

Original Article

Association between *MTHFR* polymorphisms and ankylosing spondylitis risk

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Abstract: Purpose: This study was aimed to detect the association between methylenetetrahydrofolate reductase (*MTHFR*) gene polymorphisms (C677T and A1298C) and ankylosing spondylitis (AS) risk. Methods: With matched age and gender, 113 AS patients and 120 healthy controls were recruited in this case-control study. Polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) were adopted to test the C677T and A1298C polymorphisms of *MTHFR* gene. Analyses of linkage disequilibrium and haplotypes of these polymorphisms were conducted by Haploview software. Differences in frequencies of genotypes, alleles and haplotypes in case and control groups were analyzed by chi-square test. Besides, the relative risk of AS was represented by odds ratios (ORs) with 95% confidence intervals (95% CIs). Results: Genotypes TT and CC of C677T and A1298C polymorphisms were obviously related to the occurrence of AS ($P=0.039$, $OR=2.103$, $95\% CI=1.033-4.281$; $P=0.030$, $OR=3.456$, $95\% CI=1.070-11.161$). Meanwhile, 677T was a susceptible allele for AS ($P=0.039$, $OR=1.473$, $95\% CI=1.020-2.127$). But no significant association existed between A1298C alleles and AS risk. Linkage disequilibrium and haplotype analysis indicated that T-A haplotype was more often discovered in cases, which suggested that haplotype T-C may be a risk factor for AS occurrence ($P=0.008$, $OR=1.772$, $95\% CI=1.156-2.715$). Conclusion: C677T and A1298C polymorphisms in *MTHFR* gene may increase the risk of AS.

Keywords: *MTHFR*, polymorphisms, ankylosing spondylitis, haplotypes

Introduction

Ankylosing spondylitis (AS) is a chronic systemic inflammatory disease with the symptoms of sacroiliitis, inflammation of the spine attachment points, and even the bamboo spine [1]. AS is a kind of seronegative spondyloarthropathies. Early symptoms of AS was not obviously. So, when it was diagnosed, the disease was go to an advanced stage [2]. Usually, symptoms of AS was appeared between 15 and 45 years old. AS is one of the common cause of disability, and it appears to have taken a heavy toll on family and society. Recent years, the morbidity of AS is rose, and it was higher in males than that in females. Its pathogenesis has not been clearly figured out yet, and may be related to heredity and environment. According to previous studies, human leucocyte antigen (HLA) is the main susceptible region of AS [3]. Despite HLA-B27 was associated with the AS risk, adaptive and innate immune system was also related to the susceptibility of AS [4-6]. HLA-

B27 accounts for 16% of all the genetic factors [7]. It is possible that there are other susceptible genes playing important parts in AS occurrence [8].

A number of genes which associated with the occurrence of AS have been pointed out, and methylenetetrahydrofolate reductase (*MTHFR*) gene is one of them [9, 10]. *MTHFR* gene is located in chromosome 1p36.3. Protein encoded by this gene is a rate-limiting enzyme in the methyl cycle. Methyl cycle is implicated in many metabolic reactions including synthesis and repair of DNA. Polymorphisms in *MTHFR* gene maybe alter the gene expression and protein function, leading to abnormal metabolism of methyl. C677T (rs1801133) and A1298C (rs1801131) are the two widely explored single nucleotide polymorphisms (SNPs) in *MTHFR* gene. C677T is a common thermally unstable missense mutation which could influence the activity and thermostability of *MTHFR* protein. A1298C polymorphism is a glutamate to ala-

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Table 1. Primer sequences of C677T and A1298C in *MTHFR*

Site	Primer sequence		Amplification length
C677T	For	5'-TGAAGGAGAAGGTGTCTGCGGGA-3'	198 bp
	Rev	5'-AGGACGGTGCAGAGTG-3'	
A1298C	For	5'-TTGGGGAGCTGAAGGACTAC-3'	163 bp
	Rev	5'-CTTTGTGACCATTCCGGTTT-3'	

Table 2. Comparison of distributions of genotypes and alleles of C677T and A1298C in *MTHFR*

Genotype/ Allele	Cases n=113 (%)	Controls n=120 (%)	χ^2	P	OR (95% CI)
C677T					
CC	35 (30.97)	46 (38.3)	-	-	1
CT	46 (40.71)	54 (45.0)	0.141	0.707	1.120 (0.621-2.019)
TT	32 (28.32)	20 (16.7)	4.256	0.039	2.103 (1.033-4.281)
C	116 (51.33)	146 (60.8)	-	-	1
T	110 (48.67)	94 (39.2)	4.273	0.039	1.473 (1.020-2.127)
A1298C					
AA	63 (55.75)	67 (55.8)	-	-	1
AC	37 (32.74)	49 (40.8)	0.616	0.433	0.803 (0.464-1.389)
CC	13 (11.50)	4 (3.4)	4.723	0.030	3.456 (1.070-11.161)
A	163 (72.12)	183 (76.3)	-	-	1
C	63 (27.87)	57 (23.7)	1.036	0.309	1.241 (0.819-1.881)

Table 3. Haplotype analysis of alleles in *MTHFR* C677T and A1298C polymorphisms

locus 1-locus 2	Cases 2n=226	Controls 2n=240	χ^2	P	OR (95% CI)
C-A	74	109	-	-	-
C-C	42	37	3.623	0.057	1.672 (0.983-2.845)
T-A	89	74	6.941	0.008	1.772 (1.156-2.715)
T-C	21	20	1.594	0.207	1.547 (0.784-3.052)

nine substitution showing a decrease activity of *MTHFR* enzyme.

However, results of reports on association between *MTHFR* polymorphisms and AS are varying from different races and areas. In order to detect the association between *MTHFR* gene and AS risk among Chinese population, we analyzed the C677T and A1298C polymorphisms of *MTHFR* gene in AS patients.

Materials and methods

Clinical data

Based on the New York ankylosing spondylitis revised standard in 1984 [11], 113 AS patients (aged from 16-45 years, 81 men and 32

women, duration from 1 year to 13 years) with average age of 27.6 years in Chinese People's Liberation Army General Hospital from March 2010 to March 2015 were recruited as cases in this study. AS patients all experienced routine examination, X-ray radiographic inspection, CT and nuclear magnetic resonance. All the cases didn't receive radiotherapy or chemotherapy before blood sampling. 120 healthy controls (aged from 18 to 45 years, 86 men and 34 women) with average age of 27.3 years were enrolled from health check-up center in the same hospital during same period. Controls had no histories of systemic diseases. There was no statistically significant difference in age and gender between the two groups. All participants were unrelated Han population living in north area within three generations and signed the consent forms. Besides, this study was approved by the Ethics Committee of Chinese People's Liberation Army General

Hospital, and the process of sample collection was conducted according to the human genome research ethics.

DNA extraction

6 ml fasting peripheral venous blood was collected from every participant, and then processed with ethylene diamine tetraacetic acid (EDTA), finally preserved at -20°C for spare. Genomic DNA was extracted using a TIANamp Blood DNA Kit (Beijing Tiangen Biotech Co., Ltd.) and preserved in fridge at -80°C.

PCR amplification

Primers of C677T and A1298C polymorphisms were synthesized by Shanghai Sangon Biotech

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Co., Ltd. Primer sequences were shown in **Table 1**. Total volume of reaction system was 50 μ l, including 2.5 μ l dNTP, 1 μ l Taq polymerase, 10 μ l buffer, 2 μ l forward primer, 2 μ l reverse primer, 4 μ l template DNA and 28.5 μ l sterile water. PCR reaction of *MTHFR* polymorphisms were initially performed with 4 min pre-denaturation at 94°C, followed by 35 circles of 1 min denaturation at 94°C, 61 s annealing at 61°C for C677T (1 min annealing at 55°C for A1298C), 90 s extension at 72°C; finally 7 min extension at 72°C.

Analysis of PCR amplified products

Restriction enzymes *Hinf*I and *Mbo*I were used respectively to digest the PCR amplification products of *MTHFR* C677T and A1298C polymorphisms. 2% agarose gel electrophoresis and EB staining were used to test the digested products.

Statistical analysis

PASW Statistics 18.0 software was adopted to conduct χ^2 examination in order to compare the differences of frequency distributions of genotypes, alleles and haplotypes in case and control groups ($P < 0.05$ means statistical significance). Analyses of linkage disequilibrium and haplotype were processed with Haploview software. Hardy Weinberg equilibrium (HWE) in cases and controls were tested by PLINK 1.07 software. Relative risk of AS was represented by odds ratios (ORs) with 95% confidence intervals (95% CIs).

Results

Basic features of research objects

Genotype and allele distributions of C677T and A1298C polymorphisms in *MTHFR* gene corresponded with HWE in controls. HWE result showed that the goodness of fit for every polymorphism in controls was fine ($P > 0.05$), which demonstrated that the controls were in a good balance and representativeness.

Frequencies of genotypes and alleles in *MTHFR* polymorphisms

Genotype and allele distributions of *MTHFR* C677T and A1298C polymorphisms were shown in **Table 2**. 677TT genotype and 677T

allele were obviously higher in cases than that in controls ($P < 0.05$), which suggested that 677TT genotype and 677T allele were risk factors for AS occurrence (OR=2.103, 95% CI=1.033-4.281; OR=1.473, 95% CI=1.020-2.127). Additionally, 1298CC genotype might increase the risk of AS ($P = 0.030$, OR=3.456, 95% CI=1.070-11.161). Alleles of A1298C polymorphism had no significant association with the susceptibility of AS.

Haplotype analysis of *MTHFR* gene polymorphisms

According to the linkage disequilibrium analysis, there were 4 haplotypes constituted by C677T and A1298C polymorphisms in *MTHFR* gene (**Table 3**). T-A haplotype was apparently different in cases and controls ($P = 0.008$), which made the AS risk rose (OR=1.772, 95% CI=1.156-2.715). Meanwhile, C-C and T-C haplotypes were higher in cases than in controls, but the differences was not significant ($P > 0.05$).

Discussion

AS is a multivariate chronic disease, it belongs to seronegative spondyloarthropathies. AS mainly affect the joints in spine and the sacroiliac joint, and also affect other organs. Owing to the hidden occurrence and unobvious symptoms of AS, the early aches in lumbosacral region and lower back are easily to be ignored by the patients [12]. The best time for treatment may have been lost when the symptom of bilateral sacroiliitis appears. So it has a high disability rate, and brings a heavy family and social burden. Therefore, it is significant to pay attention to early diagnosis, prevention and treatment of AS. Therefore, to find out the risk factors and select alterable factors of AS will contribute to solve these issues. In recent years, researches indicated that genetic factors play a decisive role in the occurrence of AS [13]. It was reported that *MTHFR* gene played important role in many disease [14, 15].

Kang et al. found a kind of variant which was thermolabile and low active [16], soon after they discovered that the thermolabile *MTHFR* had autosomal recessive inheritance [17]. In 1994, Goyette et al. successfully cloned and located *MTHFR* gene using cDNA technology for the first time [18]. *MTHFR* is one of the key enzymes for the remethylation of cysteine [19].

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MTHFR protein also is an important enzyme in folate pathway which relate to the synthesis of methyl donor. Methyl donor might take part in different kinds of biochemical reactions, such as the biosynthesis of purine and thymine. Besides, MTHFR supplies cymene for the methylation of DNA, RNA and proteins and then influences the normal DNA metabolism. *MTHFR* is a primordial and conservative protein in biotic community. If there exists polymorphisms in *MTHFR* gene, it might change the function of MTHFR protein, then leading to DNA defect and various diseases. Recently, candidate gene researches were performed by using polymorphisms which is the most promising molecular method.

Studies have confirmed that there are different kinds of mutation sites in *MTHFR* gene. C677T [20-23] and A1298C [24-26] are the most common mutation sites in human beings, and the variants may change the MTHFR protein activation. Recently, there are more and more studies focus on the association between *MTHFR* polymorphisms and AS risk. However, the conclusion about AS susceptibility is not unified yet. Because polymorphisms had divergences among different regions and ethnicities, association analysis between *MTHFR* polymorphisms (C677T and A1298C) and AS occurrence was detected in this case-control study.

After the detection of HWE in the cases and controls, we discovered that allele distribution of the two SNPs were in accordance with HWE in controls which showed that the controls were representative. Based on calculations, TT and CC genotype frequencies of *MTHFR* gene C677T and A1298C polymorphisms were apparently higher in case group, suggesting these two genotypes related to 2.103 and 3.456 times increased risk of AS, respectively. At the same time, 677T allele might elevate the susceptibility of AS with the OR of 1.473. However, alleles of A1298C polymorphisms had no significant association with the occurrence of AS. This study conducted haplotype analysis and linkage disequilibrium examination on the alleles of the two polymorphisms in *MTHFR* gene. Linkage disequilibrium existed in these polymorphisms. T-A haplotype, compared with C-A haplotype, may be a candidate risk factor for the occurrence of AS.

Combined with the above results, C677T and A1298C were the susceptible polymorphisms

for the onset of AS. However, the concrete pathogenesis of *MTHFR* gene in the occurrence of AS was still unknown. Therefore, well designed researches with large sample size and adjusted confounding should be carried out, so as to get a more exact evidence to certify the etiology of AS.

Disclosure of conflict of interest

None.

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