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CONCISE COMMUNICATION

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

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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 IS*6110* DNA restriction fragment length polymorphism 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 IS*6110* 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 IS*6110* 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)

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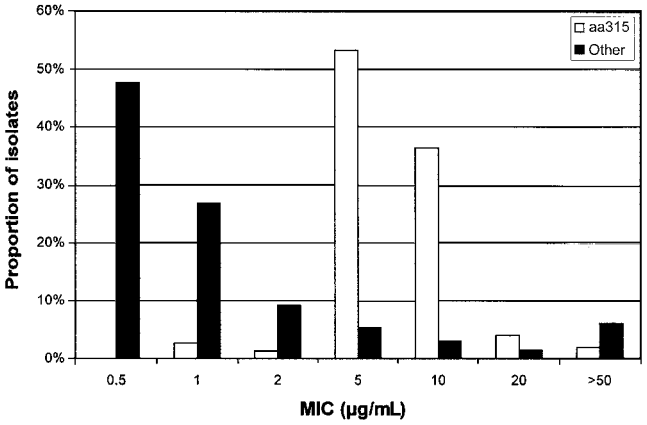

Figure 1. Association between MIC to isoniazid and amino acid (aa) 315 mutations in *katG* of *Mycobacterium tuberculosis* isolates.

[12]. Strains resistant to a concentration of 10 μg/mL INH were retested on 20 and 50 μg/mL. The strains were considered to be resistant if >1% of the bacteria of the inoculum grew on concentrations of 1, 10, and 2 μg/mL of INH, Stm, and Rif, respectively. In the daily routine of our laboratory, if >1% of the bacteria grow on a concentration of 0.5 μg INH/mL, the strain is categorized as "reduced-susceptible to INH," but in this study, we considered them "resistant." Multidrug resistance was defined, according to the definition of the World Health Organization, as resistance against at least INH and Rif.

INH-resistant isolates were investigated for the presence of the aa 315 mutation by polymerase chain reaction (PCR) amplification, followed by *Aci*I or *Msp*AI restriction endonuclease analyses, as described elsewhere [13]. On the basis of this assay, the INH-resistant isolates were divided into 2 groups—those with the aa 315 mutation and other strains.

Standard RFLP typing using insertion element IS*6110* as a probe was done as described elsewhere [10]. If <5 bands were present in the RFLP pattern, polymorphic GC-rich sequence RFLP typing was also done [11]. Patient information was obtained from the Netherlands Tuberculosis Register (NTR). The NTR lists patients anonymously; therefore, patient information was matched with laboratory information, using sex, date of birth, and postal area code to identify matches. The term "cluster" in this paper refers to ⩾2 *M. tuberculosis* isolates with completely identical RFLP patterns or to the respective patients.

Odds ratios (ORs) were used to compare groups. Adjusted ORs were calculated using logistic regression to adjust for confounders.

Results

Of 5114 isolates collected over the study period, 368 (7%) showed INH resistance: 181 were monodrug resistant, 141 were resistant to INH and Stm, 26 were resistant to INH and Rif, and 20 were resistant to INH, Stm, and Rif. NTR patient information was successfully matched for 4288 isolates (84%), 295 (7%) of which showed INH resistance. We used PCR-based testing on 278 (94%) of these 295 isolates, to identify the aa 315 mutation.

Of the 278 INH-resistant isolates tested, 148 (53%) had the aa 315 mutation, and, of those, 89% had a MIC of 5 or 10 μg/mL; the remaining 11% had MICs >10 or <5 μg/mL. Of the other 130 INH-resistant isolates, 75% had a MIC of 0.5 or 1 μg/mL; the remaining 25% had a MIC of >1 μg/mL (figure 1). Of the 148 isolates with the aa 315 mutation, 33% had monodrug resistance, compared with 69% of the other 130 isolates ($P < .0001$). Multidrug resistance was observed in 14% of isolates with the aa 315 mutation and in 7% of the other isolates ($P > .05$).

The proportion of cases belonging to an RFLP cluster was examined separately for Dutch and non-Dutch patients, because Dutch nationality was associated with increased clustering and decreased prevalence of drug resistance [5, 6]. In this study, the aa 315 mutation was significantly more common among non-Dutch than Dutch patients with INH resistance ($P < .01$). Among Dutch and non-Dutch patients, INH-resistant isolates without the aa 315 mutation were less likely to be clustered than were INH-susceptible isolates (adjusted OR 0.6, 95% confidence interval 0.4–0.9; table 1). Among the Dutch, isolates with the aa 315 mutation were slightly more likely to be clustered than were INH-susceptible isolates. Such isolates were somewhat less likely to be clustered among the non-Dutch, but neither of these differences was significant (table 1).

Discussion

This study showed that a large proportion of INH-resistant *M. tuberculosis* isolates in The Netherlands have the aa 315 mutation in the *katG* gene. This mutation was associated with relatively high levels of drug resistance (MIC of 5–10 μg/mL) and resistance to >1 drug.

In the 1950s, it was already known that INH-resistant *M.*

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

		Odds ratio (95% confidence interval)	
INH resistance status	Clustered/ total (%)	Crude	Adjusted for nationality
Dutch			
INH resistance aa315 mutation	8/13 (62)	1.6 (0.5–6.4)	
Other	13/30 (43)	0.8 (0.4–1.7)	
INH resistance not tested	14/27 (52)	1.1 (0.5–2.5)	
INH sensitive	890/1807 (49)	1	
Non-Dutch			
INH resistance aa315 mutation	52/135 (39)	0.8 (0.5–1.1)	0.8 (0.6–1.2)
Other	33/100 (33)	0.6 (0.4–0.9)	0.6 (0.4–0.9)
INH resistance not tested	15/33 (45)	1.0 (0.5–2.1)	
INH sensitive	964/2143 (45)	1	1

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 Piatek 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.

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