





Natural Polymorphisms in *Mycobacterium tuberculosis* Conferring Resistance to Delamanid in Drug-Naive Patients

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ABSTRACT Mutations in the genes of the F_{420} signaling pathway of *Mycobacterium tuberculosis* complex, including *dnn*, *fgd1*, *fbIA*, *fbIB*, *fbIC*, and *fbID*, can lead to delamanid resistance. We searched for such mutations among 129 *M. tuberculosis* strains from Asia, South America, and Africa using whole-genome sequencing; 70 (54%) strains had at least one mutation in one of the genes. For 10 strains with mutations, we determined the MIC of delamanid. We found one strain from a delamanid-naive patient carrying the natural polymorphism Tyr29del (*ddn*) that was associated with a critical delamanid MIC.

KEYWORDS *Mycobacterium tuberculosis*, delamanid, resistance, mutations, drug resistance, natural polymorphism

In 2014, the new antituberculosis (anti-TB) drug delamanid (also known as OPC-67683, or Delytba) was introduced (1). The World Health Organization (WHO) recommends the administration of delamanid if a standard effective drug regimen cannot be prescribed due to drug toxicity or resistance (2, 3). Thus, the European Medicines Agency (EMA) conditionally approved delamanid for the treatment of multidrug-resistant (MDR) TB (1, 3, 4). Of note, 6 years after its market launch, robust and widely accepted breakpoints that define susceptibility and resistance to delamanid still do not exist (5). The few available studies suggest a critical MIC between 0.125 mg/liter and 0.2 mg/liter, and an epidemiological cutoff value (ECOFF) of 0.04 mg/liter (6–9). This ECOFF is in line with the WHO technical report (10).

Delamanid is a drug of the bicyclic nitroimidazole class with potent anti-TB activity (1, 11). It is a prodrug that is activated by the deazaflavin (F_{420})-dependent nitroreduc-

Citation Reichmuth ML, Hömke R, Zürcher K, Sander P, Avihingsanon A, Collantes J, Loiseau C, Borrell S, Reinhard M, Wilkinson RJ, Yotebieng M, Fenner L, Böttger EC, Gagneux S, Egger M, Keller PM, on behalf of the International epidemiology Databases to Evaluate AIDS (IeDEA). 2020. Natural polymorphisms in *Mycobacterium tuberculosis* conferring resistance to delamanid in drug-naive patients. *Antimicrob Agents Chemother* 64:e00513-20. <https://doi.org/10.1128/AAC.00513-20>.

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Received 9 April 2020

Returned for modification 6 July 2020

Accepted 19 August 2020

Accepted manuscript posted online 31 August 2020

Published 20 October 2020

tase (*ddn*) through hydride transfer, forming unstable intermediates, which in turn lead to the formation of reactive nitrogen species (nitric oxide, nitrous acid) (12, 13). Activated delamanid thus has a dual bactericidal mode of action: the primary decomposition product prevents mycolic acid synthesis, while the reactive nitrogen species cause respiratory poisoning (12–15). Loss-of-function mutations in *ddn* or one of the genes encoding the five coenzymes (*fgd1*, *fbiA*, *fbiB*, *fbiC*, and *fbiD*) have been proposed as a mechanism of resistance to delamanid (12, 13, 16, 17). *In vitro*, the frequencies of delamanid resistance-conferring mutations in the *Mycobacterium tuberculosis* laboratory strain H37Rv and in *Mycobacterium bovis* range from 2.51×10^{-5} to 6.44×10^{-6} (13). Previous studies have found several resistance-conferring mutations, including Leu107Pro (*ddn*), 51–101del (*ddn*), Trp88STOP (*ddn*), Gly81Asp (*ddn*), Gly81Ser (*ddn*), Gly53Asp (*ddn*), c.146_147insC (*fgd1*), Gln88Glu (*fgd1*), Lys250STOP (*fbiA*), Arg175His (*fbiA*), and Val318Ile (*fbiC*) (6–8, 18–22).

This multicenter study has been described in detail elsewhere and is part of the work of the International epidemiology Databases to Evaluate AIDS (IeDEA) (23). We identified putative delamanid resistance-conferring mutations in *M. tuberculosis* strains from TB patients living with HIV (PLWH) and delamanid-naïve, HIV-negative TB patients by whole-genome sequencing (WGS) and MIC determination. We collected information on the demographic and clinical characteristics of patients who were recruited between 2013 and 2016 in Peru, Thailand, Côte d'Ivoire, the Democratic Republic of the Congo (DRC), Kenya, and South Africa (24, 25). The Cantonal Ethics Committee in Bern, Switzerland, and local institutional review boards approved the study. Written informed consent was obtained at all locations, except in South Africa, where consent was not required for archived samples.

The sequencing pipeline has been described previously (25). In brief, *M. tuberculosis* DNA was extracted and sequenced using the Illumina HiSeq 2500 system (Illumina, San Diego, CA, USA). For the analysis, we used the well-established pipeline TBProfiler (<https://github.com/jodyphelan/TBProfiler>) (26, 27). It aligns short reads to the *M. tuberculosis* reference strain H37Rv (GenBank accession no. [NC_000962.3](https://ncbi.nlm.nih.gov/nuccore/NC_000962.3)) with bowtie2 (v2.3.5), BWA (v0.7.17), or minimap2 (v2.16) and then calls variants with SAMtools (v1.9) (28–31). To identify putative delamanid resistance-conferring mutations, we analyzed *F*₄₂₀ genes (*ddn*, *fgd1*, *fbiA*, *fbiB*, *fbiC*, and *fbiD*) with variant frequencies of $\geq 75\%$. A subset of *M. tuberculosis* strains with at least one mutation in the *F*₄₂₀ genes was recultured in liquid medium and subjected to delamanid MIC determination (see Fig. S1 in the supplemental material). We assumed that 0.04 mg/liter indicates a critical MIC (9).

We included 129 *M. tuberculosis* isolates, among them 51 isolates (39.5%) from Peru, 13 (10.1%) from Thailand, 49 (38%) from Côte d'Ivoire, 14 (10.9%) from the DRC, and 1 (0.8%) each from Kenya and South Africa. We identified 70 (54.3%) isolates with polymorphisms in at least one of the six *F*₄₂₀ genes compared to the reference genome (Table S1). All patients infected with either of these strains were naïve to delamanid. We selected strains fulfilling the following criteria: (i) mutations in a part of the gene encoding regions of catalytic or structural importance predicted by ARIBA and then the PhyResSE pipeline (32, 33), (ii) availability of a culture of the strain, and (iii) bacterial growth amenable to microdilution (25). MICs were determined for 10 isolates with mutations in the *F*₄₂₀ genes. Four isolates showed MICs of >0.015 mg/liter: specifically, MICs of 0.5 (patient 1), 0.03 (patients 6 and 10), and >8 (patient 9) mg/liter (Table 1; Fig. S1). The isolate from patient 1 had a polymorphism in *fgd1* (Lys270Met) and was susceptible to the six drugs tested (isoniazid, rifampin, ethambutol, pyrazinamide, moxifloxacin, and amikacin). The patient was cured. The isolate from patient 9 had two alterations: a deletion in *ddn* (Tyr29del) and a nucleotide change in *fgd1* (T960C). The strain showed an elevated delamanid MIC and was phenotypically susceptible to six other drugs tested. The patient died. The MIC for the isolates of patients 10 and 6 was above 0.015 but below 0.04 mg/liter (Table 1). This suggests low-level resistance to delamanid (22), which could be due to the combination of various mutations: Ala416Val (*fbiC*), Trp678Gly (*fbiC*), Arg64Ser (*fgd1*), and T960C (*fgd1*).

TABLE 1 Observed polymorphisms in *F*₄₂₀ genes and MIC values of delamanid^a

Patient no. or reference	Lineage	Country	HIV status	Age (yr) at TB diagnosis	Gender	Mutation(s) in the <i>F</i> ₄₂₀ genes	Treatment outcome	MIC (mg/liter) in the microdilution
Reference	H37Rv (ATCC 27294)					Control (wt)		≤0.015
1	L4.1.2.1	Côte d'Ivoire	Negative	29	Female	<i>fgd1</i> Lys270Met	Cured	0.5
2	L4.6.2.2	Côte d'Ivoire	Negative	51	Male	<i>ddn</i> C168T	Died	≤0.015
3	L2.2.1	Kenya	Positive	40	Male	<i>fgd1</i> T960C	Died	≤0.015
4	L2.2.1	Peru	Positive	28	Male	<i>fgd1</i> T960C	Unknown	≤0.015
5	L4.3.2	Peru	Negative	21	Male	<i>fbtC</i> C1161T	Cured	≤0.015
6	L4.1.2.1	Peru	Positive	45	Male	<i>fgd1</i> Lys270Met	Unknown	0.03
7	L4.1.2.1	Peru	Positive	36	Male	<i>fbtC</i> G-11A, <i>fgd1</i> Lys270Met	Unknown	≤0.015
8	L4.1.2	South Africa	Negative	57	Female	<i>fbtA</i> Ile208Val	Cured	≤0.015
9	L2.2.1	Thailand	Unknown	76	Male	<i>fgd1</i> T960C, <i>ddn</i> 85-87del (Tyr29del)	Died	>8
10	L1.1.1	Thailand	Negative	42	Male	<i>fbtC</i> Ala416Val Trp678Gly, <i>fgd1</i> Arg64Ser T960C	Unknown	0.03

^aAll patients were treated with 2 months of daily isoniazid, rifampin, pyrazinamide, and ethambutol, followed by 4 months of daily rifampin and isoniazid. Data for isolates for which the MIC was >0.015 are shown in boldface. wt, wild type; L, lineage.

In summary, in the subset of 10 isolates with polymorphisms in the six targeted genes, six had no elevated MIC in the microdilution, while four isolates had elevated MICs (Table 1). In line with previous studies, we found that Lys270Met in *fgd1* is a natural polymorphism characteristic of *M. tuberculosis* lineage 4.1.2.1, which may (patients 1 and 6) or may not (patient 7) lead to an increased delamanid MIC (19, 34, 35). All 16 strains of lineage 4.1.2.1 showed this lineage-specific marker (Table S1). Furthermore, T960C (*fgd1*) is a synonymous substitution and was found in three other patient isolates which, as expected, did not have a critical MIC. The increase in the delamanid MIC for the isolate of patient 9 was due to the deletion in *ddn* (7). Our results thus suggest that Tyr29del is a natural polymorphism leading to an increased delamanid MIC. Our study was too small to estimate the prevalence of strains that are naturally resistant to delamanid. In 2020, Lee et al. screened 14,876 *M. tuberculosis* strains and found 2 strains with Tyr29del, for a prevalence of 0.013% (36). However, in their study, only the *ddn* gene was screened, and the prevalence of natural resistance could, therefore, be higher.

In conclusion, we confirm that mutations in *F*₄₂₀ genes can confer an elevated delamanid MIC (13, 19). Whether our findings also apply to the related drug pretomanid should be investigated in future studies. The occurrence of clinical *M. tuberculosis* isolates from previously untreated patients for which delamanid MICs are naturally elevated calls for careful drug susceptibility testing (DST) prior to delamanid treatment (5, 36). However, access to DST is limited in high-burden countries. This dilemma highlights the conflict between making new drugs available in high-burden countries and avoiding the spread of drug-resistant strains.

Data availability. WGS data from patients' *M. tuberculosis* strains shown in Table 1 have been submitted to the NCBI (BioProject accession no. PRJNA300846) (Table S1).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.02 MB.

ACKNOWLEDGMENTS

We thank those at all sites who participated, the patients whose data were used in this study, and Marie Ballif for contributing to the data collection and for critical reading of the manuscript. Calculations were performed on UBELIX (<http://www.id.unibe.ch/hpc>), the HPC cluster at the University of Bern.

This research was supported by the Swiss National Foundation (project grants 153442, 310030_166687, 310030_188888, IZRJZ3_164171, IZLSZ3_170834, and CRSII5_

177163). The International epidemiology Databases to Evaluate AIDS (IeDEA) is supported by the U.S. National Institutes of Health's National Institute of Allergy and Infectious Diseases, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Cancer Institute, National Institute of Mental Health, National Institute on Drug Abuse, National Heart, Lung, and Blood Institute, National Institute on Alcohol Abuse and Alcoholism, National Institute of Diabetes and Digestive and Kidney Diseases, Fogarty International Center, and National Library of Medicine (Asia- Pacific, U01AI069907; CCASAnet, U01AI069923; Central Africa, U01AI096299; East Africa, U01AI069911; NA-ACCORD, U01AI069918; Southern Africa, U01AI069924; West Africa, U01AI069919). R.J.W. receives support from the Francis Crick Institute, which is funded by UKRI, CRUK, and the Wellcome Trust (grants FC0010218, 104803, and 203135). This work is solely the responsibility of the authors and does not necessarily represent the official views of any of the institutions mentioned above.

We have no potential conflicts of interest to disclose.

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