Identification of mutations conferring resistance to isoniazid in multidrug-resistant *Mycobacterium tuberculosis* clinical isolates

Identyfikacja mutacji warunkujących oporność na izoniazyd w szczepach klinicznych *Mycobacterium tuberculosis* o wielolekooporności typu MDR

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Introduction. One of the major challenge in the epidemiology of tuberculosis (TB) has been the emergence and spread of multidrug-resistant (MDR)-TB, defined as resistance of tubercle bacilli to at least isoniazid (INH) and rifampicin (RMP), the two most potent anti-TB drugs. Since early 1950s INH has become a key component of drug regimens used worldwide for the treatment of TB. However, the clinical utility of the drug has recently been jeopardized by the increasing prevalence of INH resistance among *Mycobacterium tuberculosis* strains. In fact, resistance to INH is the most common form of drug resistance seen among *M. tuberculosis* isolates. Almost every seventh TB case (13.3% of all TB cases) is resistant to INH, either alone or in combination with other drugs [1]. Although the molecular mechanisms for INH resistance are not fully understood, numerous studies have linked them to distinct mutations in various genetic loci of *M. tuberculosis* genome. These mutations may potentially be used as surrogate markers in molecular assays to detect isoniazid resistance in *M. tuberculosis*, even at the clinical sample level, thus offering a rapid and effective alternative to conventional drug susceptibility testing. The diagnostic relevance of these assays depends heavily on the careful selection of mutations that would not only be strongly associated with the phenotypic resistance but also highly prevalent within the pathogen population. Therefore, the evaluation of the type and distribution frequency of INH resistance-conferring mutations in different

clinical and geographical settings is a prerequisite before genotype-based approaches for the detection of INH resistance can be established on a large scale.

The purpose of this study was to investigate the prevalence of mutations in a panel of genetic loci implicated in conferring INH resistance in MDR *M. tuberculosis* clinical isolates from Poland.

Materials and methods. A collection of 50 MDR *M. tuberculosis* isolates, housed by the National Tuberculosis and Lung Diseases Institute in Warsaw was used in the study. The isolates were recovered from 46 unrelated pulmonary TB patients from across Poland. These patients represented all bacteriologically-confirmed MDR-TB cases reported in Poland in 2004 [2]. Primary isolation, culturing, and species identification were carried out by standard mycobacteriological procedures, essentially described elsewhere [3]. Drug susceptibility testing was performed using the 1% proportion method on Löwenstein-Jensen (L-J) medium, with the following critical drug concentrations: INH, 0.2 µ/mL; RMP, 40 µg/mL; (STR), 4 µg/mL; and ethambutol (EMB), 2 µg/mL [3]. The minimal inhibitory concentration (MIC) of INH was determined in L-J medium containing twofold incremental concentrations of the drug ranging from 0.05 to 100 µg/mL. The MIC was defined as the lowest drug concentration that inhibited more than 99% of the bacterial population. Genomic DNA was extracted by the cetyl-trimethyl ammonium bromide (CTAB) method [4]. For all isolates, seven structural genes (*katG*, *inhA*, *ahpC*, *kasA*, *ndh*, *nat*, *mshA*) and two regulatory regions (the *mabA-inhA* promotor region and the *oxyR-ahpC* intergenic region) were screened for mutations by direct sequencing of each locus in its entirety. The oligonucleotide primers used for PCR amplification of the respective genetic loci were either based on previously reported studies or newly designed in the present study. Sequencing was done in both directions with the same primer pairs that were used in the amplification reactions and, occasionally, with additional, internal primers. The assembled consensus sequences were aligned against the wild-type sequences of the respective genetic loci of *M. tuberculosis* reference strain H37Rv (TubercuList; http://genolistpasteurfr/TubercuList/) using the BLASTN algorithm (http://blast.ncbi.nlm.nih.gov/).

Results. Forty-six (92%) MDR-TB isolates had mutations in the *katG* gene, and the *katG* Ser-315→Thr substitution predominated (72%). Eight (16%) isolates (six with a mutated *katG* allele) had mutations in the *inhA* promoter region and two of such isolates had also single *inhA* structural gene mutations. Mutations in the *oxyR-ahpC* locus were found in 5 (10%) isolates, of which all but one had at least one additional mutation in *katG*. Mutations in the remaining genetic loci, i.e. *kasA*, *ndh*, *nat*, and *mshA* were detected in 12 (24%), 4 (8%), 5

(10%), and 17 (34%) of MDR isolates, respectively. All non-synonymous mutants for these genes harbored mutations in *katG*. One isolate had no mutations in any of the analysed loci.

Conclusions. This study underlines the usefulness of mutations in *katG* and *inhA* promoter as predictive markers of INH resistance. Testing only for *katG* 315 and *inhA* –15 mutations would detect INH resistance in 84% of the MDR-TB sample. This percentage would increase to 96% if the sequence analysis was extended to the entire *katG* gene. Analysis of the remaining genetic loci did not contribute considerably to the identification of INH resistance. For detecting isoniazid resistance in TB, molecular approaches, based on mutation profiling, should still be a complement rather than a replacement to conventional drug susceptibility profiling. This is supported by finding of one isolate in this study, for which no mutation was found in any of the nine genetic loci analyzed.

References

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