Combined genotype and haplotype distributions of MTHFR C677T and A1298C polymorphisms

A cross-sectional descriptive study of 13,473 Chinese adult women

Shujun Fan, MDa, Boyi Yang, MD, PhDb, Xueyuan Zhi, MDc, Yanxun Wang, MD, Phdc, Quanmei Zheng, BAd, Guifan Sun, MDe,∗

Abstract
Methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms are, independently and/or in combination, associated with many disorders. However, data on the combined genotype and haplotype distributions of the 2 polymorphisms in Chinese population were limited.

We recruited 13,473 adult women from 9 Chinese provinces, collected buccal cell samples, and determined genotypes, to estimate the combined genotype and haplotype distributions of the MTHFR C677T and A1298C polymorphisms.

In the total sample, the 6 common combined genotypes were CT/AA (29.5%), TT/AA (21.9%), CC/AA (15.4%), CC/AC (14.9%), CT/AC (15.7%), and CC/CC (3.4%); the 3 frequent haplotypes were 677T-1298A (43.6%), 677C-1298A (37.9%), and 677C-1298C (17.6%). Importantly, we observed that there were 51 (0.4%) individuals with the CT/CC genotype, 92 (0.7%) with the TT/AC genotype, 17 (0.1%) with the TT/CC genotype, and that the frequency of the 677T-1298C haplotype was 0.9%. In addition, the prevalence of some combined genotypes and haplotypes varied among populations residing in different areas and even showed apparent geographical gradients. Further linkage disequilibrium analysis showed that the D’ and r² values were 0.883 and 0.143, respectively.

In summary, the findings of our study provide further strong evidence that the MTHFR C677T and A1298C polymorphisms are usually in trans and occasionally in cis configurations. The frequencies of mutant genotype combinations were relatively higher in Chinese population than other populations, and showed geographical variations. These baseline data would be useful for future related studies and for developing health management programs.

Abbreviations: Hcy = homocysteine; LD = linkage disequilibrium; MTHFR = methylenetetrahydrofolate reductase.

Keywords: A1298C, C677T, China, haplotype, methylenetetrahydrofolate reductase

1. Introduction
Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulatory form of folate in plasma and the methyl donor for the remethylation of homocysteine (Hcy) to methionine. The enzyme therefore resides at an important branch point directing the folate pool to Hcy remethylation and DNA methylation or to DNA and RNA biosynthesis.[1] Genetic defects in MTHFR gene therefore have the potential to affect the disease risk both negatively and positively.[1–3]

Many polymorphisms in the MTHFR gene have been identified. Among them, C677T and A1298C are 2 common polymorphisms that have been confirmed to reduce the enzyme activity. The C677T polymorphism decreases the enzyme activity by 70% and 35% in homozygotes (TT) and heterozygotes (CT), respectively, and thus leads to increased plasma Hcy concentrations and abnormal DNA methylation status.[1,4] The A1298C polymorphism can also reduce the enzyme activity but to a lesser extent than the C677T polymorphism.[1] Neither the homozygous nor the heterozygous of the A1298C polymorphism is associated with elevated Hcy and/or lower folate concentrations. However, combined heterozygosity for the 2 polymorphisms, the 677CT/1298AC genotype, results in an even lower MTHFR activity than heterozygosity for either of the
polymorphisms separately and results in elevated Hcy and decreased plasma folate levels as observed in homozygotes for the C677T polymorphism.\[^{[8]}\]

During the past 2 decades, numerous epidemiological studies have investigated the relationships of the MTHFR C677T and A1298C polymorphisms with various diseases, including birth defects, cardiovascular diseases, pregnancy complications, lymphoblastic leukemia, rheumatoid arthritis, and breast cancer, although the results were still inconsistent.\[^{[13–15]}\] In addition, estimation of the genotype and haplotype distributions of the 2 polymorphisms in different populations has also been a focus of considerable interest from researchers worldwide. The 677T allele frequency is often reported to be high in Europeans and North Americans, low in East Asians and Africans, and also with a high frequency in East Asia, followed by Europe, Africa, and North America.\[^{[20]}\] Our group previously recruited over 15,000 adults from 10 provinces to explore the geographical distribution of the MTHFR C677T and A1298C polymorphisms in China.\[^{[21]}\] We found that the prevalence of the 2 polymorphisms varied significantly among Han populations residing in different regions of China, and also showed apparent geographical gradients; the 677TT genotype frequencies steadily increased from southern to northern China, yet the 1298CC genotype frequencies showed a reverse geographical trend.\[^{[21]}\] The distribution of the least common mutant haplotype, 677T-1298C, also showed a large global variation: nil in Pakistan and Brazil, 2.6% in Mexico, 7.0% in Europe, and 0.5% in America.\[^{[22]}\]

Prior studies have shown that there exists an interaction between the 2 polymorphisms in vivo, and that a combined effect of the 2 would result in clinical phenotypes and carry a selective disadvantage.\[^{[8,23]}\] Therefore, understanding the haplotype and combined genotype distribution of the 2 polymorphisms is important and valuable. Several case-control studies have reported the frequencies of the combined genotype and haplotype of the MTHFR C677T and A1298C polymorphisms in Chinese population,\[^{[24-26]}\] but most of these studies were limited by a focus on the prevalence at regional levels and/or small sample sizes. In this study, we estimated and reported the combined genotype and haplotype distributions of the MTHFR C677T and A1298C polymorphisms among 13,473 Chinese adult women from 9 provinces. These regions were distributed widely from south to north of China and have nearly half of Chinese population.

2. Methods

2.1. Study subjects

From October 2008 through February 2011, a total of 13,473 healthy unrelated women within the age group of 19 to 45 years (mean age, 27.7 ± 4.4 years) who came to local maternal and children’s hospital for pre-pregnancy examination were enrolled in our study. These participants came from 9 provinces, which were located widely from southern to northern China. According to the divide represented by the Yangtze River, we further divided these individuals into 2 major groups: the northern and the southern. The northern group included participants from Shandong, Henan, and Shanxi provinces; the southern group included those from Jiangsu, Hubei, Sichuan, Yunnan, Guangdong, and Hainan provinces. The study was conducted in accordance with the World Medical Association Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects, and all procedures were approved by the Ethics Committee of China Medical University (Shenyang, China; Identification code: CMU62037324; July 13, 2008). A written informed consent form was obtained from all participants before study entry. After obtaining due informed consent, buccal smears were collected, dried at room temperature for 1 hour, and then sent to the central laboratory in Shanghai.

2.2. Genotyping

Genomic DNA was extracted from buccal cells using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA). We determined the MTHFR C677T and A1298C genotypes using the Taqman allelic discrimination assay on ABI 7900HT sequence detection system (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions. All PCR reagents were purchased from ABI company. The detailed information on primers and probes was described in our previous paper.\[^{[27]}\] PCR amplification using about 3ng/sample of genomic DNA was done in a thermal cycler (GeneAmp PCR Systeme 9700; Applied Biosystem, Foster City, CA). Cycling conditions were 95°C for 10 minutes, and 20 cycles of 92°C for 15 seconds and 60°C for 1 minute. Data analysis for allelic discrimination was performed using SDS software (Applied Biosystem, Foster City, CA).

2.3. Statistical analysis

Combined genotype frequencies were calculated by direct counting. Hardy–Weinberg equilibrium of the MTHFR polymorphisms in each population, and the difference in the prevalence of the combined genotype between the southern and northern groups were all examined by Chi-square test. The comparison of the combined genotype and haplotype frequencies among the 9 populations was examined using Kruskal–Wallis test. All the above analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). In addition, Haplovew 4.1 software (Broad Institute, Cambridge, MA) was used to estimate haplotype frequencies and to perform linkage disequilibrium (LD) test. A 2-tailed P value < 0.05 was taken as statistically significant.

3. Results

3.1. Combined genotypes

The observed genotype frequencies of the MTHFR C677T and A1298C polymorphisms were all in accordance with Hardy–Weinberg equilibrium. Table 1 presents the frequencies of the combined MTHFR C677T and A1298C genotypes in the 9 Chinese Han populations. In the total sample, the 6 common combined genotypes were CT/AA (29.5%), TT/AA (21.9%), CC/AA (15.4%), CC/AC (14.9%), CT/AC (13.7%), and CC/CC (3.4%). In addition, we observed 51 (0.38%) individuals with the CT/CC genotype, 92 (0.7%) with the TT/AC genotype, and 17 (0.1%) with the TT/CC genotype. The distribution of the combined genotypes varied among the 9 populations (P < 0.001). For example, in Shandong province, the 3 most frequent combined genotypes in sequence were TT/AA (39.9%), CT/AA (28.6%), and CT/AC (15.7%); however, in Hainan province, they were CC/AC (26.6%), CC/AA (25.4%), and CT/AA (24.0%). Furthermore, we found that the prevalence of some combined genotypes showed apparent geographical gradients.
the CC/AA, CC/AC, and CC/CC genotype frequencies steadily increased from northern to southern China, while the frequencies of the TT/AA genotype showed a reverse trend. After dividing the 9 populations into 2 groups, the northern group had significantly higher frequencies of the CT/AA (31.1% vs 27.8%), CT/AC (15.3% vs 12.1%), and TT/AA (44.0% vs 10.0%) genotypes, but lower frequencies of the CC/AA (8.7% vs 22.1%), CC/AC (8.2% vs 21.7%), and CC/CC (5.2%) genotypes. The frequencies of the 3 rare genotypes (CT/CC, 0.4% vs 0.4%; TT/CC, 0.1% vs 0.1%) were similar between the northern and southern groups.

The allelic frequencies of the 2 polymorphisms also showed apparent geographical gradients. For example, the 677T allele frequency was lowest in Hainan province (24.0%, South), intermediate in Jiangsu province (43.5%, Central), and highest in Shandong province (63.1%, North). On the contrary, the 1298C allele frequency showed a decreasing trend from Hainan province (25.7%) to Shandong province (13.1%) (Table 2).

### 3.2. Haplotypes

The LD analysis showed that the D' and r² values were 0.883 and 0.143, respectively, in the total sample, which indicates that the MTHFR C677T and A1298C polymorphisms were at high LD. Similar results were observed in the 9 populations with D' values ranging from 0.782 to 0.909 and r² values ranging from 0.077 to 0.213 (Table 2). We further performed haplotype analysis and found all the 4 haplotypes of the 2 polymorphisms. In the total sample, the most preponderant haplotype was 677T-1298A with a frequency of 43.6%, followed by 677C-1298A (37.9%), 677C-1298C (17.6%), and 677T-1298C (0.9%). The distribution of the haplotype frequencies also differed significantly among the 9 populations (P < 0.001). For example, Henan and Shaanxi provinces had the highest and lowest prevalences of the 677T-1298C haplotype (1.7% vs 0.4%), respectively. In addition, Shandong and Hainan provinces had the lowest and highest prevalences of the 677T-1298A (24.6% vs 51.0%) and the 677C-1298C haplotypes (12.4% vs 25.0%), and had the highest and lowest prevalences of the 677T-1298A haplotype (62.3% vs 20.7%).
23.3%), respectively. Geographically, the frequencies of the 677C-1298A and 677C-1298C haplotypes steadily increased from northern to southern China, whereas the frequencies of the 677T-1298A haplotype presented a reverse trend. The northern group had significantly higher frequency of the 677T-1298A haplotype (37.0% vs 29.8%), but lower frequencies of the 677C-1298A (28.7% vs 47.3%) and 677C-1298C (13.3% vs 21.9%) haplotypes. The frequencies of the 677T-1298C were similar between the 2 groups (1.0% vs 1.0%).

4. Discussion

In this study, we investigated the distribution of the combined genotypes and haplotypes of the MTHFR C677T and A1298C polymorphisms in a large sample of Chinese Han population. All 9 possible combined genotype combinations and 4 haplotypes were detected in the total sample. In addition, the genotypic and haplotypic frequencies varied among populations residing in different areas, and even showed apparent geographical gradients.

The common combined genotypes were CT/AA, TT/AA, CC/AA, CC/AC, CT/AC, and CC/CC, and the frequent haplotypes were 677T-1298A, 677C-1298A, and 677C-1298C, which were in agreement with the findings of other investigators.[17,27,28] Among these common combined genotypes, the CT/AC genotype received the most attention because it was associated with lower enzyme activity and higher plasma Hcy concentrations than heterozygosity for either variant.[7,8,29] The mean frequency of the CT/AC genotype in our study was 13.7%, which is similar to groups.[17] In this study, we observed that 51 individuals were the CC genotype, and that the frequency of 677T-1298C haplotype was 0.94%, and the 677T-1298C haplotype frequencies increased in roughly northern direction. The geographical distribution of the MTHFR polymorphisms, especially the C677T polymorphism, has been investigated in many populations worldwide. Consistent with our findings, a south to north cline of increase in the 677T allele frequency was reported among Indians and Pakistanis.[17,27] However, a reverse trend was observed in European and North America populations.[16,15] The reasons for these geographical gradients remain unclear because many factors, including migratory histories, nutrients intake (especially folic acid, vitamin B2, and vitamin B12), disease, and environmental exposures (such as ultraviolet radiation), may play a role in the propagation of the 2 alleles.[25,38]

Another important finding of the present study is that the common combined genotype and haplotype frequencies showed geographical gradients. For example, the frequencies of the CC/AA, CC/AC, and CC/CC genotypes steadily increased from northern to southern China, but the TT/AA frequencies showed a reverse trend. Correspondingly, the frequencies of 677T-1298A, 677C-1298C increased from northern to southern China, while the 677T-1298A haplotype frequencies increased in roughly northern direction. The geographical distribution of the MTHFR polymorphisms, especially the C677T polymorphism, has been investigated in many populations worldwide. Consistent with our findings, a south to north cline of increase in the 677T allele frequency was reported among Indians and Pakistanis.[17,27] However, a reverse trend was observed in European and North America populations.[16,15] The reasons for these geographical gradients remain unclear because many factors, including migratory histories, nutrients intake (especially folic acid, vitamin B2, and vitamin B12), disease, and environmental exposures (such as ultraviolet radiation), may play a role in the propagation of the 2 alleles.[25,38]

Numerous studies have explored the independent association of the MTHFR C677T or A1298C polymorphism with various clinical conditions.[9,15] The results showed that the 2 polymorphisms can affect diseases risks both positively (such as neural tube defects, coronary heart disease, hypertension, and several cancers)[12,9,10,15] and negatively (such as colorectal, colon, and prostate cancers).[9,39] The relationship between the geographical variations of the C677T polymorphism and the prevalence of related diseases is complicated in China. For example, the prevalence rates of neural tube defects and hypertension increased from south to north region,[10,41] a trend that follows the 677T allele frequencies, and also the TT/AA genotype and 677T-1298A haplotype frequencies. However, there are some exceptions. In the southern China, the prevalence rates of Alzheimer disease and nasopharyngeal cancer were high,[42,43] but the 677T allele was infrequent. With respect to the A1298C polymorphism, although it was not as extensively studied as the C677T polymorphism, epidemiological studies have linked the 1298AA genotype to an increased risk of several diseases such as lymphoblastic leukemia and rheumatoid
arthritis.[12–14] The findings remind us that not only the C677T polymorphism but also the A1298C polymorphism should warrant more attention, especially considering that in our present study, the combined TT/AA is the most represented in northern China, while CT/AA is most represented in the southern China. Also, this may partially explain the inconsistencies between geographical distribution of the C677T polymorphism and prevalence of related diseases mentioned above because the development of the 677T allele related diseases could also be affected by environmental factors and other polymorphisms, especially the A1298C polymorphism. Nevertheless, our data on the geographical distribution of the combined genotype and haplotype may be helpful for researchers seeking to investigate such relationships.

In interpreting the findings of this study, some limitations and strengths should be considered. One limitation of our study was that all study subjects are childbearing age women, which limits the generalizability to men and other age groups. However, no significant gender difference in distribution of the 2 polymorphisms was observed in our and other published studies.[21] Another limitation was that the sample sizes from some provinces were small and many regions of China were not covered, which could have compromised our estimates. A major strength of this study was that our estimates, including $D'$ and $r^2$ statistics, combined genotype and haplotype frequencies, were based on a substantial number of Chinese population, which could be used as authoritative reference data.

In conclusion, this study detected relatively higher frequencies of mutant genotype combinations in Chinese population, demonstrated significant geographical variations in the prevalence of combined genotypes and haplotypes of the MTHFR C677T and A198C polymorphisms, and reconfirmed that the 2 polymorphisms are usually in trans and occasionally in cis configurations. Because the 2 polymorphisms and their combinations are closely related to many disorders, including birth defects, vascular and neurodegenerative diseases, cancers, and pregnancy complications, our study provided important baseline data for future genetic association studies, and would be useful for government and health experts to develop regional health management programs.

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5

Fan et al. Medicine (2016) 95:48 www.md-journal.com
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