Prevalent genotypes of methylenetetrahydrofolate reductase (MTHFR C677T and A1298C) in spontaneously aborted embryos

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Objective: To assess prevalent 5,10-methylenetetrahydrofolate reductase (MTHFR) polymorphisms in spontaneously aborted embryos.
Design: A retrospectively analyzed, prospectively obtained database.
Setting: Bundang CHA General Hospital and Kangnam CHA Hospital.
Patient(s): Ninety-four spontaneously aborted embryos at <20 weeks of gestational age.
Intervention(s): None.
Main Outcome Measure: Genotype frequency of MTHFR C677T and A1298C polymorphisms in spontaneously aborted embryos.
Result(s): The aborted embryos exhibited both a significantly high frequency of the MTHFR 677CC genotype and a low frequency of the MTHFR 677CT genotype, compared to both the child and the adult control groups, respectively, whereas no significant change in the fetal MTHFR A1298C genotype frequency was observed. The combinative genotype of MTHFR 677CC/1298AC from the abortus had a high prevalence compared to the child controls. Further, the chromosomal integrity of the abortus did not affect the frequency of the MTHFR 677CC and 1298AC genotypes.
Conclusion: Spontaneously aborted embryos have a unique distribution of MTHFR C677T and A1289C polymorphisms, regardless of their chromosomal integrity. (Fertil Steril® 2007;87:351–5. ©2007 by American Society for Reproductive Medicine.)

Key Words: Methylenetetrahydrofolate reductase (MTHFR), spontaneously aborted embryo, abortion, SNP, polymorphism

Approximately 20% of recognized pregnancies end in spontaneous abortions; the genetic etiologies of these are largely unknown (1). The association of high blood homocysteine in women with recurrent spontaneous abortions was previously demonstrated (2, 3). The 5,10-methylenetetrahydrofolate reductase (MTHFR) converts 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, the prevalent form of circulating folate that provides the methyl group to homocysteine for the synthesis of methionine. The 5,10-methylenetetrahydrofolate reductase C677T, in which valine is substituted for alanine, and MTHFR A1298C, in which alanine is substituted for glutamate, showed a 20%–30% reduction in their enzyme activities (4). This reduction decreases the conversion of homocysteine into methionine, resulting in homocysteine accumulation in the circulation.

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In addition, methionine is the precursor of *S*-adenosylmethionine (SAM), the methyl group donor for the methylation of DNA, protein, and lipid. Consequently, the perturbation of methionine synthesis also affects subsequent methylation of other acceptors. It was reported that the elevation of homocysteine in the blood increases the risk of recurrent spontaneous abortions as well as of neural tube defects and placental abruption (5–8). However, many others failed to find any significant associations between maternal MTHFR C677T polymorphism and spontaneous abortions.

The 677C→T mutation is common in different populations, with reported homozygote frequencies of 5%–28% of worldwide populations (9, 10). The homozygous state for the 1298A→C transition was observed in approximately 10% of Dutch and Canadian subjects (11, 12). Recent studies reported higher incidences of MTHFR mutations in aborted embryos or fetuses based on Western populations (13, 14). Therefore, we analyzed MTHFR polymorphisms from 94 spontaneously aborted embryos to investigate the association between MTHFR mutations and fetal viability.

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MATERIALS AND METHODS

Subjects

Ninety-four spontaneously aborted embryos at <20 weeks of gestational age were obtained from the Infertility Medical Center at Bundang CHA General Hospital, Seongnam, South Korea, from June 1999 to February 2002. Controls were 100 (age range, 1–15 years) and 449 (age range, 21–86 years) normal hospital-based children and adults, respectively, collected from a sample of convenience. The institutional review board of Bundang CHA General Hospital approved the study in May 1999, and all subjects gave written informed consent.

MTHFR Genotyping

DNA was extracted from an aliquot of fetal tissues, and leukocytes were isolated from the blood of controls using a DNA extraction kit (QIAmp blood kit, Qiagen, Valencia, CA). The C677T mutation was analyzed by polymerase chain reaction (PCR) of genomic DNA, with the use of the following primer pairs: 5′-TGA AGG AGA AGG TGT CTG CGG GA-3′ (forward primer) and 5′-AGG ACG GTG CGG TGA GAG TC-3′ (reverse primer). These primers generate a 198-base pair (bp) fragment following PCR. A1298C mutation was analyzed by PCR with the following primers: 5′-GGG AGG AGC TGA CCA GTG CAG-3′ (forward primer) and 5′-GGG GTC AGG CCA GGG GCA G-3′ (reverse primer). The PCR reaction produces a 141-bp fragment. MTHFR C677T and A1298C genotypes were identified with restriction fragment length polymorphism (RFLP) analysis, as described previously (10).

Cytogenetic Analysis

To determine any association of spontaneously aborted embryos with their chromosomal abnormality, cells from the aborted fetal tissues were cultured for testing chromosomal abnormalities by the method of Evans et al. (15).

Statistical Analysis

Associations of MTHFR C677T, A1298C, or a combination of both between abortus and a normal population were analyzed by ANOVA with the use of SAS 8.2 for Windows (SAS, Inc., Cary, NC).

RESULTS

The distribution of MTHFR C677T and A1289C polymorphisms of abortus and controls is presented in Table 1. The frequencies of MTHFR C677T genotypes for normal homozygotes (677CC), heterozygotes (677CT), and variant homozygotes (677TT) of abortus was 40.4%, 41.4%, and 18.1%, respectively, whereas the child and adult groups showed 24.0%, 55.0%, and 21.0%, and 32.3%, 53.2%, and 14.5%, respectively. Spontaneously aborted embryos exhibited both a significantly higher frequency for the MTHFR 677CC genotype (odds ratio [OR], 2.149; 95% confidence interval [CI], 1.164–3.982; $P=.022$) and a lower frequency for the MTHFR 677CT genotype ($P=.071$) as compared to the child control group. In comparison with the adult controls, the MTHFR 677CT genotype was also significantly lower in the abortus (OR, 0.612; 95% CI, 0.392–0.957; $P=.041$). Additionally, the MTHFR 677TT genotype did not show any significant frequency difference with either of the control groups from the Korean population.

The fetal MTHFR A1298C genotype frequencies were 67.0%, 29.8%, and 3.2% for normal homozygotes (1298AA), heterozygotes (1298AC), and variant homozygotes (1298CC), respectively (Table 1). The distribution of the MTHFR A1289C polymorphism for the child and adult control groups were 77.0% and 69.5% for normal homozygotes, 21.0% and 28.7% for heterozygotes, and 2.0% and 1.8% for variant homozygotes, respectively. Thus, the genotypes of MTHFR A1298C exhibited no significant differences between the spontaneously aborted embryos and the controls.

The frequency of the combined genotypes of MTHFR C677T and A1298C in spontaneously aborted embryos and controls is given in Table 1. We did not find any combined genotype of MTHFR 677CT/1298CC, 677TT/1298AC, or 677TT/1298CC from either the aborted embryos or the controls. The frequency of the combined 677CC/1298AC genotype in the aborted embryos, and the child and adult controls, were 19.1%, 8.0%, and 11.6%, respectively, demonstrating a significantly higher frequency of the combined genotype in the aborted embryo group than in the child controls (OR, 2.724; 95% CI, 1.143–6.469; $P=.039$). In addition, the aborted embryos also presented a higher prevalence of the combined MTHFR 677CC/1298AC genotype in comparison with the adult control group, although this did not reach statistical significance ($P=.068$).

The spontaneously aborted embryo group consisted of an equal number of males and females, and among them 57 (60.6%), 25 (26.6%), and 12 (12.8%) subjects had a normal chromosomal structure or number, an abnormal chromosomal structure or number, and unclassified chromosomes, respectively (Table 2). The distribution of the MTHFR 677CC and 1298AC genotypes between normal and abnormal chromosome groups of the spontaneously aborted embryos was not significantly different, as shown in Table 2, and the combined MTHFR C677T and A1298C genotypes did not significantly affect the prevalence of allele distribution (data not shown).

DISCUSSION

The present study demonstrates that spontaneously aborted embryos exhibit both a significantly higher frequency of the MTHFR 677CC genotype and a lower frequency of the 677CT genotype as compared to controls. These results are of interest, because others found no significant change in frequency of the fetal MTHFR 677CC and 677CT genotypes