Clinical Impact on Tuberculosis Treatment Outcomes of Discordance Between Molecular and Growth-Based Assays for Rifampin Resistance, California 2003–2013

Neha S. Shah,[1,2] SY Grace Lin,[2] Pennan M. Barry,[2] Yi-Ning Cheng,[3] Gisela Schecter,[2,4] and Ed Desmond[2]

[1]Division of Tuberculosis Elimination, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia; [2]California Department of Health, Tuberculosis Control Branch, Richmond; [3]San Diego Health and Human Services Agency, California; and [4]University of California San Francisco

Background. Data from international settings suggest that isolates of *Mycobacterium tuberculosis* with *rpoB* mutations testing phenotypically susceptible to rifampin (RIF) may have clinical significance. We analyzed treatment outcomes of California patients with discordant molecular-phenotypic RIF results.

Methods. We included tuberculosis (TB) patients, during 2003–2013, whose specimens tested RIF susceptible phenotypically but had a *rpoB* mutation determined by pyrosequencing. Demographic data were abstracted from the California TB registry. Phenotypic drug-susceptibility testing, medical history, treatment, and outcomes were abstracted from medical records.

Results. Of 3330 isolates tested, 413 specimens had a *rpoB* mutation (12.4%). Of these, 16 (3.9%) had molecular-phenotypic discordant RIF results. Seven mutations were identified: 511Pro, 516Phe, 526Asn, 526Ser (AGC and TCC), 526Cys, and 533Pro. Fourteen (88%) had isoniazid (INH) resistance, 6 of whom were also phenotypically resistant to ethambutol (EMB) and/or pyrazinamide (PZA). Five patients (25%), 1 with 511Pro and 4 with 526Asn, relapsed or failed treatment. The initial regimen for 3 patients was RIF, PZA, and EMB; 1 patient received RIF, PZA, EMB, and a fluoroquinolone (FQN); and 1 patient received RIF, EMB, FQN, and some second-line medications. Upon retreatment with an expanded regimen, 3 (75%) patients completed treatment, 1 patient moved before treatment completion, and 1 patient continues on treatment. The remaining 11 patients had a successful outcome with 9 having received a FQN and/or a rifamycin.

Conclusions. Rifampin molecular-phenotypic discordance was rare, and most isolates had INH resistance. Patients who did not receive an expanded regimen had poor outcomes. These mutations may have clinical importance, and expanded treatment regimens should be considered.

Keywords. rifampin discordance; *rpoB* mutations; tuberculosis.

Rifampin (RIF) is one of the first-line anti-tuberculosis (TB) medications and the cornerstone of TB treatment. When RIF cannot be used, treatment duration can extend from 6 months to 2 years [1–3]. Thus, accurate and prompt diagnosis of RIF resistance is critical to ensure appropriate treatment and minimize the development of drug resistance. Phenotypic drug-susceptibility testing (DST) using growth-based systems with either solid or liquid media relies on adequate growth of *Mycobacterium tuberculosis* (Mtb) in the presence of anti-TB medications at specific concentrations. Historically, phenotypic DST has been considered the reference standard. However, DST results by growth-based systems can take 1 to 2 months, and conflicting results may occur for some medications because of differences in methodology, inoculum preparation, and critical concentrations [4–10]. In contrast, molecular tests for drug

resistance detect genetic mutations associated with drug resistance. Ninety-five percent of RIF resistance is attributed to point mutations in the 81-base pair (bp) core region of the *rpoB* gene. Detection of mutations in this region offers rapid detection of RIF resistance and serves as an indicator for multidrug-resistant (MDR) TB, because monoresistance for RIF is rare [11–14]. Testing times vary by assay, but molecular results are frequently available within 2 days, which can reduce the time to treatment initiation with an appropriate regimen by up to 6 weeks [15–18]. Recent data have shown that rapid molecular assays have detected certain *rpoB* mutations in isolates that tested RIF susceptible by certain methods of phenotypic DST resulting in discordant molecular-phenotypic results [18–22].

What is the impact of these mutations that cause molecular-phenotypic DST discordance (sometimes referred to as "disputed"; here referred to as "discordant"), and do they have clinical significance? Data from cases who failed initial treatment and were being treated with the World Health Organization (WHO) standardized retreatment regimen of isoniazid (INH), RIF, ethambutol (EMB), pyrazinamide (PZA), and streptomycin (category 2 regimen) in Bangladesh and the Democratic Republic of Congo showed no difference in the rates of treatment failure or relapse among patients

whose isolates had a *rpoB* mutations associated with discordant molecular-phenotypic results and common *rpoB* mutations associated with phenotypic resistance [21, 23, 24]. A follow-up study of untreated TB cases in Bangladesh found that 7 of 1000 (0.7%) patients had a *rpoB* mutation associated with discordant molecular-phenotypic results and 5 of 7 (71.4%) patients died, relapsed, or failed first-line treatment [20]. In a study from China of patients with discordant mutations, half of which were retreatment cases (category 2 retreatment regimen), failure or relapse was less likely if the patient was treated with an MDR TB regimen [23]. Smaller studies have also reported poor outcomes among patients with discordant mutations [25, 26]. These data suggest that mutations associated with discordant molecular-phenotypic results may have clinical implications and that a standardized category 2 retreatment regimen should not be used; however, data were limited to international settings where the management of TB may differ from the United States, and most data came from patients who had already failed 1 course of standard TB treatment. Furthermore, sample sizes were small, making generalizability of findings challenging. We reviewed cases from California to examine the clinical significance of discordance between molecular and phenotypic assays for RIF resistance.

MATERIALS AND METHODS

Detection of *rpoB* Mutations

The Microbial Diseases Laboratory (MDL) of the California Department of Public Health used a molecular beacon-based real-time polymerase chain reaction assay to detect presence or absence of mutations associated with resistance to INH and RIF during 2003–2012 [27]. Molecular beacon assays detect presence or absence of mutations without providing mutation identities. Since March of 2012, the MDL has used pyrosequencing (PSQ), a rapid sequence-based method for detection of mutations conferring drug resistance [18]. Pyrosequencing provides mutation identities and allows for recognition of mutations in *rpoB* core region that confer various levels of RIF resistance.

Specimen Identification

Tuberculosis specimens with an *rpoB* mutation detected by either molecular beacons assay or PSQ and a RIF-susceptible phenotypic DST were identified by querying the California TB registry and the MDL's TB drug-susceptibility database from 2003 to 2012. Isolates tested by molecular beacons were retested by PSQ to determine the mutation identities.

Patient Clinical Information, Treatment Regimens, and Outcomes

After identification of specimens with discordant molecular-phenotypic results for RIF, data were matched with the California TB registry to obtain demographic data. Local TB programs in which patients were treated abstracted clinical information, including medical history, treatment, and outcomes, from medical records. Comprehensive phenotypic DST data were collected from medical charts. A standardized abstraction form was used, and all data were deidentified before analysis.

Definitions

Relapse was defined as ever having a recurrent episode of TB after having been previously treated for TB with a documented cure or treatment completion. Treatment failure was defined as having a positive sputum culture after having a documented sputum culture conversion during the same course of TB treatment or failure to convert sputum cultures after 5 months of therapy [28]. Genotyping was used to confirm that a failed or relapsed patient was not infected with the same TB strain. Cure was defined as a pulmonary TB patient with initial sputum culture positive for TB with documented sputum culture negativity in the last month of treatment and at least 1 other sputum culture-negative specimen. Treatment completion was defined as patient who completed TB treatment but did not have documented sputum culture results to meet the definition of cure. Culture conversion was defined as at least 1 negative sputum culture with no further positive cultures. The time to culture conversion was defined as the time from treatment initiation to sputum culture conversion. For patients who failed treatment or relapsed, a second culture conversion time was calculated using the retreatment start date.

The severity of TB disease was stratified into 3 categories used in another study: extensive, moderate, and minimal [29]. Extensive disease was defined as having a miliary pattern on imaging study, or pathology indicating extensive pulmonary TB disease, or massive tubercular empyema or pleural effusion that opacified the entire hemithorax, or ≥2 of the following: sputum smear-positive disease, failure to convert sputum culture within 60 days, cavitary disease on imaging, bilateral disease or multilobar disease, or collapse of 1 or more lobes. Minimal disease was defined as having all of the following: sputum smear negative, noncavitary disease on imaging, and limited disease. All other situations were classified as moderate disease. For extrapulmonary TB, extensive disease was defined as mediastinal, meningeal, central nervous system, abdominal, spinal, pericardial, disseminated, or miliary TB. Minimal disease was skin, peripheral lymphadenitis, or uveitis. All other sites of extrapulmonary TB were classified as moderate.

Ethics Considerations

This analysis was conducted as part of the California Department of Public Health's mandate to routinely collect and analyze surveillance data for public health purposes. The project was reviewed by the US Centers for Disease Control and Prevention (Atlanta, GA) and the California Health and Human Services Agency and determined not to require approval by an institutional review board.

RESULTS

Between March 26, 2003 and December 31, 2013, 3330 specimens underwent molecular testing. There were 413 specimens that had an *rpoB* mutation (12.4%), 16 specimens (3.9% or 0.5% of the all specimens tested) of which had a discordant molecular-phenotypic result. Among these 16, 7 discordant mutations were identified: 511Pro (CCG, n = 3), 516Phe (TTC, n = 1), 526Asn (AAC, n = 6), 526Ser (AGC [n = 2], TCC [n = 1]), 526Cys (TGC, n = 1), and 533Pro (CCG, n = 2).

Table 1 provides the details of the 16 patients with discordant molecular-phenotypic results. All patients had pulmonary TB. The 2 patients with both extrapulmonary and pulmonary TB disease were children under 10 years of age. Half (n = 8, 50%) had extensive disease; 7 of these patients had been previously treated for TB and 4 had diabetes. On phenotypic DST, 14 (88%) patients had an isolate resistant to INH, 6 of whom had an isolate also resistant to EMB and/or PZA, and 2 patients had an isolate resistant to a fluoroquinolone (FQN). The median time to culture conversion was 42 days. Five patients (31%), 1 whose isolate had the 511Pro mutation and 4 whose isolate had the 526Asn, relapsed or failed (Table 2).

The patient (no. 1, Table 1) with a 511Pro mutation and resistance to INH was initially treated with a RIF-based regimen for 11 months and relapsed 2 years after treatment completion. Retreatment for 19 months with a 6-drug regimen that included moxifloxacin (MFX), amikacin (AMK), and rifabutin (RFB) resulted in cure. One patient with the 526Asn mutation (no. 6, Table 1) and resistance to INH and PZA was treated for 12 months with an expanded regimen, including MFX and RIF and 3 months of capreomycin and cycloserine, but relapsed 5 months after treatment completion. Retreatment for 20 months with a 6-drug regimen that included AMK, linezolid (LNZ), RIF, and MFX resulted in cure. Two patients with a 526Asn mutation treated with RIF, PZA, and EMB (nos. 8 and 9, Table 1) failed. Patient no. 8 developed resistance to PZA and EMB but was cured after being retreated with RFB, MFX, AMK, and other second-line medications. The other patient moved out of the country before treatment completion. Patient no. 10 relapsed 1 year after completing 12 months of RIF, EMB, PZA, and MFX, and at the time of publication the patient was still being treated with AMK, PZA, RIF, ethionamide, levofloxacin (LFX), and LNZ. No isolates from patients with poor outcomes acquired a new mutation within the 81-bp RIF resistance-determining region of *rpoB*.

The remaining 11 patients culture converted and either completed treatment or were cured after their initial treatment. Two patients had isolates that were sensitive to INH (nos. 2 and 5, Table 1) and were treated for 6 months with a regimen including INH and RIF. Of the remaining 9 patients, all except no. 13 had RIF or RFB in their regimen. Moxifloxacin or LFX was included in the regimen except for 2 patients; no. 7 received only 1

month of LFX and no. 16 did not receive any FQN. Five patients also had at least 1 other second-line medication included in the regimen. The median duration of treatment was 14 months (range, 6–24 months). To date, no other relapses or failures have been observed with over 1 year of follow-up after treatment completion for all patients.

DISCUSSION

In recent years, molecular assays for detection of mutations conferring drug resistance have become easy to perform and have been applied for diagnostic uses. As the use of those molecular assays increases, discordance between the results of molecular testing and growth-based DST has been encountered. How to best utilize rapid molecular test results before availability of the growth-based DST results and how to interpret them properly to aid clinical management are challenging. In this retrospective case series, we examined the treatment outcomes of 16 patients whose *Mtb* isolate had mutations that did not confer RIF resistance by growth-based DST. We provided findings related to several key factors including comorbidities, sputum smear results, drug resistance profiles, disease severity, and time to sputum culture conversion. All 5 patients who failed treatment or relapsed were INH resistant and were treated with 1 of the recommended regimens for INH resistance or intolerance but limited second-line medications [30]. Patient nos. 6, 10, and 11 received a FQN but limited other second-line medications. In addition, severity of disease was moderate or extensive for all 5, and the 4 adult cases were smear positive. Two patients also had diabetes, which has been associated with a longer time to sputum culture conversion and higher rates of relapse [31–33]. Although clinical factors may have also contributed to relapse, this case series suggests that RIF molecular-phenotypic discordance may be associated with poor outcomes among patients treated with the recommended first-line regimen (RIF, INH, EMB, PZA), specifically those with mutations 511Pro (patient no. 1) and 526Asn (patient nos. 6, 8, 9, and 10).

All patients with successful outcomes also received appropriate treatment regimens. Two patients (nos. 2 and 5) had good clinical outcomes with a standard regimen for 6 months. Both patients were smear negative and susceptible to INH. Patient no. 5 received 1 month of second-line medications that strengthened the initial regimen. Patient no. 7 was also treated with RIF, EMB, and PZA with 1 month of second-line medications but was INH resistant and received a longer duration of therapy. In terms of clinical characteristics, 2 individuals had diabetes and 7 had moderate or extensive disease but still had successful outcomes. These observations highlight the complexities of factors influencing treatment outcomes. Although we did not see the 70% relapse or failure rates noted in some international studies on molecular-phenotypic discordance, we did have a higher recurrence rate than the 0.63% California reported for TB cases overall [4, 20, 21, 25, 34, 35]. There may be several reasons for these

Table 1. Demographic, Clinical, and Microbiological Characteristics of Patients With *rpoB* Mutations Conferring Molecular-Phenotypic Discordance, California

Patient	*rpoB* Mutation	Drug Resistance on Phenotypic DST	Site(s) of Disease	Severity of Disease	Initial Smear Status	Prior TB Treatment	Medical Comorbidities	Initial Treatment Regimen	Retreatment Regimen	Time(s) to Culture Conversion (Days)[a]	(Re)Treatment Outcome(s)[a]
1	511Pro (CCG)	H	Pulmonary	1. Moderate 2. Moderate	1. Positive 2. Negative	Yes	Alcohol abuse	Total 11 mo: 2 HRZE 9 RZE	Total 19 mo 6 AMK, MFX, Z, CS, PAS, RFB 6 Z, MFX, CS, PAS, RFB 7 Z, MFX, PAS, RFB	1. 112 2. 17	1. Relapsed 2. Cured
2	511Pro (CCG)	None	Pulmonary	Moderate	Negative	Yes	Diabetes CV disease	Total 6 mo 6 HRZE		30	Cured
3	511Pro (CCG)	H, ETO, SM, EMB	Pulmonary	Minimal	Negative	No	None	Total 24 mo 6 EZ, MFX, AMK, LNZ, RFB 18 EZ, MFX, LNZ, RFB		18	Cured
4	516Phe (TTC)	H, Z	Pulmonary	Extensive	Positive	Yes	None	Total 21 mo 2 HRZE 19 E, MFX, LNZ, CM, CS, RFB		65	Cured
5	526Asn (AAC)	None	Pulmonary	Minimal	Negative	Yes	None	Total 6 mo 1 HRZE, MFX, CM 5 HRZ		23	Completed
6	526Asn (AAC)	H, Z	Pulmonary	1. Moderate 2. (2) Moderate	1. Positive 2. Negative	Yes	Diabetes Hypertension	Total 12 mo 2 HE, CM, CS PAS, MFX 1 RE, CM, CS, MFX 9 RE, MFX	Total 20 mo 9 RE, MFX, AMK, ETO, LNZ 11 RE, LNZ, MFX	1. 19 2. 45	1. Relapsed 2. Cured
7	526Asn (AAC)	H	Pulmonary	Minimal	Positive	No	None	Total 9 mo 1 RZE, CM, MFX 8 RZE		27	Completed
8	526Asn (AAC)	1. H, ETO 2. Acquired Z, E	Pulmonary	Extensive	Positive	No	Diabetes Hypertension	Total 4 mo 2 HRZE 2 RZE	Total 25 mo 3 H, CM, LFX, LNZ, CS, PAS 2 RFB, CS, PAS, MFX, CLF, LNZ 2 RFB, CS, PAS, MFX, CLF 4 RFB, CS, PAS, MFX, CLF, AUG 14 RFB, PAS, MFX, CLF, AUG	1. 61 2. 172	1. Failed 2. Cured
9	526Asn (AAC)	H	Pulmonary	Extensive	Positive	No	None	Total 12 mo 12 RZE	Total 4 mo 2 RZE, MFX 2 RZE, MFX, AMK, LNZ	1. 49 2. 59	1. Failed 2. Moved
10	526Asn (AAC)	H	Pulmonary CNS	(1) Minimal, Extensive[b] (2) Extensive	Negative (gastric wash)	No	None	Total 12 mo 12 RZE, MFX	On treatment RZ, ETO, AMK, LFX, LNZ	45	1. Relapsed 2. On treatment
11	526Ser (AGC)	H	Pulmonary	Extensive	Positive	No	None	Total 12 mo 1 HRZE 11 RZE, LFX		108	Completed

Table 1 continued.

Patient	*rpoB* Mutation	Drug Resistance on Phenotypic DST	Site(s) of Disease	Severity of Disease	Initial Smear Status	Prior TB Treatment	Medical Comorbidities	Initial Treatment Regimen	Retreatment Regimen	Time(s) to Culture Conversion (Days)[a]	(Re)Treatment Outcome(s)[a]
12	526Ser (AGC)	H, E	Disseminated	Extensive	Negative	No	None	Total 18 mo 1 E, LFX, LNZ, CS, AMK 5 E, LFX, LNZ, CS, SM 3 E, LFX, LNZ, CS 9 ETO, LFX, CS		65	Completed
13	526Ser (TCC)	H, E, SM, ETO	Pulmonary	Extensive	Positive	Yes	Hypertension	Total 12 mo 2 RZ, MFX, CS, AMK 10 RZ, MFX		40	Completed
14	526Cys (TGC)	H, Z, E, SM, ETO, MFX, CIPRO, PAS	Pulmonary	Minimal	Negative	No	None	Total 20 mo 1 HRZE 1 AK, PAS, LNZ, MFX 5 AK, CS, PAS, LNZ, RFB, MFX 13 CS, PAS, LNZ, RFB		37	Cured
15	533Pro (CCG)	H, Z, E	Pulmonary	Moderate	Negative	No	None	Total 18 mo 1 HRZE 3 R, AMK, CS, PAS, LNZ, MFX 12 R, CS, PAS, MFX 2 R, CS, PAS, MFX		28	Completed
16	533Pro (CCG)	H, SM, LFX, Clofaz, OFX, CIPRO	Pulmonary	Extensive	Positive	Yes	Diabetes Lymphoma Hepatitis C	Total 14 mo 14 RE		307	Moved

Abbreviations: AMK, amikacin; CIPRO, ciprofloxacin; CLF, clofazimine; CM, capreomycin; CNS, central nervous system; CS, cycloserine; CV, cardiovascular; DST, drug-susceptibility testing; E, ethambutol; ETO, ethionamide; H, isoniazid; LFX, levofloxacin; LNZ, linezolid; MFX, moxifloxacin; OFX, ofloxacin; PAS, para-aminosalicylic acid; R, rifampin; RFB, rifabutin; SM, streptomycin; TB, tuberculosis; Z, pyrazinamide.

[a] If 2 outcomes listed, first outcome is for initial treatment and second outcome is for retreatment outcome.

[b] Minimal pulmonary involvement but extensive extrapulmonary involvement.

Table 2. Frequency and Treatment Outcomes by _rpoB_ Mutations Conferring Molecular-Phenotypic Discordance, California

Mutation	No. Isolates With Mutation	No. Patients Failed Treatment or Relapsed After Treatment Completion
511Pro (CCG)	3	1
516Phe (TTC)	1	0
526Asn (AAC)	6	4
526Ser (AGC)	2	0
526Ser (TCC)	1	0
526Cys (TGC)	1	0
533Pro (CCG)	2	0

differences. The presence of INH resistance, and in some cases resistance to other anti-TB medications, likely contributed to providers' decision to use expanded regimens, unlike clinical management in international settings where initial and retreatment regimens are often standardized and include INH. Moreover, limited data suggest that strains with molecular-phenotypic discordance may retain RFB sensitivity or have a slightly higher RIF minimal inhibitory concentration than that of wild-type strains. Recent data have identified 10 such mutations with RIF resistance but RFB susceptibility [36]. Similar to the other studies, in our case series, RIF or RFB were often included in the regimen, suggesting that inclusion of a rifamycin in the regimen may be clinically beneficial [37, 38].

We found that mutations associated with molecular-phenotypic discordance are rare in our tested population. In our study, although 3.9% of specimens with a _rpoB_ mutation had a discordant mutation, this was only 0.5% of all specimens tested. This finding occurred despite the fact that the MDL typically tests isolates from patients at increased risk for drug resistance. With the preselected population, our observed rate of _rpoB_ mutations likely represents a higher estimate than the true population prevalence.

This was a retrospective study, and it is subject to limitations. Tuberculosis treatment failure and relapses may be associated with factors such as disease severity, comorbidities (eg, diabetes, human immunodeficiency virus infection), nutrition status, drug levels, impaired drug absorption, regimen variability, treatment duration, drug resistance profile, smear result, time to culture conversion, and _Mtb_ strain variability [32, 39]. Although we were able to collect several of these data elements, our case series was too small to identify characteristics associated with treatment failure. We also did not have a control group to assess the clinical impacts that may be associated with each mutation. Because of the small number of cases included in the study and expanded regimens being used to treat many patients, we only observed poor clinical outcomes associated with 511Pro and 526Asn mutations. This does not imply the absence of an association of poor outcomes with other mutations, rather all molecular-phenotypic discordance should be reviewed and expanded treatment regimens should be considered in conjunction with clinical factors. Furthermore, there may be other unidentified

mutations in the genome other than those associated with molecular-phenotypic discordance that could affect our ability to evaluate the impact of the mutations in our study on treatment outcomes. Lastly, because only TB isolates with a suspicion for drug resistance are sent for molecular testing, we do not have molecular results for all TB isolates during this period. Therefore, we do not know the exact prevalence of these mutations and whether there were patients who were successfully treated with the standard regimen but whose TB bacilli mutations conferred molecular-phenotypic discordance.

CONCLUSIONS

In 2013, the WHO released a policy update for new TB diagnostic tests that stated that it is uncertain whether and to what extent Xpert MTB/RIF—a molecular assay that detects the presence of _Mtb_ bacilli and RIF resistance—might outperform the phenotypic methods of DST in assessing RIF resistance [40]. As data were collected for this study, we gained a better understanding of the possible clinical implications of these mutations, and our consultations to providers on how to interpret these mutations were updated to encourage providers to consider both phenotypic and molecular DST results when determining a treatment regimen. Although molecular-phenotypic discordance is considered to be rare, data are based on small studies, and a more systematic analysis of the role of these _rpoB_ mutations is needed to confirm the prevalence of these mutations, especially among patients with INH-resistant TB bacilli, and their role in treatment outcomes. With the expansion of the use of Xpert MTB/RIF, we are likely to see more discordant results in the future. Although more data are needed, clinicians should consider expanded regimens or seek consultation from expert TB clinicians for patients with a mutation conferring molecular-phenotypic discordance especially when INH resistance is present. Tuberculosis expertise and experienced TB control programs and an understanding of these new mutations are critical components for reducing relapses or treatment failure.

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