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How to cite

FENNER, Lukas et al. Effect of mutation and genetic background on drug resistance in Mycobacterium tuberculosis. In: Antimicrobial agents and chemotherapy, 2012, vol. 56, n° 6, p. 3047–3053. doi: 10.1128/AAC.06460-11

This publication URL: https://archive-ouverte.unige.ch/unige:31638

Publication DOI: 10.1128/AAC.06460-11

AAC Journals.ASM.org

Effect of Mutation and Genetic Background on Drug Resistance in *Mycobacterium tuberculosis*

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Bacterial factors may contribute to the global emergence and spread of drug-resistant tuberculosis (TB). Only a few studies have reported on the interactions between different bacterial factors. We studied drug-resistant *Mycobacterium tuberculosis* isolates from a nationwide study conducted from 2000 to 2008 in Switzerland. We determined quantitative drug resistance levels of first-line drugs by using Bactec MGIT-960 and drug resistance genotypes by sequencing the hot-spot regions of the relevant genes. We determined recent transmission by molecular methods and collected clinical data. Overall, we analyzed 158 isolates that were resistant to isoniazid, rifampin, or ethambutol, 48 (30.4%) of which were multidrug resistant. Among 154 isoniazid-resistant strains, *katG* mutations were associated with high-level and *inhA* promoter mutations with low-level drug resistance. Only *katG*(*S315T*) (65.6% of all isoniazid-resistant strains) and *inhA* promoter −15C/T (22.7%) were found in molecular clusters. *M. tuberculosis* lineage 2 (includes Beijing genotype) was associated with any drug resistance (adjusted odds ratio [OR], 3.0; 95% confidence interval [CI], 1.7 to 5.6; $P < 0.0001$). Lineage 1 was associated with *inhA* promoter −15C/T mutations (OR, 6.4; 95% CI, 2.0 to 20.7; $P = 0.002$). We found that the genetic strain background influences the level of isoniazid resistance conveyed by particular mutations (interaction tests of drug resistance mutations across all lineages; $P < 0.0001$). In conclusion, *M. tuberculosis* drug resistance mutations were associated with various levels of drug resistance and transmission, and *M. tuberculosis* lineages were associated with particular drug resistance-conferring mutations and phenotypic drug resistance. Our study also supports a role for epistatic interactions between different drug resistance mutations and strain genetic backgrounds in *M. tuberculosis* drug resistance.

Drug resistance has a major impact on the treatment success of tuberculosis (TB). While standardized first-line treatment is highly effective in drug-susceptible TB, the treatment of multidrug-resistant (MDR) TB requires the use of second-line drugs that are less effective, more expensive, and often associated with severe side effects (20). Drug resistance to first-line drugs is emerging globally, but the extent of the MDR TB burden varies by geography. Some regions in China and the countries of the former Soviet Union show a particularly high burden of MDR-TB (39).

Strategies for controlling drug resistance in *Mycobacterium tuberculosis* include drug susceptibility testing (DST) and surveillance, as well as ensuring the completion of an adequate treatment regimen and patient follow-up (39). Bacterial factors may also contribute to the global emergence and spread of drug-resistant TB. In particular, epistatic interactions (where the effect of one gene is modified by one or several other genes) between different strain genetic backgrounds and acquired drug resistance mutations could play a role in this context (4). Indeed, among the six main *M. tuberculosis* lineages (17), there is one lineage (lineage 2, which includes Beijing strains) which has been repeatedly associated with drug resistance, for reasons that are not well understood (1, 3, 25). Some *M. tuberculosis* lineages may show a preferential association with particular drug resistance mutations (1, 14). Finally, drug resistance-conferring mutations in *M. tuberculosis* are associated with various effects on strain fitness, and the strain genetic background may modulate these effects (8, 16).

Only a few studies have explored the possible interactions between the different bacterial factors in a single study. Here, we studied drug-resistant *M. tuberculosis* isolates collected systematically during 9 years in Switzerland. We assessed the interactions between drug resistance-conferring mutations and strain genetic backgrounds and their combined effects on drug resistance levels and *M. tuberculosis* transmission.

(This work was presented in part at FEMS 2011—4th Congress

Received 12 January 2012 **Returned for modification** 7 February 2012
Accepted 26 March 2012
Published ahead of print 2 April 2012
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Supplemental material for this article may be found at http://aac.asm.org/.

doi:10.1128/AAC.06460-11

of European Microbiologists, Geneva, Switzerland, 26 to 30 June 2011, abstr. A-291-0002-00605.)

MATERIALS AND METHODS

Study setting. *M. tuberculosis* complex (MTBC) isolates were obtained by the Swiss Molecular Epidemiology of Tuberculosis (SMET) study between 2000 and 2008. SMET is a collaborative project (12) between the Swiss HIV Cohort Study (SHCS), the National Center for Mycobacteria, diagnostic microbiology laboratories, departments of respiratory medicine and public health, and the Federal Office of Public Health (FOPH). The aim was to examine the genetic population structure of *M. tuberculosis* and the associations between strain variation, patients' geographic origins, and clinical characteristics in HIV-infected compared to HIV-negative TB patients in Switzerland (www.tb-network.ch). Participating sites are listed in Acknowledgments.

Study isolates and clinical data collection. During 2000 to 2008, 256 TB cases were reported to the National TB Surveillance Registry (FOPH) as resistant to any of the three first-line drugs isoniazid, rifampin, and ethambutol that are part of the routine drug resistance surveillance. We excluded 89 cases because no MTBC isolate was available. A further nine strains were excluded for technical reasons (e.g., difficulties with subculturing). A total of 158 clinical isolates (first available isolate from each patient at time of diagnosis) with an available semiquantitative DST result (61.7% of all 256 TB cases reported to the National TB Surveillance Registry) were thus included. Of 3,965 pansusceptible TB cases notified to the FOPH during the same time period, 353 pansusceptible TB cases from the SMET study (12) for whom an MTBC isolate and additional clinical data were available were used as a control population. Clinical data were obtained by standardized questionnaires sent to the treating physicians during this study.

Phenotypic drug susceptibility testing. We used the Bactec MGIT 960 system (Becton, Dickinson Diagnostic Systems, Sparks, MD) for semiquantitative DST to first-line drugs in the included strains as previously described (5, 33). The following drug concentrations were tested: isoniazid at 0.1, 1.0, 3.0, and 10.0 μg/ml; rifampin at 1.0, 10.0, and 50.0 μg/ml; and ethambutol at 5.0, 12.5, and 50.0 μg/ml. Isolates with indeterminate results were tested again. This work was performed at the National Center for Mycobacteria, Institute of Medical Microbiology, University of Zurich, Switzerland.

Drug resistance genotyping. Culture and DNA extraction were performed according to standard laboratory procedures. Drug resistance genotypes were determined among phenotypically drug-resistant strains by amplifying and sequencing the hot-spot regions of the genes (*katG*, *inhA*, *ahpC*, *rpoB*, and *embB*) known to confer resistance to isoniazid, rifampin, and ethambutol (14, 19, 36). Mutations were compared to those reported in the TB Drug Resistance Mutation database (http://www.tbdreamdb.com). This database contains a comprehensive list of the published genetic polymorphisms associated with first- and second-line drug resistance in clinical *M. tuberculosis* isolates throughout the world (29).

Determination of the main *M. tuberculosis* lineages and molecular clusters. The main phylogenetic lineages were determined according to single-nucleotide polymorphisms (SNPs) using multiplex real-time PCR with fluorescence-labeled probes (TaqMan, Applied Biosystems, United States) as described before (13, 15). The SNP used to define lineage 4 was originally described by Sreevatsan et al. (34) and shown to be specific for this lineage (15). Region of difference (RD) deletion PCRs were performed for RD702 and RD711 which define the West African lineages (15). Lineages were categorized as phylogenetically "modern" (lineages 2, 3, and 4) and "ancient" (lineages 1, 5, and 6) as described previously (9, 17, 26). We used spacer oligonucleotide typing (spoligotyping) and 24-locus mycobacterial interspersed repetitive unit–variable-number tandem-repeat (MIRU-VNTR) analysis to identify molecular clusters, as previously described (12, 35). Molecular clusters were defined as strains with 100% identity in spoligotyping and MIRU-VNTR, as well as an identical genotypic drug resistance pattern, to explore the transmission potential of particular drug resistance mutations (14).

TABLE 1 Resistance profiles of *Mycobacterium tuberculosis* isolates systematically collected between 2000 and 2008 in a nationwide study in Switzerland

Profile	No. of isolates	% of isolates
INH	105	66.5
RIF	4	2.5
INH+EMB	1	0.6
INH+RIF	24	15.2
INH+RIF+EMB	24	15.2
Total	158	100.0

EMB, ethambutol; INH, isoniazid; RIF, rifampin.

Statistical analysis. We used the χ^2 test or Fisher's exact test to assess differences between groups in binary variables. Odds ratios (OR) were obtained from univariate or multivariate logistic regression adjusted for age, sex, and being born in Switzerland when indicated. Exact logistic models were used where necessary. Interactions between HIV status and strain lineage category or between drug resistance mutations and strain lineage category were assessed by including interaction terms in logistic regression models or by using Mantel-Haenszel procedures. All analyses were performed in Stata version 11.1 (Stata Corporation, College Station, TX).

Ethics approval. The study was approved by the Ethics Committee of the Canton of Berne, Switzerland. Informed consent was obtained from all patients enrolled in the SHCS. For all other patients, informed consent was obtained by the treating physicians. In some cases informed consent could not be obtained from the patient because he or she could not be located or was known to have died. For these cases, we obtained permission from the Federal Expert Commission on Confidentiality in Medical Research to use the data provided by the treating physician.

RESULTS

Patient characteristics. The median age of the 158 drug-resistant TB cases included in this study was 33 years (interquartile range, 27 to 42); 74 (46.8%) were male (see Table S1 in the supplemental material); and 21 (13.3%) were HIV infected. Twenty-seven (17.1%) of the cases had a history of previous TB. The TB cases with drug resistance were most frequently born in Asia (54 cases, 34.2%), followed by sub-Saharan Africa (43 cases, 27.2%); only 24 cases (15.2%) were Swiss born. Among MDR TB cases, only 4 (8.3%) were born in Switzerland.

Phenotypic drug resistance. The resistance profile is summarized in Table 1. Forty-eight of 158 isolates were multidrug resistant. Among 154 isoniazid-resistant isolates, 83 (53.9%) had a semiquantitative DST result of ≥10.0 μg/ml, and 30 isolates (19.5%) had a result of <1.0 μg/ml (see Table S2 in the supplemental material). All but two rifampin-resistant isolates (94.1%) had a high drug resistance level (≥50.0 μg/ml) (see Table S2). The 25 ethambutol-resistant strains had a semiquantitative DST result of ≤12.5 μg/ml (see Table S2 in the supplemental material).

Drug resistance-conferring mutations. The most frequent mutations conferring drug resistance to isoniazid were *katG*(*S315T*) (101 isolates, 65.6%) and *inhA* promoter −15C/T (35 isolates, 22.7%); no mutation was found in 9 isolates (5.8%). All drug resistance-conferring mutations are listed in Tables S3 and S4 in the supplemental material. When comparing MDR and non-MDR isolates, we found that *katG*(*S315T*) was more frequent among MDR isolates (85.4% versus 56.6%, $P < 0.0001$). In

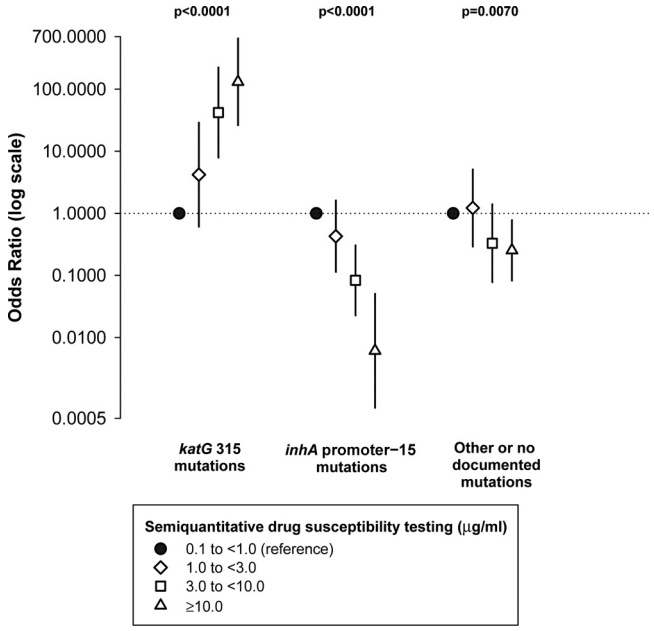

FIG 1 Association between drug resistance level and drug resistance-conferring mutations among isoniazid drug-resistant *Mycobacterium tuberculosis* strains. Each mutation category was compared to all other categories; low-level drug resistance (<1 μg/ml) is the reference. *P* values of linear tests for trend are shown. The category "*katG* 315 mutations" includes mixed *katG*/*inhA* promoter mutants (10 strains) and *katG* double mutants (3 strains).

addition, we found six new *katG* mutations [*katG*(*A256T*), *katG*(*G269S*), *katG*(*P280S*), *katG*(*M296T*), *katG*(*G297L*), and *katG*(*D329A*)] and two *inhA* promoter mutations [*inhA* promoter −113A/C and *inhA* promoter −47G/C] not previously reported in the TB Drug Resistance Mutation database (29) (http://www.tbdreamdb.com).

In 52 rifampin-resistant strains, *rpoB*(*S531L*) was the most frequent rifampin resistance-conferring mutation (30 isolates, 57.7%), followed by *rpoB*(*H526D*) (9 isolates, 17.3%) (see Table S5 in the supplemental material). We also found one new *rpoB* mutation (I572M) which had not been described before. Among the four rifampin monoresistant strains, we detected *rpoB* mutations H526D, Q513K, S531W, and L533P, respectively (see Table S5 in the supplemental material). Among 25 ethambutol-resistant strains, the most frequent mutation was *embB*(*M306V*) (44.0%) (see Table S6); in 7 strains, no mutation was found (28.0%).

Phenotypic effects of drug resistance-conferring mutations. We explored whether the different drug resistance-conferring mutations were associated with bacterial or patient phenotypes. Among isoniazid-resistant strains, we found that *M. tuberculosis* isolates with a *katG* 315 mutation (including isolates with an additional *inhA* promoter mutation [$n = 101$]) were strongly associated with high-level drug resistance. In contrast, isolates with an *inhA* promoter −15 mutation only ($n = 31$) exhibited low-level resistance. Isolates with other or undetected mutations ($n = 22$) were also associated with low-level isoniazid resistance (Fig. 1). Among rifampin-resistant strains, there was no statistically significant association between the different *rpoB* mutations and the level of rifampin resistance.

Among isoniazid-resistant isolates, *katG* 315 and *inhA* promoter −15 mutations were the only mutations found in molecular clusters, indicating successful transmission (Table 2; patient details are listed in Table S7 in the supplemental material). Swiss-born cases were more common among clustered cases (4/7 Swiss born among clustered compared to 20/147 among unclustered cases, $P = 0.01$) (Table 2).

Association between main *M. tuberculosis* lineages and drug resistance. To test whether the strain genetic background could influence drug resistance in *M. tuberculosis*, we grouped all isolates into one of the six main phylogenetic lineages of *M. tuberculosis* (17). When comparing lineage representation among the drug-resistant strains with a group of 353 pansusceptible isolates recovered during the same study (12), we found that lineage 2 (includes Beijing strains) was associated with any phenotypic resistance: the adjusted OR comparing lineage 2 with lineage 4 was 3.0 (95% confidence interval [CI], 1.7 to 5.6; $P < 0.0001$). This association was stronger for multidrug resistance (adjusted OR, 7.3; 95% CI, 3.4 to 15.8; $P < 0.0001$) and was also seen when stratifying by HIV status (Table 3).

To test whether the strain genetic background could influence the evolutionary pathway to isoniazid resistance, we compared the distribution of the different isoniazid resistance-conferring mutations across the main *M. tuberculosis* lineages. When comparing *inhA* promoter −15C/T mutations with all other mutation categories, we found that *inhA* promoter −15C/T mutations were strongly associated with lineage 1 (OR, 6.4; 95% CI, 2.0 to 20.7; $P = 0.002$) (Table 4).

Interaction between drug resistance mutations and strain genetic background. Our semiquantitative phenotypic DST showed that strains harboring the same drug resistance-conferring mutation exhibited different drug resistance levels. This was particularly true in the case of resistance to isoniazid (Fig. 1). We thus hypothesized that epistatic interactions between the strain genetic background and the particular drug resistance-conferring mutation could influence the drug resistance level of isoniazid in *M. tuberculosis*. When comparing isoniazid drug resistance levels

TABLE 2 Sociodemographic, clinical, and bacterial factors associated with molecular clustering in isoniazid-resistant TB cases in Switzerland from 2000 to 2008

Characteristic	No. (%) of cases that were:		*P* value[a]
	Clustered	Unclustered	
Presence of mutations			0.59
katG 315 and *inhA* promoter −15C/T	7 (100)	125 (85.0)	
Other or no detected mutation	0 (0)	22 (15.0)	
Age at time of TB diagnosis ≤45 yr	4 (57.1)	121 (82.3)	0.12
Male sex	3 (42.9)	71 (48.3)	0.78
Swiss born	4 (57.1)	20 (13.6)	0.012
HIV infection	1 (14.3)	19 (12.9)	0.99
Positive smear result	4 (57.1)	48 (32.7)	0.23
Cavitary disease	4 (57.1)	38 (25.9)	0.089
Pulmonary disease	7 (100)	115 (78.2)	0.35
TB in family/social surroundings[b]	2 (28.6)	14 (9.5)	0.16
Total	7	147	

[a] *P* values were obtained using Fisher's exact test.
[b] In last 2 yr.

TABLE 3 Association of drug resistance with the main *Mycobacterium tuberculosis* lineages, by HIV status

Characteristic	No. of:		Adjusted OR (95% CI)[b]				*P* value[c]	Interaction *P* value[d]
	Cases	Controls[a]	Lineage 1	Lineage 2	Lineage 3	Lineage 4		
Any resistance	158	353	1.19 (0.60–2.37)	3.03 (1.66–5.55)	0.99 (0.45–2.18)	1	0.0050	0.61
HIV infected	21	92	0.40 (0.05–3.47)	2.64 (0.65–10.70)	ND	1	0.42	
HIV negative	137	261	1.51 (0.70–3.25)	3.25 (1.61–6.55)	1.06 (0.46–2.45)	1	0.013	
Multidrug resistance	48	353	0.90 (0.24–3.29)	7.34 (3.41–15.80)	0.35 (0.04–2.79)	1	<0.0001	0.51
HIV infected	5	92	11.21 (0.44–285.32)	19.70 (1.30–298.19)	ND	1	0.097	
HIV negative	43	261	0.60 (0.12–2.85)	6.92 (2.95–16.22)	0.29 (0.03–2.38)	1	<0.0001	

[a] Patients with pansusceptible *M. tuberculosis* isolates were used as controls.
[b] Model was adjusted for age, sex and being born in Switzerland. ND, not defined; OR, odds ratio; 95% CI, 95% confidence interval; lineage 1, Indo-Oceanic lineage; lineage 2, East-Asian lineage (includes Beijing strains); lineage 3, Delhi/CAS; lineage 4, Euro-American lineage (used as reference).
[c] *P* values are model-based (Wald tests).
[d] Interaction *P* values are from logistic regression model testing interaction between HIV status and lineage category.

(categorized as <3.0 or ≥3.0 μg/ml) in strains harboring either the *katG*(*S315T*) or *inhA* promoter −15 C/T mutation, we found a statistically significant effect of strain lineage. As shown in Fig. 2, the main MTBC lineages were unequally distributed depending on the drug resistance level and drug resistance mutation. Strains harboring a *katG* 315 mutation and a semiquantitative DST result below 3.0 μg belonged more frequently to lineage 1 (60.0% among strains with a semiquantitative DST result below 3.0 μg compared to 2.1% among strains with a semiquantitative DST result above 3.0 μg) and less frequently to lineage 4 (40.0% among strains with a semiquantitative DST result below 3.0 μg compared to 67.7% among strains with a semiquantitative DST result above 3.0 μg, $P = 0.004$ by Fisher's exact test) (Fig. 2). Strains harboring any *inhA* promoter mutation and a semiquantitative DST result above 3.0 μg belonged exclusively to lineages 1 and 2 ($P = 0.01$ by Fisher's exact test). As these isolates were obtained from patients from various countries in Asia and Africa, we could not explain this finding by individual strain-specific properties.

Finally, we tested interactions using logistic regressions with interaction terms between drug resistance mutations and genetic strain background (categorized as phylogenetically "modern" and "ancient" strains [17, 26] and across all lineages). Combinations of *inhA* promoter mutations with a "modern" genetic background were associated with a lower isoniazid drug resistance level (<3.0 μg) than was found in strains with a *katG* 315 mutation (interaction *P* value is <0.0001) (see Table S8 in the supplemental material). Interaction tests of drug resistance mutations across all lineages were highly significant ($P < 0.0001$ from Mantel-Haenszel calculations), again suggesting that epistatic interactions between drug resistance-conferring mutations and strain genetic background can influence drug resistance levels.

DISCUSSION

We studied the combined effects of drug resistance-conferring mutations and strain genetic backgrounds on drug-resistant TB in a 9-year population-based study in Switzerland. We observed significant effects of interactions between the effects of drug resistance-conferring mutations and strain genetic backgrounds on the level of drug resistance.

Consistent with findings in other geographic areas (1, 6, 16, 19), we found that *rpoB*(*S531L*) (conferring resistance to rifampin) and *katG*(*S315T*) (conferring resistance to isoniazid) were the most frequent mutations in Switzerland (6, 16). We found little evidence for differences in mutation-specific resistance levels for rifampin and ethambutol, possibly because of the small sample sizes. However, for isoniazid, *katG* 315 mutations were associated with high-level drug resistance, whereas strains harboring an *inhA* promoter −15 mutation only had low-level resistance (5, 7, 22, 32). Furthermore, we found that other unidentified mutations were also associated with low-level resistance to isoniazid. These differences in isoniazid resistance levels support the broader implementation of quantitative DST for detecting specific isoniazid resistance-conferring mutations in TB, as patients infected with strains exhibiting low-level resistance could benefit from increased dosage (2, 21). Similar to previous studies (18, 23), we found that MDR isolates were more likely to carry *katG*(*S315T*) than non-MDR strains. This may reflect selection for increased levels of isoniazid resistance during treatment. Alternatively, as

TABLE 4 Association of the main *Mycobacterium tuberculosis* lineages with drug resistance-conferring mutations among isoniazid-resistant strains

Mutation category	No. of isolates in main or other lineage[a]						Comparison of each mutation category with all other categories across main lineages [OR (95% CI)][c]				
	1	2	3	4	Other	*P* value	1	2	3	4	*P* value[d]
katG(*S315T*)	5	24	5	66	1	0.022[b]	0.27 (0.08–0.87)	1.94 (0.72–5.22)	0.48 (0.13–1.80)	1	0.049
katG mutations other than S315T	0	1	0	7	0		0.70 (0.76–5.06)	0.45 (0.05–3.80)	0.99 (0.99–7.36)	1	0.42
inhA promoter −15C/T	8	4	2	17	0		6.35 (1.95–20.69)	0.73 (0.23–2.37)	1.19 (0.23–6.11)	1	0.014
No/other mutation	1	1	3	8	1		0.87 (0.10–7.49)	0.39 (0.05–3.23)	4.82 (1.04–22.35)	1	0.079

[a] Lineage 1, Indo-Oceanic lineage; lineage 2, East-Asian lineage (includes Beijing strains); lineage 3, Delhi/CAS; lineage 4, Euro-American lineage; other, West African lineages.
[b] Fisher's exact test.
[c] OR, odds ratio; 95% CI, 95% confidence interval. Lineage 4 was used as reference.
[d] *P* values are model based (maximum-likelihood estimation).

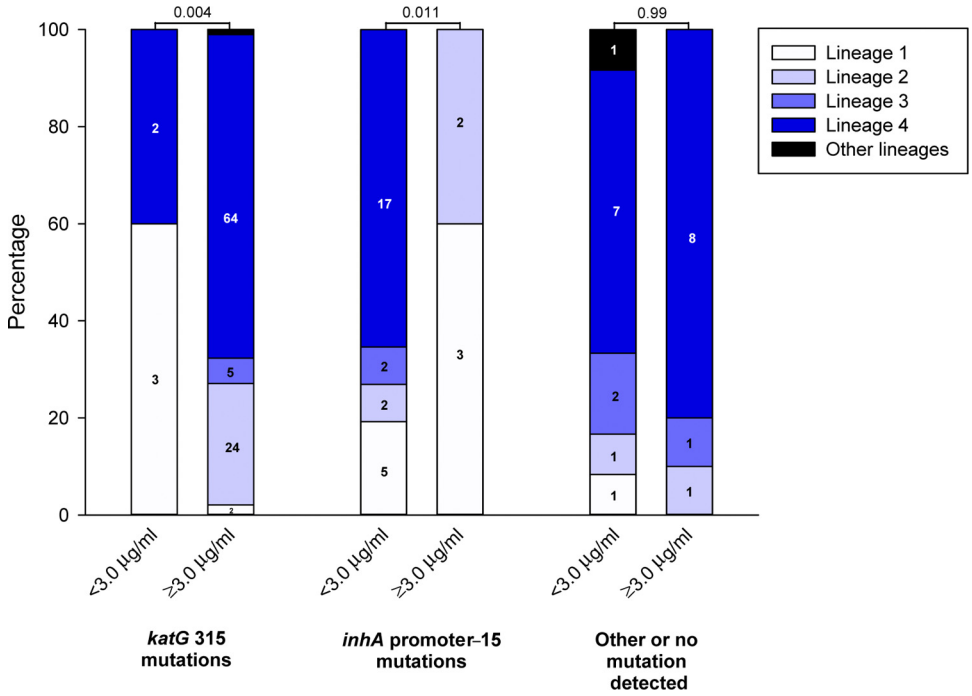

FIG 2 Distribution of main *Mycobacterium tuberculosis* lineages and semiquantitative drug susceptibility testing results (μg/ml) among isoniazid-resistant *M. tuberculosis* strains by drug resistance-conferring mutations. Numbers in bars indicate absolute numbers of strains. Lineage 1, Indo-Oceanic lineage; lineage 2, East Asian lineage (includes Beijing strains); lineage 3, CAS/Delhi; lineage 4, Euro-American lineage; other lineages, other lineages including West African lineages.

katG(*S315T*) has been associated with a limited effect on strain fitness (27), this association might indicate selection of low-cost fitness mutations in MDR strains.

We found that lineage 2 (East-Asian lineage), which includes Beijing strains, was associated with any drug resistance and MDR when comparing to a pansusceptible control population. Lineage 2 strains are most often isolated in East and Southeast Asia, in countries of the former Soviet Union, and in South Africa (17, 25, 37). The reason why Beijing strains are often (but not always) associated with drug resistance is unknown (3). Beijing strains could have a higher overall mutation rate, which could lead to an accelerated acquisition of drug resistance mutations (3, 11). Alternatively, the Beijing strain background could more efficiently compensate for the negative fitness effects of drug resistance (3).

We also found that *inhA* promoter −15C/T mutations were strongly associated with lineage 1 (Indo-Oceanic lineage). Antibiotic resistance-conferring mutations are often associated with a fitness cost: drug-resistant strains are less competitive than drug-susceptible strains (8, 16, 28). As there is evidence that lineage 1 is less virulent than other strains (24), the acquisition of additional low- or high-cost drug resistance-conferring mutations may further decrease the overall fitness of lineage 1 strains to a level that inhibits their propagation. Therefore, drug-resistant lineage 1 strains with no-cost mutations, such as *inhA* promoter −15C/T mutations, will have a selection advantage over lineage 1 strains with low- or high-cost mutations (e.g., *katG* mutations). Indeed, a recent report from India showed that mono- and multidrug resistance was less common among lineage 1 isolates than among other lineages (30). On the other hand, it remains unclear why a compensatory mechanism (10, 31) would not restore bacterial fitness in lineage 1 strains with low- or high-cost mutations. Similar to studies from San Francisco and the Netherlands (14, 38), we found that only the low and no-cost *katG* 315 and *inhA* promoter mutations were genetically clustered, indicating recent transmission of the corresponding drug-resistant strain. Therefore, *M. tuberculosis* strains with such mutations are more likely to be detected. Taken together, it thus seems unlikely that the association between lineage 1 and *inhA* promoter −15C/T mutations is due to chance, particularly because the phenomenon has now been observed independently in three distinct patient populations (1, 14).

Finally, we found that the level of resistance to isoniazid is a function of both the particular isoniazid resistance-conferring mutation and the strain genetic background as defined by the main phylogenetic lineages. Interestingly, among strains harboring *katG* mutations with unexpectedly low drug resistance levels, lineage 1 was more common. The same was true for strains harboring *inhA* promoter mutations with unexpectedly high drug resistance levels. These findings suggest that not only the drug resistance-conferring mutation (7, 22, 32) but also the genetic strain background in which the drug resistance-conferring mutation resides can modulate drug resistance levels.

One of the strengths of our study was that we were able to compare phenotypic and genotypic drug resistance with the main *M. tuberculosis* lineages and clinical phenotypes in a nationwide population-based study during 9 years. However, our study is limited by its retrospective design, which did not allow collecting all relevant clinical data, and by the sample size, which only allowed analysis of larger categories. In addition, due to the high proportion of drug-resistant TB from patients born outside Swit-

zerland, our results are influenced by the drug resistance situation in other countries.

In conclusion, the globally most frequent mutations conferring drug resistance to the first-line drugs were also the most frequently seen in Switzerland, and these mutations were associated with various levels of drug resistance and transmission. Our study provides evidence that the genetic strain background influences the level of resistance to isoniazid conveyed by particular drug resistance-conferring mutations. These findings may lead to a better understanding of the emergence of drug resistance at the population level. Further experimental studies are needed to determine the underlying mechanisms and interactions between drug resistance mutations with different fitness costs, *M. tuberculosis* lineages, and drug resistance phenotypes.

ACKNOWLEDGMENTS

We thank all tuberculosis patients participating in this study. We are grateful to the Swiss HIV Cohort Study and the participating clinics, to the treating physicians for providing clinical information, and to the Microbiology Laboratories for providing strains. We are indebted to the National TB Surveillance Registry at the Federal Office of Public Health, as well as to Christa Butz and Yvonne Bongni from the Bernese Lung Association. We also thank Claudia Ritter for technical assistance with drug susceptibility testing and Hans L. Rieder for helpful discussions on earlier versions of the manuscript.

This work was supported by the Swiss National Science Foundation (grant number 324730-12544), the Swiss HIV Cohort Study (grant number 588), and the Federal Office of Public Health (grant number 09.007368). L.F. and M.E. are also supported by the National Institute of Allergy and Infectious Diseases (IeDEA Southern Africa, grant number U01AI069924) and S.G. by the National Institutes of Health (grants number R01-AI090928-01 and HHSN266200700022C) and the Swiss National Science Foundation (grant number PP00A-119205). E.C.B. is supported by the University of Zürich, the European Community (PAR, FP7-HEALTH-2009−241476) and the Federal Office of Public Health (National Center for Mycobacteria). The Swiss HIV Cohort Study is supported by the Swiss National Science Foundation (grant number 33CS30-134277).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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The members of the Swiss HIV Cohort Study are J. Barth, M. Battegay, E. Bernasconi, J. Böni, H. C. Bucher, C. Burton-Jeangros, A. Calmy, M. Cavassini, C. Cellerai, M. Egger, L. Elzi, J. Fehr, J. Fellay, M. Flepp, P. Francioli (President of the SHCS), H. Furrer (Chairman of the Clinical and Laboratory Committee), C. A. Fux, M. Gorgievski, H. Günthard (Chairman of the Scientific Board), D. Haerry (Deputy of the Positive Council), B. Hasse, H. H. Hirsch, B. Hirschel, I. Hösli, C. Kahlert, L. Kaiser, O. Keiser, C. Kind, T. Klimkait, H. Kovari, B. Ledergerber, G. Martinetti, B. Martinez de Tejada, K. Metzner, N. Müller, D. Nadal, G. Pantaleo, A. Rauch, S. Regenass, M. Rickenbach (Head of the Data Center), C. Rudin (Chairman of the Mother & Child Substudy), P. Schmid, D. Schultze, F. Schöni-Affolter, J. Schüpbach, R. Speck, P. Taffé, P. Tarr, A. Telenti, A. Trkola, P. Vernazza, R. Weber, and S. Yerly.

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