Case Control Study

Relationship between granulomatous lobular mastitis and methylene tetrahydrofolate reductase gene polymorphism

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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 People's Hospital of Kunming in the same period were included in the control group. Deoxyribonucleic acid and MTHFR 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 (\( P > 0.05 \)).
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

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[1]. 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[2]. 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 μL 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
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 μL) 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.

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 MTHFR A1298C 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. However, the disease pathogenesis on granulomatous lobular mastitis is unclear. According to some experts, 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. 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.
Table 1 Differences in methylene tetrahydrofolate reductase C677T between the two groups

| Groups       | n  | Genotype frequency | Allele frequency, % |
|--------------|----|--------------------|---------------------|
|              |    | C/C                | C/T                 | T/T               | C                | T                |
| Experimental | 51 | 13                 | 28                  | 10                | 65.89            | 34.11            |
| Control      | 51 | 24                 | 19                  | 8                 | 51.44            | 48.56            |
| $\chi^2$     |    | 10.064             | 6.269               | 0.529             | 4.305            | 4.305            |
| $P$ value    |    | 0.002              | 0.012               | 0.467             | 0.038            | 0.038            |

Table 2 Differences in methylene tetrahydrofolate reductase A1298C between the two groups

| Groups       | n  | Genotype frequency | Allele frequency, % |
|--------------|----|--------------------|---------------------|
|              |    | A/A                | A/C                 | C/C               | A                | C                |
| Experimental | 51 | 37                 | 13                  | 1                 | 85.30            | 14.70            |
| Control      | 51 | 35                 | 14                  | 2                 | 82.35            | 17.65            |
| $\chi^2$     |    | 0.422              | 0.099               | 0.673             | 0.321            | 0.321            |
| $P$ 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[13].

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.

**REFERENCES**

1. Örşen M, Tolunay S, Gökğöz MŞ. Granulomatous Lobular Mastitis: Clinicopathologic Presentation of 90 Cases. Turk Patoloji Dergi 2018; 34: 215-219 [PMID: 29744854 DOI: 10.5146/tjpath.2018.01.431]
2. Chen L, Zhang XY, Wang YW, Zhao QF, Ding HY. [Granulomatous lobular mastitis: a clinicopathological analysis of 360 cases]. Zhonghua Bing Li Xue Za Zhi 2019; 48: 231-236 [PMID: 30831651 DOI: 10.3760/cma.j.issn.0529-5807.2019.03.012]
3. Liu L, Zhou F, Zhang X, Liu S, Liu L, Xiang Y, Guo M, Yu L, Wang F, Ma Z, Li L, Gao D, Zhang Q, Fu Q, Yu Z. Granulomatous Lobular Mastitis: Antituberculous Treatment and Outcome in 22 Patients. Breast Care (Basel) 2018; 13: 359-363 [PMID: 30498422 DOI: 10.1159/000487935]
4. Feng WQ, Ma Y. Association between methylenetetrahydrofolate reductase and gynecological diseases. Shiyong YiXue ZaZhi 2019; 35: 1677-1680
5. Wang Z, Wang N, Liu X, Wang Q, Xu B, Liu P, Zhu H, Chen J, Situ H, Lin Y. Broadleaf Mahonia attenuates granulomatous lobular mastitis-associated inflammation by inhibiting CCL5 expression in macrophages. Int J Mol Med 2018; 41: 340-352 [PMID: 29138800 DOI: 10.3892/ijmm.2017.3246]
6. Chow LW. Management of granulomatous mastitis. Zhonhua Ruxianbing Zazhi (Electronic edition) 2018; 12: 198-201
7. Abureema S, Smooker P, Malmo J, Deighton M. Molecular epidemiology of recurrent clinical mastitis due to Streptococcus uberis: evidence of both an environmental source and recurring infection with the same strain. J Dairy Sci 2014; 97: 285-290 [PMID: 24239086 DOI: 10.3168/jds.2013-7074]
8. Kazmukh A. Granulomatous lobular mastitis secondary to Mycobacterium fortuitum. World J Clin Cases 2016; 4: 409-412 [PMID: 28035314 DOI: 10.12998/wjcc.v4.i12.409]
9. Shin YD, Park SS, Song YJ, Son SM, Choi YJ. Is surgical excision necessary for the treatment of Granulomatous lobular mastitis? BMC Womens Health 2017; 17: 49 [PMID: 28738795 DOI: 10.1186/s12905-017-0412-0]
10. Zhang XY, Zhang Y. MTHFR gene polymorphism. Zhonghua Jianyan Yixue ZaZhi 2016; 39: 544-547 [DOI: 10.3760/cma.j.issn.1009-0126.2016.07.018]
11. Szweda P, Schielmann M, Frankowska A, Kot B, Zalewska M. Antibiotic resistance in Staphylococcus aureus strains isolated from cows with mastitis in eastern Poland and analysis of susceptibility of resistant strains to alternative nonantibiotic agents: lysostaphin, nisin and polymyxin B. J Vet Med Sci 2014; 76: 355-362 [PMID: 24212507 DOI: 10.1292/jvms.13-0177]
12. Chougule A, Bal A, Das A, Singh G. IgG4 related sclerosing mastitis: expanding the morphological spectrum of IgG4 related diseases. Pathology 2015; 47: 27-33 [PMID: 25475110 DOI: 10.1097/PAT.0000000000000187]
13. Zhang J, Cong HL, Cao L, Chen MY, Mao YM. Association between methylenetetrahydrofolate reductase gene A1298C polymorphism and hyperhomocysteinemia. Zhonghua Laonian Xinnao Xueguanbing ZaZhi 2017; 19: 1166-1169 [DOI: 10.3969/j.issn.1009-0126.2017.11.012]
