Predictive Power of ETRE Polymorphism and Katg463 Mutation to INH-Resistance of *M. tuberculosis*

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Abstract

**Background:** The MIRU-VNTR polymorphism and katG463 mutation are used to genotype the mycobacterium tuberculosis, but the correlation between them and INH-resistance were unknown. This study was aimed to explore whether ETRE polymorphism and katG463 mutation could predict the INH-resistance, and the relationship between ETRE polymorphism and katG463 mutation.

**Methods:** The ETRE, katG463 mutation and drug resistance information of 109 *M. tuberculosis* strains were collected from online public database. We constructed the predictive diagnostic tool of ETRE polymorphism and katG463 mutation. Chi-square test was used to analyze the relationship between ETRE polymorphism, katG463 mutation and INH-resistance. ROC curve analysis and Z-test were used to evaluate the predictive ability of ETRE and katG463. The relationship between ETRE polymorphism and katG463 mutation was analyzed with Spearman correlation analysis.

**Results:** The mutation rate of katG463 was 27.50%, and the b value of ETRE polymorphism was 0.67. KatG463 mutation was associated with INH resistance (OR=3.72). The INH drug resistance rate in VNTR≧5 group was 3.43 times higher than that in VNTR≦3 group (χ²=24.77, P<0.01), and there was no significant difference of INH resistance between the VNTR=4 group and VNTR≦3 group. The areas under the ROC curve of two loci prediction diagnostic tools were 0.64 and 0.70 respectively. The katG463 mutation was significantly related to the ETRE polymorphism (r=0.79, P<0.01).

**Conclusion:** Both katG463 mutation and the ETRE polymorphism can predict the INH-resistance of tuberculosis. The katG463 mutation was associated with ETRE VNTR polymorphism.

**Keywords:** *M. tuberculosis*, Drug resistance, ETRE, katG463, VNTR

Introduction

Drug-resistance of tuberculosis has become a serious public health problem recently. The Global Tuberculosis Report 2013 reported that about 8.6 million people had been infected with tuberculosis (TB) and 1.5 million people died from TB, moreover, 3.6% of new cases and 20.0% of retreatment were infected with MDR (1). The inadequacy of retreatment and gene mutation were the main risk factors of drug-resistant tuberculosis. The current studies mainly focus on the topic of gene mutations associated with drug resistance, such as isoniazid-resistance related gene *katG*(2), rifampicin-resistance related gene *rpoB*(3), and streptomycin-related gene *gidB A80P* (4) or efflux pump includ-
ing INH-resistance related 5 genes (5) and so on. But the gene mutations could not elucidate the mechanisms of drug resistance, which predicted only about 60% of all the anti-tuberculosis drug resistance. Further studies focusing on the molecular mechanism of drug resistance in tuberculosis is necessary. Tuberculosis of Beijing genotype is associated with drug resistance (6). This result suggests that gene loci may be associated with tuberculosis drug resistance. The Mycobacterium interspersed repetitive unit-variable number of tandem repeat (MIRU-VNTR) method is widely used in genotyping the tuberculosis strains. It is well known that gene mutation (G/T) occurs at katG463 in INH resistant strains. Zhang, et al. found that the rate of mutation of katG463 in INH-resistant strains was about 40.2%, and it was 54.4% in Chen’s experiment (7). Moreover, some TB typed by spoligotype had certain characteristics, which is closely related to the katG463 polymorphism (8). It has been confirmed that genotyping results is consistent with spoligotype and MIRU-VNTR (9). Therefore, it is possible that there is correlation between katG463 mutation and MIRU-VNTR polymorphism. Our ongoing studies found some MIRU loci may be related to INH resistance. At present, the MIRU locus was only used to analyze the epidemiology of tuberculosis. The correlation between katG463 and INH resistance was still uncertain, and whether the MIRU locus was associated with katG463 was not known yet. Therefore, we conducted a research to explore whether ETRE (Exact Tandem Repeat E) polymorphism and katG463 mutation could predict the INH-resistance of M. tuberculosis and the relationship between ETRE polymorphism and katG463 mutation.

Materials and Methods

Sample Source

One hundred and nine strains were included in the study, of which 54 strains were derived from Germany, 20 strains were from Ghana, 20 strains were from Uganda and 15 strains were from former Soviet Union.

Method

The repeat number of ETRE, katG463 mutation information and drug resistance data were derived from an online open database of MIRU-VNTR plus website (http://www.miru-vntrplus.org). The data in the reference database were provided by the Pasteur Institute in France and the German National Reference Center for Mycobacteria. Ethics approval code was obtained from Wan Nan medical college (No. 2014006).

Statistical Analysis

Descriptive statistical method was applied for the lineage information of strains. M. tuberculosis isolates were characterized by MIRU-VNTR-24. Chi-square test was used to analyze the relationship between ETRE polymorphism, katG gene codon 463 mutations and INH-resistance of M. tuberculosis. ROC curve analysis and Z-test were used to evaluate the predictive ability. Spearman correlation analysis was used to analyze the relationship between ETRE polymorphism and katG gene codon 463 mutation. There were statistically significant differences in P<0.05.

Results

Lineage information of strains

The 109 strains had 14 lineages. The number of katG463 with no mutation in Beijing, Cameroon and Delhi/CAS was 10 respectively (Table 1).

KatG463 mutation, ETRE VNTR polymorphism and INH-resistance

The INH drug resistance rate in katG463 mutation group was 3.72 times higher than that in the wild type group (χ²=24.77, P<0.01). The INH drug resistance rate in ETRE VNTR ≥ 5 group was 3.43 times higher than it in VNTR ≤3 group (χ²=7.28, P<0.01). There was no significant difference of INH resistance between the VNTR =4 group and VNTR ≤3 group (Table 2).

Predictive capability analysis of katG463 mutation and ETRE VNTR

The area under the ROC curve (AUC) of katG463 mutation and ETRE VNTR was 0.64 and 0.70 respectively (Table 3).
Table 1: Distribution of \textit{katG463} mutation in different lineages

| Lineage     | \textit{katG463} with no mutation | \textit{katG463} with mutation | INH resistance |
|-------------|-----------------------------------|-------------------------------|----------------|
| Beijing     | 0                                 | 10                            | 10             |
| Cameroon    | 10                                | 0                             | 0              |
| Delhi/CAS   | 0                                 | 10                            | 2              |
| EAI         | 0                                 | 10                            | 0              |
| Ghana       | 10                                | 0                             | 4              |
| Haarlem     | 10                                | 0                             | 1              |
| LAM         | 11                                | 0                             | 4              |
| NEW-1       | 3                                 | 0                             | 1              |
| S           | 4                                 | 0                             | 0              |
| TUR         | 4                                 | 0                             | 0              |
| URAL        | 4                                 | 0                             | 0              |
| UgandaI     | 10                                | 0                             | 1              |
| UgandaII    | 10                                | 0                             | 1              |
| X           | 3                                 | 0                             | 0              |

Table 2: Correlation of \textit{katG463} mutation and \textit{ETRE} VNTR and INH resistance

| Term       | Category | \( n \) | Resistance number | Resistance rate(%) | \( \chi^2 \) | \( P \) | OR | OR 95% CI |
|------------|----------|---------|-------------------|--------------------|-------------|--------|-----|-----------|
| Genotype*  | 0        | 79      | 12                | 0.14               | -           | -      | -   | -         |
|            | 1        | 30      | 12                | 0.40               | 7.28        | 0.01   | 3.72| 1.43-9.67 |
| \textit{ETRE} VNTR | \( \leq 3 \) | 63      | 9                 | 0.14               | 11.38       | 0.00   | 3.43| 1.30-4.10 |
|            | 4        | 21      | 3                 | 0.14               | 0.00        | 1.00   | 1   | 0.24-4.10 |
|            | \( \geq 5 \) | 25      | 12                | 0.48               | 24.77       | 0.00   | 3.43| 1.30-23.67 |

Note: *1 for the mutant (CTG), 0 for the wild type (CGG)

Correlation analysis between \textit{katG463} mutation and \textit{ETRE} VNTR

The mutation rate of \textit{katG463} was 27.50% in 109 strains. The \( b \) value of \textit{ETRE} was 0.67. The \textit{katG463} mutation was significantly related to the \textit{ETRE} polymorphism by Spearman correlation analysis \((r=0.794, P<0.01)\) (Table 4).

Table 3: The area and the sensitivity and specificity of ROC curves of \textit{katG463} and \textit{ETRE} to predict the INH resistance

| Term       | AUC | Se  | \( P \) | 95% CI for AUC | Sensitivity | 1-Specificity | Yueden index |
|------------|-----|-----|--------|----------------|-------------|---------------|--------------|
| \textit{ETRE} | 0.70 | 0.06 | 0.00   | 0.57-0.82      | 0.62        | 0.31          | 0.32         |
| \textit{katG463} | 0.64 | 0.07 | 0.03   | 0.51-0.78      | 0.50        | 0.21          | 0.29         |

Table 4: Correlation between \textit{ETRE} polymorphism and \textit{katG463} mutation

| \textit{katG463*} | \textit{ETRE} VNTR | Total |
|------------------|-------------------|-------|
| \( \leq 3 \)    | \( \geq 5 \)  | \( \leq 3 \) | \( \geq 5 \) | Total |
| 0                | 62               | 16    | 1        | 79    |
| 1                | 1                | 5     | 24       | 30    |
| Total            | 63               | 21    | 25       | 109   |

Note: *1 for the mutant (CTG), 0 for the wild type (CGG)
Discussion

All strains included in our study had 14 lineages distantly. Lineage with no katG463 mutation still had INH resistance.

The katG gene encoded a peroxide peroxidase, which could activate INH and attacked the mycolic acid of M. tuberculosis. This was the possible anti-tuberculosis mechanism of INH. Several studies confirmed that katG codon 315 mutation (AGC→AAC) was associated with INH-resistance (10-12). However, whether katG463 mutation was related to INH-resistance or not remains uncertain. Arjomandzadegan, et al.(13) identified that mutation rate of KatG463 was 57.8% in multi-drug resistant TB (MDR) and 59.2% in extensive drug resistant (XDR) isolates. It suggested that the katG codon 463 was associated with INH resistance. But some researches showed there was no correlation between katG463 mutation and INH-resistance (14-16). This study showed that the mutation rate of katG463 was 27.50% in 109 strains, and the INH drug resistance rate in katG463 mutation group was 3.72 times higher than that in the wild type group. Meanwhile, the area under the ROC curve of katG463 mutation was 0.64. It suggested that there was a certain predictive ability of katG463 mutation to INH-resistance. The mutation rate of katG463 was lower compared to other studies and the reason why this result was contradictory to previous ones may because the samples came from four different areas.

The MIRU-VNTR typing method was used for genotyping the tuberculosis and it could determine the TB epidemic circuit diagram. Through the analysis of the relationship between MIRU polymorphism and INH-resistance, we found that the INH drug resistance rate of ETRE in VNTR≥5 group was 3.43 times higher than VNTR≤3 group, but there was no significant difference between the VNTR=4 group and VNTR≤3 group. At the same time, we found the area under the ROC curve of diagnostic tool was 0.70, which indicated there may be a correlation between higher repetitions and INH-resistance. There was 0 bp between ETRE and the downstream gene frr. ETRE was in the promoter area of frr. The expressional product of gene frr is ribosome recycling factor, which is responsible for the release of ribosomes from mRNA at the termination of protein biosynthesis and may increase the efficiency of translation by recycling ribosomes from one round of translation to another(17). Meanwhile, Hosaka(18) found that increased expression of ribosome recycling factor is responsible for the enhanced mRNA-directed green fluorescent protein (GFP) synthesis in vitro protein synthesis system. Thus, ETRE may stimulate the mycolic acid related enzyme synthesis via enhancing the expression of ribosome recycling factor to increase the synthesis of mycolic acid. Simultaneously, enhanced synthesis of mycolic acid was involved in the INH-resistance (19).

This study also showed the relationship between ETRE VNTR and katG463 mutation. It was very interesting to find that the mutation rate of katG463 increased significantly with the decreased number of ETRE VNTR. We could not found any other study whose results are similar to ours. ETRE was located in 3192198 in H37Rv tuberculosis gene, It was far away from katG463 which located in 2153871. The distance was too far that the interaction between adjacent loci was unlikely to occur but the interaction between two genes in the spatial structure could not be excluded. Zhang, et al. found that there were 2 kbp in 10 kbp upstream of katG, comprising three tandem copies of a novel 75 bp repeat element flanked by multiple copies of the 10 bp major polymorphic tandem repeat (MPTR). It hypothesized that the presence of repetitive sequences may contribute to instability of gene (20).

The current early diagnosis of INH resistance was conducted by gene sequencing, the gene katG and inhA detected by using the expensive, high-end instruments, not by rapid and more practical detection methods. The treatment cost of tuberculosis with drug resistance is high and patients’ condition is worse for patients with HIV in developing countries such as South Africa (21). MIRU-VNTR was selected as the first choice for genotyping of M. tuberculosis by U.S. CDC due to simple
operation and low cost (22). Our findings confirmed that ETRE loci could predict the drug-resistant tuberculosis. Its predictive ability has no statistical difference with katG463 mutation. This study would not only expand the usage of MIRU-VNTR method, but also bring benefits to early diagnosis and treatment of the drug resistance of M. tuberculosis.

**Ethical considerations**

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

**Conclusion**

Both katG463 mutation and the ETRE polymorphism can predict the INH-resistance of tuberculosis. The katG463 mutation was associated with ETRE VNTR polymorphism.

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