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Mutations at Amino Acid Position 315 of the katG Gene Are Associated with High-Level Resistance to Isoniazid, Other Drug Resistance, and Successful Transmission of Mycobacterium tuberculosis in The Netherlands

Dick van Soolingen, Petra E. W. de Haas, H. Rogier van Doorn, Ed Kuijper, Heinz Rinder, and Martien W. Borgdorff

The prevalence of mutations at amino acid (aa) position 315 in the katG gene of isoniazid (INH)-resistant Mycobacterium tuberculosis isolates in The Netherlands and the mutation's association with the level of INH resistance, multidrug resistance, and transmission were determined. Of 4288 M. tuberculosis isolates with available laboratory results, 295 (7%) exhibited INH resistance. Of 148 aa 315 mutants, 89% had MICs of 5–10 μg/mL, whereas 75% of the other 130 INH-resistant strains had MICs of 0.5–1 μg/mL. Of the aa 315 mutants, 33% exhibited monodrug resistance, compared with 69% of other INH-resistant strains (P < .0001). Multidrug resistance was found among 14% of the aa 315 mutants and 7% of the other INH-resistant strains (P > .05). The probability of being in an IS6110 DNA restriction fragment length polymorphism (RFLP) cluster was similar for aa 315 mutants and INH-susceptible strains, but the probability was reduced in other INH-resistant strains. Thus, aa 315 mutants lead to secondary cases of tuberculosis as often as INH-susceptible strains do.

Drug-resistant tuberculosis is emerging as a major threat in various parts of the world [1, 2]. This particularly applies to combined resistance against isoniazid (INH) and rifampicin (Rif) multidrug resistance, because resistance to both of these potent antituberculosis drugs is associated with poor treatment outcome and high case-fatality rates [3]. The prospects for control of drug-resistant tuberculosis in general and multidrug resistance in particular will depend, in part, on the ability of drug-resistant strains to be transmitted and, thus, on the number of next-generation cases [4].

In The Netherlands, antituberculosis drug resistance is relatively uncommon and is associated with immigration [5]. In 1998, INH resistance was found in 6.8% of the patients with tuberculosis, Rif resistance in 1.4%, and multidrug resistance in 1.1%. In a recent study, we found that INH-resistant strains were less likely than susceptible strains to belong to an IS6110 restriction fragment length polymorphism (RFLP) cluster [6]. This suggests that INH-resistant strains have a reduced ability to generate next-generation cases, presumably because they are less infectious or less virulent (i.e., would be less likely to progress to disease after infection).

INH resistance may arise through different genetic mutations (mostly found in the inhA, katG, and ahpC loci) in Mycobacterium tuberculosis [7]. It is conceivable that different mutations lead to differences in the degree of resistance and to differences in the ability to generate next-generation cases. A common mutation in various geographic areas is found at amino acid (aa) position 315 in the katG gene [8]. In St. Petersburg, Russia, this mutation was associated with multidrug resistance [9]. The purpose of our study was to determine the prevalence of the aa 315 mutations in the katG gene of INH-resistant M. tuberculosis isolates in The Netherlands and its association with the level of INH resistance, multidrug resistance, and tuberculosis transmission.

Methods

During 1993–1997, all M. tuberculosis complex isolates from tuberculosis patients in The Netherlands were submitted to the National Institute of Public Health and the Environment for species identification, drug susceptibility testing, and IS6110 RFLP typing [10, 11]. The resistance of all isolates to INH, streptomycin (Stm), and Rif was determined by use of the MIC method, testing 0.1, 0.2, 0.5, 1, 2, 5, and 10 μg/mL in 7H10 medium (Difco, Detroit).
In the 1950s, it was already known that INH-resistant *M. tuberculosis* isolates belong to relatively high levels of drug resistance (MIC of 5–10 μg/mL) and resistance to >1 drug.

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**Table 1.** Association of isoniazid (INH)–resistant *Mycobacterium tuberculosis* genotypes with clustering.

<table>
<thead>
<tr>
<th>INH resistance status</th>
<th>Clusters/total (%)</th>
<th>Odds ratio (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Crude</td>
</tr>
<tr>
<td>Dutch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INH resistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aa315 mutation</td>
<td>8/13 (62)</td>
<td>1.6 (0.5–6.4)</td>
</tr>
<tr>
<td>Other</td>
<td>13/30 (43)</td>
<td>0.8 (0.4–1.7)</td>
</tr>
<tr>
<td>INH resistance not tested</td>
<td>14/27 (52)</td>
<td>1.1 (0.5–2.5)</td>
</tr>
<tr>
<td>INH sensitive</td>
<td>890/1007 (49)</td>
<td>1</td>
</tr>
<tr>
<td>Non-Dutch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INH resistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aa315 mutation</td>
<td>52/135 (39)</td>
<td>0.8 (0.5–1.1)</td>
</tr>
<tr>
<td>Other</td>
<td>33/100 (33)</td>
<td>0.6 (0.4–0.9)</td>
</tr>
<tr>
<td>INH resistance not tested</td>
<td>15/33 (45)</td>
<td>1.0 (0.5–2.1)</td>
</tr>
<tr>
<td>INH sensitive</td>
<td>964/2143 (45)</td>
<td>1</td>
</tr>
</tbody>
</table>
tuberculosis isolates exhibit a significantly reduced virulence for guinea pigs, compared with drug-susceptible strains [14]. Li et al. [15] recently showed that the persistence of M. tuberculosis strains in mice and guinea pigs strongly depends on the presence of particular types of mutations in the katG gene. The decrease in virulence of INH-resistant strains was recently reflected in a population-based study, using RFLP typing results of a 5-year period, on transmission of tuberculosis in The Netherlands [6]. INH-resistant strains were significantly less frequently clustered than were INH-susceptible strains. However, in the present study, INH-resistant strains with a mutation at aa 315 of the katG gene were found in clusters almost as frequently as were susceptible isolates.

The exceptional position of the aa 315 mutants among INH-resistant strains may be explained by the finding of Rouse et al. [16] that, in bacterial strains with a Ser315Thr mutation, 30%–40% of the catalase-peroxidase activity remains. In this respect, it may be worth determining the exact mutations at aa 315 of all 148 respective INH-resistant strains. This may show whether all types of mutations at aa 315, or especially Ser315Thr mutations, lead to a maintained transmissibility.

In this study, we found a significant correlation between mutations at aa 315 of the katG gene of INH-resistant strains and other drug resistance. This is in concordance with the study of Martilla et al. [9] in the St. Petersburg area of Russia, in which Ser315Thr substitutions in the katG gene were predominant (92%) among 27 multidrug-resistant M. tuberculosis isolates. The advantage of this study is the more comprehensive sampling. The importance of a population-based sampling was recently highlighted by Platek et al. [17], who showed a different relative prevalence of INH-resistant mutations, depending on whether a reference-laboratory sample or a community-wide sample was evaluated.

The observations in this study presumably reflect that strains with mutations at aa 315 of katG are more likely to gain additional resistance. Although bacteria become insensitive to INH because of the mutation at aa 315, this may not imply a decrease in virulence, as observed for other mutations in the katG gene. The combination of INH resistance and maintenance of virulence presumably offers the basis for persistence and, hence, the possibility to gain additional resistance to other drugs. An alternative, and perhaps complementary, explanation for increased resistance to other drugs is that the aa 315 mutant is the result of a second-step mutation, occurring after a prolonged period of inappropriate prior multiple drug therapy. This would be in agreement with its higher prevalence in the foreign born (non-Dutch) group.

On basis of the results in this study, reference laboratories may consider implementing a PCR–restriction endonuclease analyses test to recognize aa 315 mutants among INH-resistant strains [13]. This would facilitate the adjustment of treatment regimens in time to reduce the chances of developing further drug resistance and of transmitting resistant strains.

Acknowledgments

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References