Molecular mechanisms in microRNA-mediated TRB3 gene and hypertension left ventricular hypertrophy

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Abstract. The present study investigated the relationship between microRNA-mediated TRB3 gene and hypertension left ventricular hypertrophy at the molecular level. Polymorphic site in TRB3 gene was identified by direct PCR method, and the correlation between the SNP site and ventricular hypertrophy was determined. MicroRNAs target gene sequence interacting on the TRB3 polymorphic site was screened by bioinformatics, and the effect of microRNAs on the TRB3 polymorphic site was finally verified by luciferase test. Two polymorphic sites rs6186912 and rs6186923 were found in the TRB3 gene, and the direct relationship between rs6186923 polymorphic site and the hypertension left ventricular hypertrophy in patients with myocardial hypertrophy was compared and analyzed. Pictar software was used to analyze the effect of miR-100 on rs6186923, and the argumentation was verified by luciferase test. In conclusion, the study showed that the TRB3 gene polymorphism rs6186923 was able to affect the TRB3 gene by affecting the binding of miR-100, which indirectly caused the formation of hypertension left ventricular hypertrophy.

Introduction

Although hypertension left ventricular hypertrophy is the compensation for excessive pressure load by means of structural changes to a certain extent, more and more studies showed that continuous myocardial hypertrophy easily leads to the heart failure (1,2). In recent years, plenty of studies have analyzed mechanism of formation for left ventricular hypertrophy respectively from three aspects, including extracellular stimulation signal, intracellular signal transduction and gene transcription. Moreover, a many significant signaling pathways have been identified, such as small G proteins, mitogen-activated protein kinase (MAPK) and calcineurin (CaN). However, the specific occurrence and development mechanism of ventricular hypertrophy is still not fully clarified (3-6). Therefore, to determine the active factors that could regulate myocardial hypertrophy and reveal the cause of myocardial hypertrophy at the molecular level is important.

TRB3 gene was found in *Drosophila*, and reported to be a regulatory protein, for the process of sugar, lipid metabolism and adipocyte differentiation (7,8). In addition, relevant study results showed that the TRB3 gene could also act on the mitogen activated protein kinase signaling pathway to inhibit cell proliferation and induce apoptosis (9,10). The apoptosis of endothelial cells was closely associated with the occurrence and development of cardiac and cerebral vascular disease (11). Therefore, it was suggested that if the TRB3 gene was related to the formation of myocardial hypertrophy thereby, affecting polymorphism genetic variation of TRB3 gene expression, it may be associated with the development mechanism of myocardial hypertrophy.

MicroRNAs are a class of non-coding RNA, which have the function of regulating and controlling (12,13). The polymorphic variation in target sequence usually affects the recognition and binding of the seed sequence and target sequence, thus affecting the expression of the target gene. Therefore, we hypothesized that polymorphism in microRNAs and TRB3 gene site may be associated with the formation mechanism of hypertension left ventricular hypertrophy. In order to prove the inference, we first identified by the means of PCR, the polymorphic site of TRB3 gene SNP, and its association with hypertension left ventricular hypertrophy was investigated. After determining the polymorphic site bioinformatics was used to explore interacting microRNAs.

Patients and methods

Patient data. One hundred twenty-six patients, admitted to to the First Affiliated Hospital of Nanchang University with confirmed hypertension from January 2014 to December 2015, were selected for the present study. They were divided into two groups. The left ventricular hypertrophy group of

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Table I. Clinical data of different groups of patients with hypertension.

Clinical features	Hypertension group		Control group
	Left ventricular hypertrophy group	Non-left ventricular hypertrophy group	
Body weight (kg)	75.3±1.2	73.4±2.3	70.1±0.9
Systolic pressure (mmHg)	161.5±6.5[a]	153.1±4.3[a]	123.5±3.6[a,b]
Diastolic pressure (mmHg)	110.5±3.5[a]	100.8±5.8[a]	60.2±4.1[a,b]
Triglyceride (mmol/l)	1.93±1.2	1.88±1.5	1.42±0.5
Fasting plasma glucose (mmol/l)	6.12±0.6	6.87±0.4	5.93±0.5
Smoking rate (%)	17	22	10
Alcohol intake rate (%)	30	28	15

[a,b]Compared by the single factor inter-group variance, the letters that are the same indicate there was no significant difference (P>0.05); the letters that are different indicate there was significant difference (P<0.05).

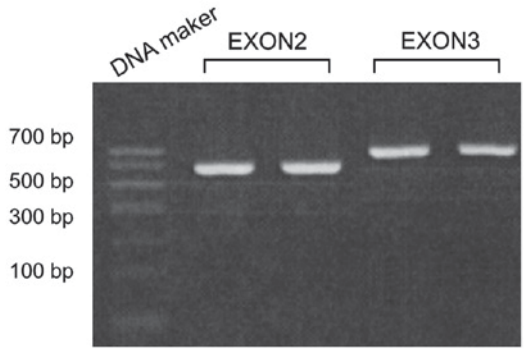

Figure 1. PCR products of exon2 and exon3.

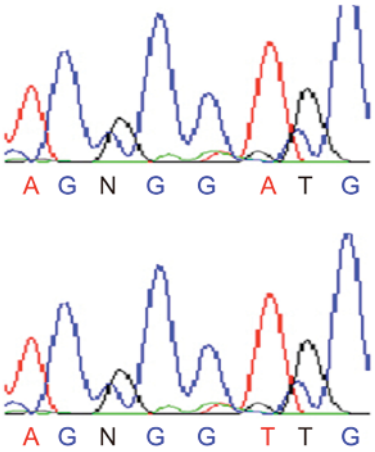

Figure 2. Gene sequencing results.

43 cases aged 55-73 years (average, 66.3 years) and non-left ventricular hypertrophy group of 83 cases aged 49-75 years (average, 58.4 years). Additionally, 31 healthy people were selected as control, aged 40-60 years (average, 55.8 years). The clinical data are shown in Table I. This study was approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University. Signed written informed consents were obtained from all participants before the study.

PCR amplification. Primers were synthesized by Shenzhen Huada Gene Technology Co., Ltd. (Shenzhen, China), and the amplification sequence was as described in literature (14), covering the second and third exon. Reaction conditions were in accordance with literature (15).

Gene sequencing. Fifteen subjects were randomly selected from the control and the two groups for a total of 45 cases, whose PCR amplification products were directly sequenced. Also, their DNA variation sequences were analyzed, according to literature (16).

Genetic balance and frequency statistical test. The analytical method was as previously described (17).

Bioinformatics analysis. The bioinformatics analytical method was as described in literature (18). Pictar database (http://www.pictar.org) was used to scan the TRB3 polymorphic site, in order to find the microRNAs that have specific binding.

Luciferase activity determinations. The specific activity determination method was selected according to literature (19).

Statistical analysis. SPSS version 13.0 statistical software (SPSS, Inc., Chicago, IL, USA) was used for data analysis. Chi-square test was adopted to compare classification statistics data. Test level was α0.05. P<0.05 was considered to indicate a statistically significanct difference.

Results

Detection of polymorphic site SNP of TRB3 gene. Direct PCR was applied to explore SNP of TRB3 gene. The electrophoretogram of exon2 and exon3 after PCR amplification is shown in Fig. 1, and sequencing results are shown in Fig. 2.

After the amplified fragments were purified forward sequencing was performed, DNA sequencing results of exon2 were: 5'-TCACTAAAATCAAATCCCTTTTTTT(A/T)AATATCCAATCNAGTATATCCCAAA-3'. The SNP site (A/T). By means of the NCBI database, it was concluded that this

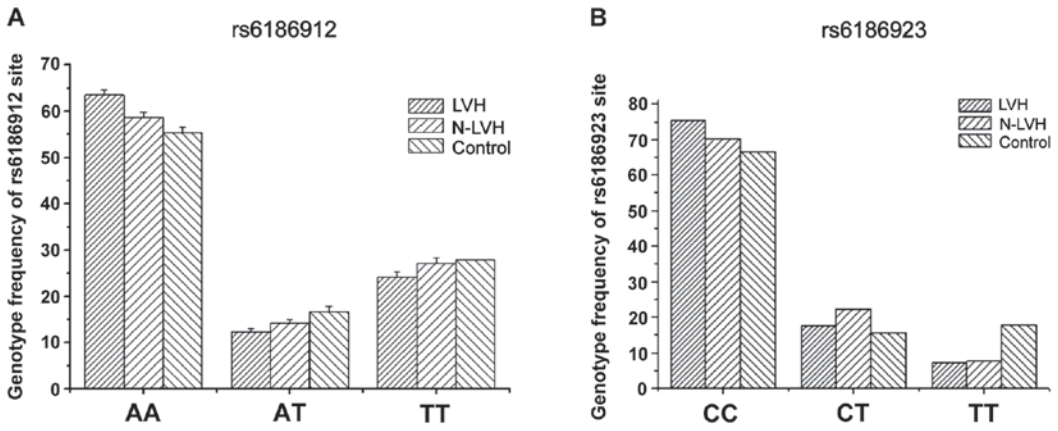

Figure 3. Gene frequency distributions in different patient groups and control group. (A) Comparison of AA, AT and TT genotypefrequency of rs6186912 site. (B) Comparison of CC, CT and TT of rs6186923 site.

Table II. SNP genotype and allele frequency distributions of gene sequencing results in different patient groups and control group.

	Patient groups (%)		
	Left ventricular hypertrophy group	Non left ventricular hypertrophy group	Control group (%)
rs6186912			
AA	63.5	58.7	55.4
AT	12.3	14.2	16.7
TT	24.2	27.1	27.9
Allele			
A allele	19.5	21.3	22.1
T allele	80.5	78.7	77.9
rs6186923			
CC	75.3	70.1	66.6
CT	17.5	22.3	15.6
TT	7.2	7.6	17.8
Allele			
C allele	84.6	77.7	74.4
T allele	15.4	22.3	25.6

gene was consistent with the SNP site rs6186912 of TRB3 gene. Similarly, DNA sequencing results of exon3 were: 5'-AATCCCTTTTTTTNAATATCCAATC(C/T)AGTATATC CCAAAATGACATGAATA-3'.

The SNP site (C/T). By means of NCBI database, it was concluded that this gene was consistent with the SNP site rs6186923 of the TRB3 gene.

Genotyping. Ten patients with hypertension of left ventricular hypertrophy, 10 cases of non left ventricular hypertrophy and 10 cases of healthy controls were randomly selected. The sequences were used as primer for PCR, and then the product was sequenced and analyzed. The results are shown in Table II. The comparison of AA, AT and TT genotype frequency of rs6186912 site between each of the two groups

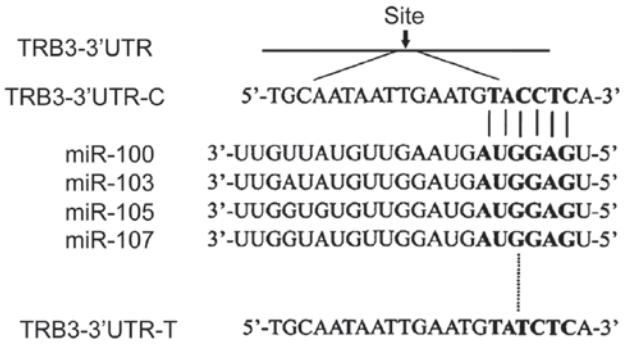

Figure 4. SNP rs6186923 locates in miR gene binding site in TRB3 gene. miR, microRNA. Bold indicates binding regions.

of hypertension and control group had no statistical significance (P>0.05) (Fig. 3A). While comparison of CC, CT and TT of rs6186923 site had significant difference (Fig. 3B). T allele of left ventricular hypertrophy group was evidently lower than that of non-left ventricular hypertrophy group and normal group (15.4 vs. 22.3%, P<0.05), but comparison of T allele between non-left ventricular hypertrophy group and normal group had no distinct difference.

Bioinformatics analysis. After determining the association between rs6186923 SNPs gene and hypertension left ventricular hypertrophy, this experiment was designed to find the corresponding microRNAs action site by bioinformatics method. Through the analysis of the online software Pictar, it was observed that TRB3 polymorphic site rs6186923 could be combined with miR-100, miR-103, miR-105 and miR-107, as shown in Fig. 4. The C allele, T allele and target sequence mRNA were conformed to Watson-Crick matching principle. According to the analysis of Pictar, it was noted that thermodynamic free energy of binding between rs6186923 and miR-100 target sequence was the highest. Therefore, to carry out the action which adopts miR-100 target sequence and rs6186923 was the next step.

Luciferase test. In order to verify the interaction between the miR-100 target sequence and TRB3 gene rs6186923, the SNP

Table III. Luciferase test results.

	Blank	Blank+miR-100	TRB3-miR100-C	TRB3-miR100-T
Fluorescence signal (%)	100	98±1.5	75±2.1	84±1.7

Compared with the blank control, the expression of TRB3-miR100-C gene luciferase was reduced by 23%, while the expression of TRB3-miR100-T gene luciferase was reduced by 14%, which were statistically significant (Fig. 5) (P<0.05).

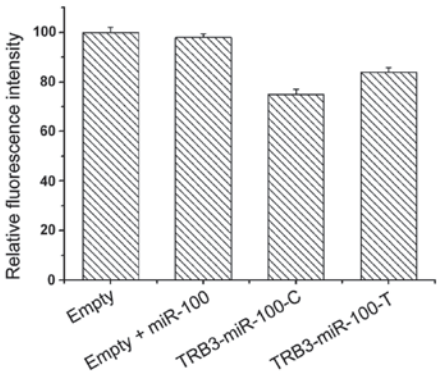

Figure 5. Luciferase test in different groups.

sequence was cloned into the 3'UTR region of the reporter gene luciferase. The principle was that if miR-100 could act on rs6186923 gene, the translation of reporter gene luciferase would be inhibited, thereby reducing the fluorescence signal, so the intensity of fluorescence signal could be used to reflect the inhibitory effect of miR-100 target sequence on rs6186923. The experiment results are shown in Table III and Fig. 5.

Discussion

In recent years, the enhancement of people's living standard and the changes in diet, have resulted in elevation of the incidences of cardiovascular disease (20). Hypertension left ventricular hypertrophy is a risk factor, which affects the incidence of cardiovascular disease (21), however, the mechanism of its formation is still not clear. Therefore, the purpose of this study was to investigate the formation mechanism of hypertension left ventricular hypertrophy at the molecular level, which would lay the foundation for further study. Prior to the beginning of this study, by the means of consulting literature it was noted that TRB3 gene is a significant regulatory factor, which may be associated with the occurrence and development of cardio-cerebral vascular disease. In recent years, microRNAs have been observed to play a key role in the regulation of myocardial hypertrophy. Based on this, we speculated that the association between TRB3 gene and ventricular hypertrophy, would allow to explore the formation mechanism of ventricular hypertrophy.

Therefore, in the present study, SNPs sites of TRB3 gene were investigated by direct PCR method. The PCR products were then purified and sequenced, and compared with NCBI database. It was observed that sequencing results of exon2 and exon3 were corresponding, respectively, with rs6186912 and rs6186923, showing that SNPs sites were accurate. Then, for the genotypes, the study investigated the association between rs6186912, rs6186923 and patients with hypertension left ventricular hypertrophy. The experiment indicated that the comparison of AA, AT and TT genotype frequency of rs6186912 site between the groups of hypertension and control had no statistical significant difference (P>0.05). This confirmed that SNP site was not directly related to myocardial hypertrophy. However, comparison of CC, CT and TT of rs6186923 site had significant differences. T allele of left ventricular hypertrophy group was evidently lower than that of non-left ventricular hypertrophy group and normal group (P<0.05), but comparison of T allele between non-left ventricular hypertrophy group and normal group showed no distinct difference, which indicated that this SNP was closely related to hypertension and myocardial hypertrophy.

After determining the association between TRB3 polymorphism gene and hypertension left ventricular hypertrophy, corresponding microRNAs were explored by bioinformatics. Through the analysis of the online software Pictar, it was concluded that there were 4 microRNAs: miR-100, miR-103, miR-105 and miR-107 that could act on the rs6186923SNP site of TRB3. Further it was noted that thermodynamic free energy of binding between rs6186923 and miR-100 target sequence was the highest. Therefore, miR-100 was used for subsequent analysis as microRNAs to interact on rs6186923. Then luciferase test was applied to investigate the effect of miR-100 on rs6186923, showing that the expression of TRB3-miR100-C gene and TRB3-miR100-T gene luciferase were significantly reduced, which confirmed the effect of miR-100 gene on rs6186923.

In conclusion, microRNA-miR100 affected hypertension left ventricular hypertrophy by mediating polymorphic site of TRB3 gene rs6186923. However, verification of miR-100 on rs6186923 by western blot needs to be further evaluated in future studies.

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