

Effect of methylenetetrahydrofolate reductase and thymidylate synthase enhancer region polymorphisms on the risk of idiopathic recurrent spontaneous abortion in a Korean population

Previous studies reported an association of methylenetetrahydrofolate reductase (MTHFR) polymorphisms and recurrent spontaneous abortion, whereas no studies are available for the association with thymidylate synthase enhancer region (TSER) genotypes. Mutations of MTHFR and TSER are not likely significant risk factors of idiopathic recurrent spontaneous abortion in Korean women. (*Fertil Steril*® 2009;91:1560–2. ©2009 by American Society for Reproductive Medicine.)

Approximately 1% to 2% of all women of reproductive age suffer from recurrent spontaneous abortion (RSA), and 40%–55% of the RSA population does not have clear etiology (1). Studies have reported that hyperhomocysteinemia is a risk factor of RSA (2, 3). 5, 10-methylenetetrahydrofolate reductase (MTHFR) is a major enzyme in homocysteine metabolism that converts 5, 10-methylenetetrahydrofolate into 5-methyltetrahydrofolate. The MTHFR 677C>T and 1298A>C polymorphisms exhibit 50%–70% reduction in enzyme activity, which leads to accumulation of homocysteine in the circulation. Recently, however, inconsistent associations have been reported between MTHFR polymorphisms and RSA (2–4).

Thymidylate synthase (TS or TYMS) is a critical enzyme for syntheses of thymidylate, requires 5, 10-methylenetetrahydrofolate as a cofactor, and competes with MTHFR for available 5, 10-methylenetetrahydrofolate (5). The TS enhancer region (TSER) contains two or three 28-base-pair tandem repeats in the 5'-untranslated region, designated 2R or 3R, respectively; the 3R allele exhibits 2.6-fold greater TS gene expression than the 2R allele, and elevated levels of plasma homocysteine have been observed in TSER 3R3R variants (6).

Here we investigated the possible association of idiopathic RSA with MTHFR and TSER polymorphisms. Two hundred twenty-two patients, who had each experienced more than two consecutive abortions, were recruited from patients who visited Bundang CHA General Hospital

from March 1999 to February 2005. Their average age was 32.6 years (range, 22–44 years). Controls were 122 normal hospital-based women with a mean age of 31.2 years (range, 23–43 years), each of whom had had at least one live birth and no history of abortion and other thrombotic diseases. The institutional review board of Bundang CHA General Hospital approved this genetic study in 1999. All patients gave written informed consent.

Leukocyte DNA was extracted with the QIAmp Blood Kit (Qiagen, Valencia, CA), and the MTHFR 677C>T and 1298A>C genotypes and TSER mutations were identified with restriction fragment length polymorphism analysis after polymerase chain reaction of genomic DNA, as previously described (3). Statistical analyses were performed with GraphPad Prism 4.0 (GraphPad Software, San Diego, CA) and SNPalyze 5.10 (DYNACOM, Yokohama, Japan).

The genotype distribution of MTHFR 677C>T and 1298A>C, TSER polymorphic loci did not deviate significantly from the Hardy-Weinberg equilibrium in either group. The association of the combinational polymorphisms of TSER either with MTHFR 677C>T or 1298A>C in RSA patients was further determined. Neither the genotype frequencies of MTHFR 677C>T nor the haplotype analysis of MTHFR 677C>T and 1298A>C in RSA patients showed any significant difference compared with the control group (Table 1). The genotypic frequencies of TSER polymorphism among RSA patients showed a distribution frequency similar to that in the control population, and the combinational genotype distributions of TSER with MTHFR 677C>T or MTHFR 1298A>C were not significantly different compared with control.

Nelen et al. (2) performed a meta-analysis of 10 case-control studies to assess the risks of hyperhomocysteinemia and of the MTHFR 677C>T mutation in recurrent early pregnancy loss and found that elevated blood homocysteine and the variant homozygous genotype (677TT) of MTHFR 677C>T polymorphism are risk factors for recurrent early pregnancy loss. According to a more recent meta-analysis

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TABLE 1

Risk assessment of MTHFR and TSER genotypes in idiopathic recurrent spontaneous abortion.

Genotype/Haplotype	Control	Case	OR (95% CI)	P value
Genotype				
MTHFR 677C>T				
CC	45 (36.9)	82 (36.9)	1.0	—
CT	63 (51.6)	104 (46.8)	0.906 (0.561-1.464)	.687
TT	14 (11.5)	36 (16.2)	1.411 (0.689-2.889)	.346
MTHFR 1298A>C				
AA	75 (61.5)	144 (65.2)	1.0	—
AC	43 (35.2)	68 (30.8)	0.824 (0.513-1.322)	.421
CC	4 (3.3)	9 (4.1)	1.172 (0.349-3.933)	.797
TSER				
2R2R	6 (4.9)	12 (5.4)	1.0	—
2R3R	36 (29.5)	61 (27.6)	0.847 (0.293-2.453)	.760
3R3R	78 (63.9)	148 (67.0)	0.949 (0.343-2.625)	.919
TSER/MTHFR 677				
3R3R/TT	11 (9.2)	19 (8.6)	1.0	—
2R2R+2R3R/TT	3 (2.5)	17 (7.7)	3.281 (0.781-13.78)	.105
3R3R/CC+CT	67 (55.8)	129 (58.4)	1.115 (0.501-2.479)	.790
2R2R+2R3R/CC+CT	39 (32.5)	56 (25.3)	0.831 (0.356-1.941)	.669
TSER/MTHFR 1298				
3R3R/AA	49 (40.8)	94 (42.5)	1.0	—
2R2R+2R3R/AA	24 (20.0)	50 (22.6)	1.086 (0.598-1.973)	.786
3R3R/AC+CC	29 (24.2)	54 (24.4)	0.971 (0.550-1.714)	.918
2R2R+2R3R/AC+CC	18 (15.0)	23 (10.4)	0.666 (0.328-1.351)	.260
Haplotype				
MTHFR 677/1298				
C-A	0.418	0.414	—	.928
C-C	0.209	0.187	—	.494
T-A	0.373	0.391	—	.643
T-C	1.519E-8	0.007	—	.185

Note: Values are number (percentage) unless otherwise noted. OR = odds ratio; CI = confidence interval.

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of 26 case-control studies by Ren and Wang (4), the genotypes of MTHFR 677C>T had overall significant associations with RSA. However, they attributed the positive association to five Chinese studies that exhibited very strong correlation between MTHFR 677C>T polymorphism and RSA, because none of the other 21 studies showed any association. In this study, we failed to find any association of MTHFR and TSER polymorphisms with idiopathic RSA, regardless of any combinational and haplotypic analyses (Table 1). Thus, the present study indicates that MTHFR and TSER mutations in Korean women are not likely significant risk factors for idiopathic RSA.

In our previous abortus study, we found that the frequencies of the 677CT and 677CT+TT genotypes of the MTHFR 677C>T polymorphism were significantly lower in spontaneously aborted embryos, and the combined MTHFR 677CC and TSER 2R2R+3R3R genotype frequencies in the aborted embryo group was higher than

that in the adult control group (7). Therefore, these findings suggest that spontaneous abortion likely occurs as the result of a complex association between parental and fetal factors. Because we were the first group to attempt to assess the association of both MTHFR and TSER in idiopathic RSA, additional studies from diverse ethnic and geographic distributions are needed to draw any further conclusions in conjunction with consideration of other factors. Future studies involving a larger number of samples will be beneficial to firmly validate the association with RSA and to provide a potential tool for risk assessment in patients who are at risk of developing RSA.

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