DETECTION OF MUTATIONS ASSOCIATED WITH RESISTANCE TO ISONIAZID IN *MYCOBACTERIUM TUBERCULOSIS* CLINICAL ISOLATES

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INTRODUCTION

Tuberculosis (TB) still represents a significant global health problem, with an estimated 9 million new cases and 1.5 million deaths reported in 2013 [1]. One of the main reasons for the continuing TB prevalence is the emergence and spread of drug-resistant (DR) *Mycobacterium tuberculosis* strains, including multidrug-resistant (MDR) strains, defined as resistant to at least isoniazid [INH] and rifampicin [RMP], the two most potent anti-TB drugs. A total of 480,000 cases of MDR-TB were estimated to have occurred in 2013, equivalent to 5% of all TB cases in that year [1].

Progress in the detection of DR-TB has been underpinned by the development and implementation of new, reliable and rapid diagnostic tools. Most of these tools detect specific mutations, in different genetic loci, that confer resistance to particular anti-TB drugs. The aim of this study was to search for mutations associated with resistance to INH among a large sample of *M. tuberculosis* clinical isolates.

MATERIALS AND METHODS

A collection of 150 *M. tuberculosis* isolates, housed by the National TB and Lung Diseases Research Institute in Warsaw, was used for the study. Included in that number were 50 MDR, 50 INH-monoresistant, and 50 pan-susceptible isolates. The isolates were obtained from pulmonary TB patients diagnosed in different parts of Poland and collected during the third national survey of DR-TB (i.e. from 1 January to 31 December 2004).

Primary isolation, cultivation, and species identification were done by using standard mycobacteriological methods [2].

Drug susceptibility testing was performed with the proportion method on Löwenstein-Jensen (L-J) medium, strictly according to the WHO guidelines [3].

For all the isolates, nine genetic loci, including structural genes (*katG, inhA, ahpC, kasA, ndh, nat*, and *mshA*) and regulatory regions (i.e. the *mabA-inhA* promoter and *oxyR-ahpC* intergenic region) were PCR-amplified and sequenced in their entirety.

Genomic DNA was extracted from *M. tuberculosis* cultures on L-J slants by the lysozyme/proteinase K cetyl-trimethyl ammonium bromide (CTAB) method [4].

The PCR amplification, sequencing, and data processing were performed as described elsewhere [5].

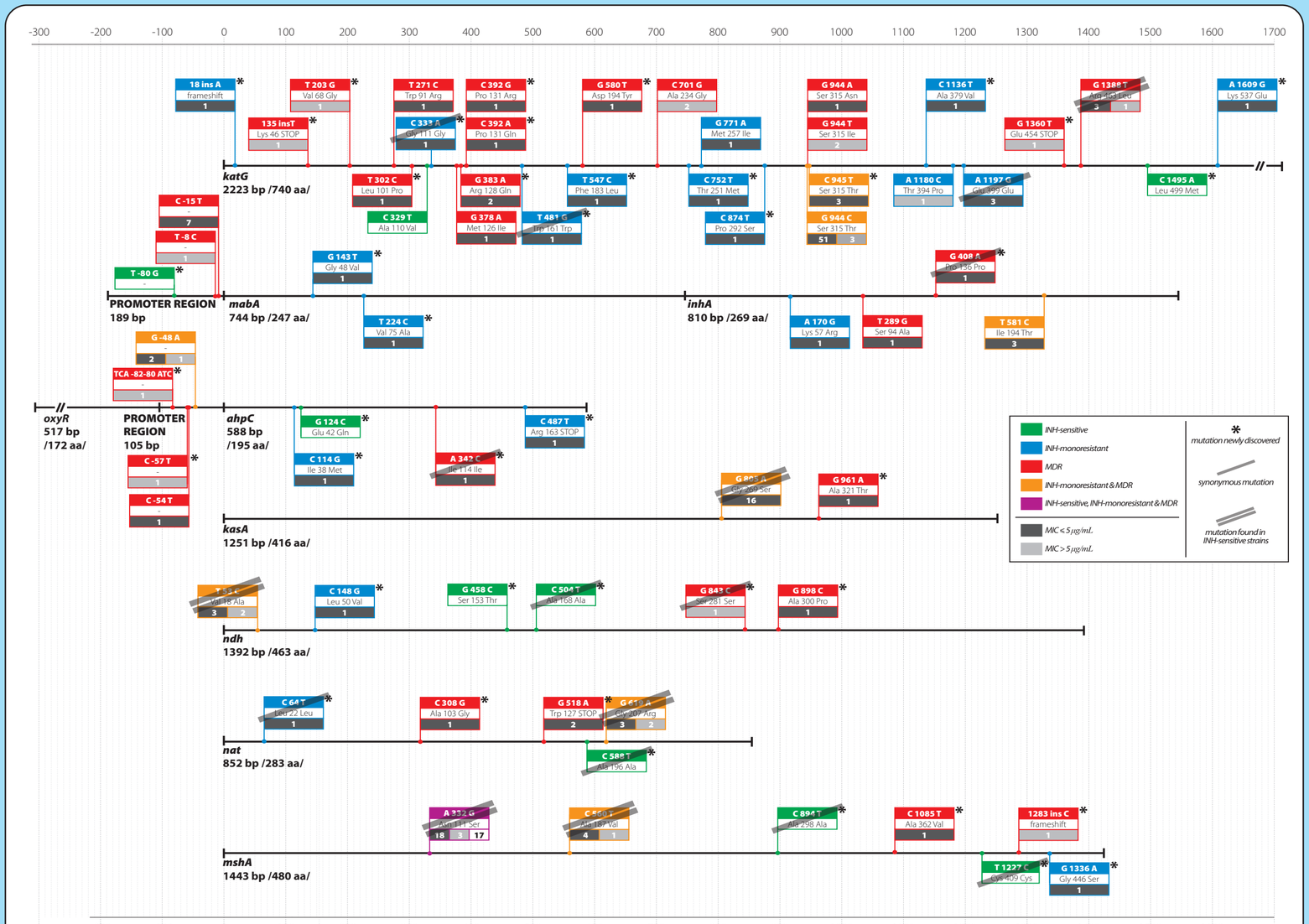

Figure. Schematic representation of the distribution of mutations in 9 genetic loci investigated, with regard to MIC values of INH. ins, insertion; fs, frameshift mutation. Numbers in light or dark grey-shaded boxes, below mutation designations, represent isolates with a particular mutation type and MIC of INH.

RESULTS

A total of 66 distinct mutations were detected at all nine loci investigated, accounting for 109 (72.7%) of the strains tested. The number of strains with any mutation within the MDR, INH-monoresistant, and pan-susceptible strain subsets was 49 (98%), 37 (74%), and 23 (46%), accordingly. Mutations in the *katG* gene predominated, with 29 different types, distributed among 46 (92%) MDR, 31 (64%) INH-monoresistant, and 2 (4%) pan-susceptible strains. None of the pan-susceptible isolates had an altered *inhA, ahpC*, or *kasA* allele. Of 10 different mutations found in the remaining loci in pan-susceptible strains, all but one (at *mshA* gene) were observed in susceptible isolates only. Twenty-nine and 19 mutations were found exclusively in MDR and INH-monoresistant strains, respectively. Eight mutations were demonstrated for both MDR and INH-monoresistant strains. Four of these mutations had previously been observed in INH-sensitive strains.

CONCLUSIONS

- The study revealed several mutations, not previously reported, that might be of potential use as new surrogate markers of INH resistance in TB.
- Twenty-six and 15 non-synonymous mutations were exclusively found in MDR or INH-monoresistant strains, respectively; their accuracy as predictive markers of MDR status or INH monoresistance, respectively, requires further investigation on much larger strain samples.
- For detecting INH resistance in TB, molecular approaches should still be a complement rather than a replacement to conventional drug susceptibility profiling. This is supported by the lack of mutations in any of the nine genetic loci analyzed in 14 INH-resistant strains from this study.

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