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#### **ABOUT COVER**

Editorial board member of World Journal of Clinical Cases, Dr. Li is a Professor at the Nanjing University Medical School in Nanjing, China. Having received his Bachelor's degree from Xuzhou Medical College in 1997, Dr. Li undertook his postgraduate training first at Nanjing Medical University, receiving his Master's degree in 2004, and then at Fudan University, receiving his PhD in 2007. He advanced to Chief Physician in the Department of Anesthesiology at The Affiliated Hospital of Nanjing University Medical School in 2017 and has held the position since. His ongoing research interests involve ultrasound (transthoracic echo and transesophageal echo) in clinical anesthesia and ultrasound-guided limb and trunk nerve block in postoperative pain management. (L-Editor: Filipodia)

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ORIGINAL ARTICLE

# **Case Control Study** Relationship between granulomatous lobular mastitis and methylene tetrahydrofolate reductase gene polymorphism

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## Abstract

#### BACKGROUND

Variations in the methylene tetrahydrofolate reductase (MTHFR) gene have been reported as risk factors for numerous conditions, including cardiovascular disease, thrombophilia, stroke, hypertension and pregnancy-related complications. Moreover, it was reported there is an association between breast cancer and mutations in MTHFR-C677T. However, whether there is an association between MTHFR gene polymorphism and granulomatous lobular mastitis or not has been rarely investigated.

#### AIM

To analyze the association between MTHFR gene polymorphism and granulomatous lobular mastitis.

#### **METHODS**

Fifty-one patients with granulomatous lobular mastitis admitted to The First Hospital of Kunming were selected as study samples. Their hospitalization time ranged from February 2018 to February 2019. The 51 patients were included in the experimental group, and another 51 women who underwent physical examination at The First Hospital of Kunming in the same period were included in the control group. Deoxyribonucleic acid and MTFR genetic polymorphism testing were performed in each group. The association between MTHFR gene polymorphism and granulomatous lobular mastitis was observed.

### RESULTS

There were significant differences in genotype frequency and allele frequency of C/C and C/T between the experimental group and the control group (all P <0.05). However, there was no significant difference in frequency of T/T genotype between the two groups (P > 0.05). In addition, there was no significant difference in genotype frequency and allele frequency of A/A, A/C and C/C between the two groups (P > 0.05).



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#### CONCLUSION

MTHFR gene C677T locus polymorphism is closely related to granulomatous lobular mastitis.

Key Words: Methylene tetrahydrofolate reductase; Gene polymorphism; Granulomatous lobular mastitis; Association; C677T; Factor

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**Core Tip:** Methylene tetrahydrofolate reductase (*MTHFR*) gene polymorphism is associated with several diseases. It was reported that mutations in MTHFR-C677T is associated with breast cancer. We tested the association between MTHFR gene polymorphism and granulomatous lobular mastitis in patients with this disease. The results supported our hypothesis that MTHFR C677T is a susceptibility factor that may influence the occurrence of granulomatous lobular mastitis.

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## INTRODUCTION

Granulomatous lobular mastitis is a chronic inflammatory response caused by granulomatous changes around lobules and ducts of the breast. It often occurs in young women, and the disease course is long. If patients are not treated effectively, they are likely to relapse. In addition, the rate of misdiagnosis is high because the clinical manifestations are similar to those of plasma cell mastitis. The diagnosis error leads to delayed treatment and tremendous physical and emotional damage in patients<sup>[1]</sup>. Although some research reported that granulomatous lobular mastitis is associate with methylene tetrahydrofolate reductase (MTHFR) gene, the research is limited and the relationship is not clear<sup>[2]</sup>. Thus, we examined MTHFR gene polymorphism in 51 patients with granulomatous lobular mastitis.

### MATERIALS AND METHODS

#### Materials at baseline

Fifty-one patients with granulomatous lobular mastitis who were admitted to The First Hospital of Kunming from February 2018 to February 2019 were included in the experimental group. Another 51 women who underwent physical examination in our hospital in the same period were included in the control group. The average age of the experimental group and the control group was 32.19 ± 3.28 years and 32.26 ± 3.30 years, respectively. Data at baseline were analyzed by statistical software. A P value < 0.05 was considered statistically significant.

#### Methods

Two milliliters of blood specimen was collected from the patients' upper arm. Deoxyribonucleic acid (DNA) was extracted and following DNA extraction, samples were tested for MTHFR A1298C and C677T gene polymorphisms. Then, 200 µL of blood specimen was drawn, and 200 µL of GB buffer and 20 µL of Proteinase K mix were added. The sample was left at room temperature 2-5 min, and then 350  $\mu$ L of BD buffer was added. After centrifugation at 12000 rpm for 30 min, 500 µL of PWB buffer was added to the CG2 adsorption column. Then, 600 µL of PWB rinsing liquids were added, and an equivalent amount of rinsing was added repeatedly followed by centrifugation for 2 min. The waste was discarded. The CG2 adsorption column was left for 2 min at room temperature. The remaining rinses were dried in adsorption columns that were then transferred to centrifuge tubes with 1.5 mL volume. Fifty



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microliters of elution buffer TB was added and left for 2 min at room temperature. The sample was centrifuged at 12000 rpm for 2 min, and the solution was collected.

Kits manufactured by Kuangyuan Molecular (Suzhou, China) were used for MTHFR gene polymorphism detection. Fluorophore solution (5  $\mu$ L) was placed into each reaction hole, and the samples, including positive samples 1-A, 1-B and 1-C, were added to each reaction hole. The above steps were repeated, and the samples, including positive samples 2-A, 2-B and 2-C, were added to each reaction hole. Then, 1 µL of purified water was dripped into the holes. The above reaction plates were centrifuged and brought to a standstill. Quantitative polymerase chain reaction device manufactured by Beijing Keyu Xingye Science and Technology Development Co. Ltd (Beijing, China) was set to the heating mode. The fluorophore was activated at 95°C for 10 min, and the samples were denatured at 95°C for 15 min. Complete integration of samples and fluorescence were acquired at 95°C for 40 min. The steps of denaturation and integration were repeated 40 times and then the samples were cooled down. VIC and FAM signals were acquired and recorded at 60°C. Reaction plates were embedded in the device. Scale reading was conducted, and data were acquired.

#### Measurements

The expression of C677T and A1298C MTHFR genes were observed at different loci. The transcript sequences for C677T and A1298C MTHFR genes were as follows: Mutant type C/C and wild type C/C; mutant type C/C and heterozygous C/C; mutant type C/C and mutant type T/T; heterozygous A/C and wild type C/C; heterozygous A/C and heterozygous C/T; heterozygous A/C and mutant type T/T; wild type A/A and wild type C/C; wild type A/A and heterozygous C/T; wild type A/A and mutant type  $T/T^{[3,4]}$ .

#### Data processing

The results in this study are presented using "%". SPSS 20.0 statistical software (Armonk, NY, United States) was used, and chi-square test was performed to compare the differences between the two groups. P < 0.05 indicated that the differences between the two groups were significant.

#### RESULTS

#### Genotypic and allelic frequencies of MTHFR C677T

There were significant differences in C/C and C/T genotype frequency between the experimental group and the control group (all P < 0.05). However, the difference in T/T genotype frequency was not significant between the two groups (P > 0.05). There were significant differences in allele frequency between the two groups (all P < 0.05, Table 1).

#### Genotypic and allelic frequencies of MTHFR A1298C

The differences in A/A, A/C and C/C genotypic and allelic frequencies of MTHFRA1298C were not significant between the two groups (P > 0.05, Table 2).

#### DISCUSSION

Granulomatous lobular mastitis is very common in breast-feeding women. Mostly, it occurs in women within 6 years after giving birth<sup>[4,5]</sup>. However, the disease pathogenesis on granulomatous lobular mastitis is unclear. According to some experts<sup>[6-8]</sup>, it is associated with *MTHFR* gene, to be specific, *MTHFR* C677T. Generally, autoimmune disease and malignant diseases have genetic susceptibility, which may be due to gene mutations. Accordingly, the present study discussed the relationship between granulomatous lobular mastitis and MTHFR gene polymorphism.

MTHFR gene polymorphism may cause decreased thermostability and bioactivity of an enzyme. C677T and A1298C are two important loci to test the relevant diseases in the clinical practice. C677T is associated with autosomal recessive inheritance. Similarly, A1298C mutation may also cause decreased thermostability and bioactivity of an enzyme. Comparatively, the effect of A1298C is less serious than that of A1298C<sup>[9,10]</sup>. Reduced enzymatic activity of *MTHFR* results in high homocysteine level and abnormal folic acid levels, which in turn has an adverse effect on DNA formation and causes chromosome disorder<sup>[11,12]</sup>.



#### Lei QR et al. MTHFR C677T and granulomatous lobular mastitis

Table 1 Differences in methylene tetrahydrofolate reductase C677T between the two groups								
<b>C</b>	_	Genotype frequer	ncy	Allele frequency, %				
Groups	n	C/C	C/T	T/T	C	Т		
Experimental group	51	13	28	10	65.89	34.11		
Control group	51	24	19	8	51.44	48.56		
<i>x</i> <sup>2</sup>		10.064	6.269	0.529	4.305	4.305		
<i>P</i> value		0.002	0.012	0.467	0.038	0.038		

Table 2 Differences in methylene tetrahydrofolate reductase A1298C between the two groups								
Crowne	_	Genotype freque	ency	Allele frequency, %				
Groups	п	A/A	A/C	C/C	Α	C		
Experimental group	51	37	13	1	85.30	14.70		
Control group	51	35	14	2	82.35	17.65		
<i>x</i> <sup>2</sup>		0.422	0.099	0.673	0.321	0.321		
<i>P</i> value		0.516	0.753	0.412	0.571	0.571		

In the current study, there were significant differences in C/C and C/T genotype frequency between the experimental group and the control group (all P < 0.05). However, the difference in T/T genotype frequency was not significant between the two groups (P > 0.05). In addition, the differences in A/A, A/C and C/C genotypic and allelic frequencies of MTHFR A1298C between the two groups were not significant (P > 0.05). Nevertheless, there were significant differences in allele frequency between the two groups (all P < 0.05). The results suggest that the *MTHFR* gene is a susceptible factor for granulomatous lobular mastitis, and it causes specificity of genetic loci. Of them, loci C677T may cause diseases but A1298C may not<sup>[13]</sup>.

#### CONCLUSION

In summary, MTHFR C677T is one of the factors that induce granulomatous lobular mastitis. The relationship between MTHFR polymorphism and granulomatous lobular mastitis need to be studied further in large studies.

### ARTICLE HIGHLIGHTS

#### Research background

Granulomatous lobular mastitis is a chronic disease of the breast. Its clinical and radiological features are similar to breast cancer, which makes its diagnosis and treatment complicated. To date, there is no obviously effective treatment for it because its etiology and pathogenesis remain unclear. Several potential reasons include autoimmunity, hormonal disorders, gene polymorphisms, etc. For polymorphisms, the relationship between common variants of MTHFR, e.g., 677T and 1298C, and granulomatous lobular mastitis was not fully understood.

#### **Research motivation**

Identification of the pathogenic genes for granulomatous lobular mastitis will aid in the diagnosis of this disorder by using gene testing. In addition, it will help in the development of targeted therapy, which will allow for better clinical outcomes.

#### Research objectives

This study aimed to analyze the association between MTHFR gene polymorphism and granulomatous lobular mastitis.



#### Research methods

Participants were enrolled and divided into two groups, an experimental group and a control group. The experimental group included patients with granulomatous lobular mastitis. Participants in the control group were women who underwent physical examination. Blood specimen was collected for *MTHFR* A1298C and C677T gene polymorphisms. The expression of C677T and A1298C *MTHFR* genes were observed at different loci.

#### Research results

The results revealed that there were significant differences in C/C and C/T genotype frequency between the experimental group and the control group (all P < 0.05). In addition, there were significant differences in allele frequency between the two groups (all P < 0.05).

#### Research conclusions

*MTHFR* gene was a susceptible factor for granulomatous lobular mastitis, and it causes specificity of genetic loci.

#### Research perspectives

The relationship between common variants of *MTHFR* and the incidence of granulomatous lobular mastitis should be further identified in clinical practice with a large number of patients.

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