



Correlation between GenoType MTBDR_{plus} Assay and Phenotypic Susceptibility Test for Prothionamide in Patients with Genotypic Isoniazid Resistance

Joo Hee Lee, M.D.¹, Kyung-Wook Jo, M.D., Ph.D. and Tae Sun Shim, M.D., Ph.D.¹

Department of Pulmonary and Critical Care Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

Background: The purpose of this study was to analyze the relationship between the gene mutation patterns by the GenoType MTBDR_{plus} (MTBDR_{plus}) assay and the phenotypic drug susceptibility test (pDST) results of isoniazid (INH) and prothionamide (Pto).

Methods: A total of 206 patients whose MTBDR_{plus} assay results revealed *katG* or *inhA* mutations were enrolled in the study. The pDST results were compared to mutation patterns on the MTBDR_{plus} assay.

Results: The *katG* and *inhA* mutations were identified in 68.0% and 35.0% of patients, respectively. Among the 134 isolated *katG* mutations, three (2.2%), 127 (94.8%) and 11 (8.2%) were phenotypically resistant to low-level INH, high-level INH, and Pto, respectively. Among the 66 isolated *inhA* mutations, 34 (51.5%), 18 (27.3%) and 21 (31.8%) were phenotypically resistant to low-level INH, high-level INH, and Pto, respectively. Of the 34 phenotypic Pto resistant isolates, 21 (61.8%), 11 (32.4%), and two (5.9%) had *inhA*, *katG*, and both gene mutations.

Conclusion: It is noted that Pto may still be selected as one of the appropriate multidrug-resistant tuberculosis regimen, although *inhA* mutation is detected by the MTBDR_{plus} assay until pDST confirms a Pto resistance. The reporting of detailed mutation patterns of the MTBDR_{plus} assay may be important for clinical practice, rather than simply presenting resistance or susceptibility test results.

Keywords: *katG* Protein; *InhA* Protein; Isoniazid; Prothionamide; Genotype; Biological Assay; Disease Susceptibility; Research Design; *Mycobacterium tuberculosis*; *Mycobacterium*

Introduction

Tuberculosis (TB) is a global health problem. According to the World Health Organization (WHO) report, there were an estimated 10.4 million new TB cases worldwide in 2015, of which 480,000 cases were multidrug-resistant TB (MDR-TB), and an additional 100,000 cases were rifampin (RIF)-resistant TB that were also newly eligible for MDR-TB treatment¹. In Korea, isoniazid (INH) resistance rates were 9.9% in new patients and 24.1% in retreatment patients². The rate of MDR-TB among new TB cases increased from 2.7% in 2004 to 2.9% in 2008², and there were 30,892 new TB cases in 2016, of which 852 cases were MDR-TB³.

It is important to quickly identify the results of drug susceptibility testing (DST) in patients at increased risk of drug resistance and to choose individualized treatment regimens based on DST profiles. Korean guidelines for TB, issued in

Address for correspondence: Tae Sun Shim, M.D., Ph.D.

Department of Pulmonary and Critical Care Medicine, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Korea

Phone: 82-2-3010-3892, **Fax:** 82-2-3010-6968

E-mail: shimts@amc.seoul.kr

Received: Mar. 28, 2018

Revised: Apr. 28, 2018

Accepted: Jun. 25, 2018

Published online: Sep. 28, 2018

© It is identical to the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>).



Copyright © 2019

The Korean Academy of Tuberculosis and Respiratory Diseases.

2017, recommend that genotypic RIF resistance should be treated as MDR-TB until conventional DST results are identified⁴. Early detection of drug resistance allows immediate and effective treatment, which improves the prognosis and prevents MDR strains from transmission as well as acquisition of additional resistance. Although conventional DST using liquid media culture has shortened the time to 2 weeks compared to solid media culture using Löwenstein-Jensen (LJ) medium, recent advances in molecular tools, such as the detection of mutations associated with drug resistance, have helped to detect drug resistance earlier. Based on this, the WHO recommended the use of molecular line probe assays (LPAs) for rapid detection of MDR-TB in 2016⁵.

The GenoType MTBDR*plus* assay (MTBDR*plus* assay; Hain Lifescience, Nehren, Germany) is a rapid molecular diagnostic test that simultaneously detects the presence or absence of *Mycobacterium tuberculosis* and mutations causing resistance to specific drugs⁶. This assay is one of the commercial versions of LPAs that detect drug resistance to RIF and INH relying on specific probes immobilized on nitrocellulose strips. INH resistance is known to be mostly related with mutations in *katG* and *inhA* gene⁷⁻⁹. Mutations in *katG* have been found to be associated with a high-level INH resistance, while *inhA* mutations produce both low-level resistance to INH and high-level resistance to prothionamide (Pto), which is used for the treatment of MDR-TB¹⁰⁻¹⁴. Cross-resistance to Pto may occur in patients who had received INH but had never been treated with Pto¹⁵, and the frequency of cross-resistance in Korea has been reported to be 50%¹⁶.

Currently, Pto resistance can only be detected by phenotypic susceptibility test; however, the result of this test is often questioned due to low reliability and reproducibility¹⁷. Therefore, verifying the presence of *inhA* mutation helps determine the use of Pto in cases of MDR-TB before conventional DST is confirmed. Nevertheless, few studies have reported the correlation between the results of the MTBDR*plus* assay and phenotypic DST for Pto resistance.

In this study, we sought to analyze the relationship between gene mutation pattern according to the MTBDR*plus* assay and phenotypic INH and Pto susceptibility by conventional DST in patients with mutations on *katG* or *inhA* genes.

Materials and Methods

1. Study population

This study retrospectively enrolled patients whose MTBDR*plus* assay results revealed *katG* or *inhA* gene mutations from September 2009 to October 2016 at a single tertiary referral center, Asan Medical Center, Seoul, Korea. Baseline characteristics at the time of diagnosis were gathered, including age, sex, physical examination findings, body mass index (BMI),

and medical history (previous anti-TB treatment, diabetes mellitus, chronic liver disease, chronic kidney disease stage ≥ 2 , respiratory disease, transplantation, human immunodeficiency virus infection, anti-tumor necrosis factor [TNF]- α therapy, and hematologic malignancy). The study was approved by the Institutional Review Board of Asan Medical Center (approval number: 2017-1012). The requirement for informed consent was waived due to the retrospective nature of the study.

2. Collection of specimens and drug susceptibility test

Specimens from lung (sputum, bronchial washing by bronchofibroscope, and lung tissue by percutaneous needle aspiration) and other involved organs (cerebrospinal fluid, intestine, lymph nodes, pleural fluid, kidney, bone, and muscle) were included. All specimens were subjected to Ziehl-Neelsen staining for examination of acid-fast bacilli (AFB) on smear and culture using both solid (Ogawa medium; Korean Institute of Tuberculosis, Cheongju, Korea) and liquid (BACTEC 960 Mycobacterial Growth Indicator Tube; Becton Dickinson, Sparks, MD, USA) media. MTBDR*plus* assay was performed on AFB-positive specimens or clinical isolates in patients with increased risk of drug resistance. Cultured clinical isolates were subjected to conventional DST. Conventional DST was evaluated by the absolute concentration method with LJ media at the Korean Institute of Tuberculosis, a Supranational TB Reference Laboratory. Growth exceeding that of the control wells by $>1\%$ was considered to indicate drug resistance. A low-level resistance to INH was defined as resistance to 0.2 $\mu\text{g}/\text{mL}$ but susceptible to 1 $\mu\text{g}/\text{mL}$ of INH, while a high-level resistance was defined as resistance to INH of 1 $\mu\text{g}/\text{mL}$. Isolates were tested for resistance to RIF using concentrations of 40 $\mu\text{g}/\text{mL}$. The MTBDR*plus* assay was performed according to the manufacturer's instructions. The assay detects the presence or absence of wild-type (WT) and mutant DNA sequences in *rpoB*, *katG*, and *inhA*, which are known to be associated with RIF and INH resistance. There are 27 reaction lines comprising six control lines and 21 WT or mutation reaction lines (MUT) as follows: eight *rpoB* WT (codon 505 to 533) and four MUT bands (MUT1, D516V; MUT2A, H526Y; MUT2B, H526D; MUT3, S531L), one *katG* WT (S315 region), and two MUT bands (MUT1, S315T[AGC315ACC]; MUT2, S315T[AGC315ACA]), two *inhA* WT (WT1, positions -15 to -16; WT2, positions -8), and four MUT bands (MUT1, C15T; MUT2, A16G; MUT3A, T8C; MUT3B, T8A). A gene mutation indicates the presence of MUT band and/or loss of WT band.

3. Statistical analysis

Descriptive statistics were used to describe patient characteristics. Categorical variables were reported as counts and percentages and compared using the Pearson's chi-square test or Fisher exact test. Continuously measured parameters were

reported as means±standard deviation and were compared using student's t-test. Statistical significance was indicated for p-values of <0.05. All statistical analyses were completed using SPSS version 21.0 (IBM Corp., Armonk, NY, USA).

Results

1. Baseline clinical characteristics

In this retrospective study, the authors reviewed 206 patients whose MTBDRplus assay results revealed *katG* or *inhA* gene mutations. The most common type of infection was

pulmonary TB (n=185, 89.8%) followed by disseminated TB (n=12, 5.8%) and extrapulmonary TB (total n=9, 4.4%; lymphadenitis, n=5; intestine, n=2; kidney, n=1; osteomyelitis, n=1). Specimens were obtained from sputum (n=185), bronchial washing (n=3), lung tissue (n=1), and other involved organs (lymph node, n=6; pleural fluid, n=4; cerebrospinal fluid, n=2; intestine, n=2; renal abscess, n=1; spine, n=1; muscle, n=1).

Stratifying by genotypic resistance to RIF, 54.9% isolates were found to be MDR and 45.1% were found to be INH mono-resistant by MTBDRplus assay (Table 1). Patients with MDR-TB were younger (43.0±16.1 vs. 50.9±16.9 years, p=0.001) and had a more frequent history of previous TB treatment (53.1% vs. 20.4%, p<0.001) than those with INH mono-resistance. There

Table 1. The baseline characteristics of 206 patients with a genotypic resistance to isoniazid, stratified by the genotypic rifampin resistance

Variable	INH mono-resistance	MDR-TB	Total	p-value
No. of subjects	93 (45.1)	113 (54.9)	206 (100)	
Age, yr	50.9±16.9	43.0±16.1	46.6±17.0	0.001
Male sex	62 (66.7)	70 (61.9)	132 (64.1)	0.482
BMI, kg/m ²	21.3±3.4	21.1±3.0	21.2±3.2	0.745
Previous TB history	19 (20.4)	60 (53.1)	79 (38.3)	<0.001
Concomitant disease				
Diabetes mellitus	12 (12.9)	19 (16.8)	31 (15.0)	0.435
Chronic liver disease*	10 (10.8)	3 (2.7)	13 (6.3)	0.017
Chronic kidney disease	4 (4.3)	1 (0.9)	5 (2.4)	0.177
Respiratory disease [†]	7 (7.5)	5 (4.4)	12 (5.8)	0.344
Transplantation	5 (5.4)	2 (1.8)	7 (3.4)	0.248
Others [‡]	7 (7.5)	5 (4.4)	12 (5.8)	0.344
Positive HIV test	1 (1.1)	0 (0)	1 (0.5)	0.451
Phenotypic DST results				
INH-mono resistance	75 (80.6)	6 (5.3)	81 (39.3)	
High-level resistance	54 (58.1)	4 (3.5)	58 (28.2)	
Low-level resistance	21 (22.6)	2 (1.8)	23 (11.2)	
MDR-TB	2 (2.2)	105 (92.9)	107 (51.9)	
RIF-mono resistance	0 (0)	2 (1.8)	2 (1.0)	
Susceptible to INH/RIF	16 (17.2)	0 (0)	16 (7.8)	

Values are presented as number (%).

*Chronic liver disease included alcoholic LC and chronic hepatitis B and C. [†]Respiratory disease included lung/tracheal cancer (n=4), COPD (n=2), and pneumoconiosis (n=1) in INH-mono resistance, and lung cancer (n=3), COPD (n=1), and IPF (n=1) in MDR-TB. [‡]Other comorbidities in INH-mono resistant TB patients included Crohn's disease (n=2; one with anti-TNF-α therapy and one without), hematologic malignancy without bone marrow transplantation (n=2; one with myelodysplastic syndrome and one with lymphoma), adrenal insufficiency (n=1), c-ANCA associated vasculitis (n=1), and IgA nephropathy with normal kidney function (n=1). MDR-TB patients had rheumatoid arthritis on anti-TNFα therapy (n=1), hematologic malignancy without bone marrow transplantation (n=2; one with acute myeloid leukemia and one with lymphoma), and adrenal insufficiency (n=2).

INH: isoniazid; MDR-TB: multidrug resistant tuberculosis; BMI: body mass index; TB: tuberculosis; HIV: human immunodeficiency virus; DST: drug sensitivity test; RIF: rifampin; LC: liver cirrhosis; COPD: chronic obstructive pulmonary disease; IPF: idiopathic pulmonary fibrosis; TNF-α: tumor necrosis factor α.

was no difference in sex and BMI. Comorbidities were similar in both groups except for chronic liver disease (2.7% in MDR-TB vs. 10.8% in INH mono-resistance, $p=0.017$).

2. Mutation patterns of *katG* and *inhA* genes by MTBDRplus assay

Table 2 shows mutation patterns of *katG* and *inhA* genes detected by the MTBDRplus assay. *katG* mutations were identified in 140 of 206 isolates (68.0%) and *inhA* mutations were identified in 72 of 206 isolates (35.0%). Except for six patients with both *katG* and *inhA* mutations, MDR-TB patients had more frequent mutations in *katG* than INH mono-resistant TB patients (69.9% vs. 59.1%, $p=0.107$), although this difference was not statistically significant. In contrast, MDR-TB patients had significantly less frequent *inhA* mutations (25.7% vs. 39.8%, $p=0.031$).

3. Correlation between phenotypic and genotypic susceptibility results based on mutation patterns of *katG* and *inhA* genes

The concordances of the MTBDRplus assay and the conventional DST for the detection of INH and Pto susceptibility are shown in Table 3. Strains with isolated *katG* mutation presented phenotypic INH resistance in 97.0% and phenotypic Pto resistance in 8.2% of cases. Among the isolates with isolated *inhA* mutation, 78.8% were identified as phenotypic INH resistant and 31.8% as phenotypic Pto resistant. Of all genotypic INH resistant patients, 2.9% had mutations in both the

katG and *inhA* genes, all of which were resistant to INH, and 33.3% were resistant to Pto. Except for six patients with both *katG* and *inhA* mutations, the presence of *katG* mutation presented high-level INH resistance in most cases (94.8%) while over half of the patients with *inhA* mutation (51.5%) showed low-level resistance. Of the 34 phenotypic Pto resistant isolates, 21 (61.8%) had an *inhA* mutation, 11 (32.4%) had a *katG* mutation, and two (5.9%) had both mutations.

Among the 134 strains with isolated *katG* mutation, MUT1 band was observed in 119 (88.8%) isolates, and positive MUT2 band and WT loss alone were observed in eight (6.0%) and seven (5.2%), respectively. Among the 66 isolates with isolated *inhA* mutation, the most common type was MUT1 ($n=60$, 91.0%), followed by WT1 loss alone ($n=4$, 6.1%) and MUT3A ($n=1$, 1.5%), and MUT3B ($n=1$, 1.5%). Among 127 strains with isolated *katG* mutant band (MUT1 or MUT2), 123 (96.9%) presented resistance to INH and 11 (8.7%) to Pto. Isolates with *katG* WT loss alone were all high-level INH resistant and Pto susceptible ($n=7$). Among 62 strains with isolated *inhA* mutant band (MUT1, MUT2, MUT2A, or MUT3B), 51 (82.3%) were INH resistant and 21 (33.9%) were Pto resistant. *inhA* WT loss alone was found in four isolates, of which one (25%) showed high-level INH-resistance and none showed Pto resistance.

Discussion

This study analyzed the relationship between gene mutation pattern and phenotypic INH and Pto resistance using the

Table 2. The mutation patterns of the *katG* and *inhA* genes in 206 patients with a genotypic INH resistance

Group	INH mono-resistance	MDR-TB	Total	p-value
No. of subjects	93 (45.1)	113 (54.9)	206 (100)	
<i>katG</i> mutation alone	55 (59.1)	79 (69.9)	134 (65.0)	0.107
Mutant probe (+)	50 (53.8)	77 (68.1)	127 (61.7)	0.035
Wild-type band loss alone	5 (5.4)	2 (1.8)	7 (3.4)	0.248
<i>inhA</i> mutation alone (%)	37 (39.8)	29 (25.7)	66 (32.0)	0.031
Mutant probe (+)	33 (35.5)	29 (25.7)	62 (30.1)	0.126
Wild-type band loss alone	4 (4.3)	0 (0)	4 (1.9)	0.040
Both gene mutation	1 (1.1)	5 (4.4)	6 (2.9)	0.225
<i>katG</i> (MUT1), <i>inhA</i> (MUT1)	0 (0)	2 (1.8)	2 (1.0)	
<i>katG</i> (MUT1), <i>inhA</i> (WT1 loss)	1 (1.1)	0 (0)	1 (0.5)	
<i>katG</i> (MUT1), <i>inhA</i> (WT2 loss)	0 (0)	1 (0.9)	1 (0.5)	
<i>katG</i> (WT loss), <i>inhA</i> (MUT1)	0 (0)	1 (0.9)	1 (0.5)	
<i>katG</i> (WT loss), <i>inhA</i> (WT2 loss)	0 (0)	1 (0.9)	1 (0.5)	

Values are presented as number (%).

INH: isoniazid; MDR-TB: multidrug-resistant tuberculosis; *katG* WT: S315 region; *katG* MUT1: S315T[AGC315ACC]; *inhA* WT1: positions -15 to -16; *inhA* WT2: positions -8; *inhA* MUT1: C15T.

Table 3. The phenotypic susceptibility to INH and Pto based on the specific mutation patterns of *katG* and *inhA*

Mutation	Total	Phenotypic INH resistance			Phenotypic Pto resistance
		High-level	Low-level	Total	
No. of subjects	206 (100)	149 (72.3)	39 (18.9)	188 (91.3)	34 (16.5)
<i>katG</i> gene alone	134 (65.0)	127 (94.8)	3 (2.2)	130 (97.0)	11 (8.2)
MUT1 (+)	119 (57.8)	112 (94.1)	3 (2.5)	115 (96.6)	9 (7.6)
MUT2 (+)	8 (3.9)	8 (100)	0 (0)	8 (100)	2 (25)
WT (-)/MUT1 (-)/MUT2 (-)	7 (3.4)	7 (100)	0 (0)	7 (100)	0 (0)
<i>inhA</i> gene alone	66 (32.0)	18 (27.3)	34 (51.5)	52 (78.8)	21 (31.8)
MUT1 (+)	60 (29.1)	17 (28.3)	34 (56.7)	51 (85)	21 (35)
MUT2 (+)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
MUT3A (+)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)
MUT3B (+)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)
WT1 (-) and all MUT (-)	4 (1.9)	1 (25)	0 (0)	1 (25)	0 (0)
WT2 (-) and all MUT (-)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Both gene mutation	6 (2.9)	4 (66.7)	2 (33.3)	6 (100)	2 (33.3)
<i>katG</i> (MUT1), <i>inhA</i> (MUT1)	2 (1.0)	2 (100)	0 (0)	2 (100)	0 (0)
<i>katG</i> (MUT1), <i>inhA</i> (WT1 loss)	1 (0.5)	0 (0)	1 (100)	1 (100)	0 (0)
<i>katG</i> (MUT1), <i>inhA</i> (WT2 loss)	1 (0.5)	0 (0)	1 (100)	1 (100)	0 (0)
<i>katG</i> (WT loss), <i>inhA</i> (MUT1)	1 (0.5)	1 (100)	0 (0)	1 (100)	1 (100)
<i>katG</i> (WT loss), <i>inhA</i> (WT2 loss)	1 (0.5)	1 (100)	0 (0)	1 (100)	1 (100)

Values are presented as number (%).

INH: isoniazid; Pto: prothionamide; *katG* WT: S315 region; *katG* MUT1, S315T[AGC315ACC]; *katG* MUT2, S315T[AGC315ACA]; *inhA* WT1: positions -15 to -16; *inhA* WT2: positions -8; *inhA* MUT1: C15T; *inhA* MUT2, A16G; *inhA* MUT3A: T8C; *inhA* MUT3B: T8A.

MTBDR_{plus} assay. We analyzed 206 genotypic INH-resistant strains and found that 68.0% had a *katG* mutation and 35.0% had an *inhA* mutation. The presence of *katG* mutation presented high-level INH resistance in most case (94.8%), while over half of the patients with *inhA* mutation (51.5%) showed low-level resistance, which is consistent with previous studies¹⁰⁻¹². However, only 31.8% showed phenotypic Pto resistance in 66 patients with *inhA* mutations.

In our study, *katG* mutations were more frequent in MDR-TB isolates, and *inhA* mutations were significantly more frequent in INH mono-resistant isolates. According to previous studies, the frequency of S315T substitution in the *katG* gene is higher in MDR-TB than in INH mono-resistant TB^{18,19} and might be related to the higher transmission capabilities of MDR-TB²⁰.

Among the isolates with *katG* mutation, MUT1 (S315T) was the most common mutation. This is a transitional mutation G→C (AGC→ACC), resulting in the substitution of serine by threonine (S→T) in the amino acid chain. This S315T mutation has been found in up to 94% of the INH-resistant TB²¹, which is in agreement with our results. Among the isolates

with *inhA* mutation, MUT1 (-15C→T) in the *inhA* promoter was the most common mutation. This mutation is found in up to 35% of INH-resistant and 55% of Pto-resistant TB²², which is also in agreement with our results.

Phenotypic Pto resistance presented in 8.2% of isolated *katG* mutations and 31.8% of isolated *inhA* mutations. In phenotypic Pto resistant isolates, 61.8% had *inhA* mutation, which agrees with the results of previous studies. Vilcheze and Jacobs²² mentioned that the proportion of *inhA* mutations in Pto-resistant TB is 68% on average, suggesting that this is the main mechanism of Pto resistance in *M. tuberculosis*. Pto is a structural analog of INH used as a second-line drug in the treatment of MDR-TB. Both INH and Pto are classified as pro-drugs that need to be activated by different mycobacterial enzymes. INH is activated by the *katG*-encoded catalase-peroxidase²³, and Pto is activated by the NADPH-specific flavin adeninedinucleotide-containing monooxygenase EthA, which is regulated by EthR²⁴. Once activated, the mode of action of each drug is very similar such that the active forms of the drugs react with NAD⁺ to yield an INH-NAD or ETH-NAD adduct, which inhibits *inhA*, leading to disruption of mycolic

acid biosynthesis and cell death²². Therefore, mutations at the *inhA* gene can cause cross-resistance to INH and Pto by resulting in the overexpression or modification of the *inhA* target^{25,26}. In addition to the *inhA* mutation, *ndh* mutation causes cross-resistance to both drugs^{27,28}, and *ethA*, *ethR*, or *mshA* gene mutation also results in Pto mutation²². Because *inhA* mutation is considered as the major mechanism of Pto resistance in TB as previously mentioned, the use of LPAs would help physicians to get information about the use of Pto as well as RIF and INH. Nevertheless, few studies have reported the correlation between the results of the MTBDR*plus* assay and phenotypic DST for Pto resistance. Machado et al.²⁵ examined 17 MDR-TB isolates and found that the presence of a mutation in the *inhA* regulatory region together with a mutation in the *inhA* coding region can lead to the development of high-level INH resistance and cross-resistance to Pto. Niehaus et al.²⁹ sought to determine the proportion of patients who could potentially benefit from high-dose INH and who may be resistant to Pto. However, phenotypic Pto resistance was deduced from the *inhA* mutation because phenotypic DST was not performed. Rueda et al.³⁰ examined the relationship between mutations and phenotypic resistance; however, they used whole genome sequencing, which is generally difficult for widespread use in clinical fields. In this study, only 31.8% showed phenotypic Pto resistance in 66 patients with *inhA* mutations, suggesting that Pto may be selected as one of MDR-TB regimen in patients with *inhA* mutation detected by MTBDR*plus* assay until phenotypic DST confirms Pto resistance. There could be two main speculations for the discrepancy between our hypothesis and the results. First, the low reliability and reproducibility of conventional DST of Pto might be the cause of the discrepant results¹⁷. Secondly, other compensatory mechanisms might play a role in maintaining Pto susceptibility in *M. tuberculosis* strains. Tan et al.³¹ found that mutations in the promoter region of *inhA* and *inhA* gene in Pto-susceptible isolates were different from those in Pto-resistant isolates, and these mutations are more likely to appear together with other mutations in Pto-resistant isolates. They suggested that these mutations may show cumulative effect on increasing minimum inhibitory concentration values of *M. tuberculosis* against Pto. We also speculate that mutations in specific parts of *inhA* may not affect Pto susceptibility.

Although the number of cases in this study was small, isolates with *katG* WT loss alone were all high-level INH resistant, while isolates with *inhA* WT loss alone were INH-resistant in only 25% of cases. Han et al.³² found one of 144 isolates showed low-level INH resistance with *ahpC* mutation confirmed by other LPA (AdvanSure MDR-TB GenoBlot assay), which revealed susceptibility to INH by conventional DST. The authors interpreted that the discrepancy might result from false positive of LPA, low-level resistance suppressing growth of *M. tuberculosis* at 0.2 µg/mL of INH, or heteroresistance in which susceptible and resistant strains coexist.

Therefore, in the case of genotypic INH resistance with isolated *inhA* wild type loss, we recommend that the possibility of an INH susceptible strain should not be ruled out before the final phenotypic DST results are obtained. None of four patients with loss of WT *inhA* band alone showed Pto resistance. Due to the small number of subjects and the absence of gene sequencing data, we could not draw any conclusion from this data. Further studies are needed to reveal the real meaning of this finding.

This study has several limitations. In patients with discrepant results between conventional and molecular DST, gene sequencing, a gold standard method of molecular test, was not performed. The phenotypic DST of second-line drugs such as Pto is considered as an unreliable test, but this test, as a gold standard method, was compared to MTBDR*plus* assay results. Finally, even though we enrolled a large number of isolates with *katG* or *inhA* mutations, the number of isolates with *inhA* WT loss alone was too small to draw any firm conclusion.

The *katG* and *inhA* mutations detected by MTBDR*plus* assay are good indicators of phenotypic INH resistance. However, only about one-third of *inhA* mutations revealed phenotypic Pto resistance, suggesting that Pto may be selected as one of MDR-TB regimen in patients with *inhA* mutation detected by MTBDR*plus* assay until conventional DST confirms phenotypic Pto resistance. We suggest that the reporting of detailed mutation patterns of MTBDR*plus* assay would be important for clinical practice, rather than simply presenting the resistance or susceptibility results.

Authors' Contributions

Conceptualization: Shim TS. Methodology: Shim TS. Formal analysis: Lee JH, Jo KW. Data curation: Shim TS. Software: Lee JH. Validation: Jo KW, Shim TS. Investigation: Lee JH. Writing - original draft preparation: Lee JH. Writing - review and editing: Jo KW, Shim TS. Approval of final manuscript: all authors.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

References

1. World Health Organization. Global tuberculosis report 2016. WHO/HTM/TB/2016.13. Geneva: World Health Organization; 2016.
2. Bai GH, Park YK, Choi YW, Bai JI, Kim HJ, Chang CL, et al. Trend of anti-tuberculosis drug resistance in Korea, 1994-2004. *Int J Tuberc Lung Dis* 2007;11:571-6.

3. Korea Centers for Disease Control and Prevention. Annual report on the notified tuberculosis in Korea, 2016. Cheongju: Korea Centers for Disease Control and Prevention; 2016.
4. Korea Centers for Disease Control and Prevention. Korean guidelines for tuberculosis third edition, 2017. Cheongju: Korea Centers for Disease Control and Prevention; 2017.
5. World Health Organization. WHO treatment guidelines for drug-resistant tuberculosis, 2016 update. WHO/HTM/TB/2016.04. Geneva: World Health Organization; 2016.
6. Leylabadlo HE, Kafil HS, Yousefi M, Aghazadeh M, Asgharzadeh M. Pulmonary tuberculosis diagnosis: where we are? *Tuberc Respir Dis* 2016;79:134-42.
7. Rouse DA, Li Z, Bai GH, Morris SL. Characterization of the *katG* and *inhA* genes of isoniazid-resistant clinical isolates of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 1995;39:2472-7.
8. Guo H, Seet Q, Denkin S, Parsons L, Zhang Y. Molecular characterization of isoniazid-resistant clinical isolates of *Mycobacterium tuberculosis* from the USA. *J Med Microbiol* 2006;55(Pt 11):1527-31.
9. Seifert M, Catanzaro D, Catanzaro A, Rodwell TC. Genetic mutations associated with isoniazid resistance in *Mycobacterium tuberculosis*: a systematic review. *PLoS One* 2015;10:e0119628.
10. Brossier F, Veziris N, Truffot-Pernot C, Jarlier V, Sougakoff W. Performance of the genotype MTBDR line probe assay for detection of resistance to rifampin and isoniazid in strains of *Mycobacterium tuberculosis* with low- and high-level resistance. *J Clin Microbiol* 2006;44:3659-64.
11. Gali N, Dominguez J, Blanco S, Prat C, Alcaide F, Coll P, et al. Use of a mycobacteriophage-based assay for rapid assessment of susceptibilities of *Mycobacterium tuberculosis* isolates to isoniazid and influence of resistance level on assay performance. *J Clin Microbiol* 2006;44:201-5.
12. Lavender C, Globan M, Sievers A, Billman-Jacobe H, Fyfe J. Molecular characterization of isoniazid-resistant *Mycobacterium tuberculosis* isolates collected in Australia. *Antimicrob Agents Chemother* 2005;49:4068-74.
13. Morlock GP, Metchock B, Sikes D, Crawford JT, Cooksey RC. *ethA*, *inhA*, and *katG* loci of ethionamide-resistant clinical *Mycobacterium tuberculosis* isolates. *Antimicrob Agents Chemother* 2003;47:3799-805.
14. Engohang-Ndong J, Baillat D, Aumercier M, Bellefontaine F, Besra GS, Locht C, et al. EthR, a repressor of the TetR/CamR family implicated in ethionamide resistance in mycobacteria, octamerizes cooperatively on its operator. *Mol Microbiol* 2004;51:175-88.
15. Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um KS, Wilson T, et al. *inhA*, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*. *Science* 1994;263:227-30.
16. Lee H, Cho SN, Bang HE, Lee JH, Bai GH, Kim SJ, et al. Exclusive mutations related to isoniazid and ethionamide resistance among *Mycobacterium tuberculosis* isolates from Korea. *Int J Tuberc Lung Dis* 2000;4:441-7.
17. Lin SY, Desmond E, Bonato D, Gross W, Siddiqi S. Multicenter evaluation of Bactec MGIT 960 system for second-line drug susceptibility testing of *Mycobacterium tuberculosis* complex. *J Clin Microbiol* 2009;47:3630-4.
18. Unissa AN, Selvakumar N, Narayanan S, Suganthi C, Hanna LE. Investigation of Ser315 substitutions within *katG* gene in isoniazid-resistant clinical isolates of *Mycobacterium tuberculosis* from south India. *Biomed Res Int* 2015;2015:257983.
19. van Doorn HR, de Haas PE, Kremer K, Vandenbroucke-Grauls CM, Borgdorff MW, van Soolingen D. Public health impact of isoniazid-resistant *Mycobacterium tuberculosis* strains with a mutation at amino-acid position 315 of *katG*: a decade of experience in The Netherlands. *Clin Microbiol Infect* 2006;12:769-75.
20. Gagneux S, Burgos MV, DeRiemer K, Encisco A, Munoz S, Hopewell PC, et al. Impact of bacterial genetics on the transmission of isoniazid-resistant *Mycobacterium tuberculosis*. *PLoS Pathog* 2006;2:e61.
21. Mokrousov I, Narvskaya O, Otten T, Limeschenko E, Steklova L, Vyshnevskiy B. High prevalence of KatG Ser315Thr substitution among isoniazid-resistant *Mycobacterium tuberculosis* clinical isolates from northwestern Russia, 1996 to 2001. *Antimicrob Agents Chemother* 2002;46:1417-24.
22. Vilcheze C, Jacobs WR Jr. Resistance to isoniazid and ethionamide in *Mycobacterium tuberculosis*: genes, mutations, and causalities. *Microbiol Spectr* 2014;2:MGM2-0014-2013.
23. Lei B, Wei CJ, Tu SC. Action mechanism of antitubercular isoniazid: activation by *Mycobacterium tuberculosis* KatG, isolation, and characterization of *inhA* inhibitor. *J Biol Chem* 2000;275:2520-6.
24. Vannelli TA, Dykman A, Ortiz de Montellano PR. The anti-tuberculosis drug ethionamide is activated by a flavoprotein monooxygenase. *J Biol Chem* 2002;277:12824-9.
25. Machado D, Perdigao J, Ramos J, Couto I, Portugal I, Ritter C, et al. High-level resistance to isoniazid and ethionamide in multidrug-resistant *Mycobacterium tuberculosis* of the Lisboa family is associated with *inhA* double mutations. *J Antimicrob Chemother* 2013;68:1728-32.
26. Larsen MH, Vilcheze C, Kremer L, Besra GS, Parsons L, Salfinger M, et al. Overexpression of *inhA*, but not *kasA*, confers resistance to isoniazid and ethionamide in *Mycobacterium smegmatis*, *M. bovis* BCG and *M. tuberculosis*. *Mol Microbiol* 2002;46:453-66.
27. Vilcheze C, Weisbrod TR, Chen B, Kremer L, Hazbon MH, Wang F, et al. Altered NADH/NAD⁺ ratio mediates co-resistance to isoniazid and ethionamide in mycobacteria. *Antimicrob Agents Chemother* 2005;49:708-20.
28. Cardoso RF, Cardoso MA, Leite CQ, Sato DN, Mamizuka EM, Hirata RD, et al. Characterization of *ndh* gene of isoniazid resistant and susceptible *Mycobacterium tuberculosis* isolates from Brazil. *Mem Inst Oswaldo Cruz* 2007;102:59-61.

29. Niehaus AJ, Mlisana K, Gandhi NR, Mathema B, Brust JC. High prevalence of *inhA* promoter mutations among patients with drug-resistant tuberculosis in KwaZulu-Natal, South Africa. *PLoS One* 2015;10:e0135003.
30. Rueda J, Realpe T, Mejia GI, Zapata E, Rozo JC, Ferro BE, et al. Genotypic analysis of genes associated with independent resistance and cross-resistance to isoniazid and ethionamide in *Mycobacterium tuberculosis* clinical isolates. *Antimicrob Agents Chemother* 2015;59:7805-10.
31. Tan Y, Su B, Zheng H, Song Y, Wang Y, Pang Y. Molecular characterization of prothionamide-resistant *Mycobacterium tuberculosis* isolates in southern China. *Front Microbiol* 2017;8:2358.
32. Han SB, Jo Y, Yu JK, Kim Y, Park YJ. Performance assessment of Advansure MDR-TB Genoblot Assay Kit for anti-tuberculosis drug susceptibility test. *Lab Med Online* 2012;2:34-40.