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# Epidemiology of Isoniazid Resistance Mutations and Their Effect on Tuberculosis Treatment Outcomes

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**Isoniazid resistance is highly prevalent in Vietnam. We investigated the molecular and epidemiological characteristics and the association with first-line treatment outcomes of the main isoniazid resistance mutations in *Mycobacterium tuberculosis* in codon 315 of the *katG* and in the promoter region of the *inhA* gene. *Mycobacterium tuberculosis* strains with phenotypic resistance to isoniazid from consecutively diagnosed smear-positive tuberculosis patients in rural Vietnam were subjected to GenoType MTBDR*plus* testing to identify *katG* and *inhA* mutations. Treatment failure and relapse were determined by sputum culture. In total, 227 of 251 isoniazid-resistant strains (90.4%) had detectable mutations: 75.3% in *katG* codon 315 ($katG_{315}$) and 28.2% in the *inhA* promoter region. $katG_{315}$ mutations were significantly associated with pretreatment resistance to streptomycin, rifampin, and ethambutol but not with the Beijing genotype and predicted both unfavorable treatment outcome (treatment failure or death) and relapse; *inhA* promoter region mutations were only associated with resistance to streptomycin and relapse. In tuberculosis patients, *M. tuberculosis* $katG_{315}$ mutations but not *inhA* mutations are associated with unfavorable treatment outcome. *inhA* mutations do, however, increase the risk of relapse, at least with treatment regimens that contain only isoniazid and ethambutol in the continuation phase.**

With 8.8 million cases notified and 1.4 million deaths in 2010, tuberculosis (TB) remains a major burden to global health (1). In addition to rifampin, isoniazid is an important drug in first-line anti-TB treatment (2). *Mycobacterium tuberculosis* strains resistant to at least both rifampin and isoniazid are referred to as multidrug resistant (MDR). Both multidrug resistance and resistance to isoniazid without concomitant rifampin resistance are associated with poor response to first-line treatment (3, 4). Whereas rifampin resistance is usually encoded in a part of the *rpoB* gene, the mechanism of resistance to isoniazid is more complex, with mutations conferring resistance in several genomic loci, such as *katG*, *inhA*, *ahpC*, and, potentially, *ndh* (5–8). Mutations in codon 315 of the *katG* gene ($katG_{315}$) and in the promoter region of the *inhA* gene are by far the most common. $katG_{315}$ mutations occur in 50 to 95% of isoniazid-resistant strains (6, 9, 10), whereas 20 to 42% of such strains have mutations in the promoter region of the *inhA* gene (6, 10, 11), depending on the geographic region studied.

Isoniazid is activated by the enzyme catalase peroxidase, encoded by *katG* (12). *katG* mutations lead to high-level isoniazid resistance (to ≥1.0 μg/ml in 7H10 agar) (13). The *inhA* gene encodes an enoyl acyl carrier protein reductase involved in fatty acid synthesis. These fatty acids are the target of the active derivative of isoniazid. *inhA* mutations usually lead to low-level isoniazid resistance (resistant to 0.2 μg/ml in 7H10 agar) (13, 14).

$katG_{315}$ mutations have been shown to be associated with MDR-TB and TB transmission (15, 16). Such mutations were more frequent among patients infected with Beijing genotype strains (17, 18), which are common in East Asia, including Vietnam (19, 20), and related to drug resistance (20, 21), as well as to relapse in various areas (22, 23). These differences in isoniazid resistance-conferring mutations may also be related to other characteristics of *M. tuberculosis* strains, as well as to treatment outcomes. There are, however, very few studies on the mutations underlying resistance to anti-TB drugs and treatment outcome. We therefore studied the epidemiology of *katG* and *inhA* mutations in *M. tuberculosis* isolates and the clinical characteristics of the respective patients in Vietnam, where the prevalence of smear-positive TB was 197/100,000 in 2006-2007 (24) and resistance to isoniazid is common (16 to 25% in new patients) (25). For this we assessed *M. tuberculosis* genotype and TB treatment outcomes in association with *katG* and *inhA* mutations in a prospective, population-based study.

## MATERIALS AND METHODS

**Study subjects.** The study area consisted of three adjacent rural districts in Tiengiang Province in the Mekong River Delta in Southern Vietnam. Details of the study have been described elsewhere (26).

From 1 July 2005 to 30 June 2007, all patients aged ≥15 years, resident in the study area and registered for treatment of smear-positive pulmonary TB, were eligible for inclusion after provision of written informed consent. Excluded were patients who received treatment for more than 2 weeks before registration. Ethical clearance for the study was obtained from the ethical health committee of the Ho Chi Minh City Council.

According to the guidelines of the Vietnam National TB Control Program (27) patients with no history of treatment with anti-TB drugs for >1

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month (i.e., new TB cases) were treated with 2 months of daily streptomycin (S), isoniazid (H), rifampin (R), and pyrazinamide (Z), followed by daily ethambutol (E) and isoniazid for 6 months (2SHRZ/6EH). Previously treated patients were given all five drugs (SHRZE) daily for 2 months and then four drugs (HRZE) for one more month, followed by RHE 3 days per week for 5 months. All doses were given under directly observed treatment as long as patients were given rifampin, irrespective of treatment phase or regimen. Drug susceptibility testing was done later, and results were not used to modify treatment regimens. Treatment adherence was confirmed from the treatment cards kept at the district tuberculosis unit (DTU).

**Study design.** The purpose of the present study was to quantify possible associations between isoniazid resistance-conferring mutations in the *M. tuberculosis* strain isolated before treatment and *M. tuberculosis* genotype and patient characteristics among all TB patients, and between pretreatment isoniazid resistance-conferring mutations and treatment outcomes (treatment failure and relapse) among new TB cases. New TB patients were followed up during standard first-line treatment with sputum smear microscopy at months 3, 5, and 8 and with sputum culture at the end of treatment (after 8 months, or after 5 months if the month 5 smear was positive). Participants whose sputum smear and culture were negative for *M. tuberculosis* at the end of treatment were visited by study staff twice thereafter, at around 9 and 18 months after treatment completion, or later if not encountered. In addition, data were collected during this period on study participants reporting with TB symptoms at any of the study clinics. Participants who had any complaints suggesting recurrent TB during these visits, or when they themselves consulted a participating clinic, provided two sputum specimens for smear and culture. The data were also collected on any intermediate TB treatment elsewhere, and on causes of death among the study patients based on clinic reports, death certificates and interviews with family members.

**Laboratory methods.** Sputum specimens were kept refrigerated and transported to Pham Ngoc Thach Hospital in Ho Chi Minh City within 72 h after collection. They were decontaminated and liquefied with 1% *N*-acetylcystine–2% NaOH, inoculated on modified Ogawa medium, and incubated at 37°C. Cultures were examined for growth after 1, 2, 4, 6, and 8 weeks of incubation. Cultures with no growth after 8 weeks were reported as negative. *M. tuberculosis* was identified using the niacin and the nitrate tests.

Drug susceptibility testing (DST) was performed using the proportion method on Löwenstein-Jensen (LJ) medium (28). Criteria for drug resistance were ≥1% of the CFU grown at 28 or 42 days compared to the drug-free control medium at the following drug concentrations: isoniazid, 0.2 μg/ml; rifampin, 40 μg/ml; streptomycin, 4 μg/ml; and ethambutol, 2 μg/ml (28). All isoniazid-resistant *M. tuberculosis* strains that were isolated were subjected to testing by GenoType MTBDR*plus* that combines detection of *M. tuberculosis* complex with detection of mutations in the 81-bp hot spot region of *rpoB*, at codon 315 of the *katG* gene and in the *inhA* promoter region (15). All baseline and follow-up isolates from patients with positive follow-up *M. tuberculosis* cultures were subjected to molecular typing by spoligo and variable number of tandem repeats (VNTR) typing. Bacterial DNA was extracted from positive cultures using an earlier described method (29). Spoligotyping was performed according to the internationally standardized method (30), and VNTR typing was done using15 loci (31).

**Definitions.** Previously treated patients were those who received 1 month or more of anti-TB drugs in the past. Cure was defined as a negative sputum smear examination and culture in the last month of treatment and on at least one previous occasion, and treatment failure was defined as any positive sputum smear or culture at 5 months or later during treatment. Treatment completion was defined as having completed treatment without meeting the criteria for being classified as cure or failure.

Recurrent TB was defined as any case of positive smear and/or culture during the follow-up period among the cured patients (32). We defined a case of recurrent TB as relapse if the initial and follow-up *M. tuberculosis* isolates had identical spoligotypes and VNTR patterns, or if the VNTR patterns differed by ≤1 locus, and as reinfection if otherwise (31). Unfavorable treatment outcome was referred to as treatment failure or death and related to the treatment period only.

Genotypes were based on spoligotyping. The Beijing genotype was defined as any isolate without direct-repeat spacers 1 to 34 and the presence of at least three of the spacers from 35 to 43 (33). Other genotypes, including the East African-Indian (EAI) genotype that is predominant in Vietnam, were defined as described by Brudey et al. (34).

**Data analysis.** Data were double entered in Epi-Info (version 6.04; Centers for Disease Control and Prevention, Atlanta, GA); discrepancies were corrected based on the raw data. Analyses were performed in Stata (version 10SE; Stata Corp., College Station, TX).

For comparison of categorical variables we used the chi-squared and two-sided Fisher exact tests as appropriate. Associations of $katG_{315}$ or *inhA* mutations with explanatory variables before start of treatment were expressed as odds ratios (ORs); confounding effects were investigated by multivariable logistic regression modeling. In the analysis of treatment failure, mutations in *katG* and *inhA* were assessed by multivariable logistic regression as explanatory variables, along with covariates that showed confounding effects, potentially including age, sex, residence, resistance to other drugs, the *M. tuberculosis* genotype, pretreatment smear grading and the extent of chest X-ray abnormalities, and treatment adherence. Only variables that showed confounding effects for the association between resistance mutations and the outcome were retained in the final model. Since patients who died during treatment may reflect treatment failures, we repeated this analysis taking failure or death as unfavorable treatment outcome. For the association with relapse, we did a similar analysis using multivariable Cox' proportional hazard modeling. *P* values for contribution to multivariate models, including interaction, were based on the likelihood ratio test. All tests were done at the 5% significance level.

## RESULTS

After excluding 151 patients (Fig. 1), pretreatment data were available for analysis for 1,213 (88.9%) of 1,364 registered patients. Of these, 924 were male (76.2%); the mean age was 50 years (standard deviation [SD] = 18.3; range, 15 to 102). There were 1,102 (90.9%) new patients and 111 (9.1%) patients previously treated for TB.

Of 1,213 *M. tuberculosis* pretreatment isolates, 69 (5.7%) were monoresistant to isoniazid, 146 (12.4%) were monoresistant to streptomycin, 128 (10.6%) were resistant to isoniazid and streptomycin, and 47 (3.9%) were multidrug resistant (see Table S1 in the supplemental material). Monoresistance to isoniazid was more frequent among previously treated patients than among new TB patients (12.6% versus 5.0%, $P < 0.05$), whereas the proportion of other monoresistance patterns did not significantly differ between previously treated and new patients.

**Isoniazid resistance-conferring mutations.** Of the 251 (20.7%) phenotypically isoniazid-resistant *M. tuberculosis* strains, 227 (90.4%) exhibited mutations by GenoType MTBDR*plus* testing; 171 (75.3%) had mutations or no reaction on wild-type (WT) probes in $katG_{315}$, including 167 (97.7%) with *katG* S315T1 mutations. Sixty-four (28.2%) had mutations in the *inhA* promoter region, including 61 (95.3%) involving *inhA* C15T mutations. Only 8 of 227 (3.5%) strains with a $katG_{315}$ mutation had an additional mutation in the *inhA* promoter region (Table 1).

**Characteristics for $katG_{315}$ mutations.** There were no significant associations between the probability of having a strain with a $katG_{315}$ mutation and the patient's district, type of residence, age or presence of mutations in the *inhA* promoter region. However, $katG_{315}$ mutations were significantly more frequent among women (odds ratio [OR] 1.4), among patients previously treated

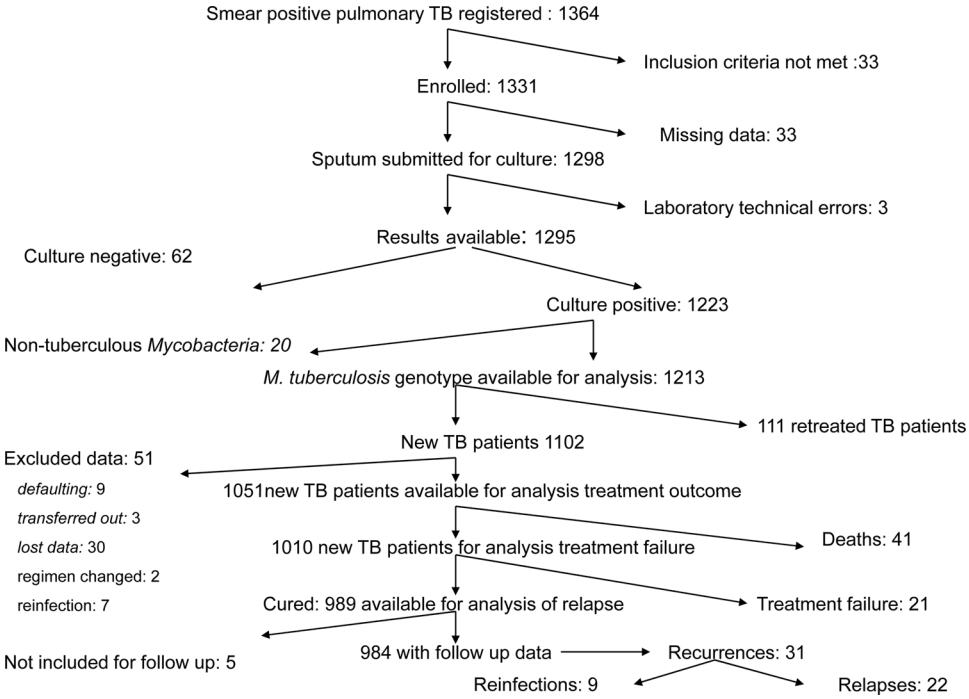


**FIG 1** Schematic presentation of enrollment of the study population. TB, tuberculosis.

for TB (OR 5.3), among strains that were resistant to rifampin (OR 27.7), streptomycin (OR 16.9), or ethambutol (OR 62.1), and among strains that belonged to the Beijing genotype (OR 3.2).

In a multivariable model $katG_{315}$ mutations remained associated (adjusted OR [$OR^{adj}$]; 95% confidence interval [CI]) with previous TB treatment (2.6; 1.4 to 4.8), resistance to rifampin (4.5; 1.8 to 11.1), ethambutol (12.0; 2.7 to 54.4) or streptomycin (15.0; 9.4 to 23.9), as well as with female sex (1.9; 1.2 to 3.0), but not with the Beijing genotype (1.3; 0.8 to 2.2) or EAI genotype (1.7; 0.9 to 3.0) compared to all other genotypes together (Table 2). When leaving resistance to other drugs than isoniazid out of the model, the Beijing genotype (2.6; 1.7 to 4.1) but not the EAI genotype was associated with $katG_{315}$ mutation. When adding resistance to only one of the three other drugs to the model, the Beijing genotype was still significantly associated with $katG_{315}$ mutations after adjustment for rifampin (2.2; 1.4 to 3.4) or ethambutol resistance (2.5; 1.6 to 4.0), whereas this association disappeared after adjustment for streptomycin resistance (1.5; 0.9 to 2.5).

**TABLE 1** Results of isoniazid resistance mutations detected by MTBDR*plus* test among 227 TB patients with phenotypic resistance to isoniazid in Vietnam

| Mutation(s) | | Frequency | |
|---|---|---|---|
| *katG* | *inhA* | No. of patients | % Total |
| WT (315) absent | | 171 | 75.3 |
| MUT1 (S315T1) | | 167 | 73.6 |
| MUT2 (S315T2) | | 0 | 0 |
| | WT (−15/−16) absent | 56 | 24.7 |
| | WT (−8) absent | 5 | 2.2 |
| | MUT1 (C15T) | 61 | 26.9 |
| | MUT2 (A16G) | 0 | 0 |
| | MUT3A (T8C) | 0 | 0 |
| | MUT3B (T8A) | 3 | 1.3 |
| MUT1 (S315T1) | MUT1 (C15T) | 8 | 3.5 |

**Characteristics for *inhA* promoter region mutations.** In univariate analysis, *inhA* mutations were associated with previous TB treatment (OR 2.7), with resistance to rifampin (OR 3.2), streptomycin (OR 3.1), or ethambutol (OR 4.0) and with living in one of the districts (Caibe, OR 2.4), but not with sex, age, residence, or genotype. In multivariate analysis also including resistance to other drugs, *inhA* mutations remained significantly associated with resistance to streptomycin ($OR^{adj}$ 4.4; 95% CI = 2.4 to 8.1) and previous TB treatment ($OR^{adj}$ 2.5; 95% CI = 1.2 to 5.4), and near-significantly associated with the Caibe district ($OR^{adj}$ 2.1; $P = 0.066$) but not with resistance to rifampin or ethambutol (Table 3). There was significant interaction between previous TB treatment and streptomycin resistance: *inhA* mutations were significantly associated with streptomycin resistance among new TB patients ($OR^{adj}$ 6.5, $P < 0.001$) but not among previously treated patients ($OR^{adj}$ 0.36, $P > 0.05$).

**Predictors of treatment failure.** Of 1,102 new TB patients, 51were excluded due to loss of data (30), reinfection (7), defaulting (9), transfer-out (3), and changed treatment regimen because of side effects (2). Furthermore, we excluded 41 patients who died during treatment, leaving 1,010 new patients for this analysis (Fig. 1). Of these, 21 (2.1%) had a treatment failure (Table 4).

In univariate analysis, the risk of treatment failure was significantly increased for isoniazid-resistant strains having at least a $katG_{315}$ mutation (OR 13.6; 95% CI = 5.3 to 35.4) but not for isoniazid-resistant strains having *inhA* mutations only (OR 2.9; 95% CI = 0.3 to 14.0) or no mutations (OR 6.9; 95% CI = 0.8 to 59.6). After multivariable adjustment for district and resistance to rifampin,

**TABLE 2** Univariable and multivariable associations with patient characteristics, genotype, and anti-TB drug resistance for *katG* codon 315 mutations at the start of treatment (baseline)

| Characteristic or parameter | No. of patients | *katG* mutations | | OR (95% CI)[a] | | *P* |
|---|---|---|---|---|---|---|
| | | No. | % | Crude | Adjusted | |
| Total no. of subjects | 1,213 | | | | | |
| Sex | | | | | | 0.009 |
| Male | 924 | 120 | 13 | 1 | 1 | |
| Female | 289 | 51 | 17.7 | 1.4 (1.0–2.05) | 1.9 (1.2–3.0) | |
| Age (yrs) | | | | | | 0.349 |
| <25 | 119 | 16 | 13.5 | 1 | 1 | |
| 25 to 49 | 528 | 79 | 15 | 1.1 (0.6–2.0) | 1.7 (0.8–3.7) | |
| ≥50 | 566 | 76 | 13.4 | 1.0 (0.6–1.8) | 1.7 (0.8–3.7) | |
| History of TB | | | | | | 0.002 |
| New | 1,102 | 126 | 11.4 | 1 | 1 | |
| Previously treated | 111 | 45 | 40.5 | 5.3 (3.4–8.2) | 2.6 (1.4–4.8) | |
| District | | | | | | |
| Cailay | 440 | 60 | 13.6 | 1 | | |
| Caibe | 418 | 57 | 13.6 | 1.0 (0.7–1.5) | | |
| Chauthanh | 355 | 54 | 15.2 | 1.1 (0.8–1.7) | | |
| Residence | | | | | | |
| On waterway | 457 | 67 | 14.7 | 1 | | |
| On provincial road | 624 | 91 | 14.6 | 1.0 (0.7–1.4) | | |
| On national road | 132 | 13 | 9.9 | 0.6 (0.3–1.2) | | |
| Genotype family | | | | | | 0.203 |
| East African-Indian | 461 | 40 | 8.7 | 0.9 (0.6–1.5) | 1.7 (0.9–3.0) | |
| Beijing | 407 | 98 | 24.1 | 3.0 (2.0–4.6) | 1.3 (0.8–2.2) | |
| Other | 345 | 33 | 9.6 | 1 | 1 | |
| Rifampin resistance | | | | | | <0.001 |
| No | 1,163 | 132 | 11.4 | 1 | 1 | |
| Yes | 50 | 39 | 78 | 27.7 (13.1–58.6) | 4.5 (1.8–11.1) | |
| Streptomycin resistance | | | | | | <0.001 |
| No | 889 | 36 | 4.1 | 1 | 1 | |
| Yes | 324 | 135 | 41.7 | 16.9 (10.7–26.7) | 15.0 (9.4–23.9) | |
| Ethambutol resistance | | | | | | <0.001 |
| No | 1,184 | 145 | 12.3 | 1 | 1 | |
| Yes | 29 | 26 | 89.7 | 62.1 (17.2–224.4) | 12.0 (2.7–54.4) | |
| InhA mutation | | | | | | <0.001 |
| No | 1149 | 163 | 14.2 | 1 | 1 | |
| Yes | 64 | 8 | 12.5 | 0.9 (0.4–1.9) | 0.2 (0.1–0.5) | |

[a] "Adjusted" means adjusted for all other variables in the model. CI, confidence interval; OR, odds ratio.

streptomycin, or ethambutol, the association between $katG_{315}$ mutations and treatment failure ($OR^{adj}$; 95% CI) was no longer significant (3.2; 0.8 to 12.8; Wald test, $P = 0.102$). Similarly, neither *inhA* mutations only (1.0; 0.1 to 10.1) nor isoniazid resistance without mutations detectable by the MTBDR*plus* assay (1.8; 0.1 to 24.1) showed significant association with treatment failure (Table 4). There was no significant difference in failure between patients with $katG_{315}$ and patients with *inhA* mutated strains. Isoniazid resistance mutations showed no association with treatment adherence, pretreatment smear grade, and the extent of pretreatment abnormalities, and none of these variables confounded the observed associations between resistance mutation and treatment failure.

When the 41 patients who had died during treatment were included in the analysis and failure or death (62 of 1,051 patients; 5.9%) was combined as unfavorable treatment outcome, its risk was significantly increased for isoniazid-resistant strains having at least a $katG_{315}$ mutation (OR 3.7; 95% CI = 2.0 to 6.7) or no mutations detectable by the MTBDR*plus* assay (OR 3.7; 95% CI = 1.1 to 13.3) but not for *inhA* mutations only (OR 1.0; 95% CI = 0.2 to 4.4). In a multivariate model adjusting for covariates that appeared to confound this association (resistance to rifampin or resistance to streptomycin) unfavorable treatment outcome remained significantly associated with the $katG_{315}$ mutations ($OR^{adj}$ 3.0; 95% CI = 1.4 to 6.8; Wald test $P = 0.007$) but not with no

**TABLE 3** Univariable and multivariable associations with patient characteristics, genotype, and anti-TB drug resistance for the *inhA* promoter region mutations at the start of treatment (baseline)

| Characteristic or parameter | No. of patients | *inhA* mutations | | OR (95% CI)[a] | | *P* |
|---|---|---|---|---|---|---|
| | | No. | % | Crude | Adjusted | |
| Total | 1,213 | | | | | |
| Sex | | | | | | 0.184 |
| Male | 924 | 46 | 5 | 1 | 1 | |
| Female | 289 | 18 | 6.2 | 1.3 (0.7–2.2) | 1.5 (0.8–2.7) | |
| Age group (yrs) | | | | | | 0.453 |
| <25 | 119 | 4 | 3.4 | 1 | 1 | |
| 25 to 49 | 528 | 30 | 5.7 | 1.7 (0.6–5.0) | 1.9 (0.6–5.9) | |
| ≥50 | 566 | 30 | 5.3 | 1.6 (0.6–4.7) | 1.7 (0.6–5.1) | |
| History of TB | | | | | | 0.025 |
| New | 1,102 | 51 | 4.6 | 1 | 1 | |
| Previously treated | 111 | 12 | 11.7 | 2.7 (1.4–5.2) | 2.5 (1.2–5.4) | |
| District | | | | | | 0.066 |
| Cailay | 440 | 14 | 3.2 | 1 | 1 | |
| Caibe | 418 | 31 | 7.4 | 2.4 (1.3–4.7) | 2.1 (1.1–4.1) | |
| Chauthanh | 355 | 19 | 3.4 | 1.7 (0.9–3.5) | 1.3 (0.6–2.8) | |
| Residence | | | | | | |
| On waterway | 457 | 25 | 5.5 | 1 | | |
| On provincial road | 624 | 32 | 5.1 | 0.9 (0.6–1.6) | | |
| On national road | 132 | 7 | 5.3 | 1.0 (0.4–2.3) | | |
| Genotype family | | | | | | 0.276 |
| East African-Indian | 461 | 21 | 4.6 | 0.7 (0.4–1.4) | 1.0 (0.5–2.0) | |
| Beijing | 407 | 22 | 5.4 | 0.9 (0.5–1.6) | 0.6 (0.3–1.2) | |
| Other | 345 | 21 | 6.1 | 1 | 1 | |
| Rifampin resistance | | | | | | 0.496 |
| No | 1,163 | 57 | 4.9 | 1 | 1 | |
| Yes | 50 | 7 | 14 | 3.2 (1.4–7.4) | 1.6 (0.5–5.4) | |
| Streptomycin resistance | | | | | | <0.001 |
| No | 889 | 31 | 3.5 | 1 | 1 | |
| Yes | 324 | 33 | 10.2 | 3.1 (1.9–5.2) | 4.4 (2.4–8.1) | |
| Ethambutol resistance | | | | | | 0.122 |
| No | 1,184 | 59 | 5 | 1 | 1 | |
| Yes | 29 | 5 | 17.2 | 4.0 (1.5–10.8) | 3.3 (0.8–14.3) | |
| *katG* mutation | | | | | | <0.001 |
| No | 1,042 | 56 | 5.4 | 1 | 1 | |
| Yes | 171 | 8 | 4.7 | 0.9 (0.4–1.8) | 0.2 (0.1–0.6) | |

[a] "Adjusted" means adjusted for all other variables in the model.

detectable mutations ($OR^{adj}$ 3.4; 95% CI = 0.9 to 13.4; $P = 0.081$) (Table 4).

**Predictors of relapse.** For this analysis, we included all 984 new patients who were smear and culture negative at the end of treatment and available for follow-up (Fig. 1). We observed 31 cases of recurrent TB, of which 9 were classified as reinfections and 22 (2.2%) were classified as relapse. There were no relapses among the 17 participants with isoniazid-resistant isolates that did not display any mutation in the MTBDR*plus* assay. The three strains that displayed both a $katG_{315}$ and an *inhA* mutation were included in the $katG_{315}$ mutation category.

In univariate analysis, both $katG_{315}$ and *inhA* mutations were strongly associated with relapse; hazard ratios (HR) were 6.7 (95% CI = 2.6 to 16.9) and 8.3 (95% CI = 2.6 to 26.4), respectively (Fig. 2). Relapse was also significantly more frequent among participants harboring strains that were of the Beijing genotype (HR 6.2) or streptomycin resistant (HR 4.0) and among those with MDR-TB (HR 7.4).

After multivariable adjustment for genotype and resistance to streptomycin or rifampin, relapse remained strongly associated with $katG_{315}$ mutations ($HR^{adj}$ 4.3; 95% CI = 1.4 to 13.6, $P = 0.013$) and *inhA* mutations ($HR^{adj}$ 8.7; 95% CI = 2.5 to 30.0, $P = 0.001$) compared against isoniazid-susceptible strains (Table 5). The relapse rate did not differ significantly between strains with

**TABLE 4** Multivariable associations for treatment failure or treatment failure and death during treatment combined

| Resistance | Failure[a] | | | | | Failure or death[b] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Total | No. | % | Adjusted OR (95% CI) | *P* | Total | No. | % | Adjusted OR (95% CI) | *P* |
| Isoniazid resistance | | | | | 0.370 | | | | | 0.046 |
| Susceptible | 834 | 7 | 0.8 | 1 | | 866 | 39 | 4.5 | 1 | |
| Any *katG* mutation | 116 | 12 | 10.3 | 3.2 (0.8–12.8) | 0.102 | 122 | 18 | 14.8 | 3.0 (1.4–6.8) | 0.007 |
| InhA mutations only | 42 | 1 | 2.4 | 1.0 (0.1–10.0) | 0.974 | 43 | 2 | 4.7 | 1 (0.2–4.5) | 0.994 |
| No mutations | 18 | 1 | 5.6 | 1.8 (0.1–24.1) | 0.657 | 20 | 3 | 15.0 | 3.4 (0.9–13.4) | 0.081 |
| Rifampin resistance | | | | | 0.001 | | | | | <0.001 |
| No | 986 | 12 | 1.2 | 1 | | 1027 | 53 | 5.2 | 1 | |
| Yes | 24 | 9 | 37.5 | 7.6 (1.7–34.1) | | 24 | 9 | 37.5 | 6.3 (2.3–17.1) | |
| Streptomycin resistance | | | | | 0.191 | | | | | 0.348 |
| No | 760 | 7 | 0.9 | 1 | | 794 | 41 | 5.2 | 1 | |
| Yes | 250 | 14 | 5.6 | 2.3 (0.7–8.2) | | 257 | 21 | 8.2 | 0.7 (0.3–1.5) | |
| Ethambutol resistance | | | | | 0.056 | | | | | |
| No | 997 | 15 | 1.5 | 1 | | 1038 | 56 | 5.4 | – | |
| Yes | 13 | 6 | 46.2 | 5.9 (1.0–32.3) | | 13 | 6 | 46.2 | | |

[a] The model for the OR and *P* values includes the following covariates: district, isoniazid resistance, rifampin resistance, streptomycin resistance, and ethambutol resistance.
[b] The model for the OR and *P* values includes the following covariates: isoniazid resistance, rifampin resistance, and streptomycin resistance.

*katG*$_{315}$ mutations, and strains with *inhA* mutations. We found no significant interactions.

## DISCUSSION

In this population-based study conducted in Vietnam, *katG*$_{315}$ mutations occurred in 75% of the isoniazid-resistant strains and were more often found in strains resistant to rifampin, streptomycin, or ethambutol. In contrast, the *inhA* promoter region mutations were less frequent among isoniazid-resistant strains and only associated with streptomycin resistance. Follow-up of new TB patients on standard first-line treatment showed that *katG*$_{315}$ and *inhA* promoter region mutations were both strongly associated with relapse. *katG*$_{315}$ mutations showed a 3-fold but nonsignificant association with treatment failure, while *inhA* promoter region mutations showed no such association at all. Unfavorable treatment outcome was, however, significantly associated with *katG*$_{315}$ mutations, as previously reported by Tolani et al. (35). This was expected because these mutations confer high-level isoniazid resistance (13). The independent association between *katG*$_{315}$ and failure was nonsignificant, probably because of small numbers, which is supported by the finding that the association was of similar magnitude but now significant when treatment failure and death were combined. We found no associations between *katG*$_{315}$ or *inhA* mutations and the Beijing genotype.

A strong association between *katG*$_{315}$ and *inhA* mutations and relapse may be caused by the 8-month 2SHRZ/6EH regimen used for new TB patients in Vietnam. This means that not only with high-level but also with low-level isoniazid resistance the supplementation of isoniazid with only ethambutol in the continuation phase of treatment is not effective in sterilizing *M. tuberculosis*.

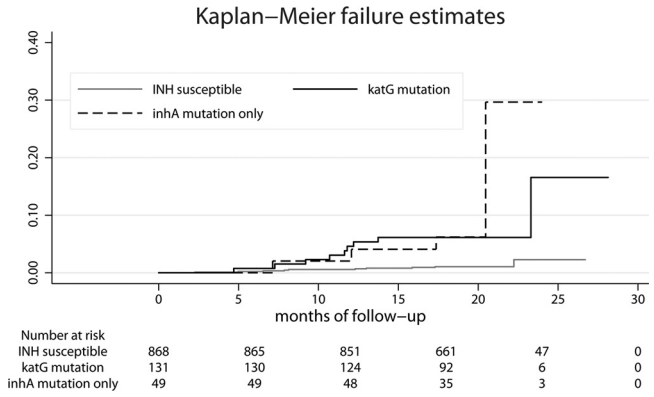


**FIG 2** Inverted survival curve for tuberculosis relapse cases among 984 patients after first line TB treatment, isoniazid resistance-conferring mutations. Log-rank test, $P < 0.001$. Solid black line, cases due to *katG* codon 315 mutated strains. Interrupted black line, cases due to *inhA* promoter region-only mutated strains. Gray line, cases due to isoniazid-susceptible strains. *y* axis, proportion of relapse cases. INH, isoniazid.

**TABLE 5** Multivariable associations for relapse in 967 new TB patients[a]

| Resistance | No. of relapses | Incidence per 100 person-years of follow-up | Adjusted hazard ratio | 95% CI | *P* |
|---|---|---|---|---|---|
| Total | 22 | | | | |
| Isoniazid resistance | | | | | 0.004 |
| Susceptible | 10 | 0.78 | 1 | | |
| Any *katG* mutations | 8 | 5.14 | 4.3 | 1.4–13.6 | 0.013 |
| *inhA* mutations only | 4 | 6.34 | 8.7 | 2.5–30 | 0.001 |
| Rifampin resistance | 2 | 9.21 | 1.2 | 0.3–5.7 | 0.821 |
| Streptomycin resistance | 12 | 3.5 | 1.1 | 0.4–3.1 | 0.900 |
| Genotype | | | | | <0.001 |
| Beijing | | 3.46 | 5.1 | 1.9–14 | |
| Non-Beijing | | 0.58 | 1 | | |

[a] Variables in the model: isoniazid resistance mutations, Beijing genotype, streptomycin resistance, and rifampin resistance. A total of 17 patients with isoniazid-resistant strains for which no mutation was found were excluded from the analysis.

Interestingly, the association between isoniazid resistance and relapse was most pronounced for *inhA* mutations. Since the catalase-peroxidase release is a component of the bacterial *oxyR* response, this helps the bacteria to survive inside macrophages (36). Hence, the probability of survival of bacteria with an *inhA* mutation inside macrophages is higher than for the $katG_{315}$ mutant strains because they still have full catalase-peroxidase expression. Whether the increased risk of relapse, in particular for *inhA* mutations, also exists with the World Health Organization (WHO)-recommended 6-month regimen (2RHEZ/4RH) remains to be studied.

In the univariate analysis $katG_{315}$ mutations were strongly associated with the Beijing genotype. Although in accordance with previous studies (9, 17, 18), this association completely disappeared after adjustment for streptomycin resistance. In our study 47% of the Beijing strains were resistant to streptomycin of which 48% also had $katG_{315}$ mutations, suggesting that in the Beijing strains streptomycin resistance and $katG_{315}$ mutations are often present simultaneously. This correlation is not unexpected; streptomycin resistance was also associated with the Beijing genotype, MDR-TB and increased transmission in the same study area (37). The association between streptomycin resistance and high-level isoniazid resistance and MDR, especially among Beijing strains, needs further study. It might be related to a specific combination of low-fitness cost mutations conferring these resistances and the strain's genetic background. In addition, yet-unknown compensatory mutations may contribute to the strain's fitness.

Our findings also have consequences for the choice of the standard first-line regimen in Vietnam. In line with WHO recommendations, the 8-month regimen for the treatment of new TB patients should be replaced by the 6-month regimen, including rifampin in the continuation phase.

There were limitations to our study. We only tested isolates for mutations that showed phenotypic resistance to isoniazid and may have missed genotypically isoniazid-resistant isolates. However, drug susceptibility testing was done by an internationally recognized reference laboratory that has consistently shown high concordance rates in proficiency testing therefore this risk is small. We did not determine the MICs of isoniazid for the *M. tuberculosis* isolates. We also did not use other genotyping methods to assess the type of mutations conferring isoniazid resistance other than those included in the MTBDR*plus* test. However, the MTBDR*plus* test covers ≥90% of the isoniazid mutations in *M. tuberculosis* isolates in Vietnam, as shown previously (15). HIV testing was not routinely performed for all patients. However, the HIV infection prevalence in Vietnam is estimated to be 0.4% of the adult population, with substantially lower prevalence in rural provinces than in major cities. We collected no data on clinical characteristics known to predict treatment failure or relapse, such as the presence of comorbidities or cavities on the chest X-ray. Since it is unlikely that these would be associated with specific isoniazid resistance-conferring mutations before treatment, we do not expect that this resulted in uncontrolled confounding of the observed associations.

In conclusion, isoniazid resistance was most frequently due to mutations in the $katG_{315}$ gene, and these mutations were associated with multidrug and polydrug resistance, whereas *inhA* mutations were less frequent and were only associated with streptomycin resistance. Both $katG_{315}$ and *inhA* mutations increased the risk of relapse. Our results also suggest that in Vietnam the 8-month regimen should be discontinued and be replaced by the WHO-recommended 6-month regimen for the treatment of new TB patients.

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