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Short Communication

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 *h* 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 (χ^2=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.t uberculosis*, 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 1.3 million people died from TB, moreover, 3.6% of new cases and 20.0% of r*etre*atment 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-resistance 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 ($\chi^2=7.28$, $P<0.01$). The INH drug resistance rate in *ETRE* VNTR ≧ 5 group was 3.43 times higher than it in VNTR ≦3 group ($\chi^2=24.77$, $P<0.01$). 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).

Table1: Distribution of *katG463* mutation in different lineages

Lineage	*katG463* with no mutation	*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 *katG463* mutation and *ETRE* VNTR and INH resistance

Term	Category	*n*	Resistance number	Resistance rate(%)	χ^2	*P*	*OR*	*OR*95%*CI*
Genotype*	0	79	12	0.15	-	-	-	-
	1	30	12	0.40	7.28	0.01	3.72	1.43-9.67
ETRE VNTR	≦3	63	9	0.14	11.38	0.00	-	-
	4	21	3	0.14	0.00	1.00	1	0.24-4.10
	≧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 katG463 mutation and ETRE VNTR

The mutation rate of *katG463* was 27.50% in 109 strains. The *h* value of *ETRE* was 0.67. The *katG463* mutation was significantly related to the *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 *katG463* and *ETRE* to predict the INH resistance

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

Table 4: Correlation between *ETRE* polymorphism and *katG463* mutation

*katG463**	*ETRE* VNTR			Total
	≦3	4	≧5	
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 mycolicacid of *M. tuberculosis*. This was the possible antituberculosis 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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