Sequence analysis of the *embB* gene for identification of mutations associated with resistance of multidrug-resistant *Mycobacterium tuberculosis* strains to ethambutol

Zofia Bakuła[1], Agnieszka Napiórkowska[2], Katarzyna Roeske[1], Michał Kamiński[1], Radosław Stachowiak[1], Jacek Bielecki[1], Ewa Augustynowicz-Kopeć[2], and Tomasz Jagielski[1]

[1] Department of Applied Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw

[2] Department of Microbiology, National Tuberculosis and Lung Diseases Research Institute, Warsaw

BACKGROUND

One of the greatest challenges in the fight against tuberculosis (TB) has been the emergence and spread of drug-resistant (DR), and multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis*.

Ethambutol (EMB) is a first-line drug for the treatment of tuberculosis that targets the cell wall of tubercle bacilli through interfering with arabinosyl transferases, encoded by the genes of the *embCAB* operon and involved in the biosynthesis of arabinogalactan and lipoarabinomannan, the key structural components of the mycobacterial cell wall. Mutations in the *embB* gene, and those within its conserved EMB resistance determining region (ERDR) in particular, have been associated with resistance to EMB in *Mycobacterium tuberculosis*. Analysis of mutations in the *embB* gene in *M. tuberculosis* strains may contribute to the development of new tests for rapid detection of EMB resistance.

OBJECTIVE

The aim of this study was to examine the mutational "hot spots" in the *embB* gene, including the ERDR, among multidrug-resistant (MDR) *M. tuberculosis* clinical isolates and to find a possible association between *embB* mutations and resistance to EMB.

METHODS

A 863-bp region of the *embB* gene of 48 clinical isolates of *M. tuberculosis* (21 EMB-resistant, 27 EMB-susceptible), recovered from as many MDR-TB patients in Poland in 2004 was sequenced and screened for mutations linked to EMB resistance. Mutations in the *embB* gene were detected by comparing the assembled sequences with the corresponding sequences of a wild-type reference laboratory strain *M. tuberculosis* H37Rv. The obtained results were interpreted in the context of EMB susceptibility profile of the strains tested.

RESULTS

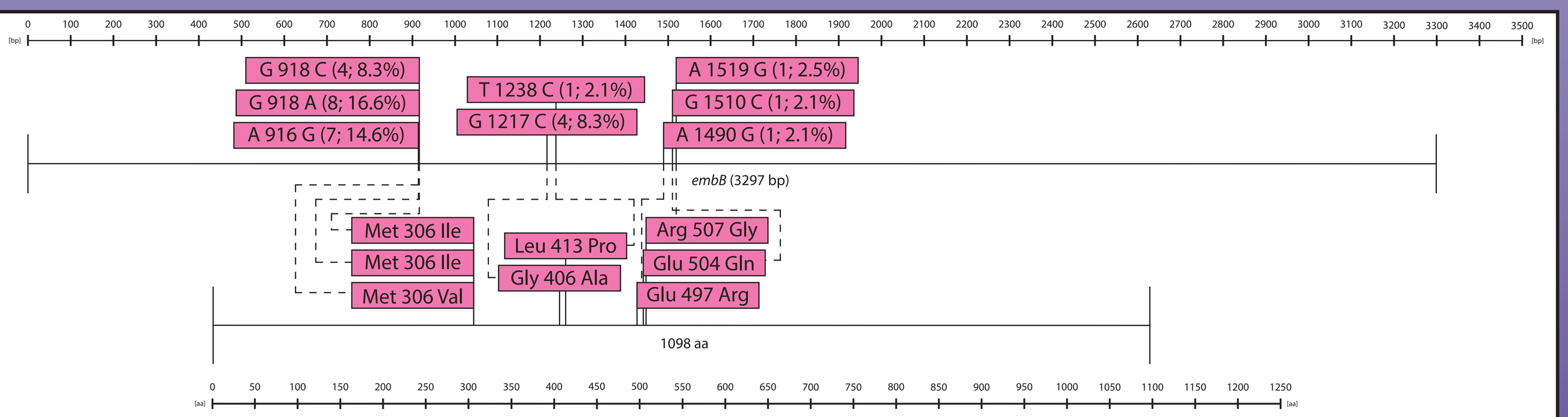

Fig. 1. Schematic representation of mutations in the *embB* gene identified among 48 MDR *M. tuberlculosis* strains tested. Nucleotide positions at codons, and their corresponding amino acid residues affected by mutations are given in frames. Number and percentage of strains presenting with a given type of mutation are given in brackets.

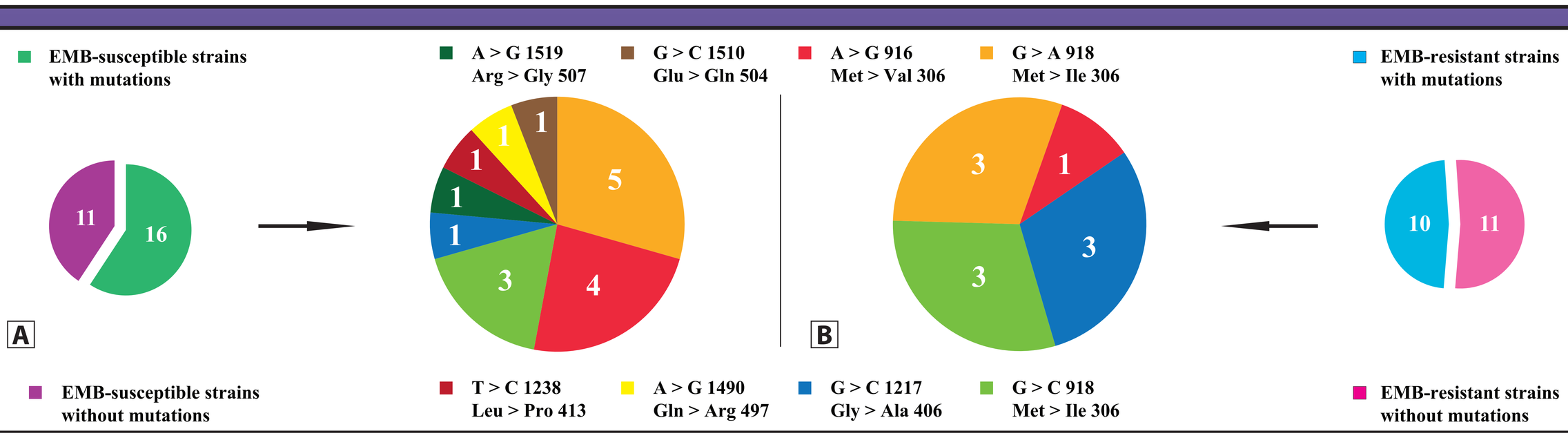

Fig. 2. A - number of EMB-susceptible strains with confirmed mutational changes; type and number of mutations detected among EMB-susceptible strains tested; **B** - number of EMB-resistant strains with confirmed mutational changes; type and number of mutations detected among EMB-resistant strains tested.

CONCLUSIONS

- Eight *embB* mutation types were detected in 6 distinct codons in 26 (54.2%) *M. tuberculosis* strains
- Only 10 (38.5%) of those strains were EMB-resistant and had mutations either in codon 306 (7 strains) or 406 (3 strains)
- None of the two mutation types were found exclusively in EMB-resistant strains
- Analysis of other genetic loci is needed for the identification of more specific mutations associated with EMB resistance