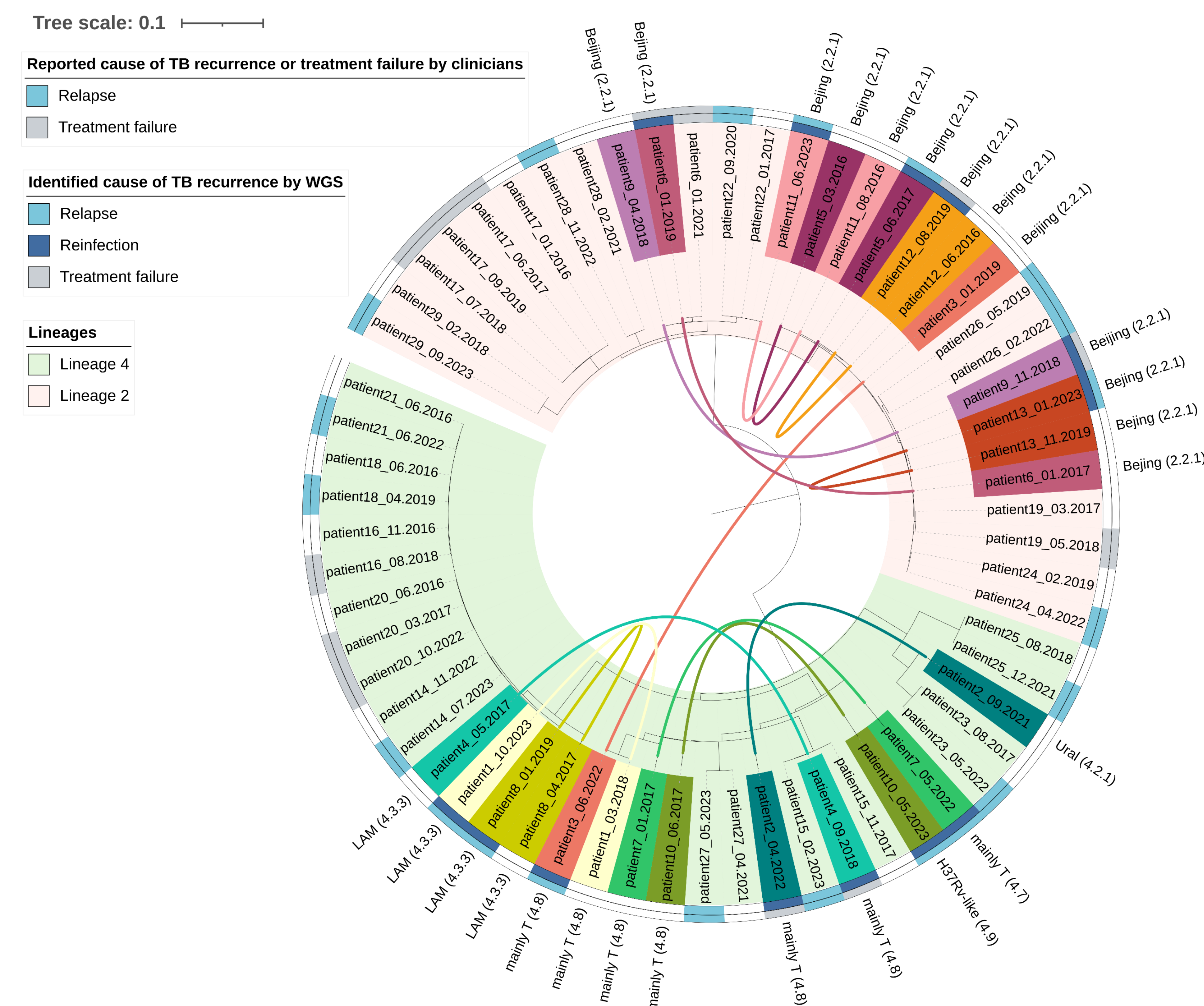


Figure 1. Phylogenetic tree of 62 *Mycobacterium tuberculosis* strains from 29 recurrent/treatment failure patients.

ID of every strain consists of patients' number, month and year of the isolate collection. Inner band indicates identified cause of TB recurrence/treatment failure (reinfection represents strain pairs differences >5 SNPs, whereas relapse/treatment failure represents strain pairs differences ≤5 SNPs) by WGS and the outer band indicates TB recurrence cause or treatment failure reported by clinicians (see legend). White bands represent first available isolate of the patient that was available for sequencing. Strains of reinfected patients are highlighted with colored rectangles and connected by curved lines, indicating paired strains. The identified *Mycobacterium tuberculosis* genotype is specified for strains in reinfection case pair.

REFERENCES

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