

## 한국인의 반복자연유산 환자에서 Thymidylate Synthase Enhancer Region (TSER) 돌연변이형의 혈중 호모시스테인 양과의 관련성

포천중문 의과대학교 분당 차병원 진단검사의학과<sup>1</sup>, 임상의학연구소<sup>2</sup>,  
차병원 산부인과 및 여성의학연구소<sup>3</sup>

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### Contribution of Thymidylate Synthase Enhancer Region (TSER) Polymorphism to Total Plasma Homocysteine Levels in Korean Patients with Recurrent Spontaneous Abortion

Yoon Kyung Choi<sup>1</sup>, Myung Seo Kang<sup>1</sup>, Nam Keun Kim<sup>2</sup>, Sun Hee Kim<sup>2</sup>,  
Dong Hee Choi<sup>3</sup>, Myung Ok An<sup>3</sup>, Suman Lee<sup>3</sup>

<sup>1</sup>Department of Diagnostics, <sup>2</sup>Institute for Clinical Research, Department of Obstetrics & Gynecology and <sup>3</sup>Infertility Medical Center, Bundang CHA General Hospital, College of Medicine, Pochon CHA University, Seongnam 463-712, South Korea

**Objectives:** Methylenetetrahydrofolate reductase (MTHFR) mutation are commonly associated with hyperhomocysteinemia, and through their defects in homocysteine metabolism, they have been implicated as a risk factor for recurrent spontaneous abortion. Recent report describe that 28-bp tandem repeat polymorphism in thymidylate synthase enhancer region (TSER) that influence enzyme activity would affect plasma homocysteine level. We have investigated the relationship between TSER genotype and plasma homocysteine level in 54 patients with recurrent spontaneous abortion.

**Methods:** Plasma homocysteine level was measured by fluorescent polarizing immunoassay. MTHFR mutation (C677T and A1298C) was identified by PCR-restriction fragment length polymorphism assay and TSER mutation was analyzed by PCR method. The data were analyzed using the program SAS 8.2 for Windows.

**Results:** Total homocysteine level was significantly higher in MTHFR 677TT genotype ( $9.80 \pm 3.87$   $\mu\text{mol/L}$ ) than MTHFR 677CC genotype ( $8.14 \pm 1.74$   $\mu\text{mol/L}$ ) in Korean patients with unexplained recurrent spontaneous abortion ( $p=0.0143$ ). However, the plasma homocysteine level was not significantly different in the MTHFR 1298AA ( $8.42 \pm 2.65$   $\mu\text{mol/L}$ ) and 1298CC ( $6.09 \pm 0.32$   $\mu\text{mol/L}$ ;  $p=0.2058$ ) and, TSER 2R2R ( $8.61 \pm 1.68$   $\mu\text{mol/L}$ ) and 3R3R ( $8.05 \pm 2.81$   $\mu\text{mol/L}$ ;  $p=0.9319$ ) mutant genotypes, respectively. In this study, we found the combination effects of TSER and MTHFR C677T genotypes. Plasma homocysteine levels were the highest ( $11.47 \pm 4.66$   $\mu\text{mol/L}$ ) in individuals with TSER 3R3R ( $8.05 \pm 2.81$   $\mu\text{mol/L}$ ) and MTHFR 677TT ( $9.80 \pm 3.87$   $\mu\text{mol/L}$ ) genotypes. Individuals with a combination of both TSER 2R2R/2R3R and MTHFR 677CC/CT genotypes ( $7.69 \pm 1.77$   $\mu\text{mol/L}$ ) had

lower plasma homocysteine levels than TSER 2R2R (8.61±1.68 μmol/L) and MTHR 677CC (8.14±1.74 μmol/L) genotypes, respectively. The effect of MTHFR polymorphism in the homocysteine metabolism appears to be stronger than that of TSER polymorphism.

**Conclusion:** Although statistically not significant, we found the elevated level of plasma homocysteine in combined genotypes with TSER and MTHFR (C677T and A1298C) in Korean patients with unexplained habitual abortion. In this study, we reported the possibility that TSER polymorphism is a genetic determinant of plasma homocysteine levels in the Korean patients as well as MTHFR C677T polymorphism. A large prospective study is needed to verify our findings.

**Key Words:** MTHFR, TSER, Homocysteine, Habitual abortion

1~5% . TSER 3R3R

40~55% .<sup>1-3</sup>  
(homocysteine) 가 가  
가 .<sup>2,4</sup> 5,10-methylenetetrahydrofo-  
late reductase (MTHFR) 5,10-methylenetetrahydro-  
folate 5-methyltetrahydrofolate , MTHFR (C677T, A1298C)  
가  
MTHFR C677T  
(alanine; A) (valine; V) , TSER  
, MTHFR A1298C (glutamate; MTHFR (C677T, A1298C)  
G)가 (alanine; A) ,  
20~30% .<sup>5</sup>  
. <sup>6-8</sup>  
가 , 1.  
, , , 1999 3 2001 2  
가 가 .<sup>4,9-12</sup> 2  
Thymidylate synthase (TS) gene 18 54  
, 5,10-methylenetetrahydrofolate . 31.9 (age range,  
pyrimidine , DNA de- 23~42) .  
oxynucleotide dUMP가 dTMP 2.  
, TS 5-fluorouracil (5-FU) 1)  
raltitexed (RTX) 1)  
target .<sup>13</sup> TS 5'-untranslated region EDTA 가 tube  
(5'-UTR) 2 (2R) 3 (3R) 28-bp 1,000 g 15  
tandem repeat 가 3R 2R mRNA 가 가  
2.6~3.6 .<sup>14</sup> 2R 3R -70 12  
가 3R  
, TSER tandem repeat (fluorescent polarizing immunoassy) FPIA  
IMx

(Abbott, USA) (Perkin Elmer 2400) Ho-

2) MTHFR TSER rie (1995) <sup>14</sup>

DNA DNA (extraction column, 3.

QIAmp blood kit, Qiagen

MTHFR C677T MTHFR 677 MTHFR, TSER

(pri- Haenszel Cochran-Mantel-

mer set) sense primer (5'-TGA AGG AGA AGG TGT A1298C TSER , MTHFR C677T,

CTG CGG GA-3') antisense primer (5'-AGG ACG GeneAmp ANOVA

GTG CGG TGA GAG TC-3') GeneAmp SAS 8.2 for Windows

PCR machine (Perkin Elmer 2400) ,

MTHFR A1298C MTHFR 1298

(primer set) 1. MTHFR C677T, A1298C TSER

sense primer (5'-GGG AGG AGC TGA CCA GTG MTHFR C677T (homozygote;

CAG-3') antisense primer (5'-GGG GTC AGG CCA CC)가 20 , (heterozygote; CT)가 23 ,

GGG GCA G-3') GeneAmp PCR machine (TT)가 11 ,

(Perkin Elmer 2400) . 8.14, 7.04, 9.80

(2002) <sup>16</sup> TSER

Genomic DNA 10 mM Tris/1 mM sense primer  $\mu\text{mol/L}$  TT 가

EDTA pH 8.0 sense primer (5'-GTG GCT CCT GCG TTT CCC CC-3') antisense (p=0.0143;

primer (5'-GCT CCG AGC CCG GCC ACA GGC ATG Table 1). MTHFR A1298C 54 52

GCG CGG-3') GeneAmp PCR machine , (AA)가

**Table 1.** MTHFR C677T, A1298C, TSER genotype and gene frequencies with homocysteine concentration in patients with habitual abortion

Genotype	No. of case (%)	Homocysteine ( $\mu\text{mol/L}$ )	p-value for homocysteine comparison
<b>MTHFR C677T</b>			
CC	20 (37.04)	8.14 $\pm$ 1.74*	0.0143**
CT	23 (42.59)	7.04 $\pm$ 1.99	
TT	11 (20.37)	9.80 $\pm$ 3.87	
<b>MTHFR A1298C</b>			
AA	37 (71.15)	8.42 $\pm$ 2.65	0.2058**
AC	13 (25.00)	6.98 $\pm$ 2.44	
CC	3 ( 3.85)	6.09 $\pm$ 0.32	
<b>TSER</b>			
2R2R	2 ( 3.70)	8.61 $\pm$ 1.68	0.9319**
2R3R	16 (29.63)	7.84 $\pm$ 2.16	
3R3R	36 (66.67)	8.05 $\pm$ 2.81	

\* mean  $\pm$  SD, \*\* Analysis by Cochran-Mantel-Haenszel Statistics

**Table 2.** Mean plasma homocysteine concentration associated with TSER and MTHFR C677T genotype in habitual abortion

TSER genotype	MTHFR genotype	Frequency (%)	Plasma homocysteine (μmol/L)	p-value for homocysteine comparison
MTHFR C677T: All subject (n=54)			8.01 ± 2.57*	
3R3R	TT	5 ( 9.26)	11.47 ± 4.66	0.0534**
2R2R/2R3R	TT	6 (11.11)	8.41 ± 2.73	
3R3R	CC/CT	31 (57.41)	7.50 ± 2.02	
2R2R/2R3R	CC/CT	12 (22.22)	7.69 ± 1.77	
MTHFR A1298C: All subject (n=52)			7.97 ± 2.60*	
3R3R	AA	22 (42.31)	8.54 ± 3.05	0.8537**
2R2R/2R3R	AA	15 (28.85)	8.25 ± 2.00	
3R3R	AC/CC	12 (23.07)	6.99 ± 2.30	
2R2R/2R3R	AC/CC	3 ( 5.77)	6.35 ± 2.06	

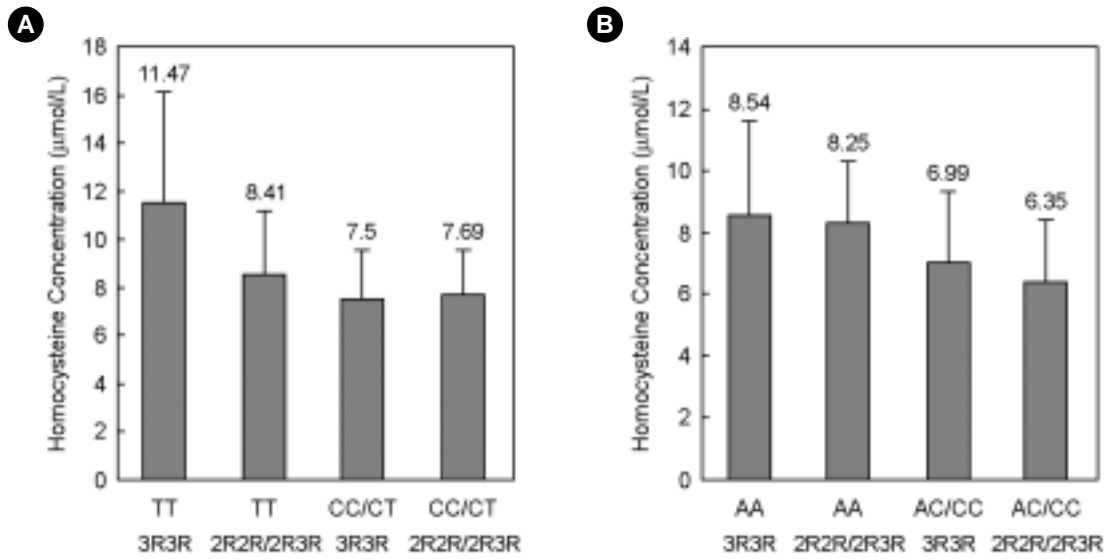
\* mean ± SD, \*\* Analysis by ANOVA Statistics

37 , (AC)가 13 , (CC)가 2 . 8.42, 6.78, 6.09 μmol/L AA 가 , (p=0.2058; Table 1). TSER (2R2R)가 2 , (3R3R) (2R3R) 16 , (3R3R) 가 36 , 8.61, 7.84, 8.05 μmol/L 2R2R 가 (p=0.9319; Table 1).

2. MTHFR C677T TSER MTHFR C677T TT CC/CT , TSER 3R3R 2R2R/2R3R 4 . TSER 3R3R MTHFR C677T TT 11.47 μmol/L, 2R2R/2R3R TT 8.41 μmol/L, 3R3R CC/CT 7.50 μmol/L, 2R2R/2R3R CC/CT 7.69 μmol/L 3R3R TT 가 (p=0.0534; Table 2; Figure 1A).

3. MTHFR A1298C TSER MTHFR A1298C CC AA/AC , TSER 3R3R 2R2R/2R3R 4 . TSER 3R3R MTHFR A1298C AA 8.54 μmol/L, 2R2R/2R3R AA 8.25 μmol/L, 3R3R AC/CC 6.99 μmol/L, 2R2R/2R3R AC/CC 6.35 μmol/L , 2R2R/2R3R AA 3R3R AC/CC 3R3R AA 2R2R/2R3R AC/CC (p=0.8537; Table 2; Figure 1B).

1997 Nelen MTHFR C677T 가 2~3 , MTHFR C677T .<sup>17</sup> MTHFR C677T TT (Table 1), 가 가



**Figure 1.** Distribution of plasma homocysteine concentration of TSER & MTHFR C677T (A), TSER & MTHFR A1298C (B) genotype combination

(p=0.0143; Table 1) MTHFR C677T  
 Kutteh (1999)  
 Brenner (1999) MTHFR C677T  
 가 가  
<sup>18,19</sup>  
 MTHFR 가 MTHFR  
<sup>20,21</sup>  
 Trinh (2002) TSER 3R 가  
 가 , MTHFR  
<sup>15</sup>  
 3R3R MTHFR 677TT 11.1 μmol/L  
 12.1 μmol/L , TSER MTHFR  
 C677T TSER  
 3R3R & MTHFR 677TT 12.7 μmol/L  
 가 가 ,  
 가 가  
 TSER 2R2R, MTHFR 677TT,  
 MTHFR 1298AA 8.61±1.68, 8.14±1.74,  
 8.42±2.65 μmol/L (Table 1),  
 TSER MTHFR TSER 3R3R &  
 MTHFR 677TT TSER 3R3R & MTHFR 1298AA  
 11.47±4.66, 8.54±3.05 μmol/L 가  
 (Table 2).  
 TSER MTHFR TSER 3R3R  
 MTHFR 가  
 가  
 Trinh (2002) 가  
 가  
<sup>15</sup>  
 TSER MTHFR 3R3R & 677TT ,  
 2R2R/2R3R & 677TT , 3R3R & CC/CT ,  
 2R2R/2R3R & CC/CT 4  
 4  
 (p=0.0534; Table  
 1; Figure 1A). p  
 가  
 가  
 , TSER 2R2R/2R3R &

MTHFR 677CC/CT  
(7.69±1.77 μmol/L) TSER 2R2R (8.61±1.68 μmol/L)  
MTHFR 677CC (8.14±1.74 μmol/L)

2R2R/2R3R

MTHFR A1298C

C677T TSER  
가 (Table 2; Figure 1B).  
MTHFR A1298C TSER

2R3R

가

MTHFR TSER  
가

B6, B12

49

500 μg

677CC

가

가

μg

1)

B6 B12

1) 1 2000 7 ; ( )

MTHFR C677T

, A1298C TSER

C677T TSER  
MTHFR 677TT 가

(p=0.0534), MTHFR A1298C TSER

(p=0.8537). MTHFR C677T  
TSER MTHFR A1298C TSER

MTHFR

(Table 2).

가

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