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Mutation Characteristics of *inhA* and *katG* Genes in Isoniazid-Resistant Mycobacterium Tuberculosis Patients in Xinjiang

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Abstract: *Objective:* To analyze the mutation characteristics of *inhA* and *katG* genes in isoniazid-resistant Mycobacterium tuberculosis in Xinjiang. *Methods*: The *katG* and *inhA* in 148 strains of isoniazid-resistant Mycobacterium tuberculosis were amplified through fluorescence quantitative PCR, and the amplified products were sequenced and compared. *Results:* The *inhA* gene mutation rate of 148 strains of isoniazid-resistant mycobacterium tuberculosis was 13.51% (20/148), among which the inhA gene mutation rate among patients of Han, Uygur, and Kazakh ethnicity were 15.87%, 13.21%, and 17.65%, respectively. There was no significant difference in the *inhA* mutation rate among ethnic groups (c^2 = 2.897, $P > 0.05$). The mutation rate of the *katG* gene was 84.46% (125/148), among which the mutation rates of patients of Han, Uyghur, and Kazak ethnicities were 82.54%, 84.91%, and 76.47%, respectively. The Hui and other ethnic groups were all affected by the *katG* gene mutation. There was no significant difference in the mutation rate of the *katG* gene among different ethnicities (c^2 = 3.772, $P > 0.05$). The mutation rates of the *inhA* gene in southern Xinjiang, northern Xinjiang, and other provinces were 18.60%, 9.28%, and 37.50%, respectively. The mutation rates of the *inhA* gene in different regions were statistically different (c^2 = 6.381, $P < 0.05$). There was no significant difference in the *inhA* mutation rate between patients from southern and northern Xinjiang (c^2 = 2.214, $P > 0.05$) and between southern Xinjiang and other provinces (c^2 =1.424, $P > 0.05$). However, the mutation rate of the *inhA* gene in patients from other provinces was higher than that in northern Xinjiang (c^2 = 5.539, $P < 0.05$). The mutation rates of the *katG* gene in southern Xinjiang, northern Xinjiang, and other provinces were 81.40%, 87.63%, and 62.50%, respectively. There was no significant difference in the mutation rates of the *katG* gene among different regions (c^2 = 3.989, $P > 0.05$). *Conclusion: katG* gene mutation was predominant in isoniazid-resistant tuberculosis patients in Xinjiang Uygur Autonomous Region, and *inhA* and *katG* gene mutation were no different among different ethnic groups.

Keywords: Mycobacterium tuberculosis; Drug resistance; Isoniazid; Gene mutation

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1. Introduction

The 2021 Global Tuberculosis Report estimated that there were 9.9 million (95% CI: 8.9 million to 11 million) new tuberculosis cases in 2020, and the estimated global number of tuberculosis deaths was about 1.5 million. Among them, 1.3 million were HIV-negative tuberculosis deaths and 214,000 were HIV-positive patients, both higher than the 1.2 million and 209,000 in 2019, respectively [1]. Tuberculosis ranks first among the causes of death due to infectious diseases, making it a major global public health problem [2]. As a developing country with a large population, China ranks second among the 30 tuberculosis and multidrug-resistant countries with a high burden, further highlighting the severity of the situation [3]. Drug-resistant tuberculosis, especially multidrug-resistant TB, remains a difficult challenge for tuberculosis prevention and control.

Isoniazid (INH) is one of the most effective drugs for treating sensitive tuberculosis. The emergence of INH-resistant strains is detrimental to the efficacy of tuberculosis treatment. The main resistance mechanism of INH is *katG* and/or *inhA* gene mutation. The mutation rates of *katG* and *inhA* in INH-resistant Mycobacterium tuberculosis strains are affected by regional factors. In order to understand the gene mutations of INH-resistant Mycobacterium tuberculosis in the Xinjiang Uygur Autonomous Region, INH-resistant Mycobacterium tuberculosis samples that were isolated from the Eighth Affiliated Hospital of Xinjiang Medical University were tested for *katG* gene and *inhA* gene mutation. The prevalence of hydrazine resistance provides a technical basis for the rapid diagnosis of drug-resistant tuberculosis and the formulation of prevention and control policies in this region.

2. Materials and methods

INH-resistant tuberculosis mycobacterial strains were isolated from sputum samples of 148 pathogen-positive pulmonary tuberculosis patients from all over Xinjiang who were treated at the Eighth Affiliated Hospital of Xinjiang Medical from January 1, 2021, to August 8, 2022.

2.1. Experimental methods

2.1.1. Mycobacterium tuberculosis culture

First, the patient's sputum or bronchoalveolar lavage fluid specimens were preprocessed, and 0.5 mL of the processed specimen was placed into a BD960 special tube and cultured in the BD960 cell culture instrument.

2.2.2. Isoniazid susceptibility test of Mycobacterium tuberculosis

Use the BD960 drug susceptibility test to detect the sensitivity to INH. The minimum inhibitory concentration of isoniazid was 0.1 μg/ml. The resistance percentage was used as the criterion: those with a percentage < 1% were considered sensitive, while those with a percentage ≥ 1% were classified as resistant.

2.2.3. Gene mutation detection

The sputum was first decontaminated using N-acetyl-L-cysteine-sodium hydroxide (NALC-NAOH); the bronchoalveolar lavage fluid and its culture were directly sampled and added to the extraction solution, and DNA was extracted by heating and centrifugation. An INH-resistance gene mutation detection kit (purchased from Xiamen Zeesan Biotechnology Co., Ltd.) was used for fluorescence quantitative PCR melting curve analysis, and the *inhA* and *katG* gene mutation results were recorded.

2.2.4. Sequence alignment

The *inhA* and *katG* gene sequencing results were analyzed using BioEdit V7.2.5 software. The sequencing results were consistent with those of standard bacteria. The gene sequence of H37Rv was compared to determine the gene mutation site and gene mutation status.

2.3. Statistical analysis

The experimental data were input into an Excel sheet, and SPSS 22.0 was used for statistical analysis. The mutation rate was compared using the chi-square test, and $P < 0.05$ was considered a statistically significant difference.

2.4. Quality control

H37Rv is used as the control for the INH drug sensitivity test. The standard strain of Mycobacterium tuberculosis (H37Rv) was provided by the National Tuberculosis Reference Laboratory, Center for Tuberculosis Prevention and Control, Chinese Center for Disease Control and Prevention.

3. Results

3.1 General information

A total of 148 INH-resistant Mycobacterium tuberculosis strains were collected and isolated. The samples were extracted from patients aged 18–83 years old, involving 6 ethnic groups (63 persons of Han ethnicity, 53 persons of Uyghur ethnicity, 17 persons of Kazakh ethnicity, 13 persons of Hui ethnicity, 1 person of Mongolian ethnicity, and 1 person of Kirgiz ethnicity). Xinjiang is divided into Southern Xinjiang and Northern Xinjiang by the Tianshan Mountains. Among the 148 cases of INH resistance, 97 came from Northern Xinjiang, 43 came from Southern Xinjiang, and 8 came from other provinces.

3.2. Characteristics of INH resistance-related gene mutations

A total of 148 isoniazid-resistant Mycobacterium tuberculosis strains were collected and isolated, and their *inhA* and *katG* genes were amplified through PCR. The sequencing results showed that INH resistance-related gene mutations were detected in 147 strains (0.68%). The coincidence rate between INH resistance genotype and phenotype was 99.32% (147/148). Among them, 20 strains (13.51%) had the *inhA* gene mutation, 125 strains (84.46 %) had the *katG* gene mutation, and 2 strains (1.35%) had combined mutations of the *inhA* and *katG* genes. Further details are shown in **Table 1**.

Table 1. Mutation status of *inhA* and *katG* genes in 148 strains of isoniazid-resistant Mycobacterium tuberculosis

Type of gene mutation	Number of strains (strains)	Proportion (%)
inhA gene	20	13.51
katG gene	125	99.32
Combined mutation of the *inhA* and *katG* genes	2	1.35
None	1	0.68

3.3. Characteristics of INH resistance-related gene mutations in different ethnic groups in Xinjiang

As described in the previous section, the *inhA* gene mutation rate of the 148 isoniazid-resistant Mycobacterium tuberculosis strains was 13.51% (20/148). Among them, the *inhA* gene mutation rates of Han, Uyghur, and Kazakh were 15.87%, 13.21%, and 17.65%, respectively, and no *inHA* gene mutation was detected in the Hui and other ethnic groups. There was no significant difference in the *inhA* gene mutation rate among the different ethnic groups ($c^2 = 2.897$, $P > 0.05$); the *katG* gene mutation rate was 84.46% (125/148). Among them, the *katG* gene mutation rates of Han, Uyghur, and Kazakhs were 82.54%, 84.91%, and 76.47%, respectively. Meanwhile, *katG* gene mutation was found in all samples of the Hui ethnic group and other ethnic groups. There was no significant difference between the *katG* gene mutation rates of various ethnic groups ($c^2 = 3.772$, $P > 0.05$). Further details are shown in **Table 2**.

Table 2. *inhA* and *katG* gene mutations of INH-resistant Mycobacterium tuberculosis patients of different ethnic groups

Ethnic groups	Number of people	***inhA* gene**				***katG* gene**			
		Number of strains	**Mutation rate (%)**	x^2	***P***	**Number of strains (strains)**	**Mutation rate (%)**	x^2	***P***
Han	63	10	15.87			52	82.54		
Uighur	53	7	13.21			45	84.91		
Kazakh	17	3	17.65	2.897	0.575	13	76.47	3.772	0.438
Hui	13	0	0			13	100		
Others	2	0	0			2	100		
Total	148	20	13.51			125	84.46		

3.4. Characteristics of INH resistance-related gene mutations in different regions of Xinjiang

The *inhA* gene mutation rates of cases in southern Xinjiang, northern Xinjiang, and other provinces were 18.60%, 9.28%, and 37.50% respectively. There were statistical differences in the *inhA* gene mutation rates of cases between different regions ($c^2 = 6.381$, $P < 0.05$). After pairwise comparison, there was no statistical difference in the *inhA* gene mutation rate between cases in southern Xinjiang and northern Xinjiang ($c^2 = 2.214$, $P > 0.05$). There was no statistical difference in the mutation rate of the *inhA* gene between southern Xinjiang and other provinces ($c^2 = 1.424$, $P > 0.05$); the *inhA* gene mutation rate of cases from other provinces was higher than that of cases from northern Xinjiang, and the difference was statistically significant ($c^2 = 5.539$, $P < 0.05$); the *katG* gene mutation rates of cases in southern Xinjiang, northern Xinjiang, and other provinces were 81.40%, 87.63%, and 62.50%, respectively. There was no statistical difference in the *katG* gene mutation rates between different regions ($c^2 = 3.989$, $P > 0.05$). Further details are shown in **Table 3**.

Table 3. Mutations of *inhA* and *katG* genes of INH-resistant Mycobacterium tuberculosis in different regions

Area	Number of samples	***inhA* gene**				***katG* gene**			
		Number of strains (strains)	**Mutation rate (%)**	x^2	***P***	**Number of strains (strains)**	**Mutation rate (%)**	x^2	***P***
Southern Xinjiang	43	8	18.60			35	81.40		
Northern Xinjiang	97	9	9.28	6.381	0.041	85	87.63	3.989	0.136
Other provinces	8	3	37.50			5	62.50		
Total	148	20	13.51			125	84.46		

4. Discussion

INH is one of the most effective first-line drugs for active tuberculosis and latent tuberculosis infection (LTBI) due to its strong bactericidal activity and good safety [4]. The mechanism of INH resistance is very complex, involving the mutations of multiple genes such as *katG*, *inhA*, *kasA*, ahpC, etc. Among them, *katG* and *inhA* mutations are the most common [5,6]. In China, the most common gene mutations that occur in INH-resistant Mycobacterium tuberculosis patients are *katG*, *inhA*, and *ahpC* mutations, accounting for 77.57 %, 15.20%, and 3.69 % respectively. The incidence of *inhA* mutation is the highest in east China, followed by the middle region and the northern region [7]. In this study, 148 INH-resistant Mycobacterium tuberculosis strains isolated from the Eighth Affiliated Hospital of Xinjiang Medical University were analyzed for *katG* and *inhA* gene mutations. The results showed 99.32% of the strains had *katG* and/or *inhA* mutations. Only one of the strains had none of these mutations (0.68%). It is speculated that this strain is associated with other forms of genetic mutations of INH resistance-related genes. It has been reported that the *ahpC* mutation rate among INH-resistant strains can reach 1%, 5%, to 10%, which is basically consistent with our country's data [7-10]. In this study, *katG* gene mutation was detected in 84.46% of the INH-resistant strains. This percentage was lower than that of Chongqing (96.5%) [11], similar to that of Sichuan (83.53%) [12], and higher than that of Yunnan (73.86%) [13], Jiangxi (73.25%) [14], and Jilin (70.60%) [15]. The proportion of *katG* mutation seems to be higher in the east and lower in the west. However, other studies have shown different results [7].

The *inhA* gene mutation rate of this study's samples was 13.51%, which was higher than Chongqing (4.72 %) [11], and close to Sichuan (15.48%) [12] and Jiangxi (15.30%) [14], but lower than Shanghai (16.9%) [16] and Beijing (19.5%) [17]. The mutation rate of the *inhA* gene was found to be higher in patients who relocated to Xinjiang from other provinces compared to cases in northern Xinjiang. However, there was no significant difference in mutation rates when compared to cases in southern Xinjiang. This observation is attributed to the relatively smaller number of cases from other provinces. Notably, in this study, no statistically significant differences were observed in the mutation rates of the *inhA* and *katG* genes among various ethnic groups, including Han, Uyghur, Hui, and others. This suggests that mutations in the *katG* and *inhA* genes may be influenced primarily by regional factors rather than ethnic factors.

5. Conclusion

Genetic testing was performed to reveal the INH resistance characteristics of Mycobacterium tuberculosis in Xinjiang. The results showed that INH resistance in Xinjiang Uygur Autonomous Region was mainly associated with the mutation of the *katG* gene. Therefore, isoniazid resistance gene testing should be conducted before formulating a short-course treatment plan. This study can be used as a guide for the clinical treatment of drug-resistant tuberculosis in Xinjiang.

However, there are certain limitations to this study. Firstly, this study only identified the presence of *katG* and *inhA* mutations without describing the specific mutation sites. Therefore, the specific mutation sites like the regulatory or coding regions of these two genes remain unknown. Secondly, the number of research subjects is limited, and the data results may be biased. Nevertheless, it is important to note that patients seeking medical treatment in this study were drawn from various regions across Xinjiang. This diverse patient population offers insights into the geographical distribution characteristics of isoniazid-resistant tuberculosis bacteria in the entire Xinjiang region to a certain extent. Further research with a larger sample size is needed to verify our results.

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Disclosure statement

The authors declare no conflict of interest.

References

[1] World Health Organization, Global Tuberculosis Report 2021, viewed October 26, 2023, Decemhttps://www.who.int/publications/digital/global-tuberculosis-report-2021

[2] World Health Organization, 2020, Global Tuberculosis Report 2020, viewed October 26, 2023, https://www.who.int/tb/publications/global report/en/

[3] Liu J, Zhong Q, 2020, Challenges and Response Strategies for "Ending Tuberculosis". Chinese Journal of Anti-Tuberculosis, 42(04): 308–310.

[4] Fu L, Gao J, Deng G, et al., 2018, WHO Treatment Guidelines for Isoniazid-Resistant Tuberculosis: Supplement to the WHO Treatment Guidelines for Drug-Resistant Tuberculosis. International Respiratory Journal, 38(24): 1841–1845.

[5] Chen S, Liu H, Dan W, et al., 2016, Advances in Correlation Between Drug Resistance Phenotype and Genotype of Mycobacterium Tuberculosis. Chinese Journal of Infection Control, 15(11): 883–886.

[6] Song Y, Wang G, Huo F, et al., 2018, Correlation Analysis Between *inhA* Gene Mutations and Resistance to Prothionamide in Multidrug-Resistant and Extensively Drug-Resistant MTB. Chinese Journal of Anti-Tuberculosis, 40(8): 821–824.

[7] Tian L, Zhou W, Huang X, et al. 2022, Analysis of Gene Mutation Characteristics of Isoniazid-Resistant Mycobacterium Tuberculosis in China. Chinese Journal of Antituberculosis, 44(04): 354–361.

[8] Miotto P, Zhang Y, Maria D, et al., 2018, Drug Resistance Mechanisms and Drug Susceptibility Testing for Tuberculosis. Respirology, 23(12): 1098–1113.

[9] Zhao LL, Chen Y, Chen ZN, et al., 2014, Prevalence and Molecular Characteristics of Drug-Resistant Mycobacterium Tuberculosis in Hunan, China. Antimicrobial Agents and Chemotherapy, 58(6): 3475–3480.

[10] Victor TC, van Helden PD, Warren R, 2002, Prediction of Drug Resistance in M. Tuberculosis: Molecular Mechanisms, Tools, and Applications. IUBMB Life, 53(4): 231–237.

[11] Zhu D, Liu W, Shen J, et al., 2022, Research on Isoniazid and Prothionamide Resistance and Cross-Resistance-Related Gene Mutations of Mycobacterium Tuberculosis in Chongqing. Chinese Journal of Zoonosis, 38(05): 405–409.

[12] Chen L, 2022, Analysis of Resistance Levels and Mutation Frequency Characteristics of *katG*, *inhA* and *rpoB* Gene Mutation Sites of Mycobacterium Tuberculosis in Sichuan. Journal of Clinical Pulmonology, 27(02): 242–246.

[13] Chen L, Ru H, Yang X, et al., 2022, Characteristics of *katG* and *inhA* Gene Mutations in isoniazid-Resistant Mycobacterium Tuberculosis in Yunnan Province. Journal of Kunming Medical University, 43(08): 28–33.

[14] Dong L, Qiang C, Xiong G, et al., 2019, Prevalence and Molecular Characterization of Multidrug-Resistant M. Tuberculosis in Jiangxi Province, China. Scientific Reports, 9(1): 7315.

[15] Zhang W, Yang X, Zhang Li, et al., 2017, Analysis of *rpoB* and *katG* Gene Mutation Characteristics of Multidrug-Resistant Mycobacterium Tuberculosis in Jilin Province. Chinese Experimental Diagnostics, 21(10): 1731–1735.

[16] Wen S, Lin Z, Liu D, et al., 2019, Isoniazid-Resistant Mycobacterium Tuberculosis and Mutations in its *katG* and *inhA* Genes. Chinese Thermal Medicine, 19(08): 723–726.

[17] Jia H, Xu Y, Sun Z, 2021, Analysis on Drug-Resistance-Associated Mutations Among Multidrug-Resist-Ant Mycobacterium Tuberculosis Isolates in China. Antibiotics, 10(11): 1367–1377.