

한국인 남성 불임 환자에서 5,10-Methylenetetrahydrofolate Reductase (MTHFR) 유전자의 1298번의 다형성과의 관련성에 관한 연구

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Genetic analysis for Polymorphism of 5, 10-Methylenetetrahydrofolate Reductase (MTHFR) A1298C and Infertile Males in Korea

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Objective: To investigate the association of genetic background between MTHFR A1298C genotype and male infertility.

Materials and Methods: We compared 377 infertile males with 396 healthy fertile males with one or more offspring. Infertile males were classified into four subtypes (281 azoospermia, 26 oligoasthenoteratozoospermia (OAT), 59 severe OAT and 11 remnants) by World Health Organization (WHO). Pyrosequencing analysis for MTHFR (methylenetetrahydrofolatereductase) A1298C variation was performed on polymerase chain reaction (PCR) product of study group. To validate pyrosequencing data of A1298C variation for randomly selected 50 samples, we compared the pyrosequencing result with the PCR-RFLP (Restriction Fragment Length Polymorphism) result of MTHFR A1298C genotype.

Results: We studied MTHFR A1298C variation by pyrosequencing. A1298C variation data (1298 AC; p=0.2166 and 1298 CC; p=0.5056) of MTHFR gene was no significant difference in between fertile and infertile males.

Conclusion: The genetic analysis in MTHFR gene didn't appear genetic difference in Korean fertile and infertile males. We require further study for MTHFR gene in infertile males.

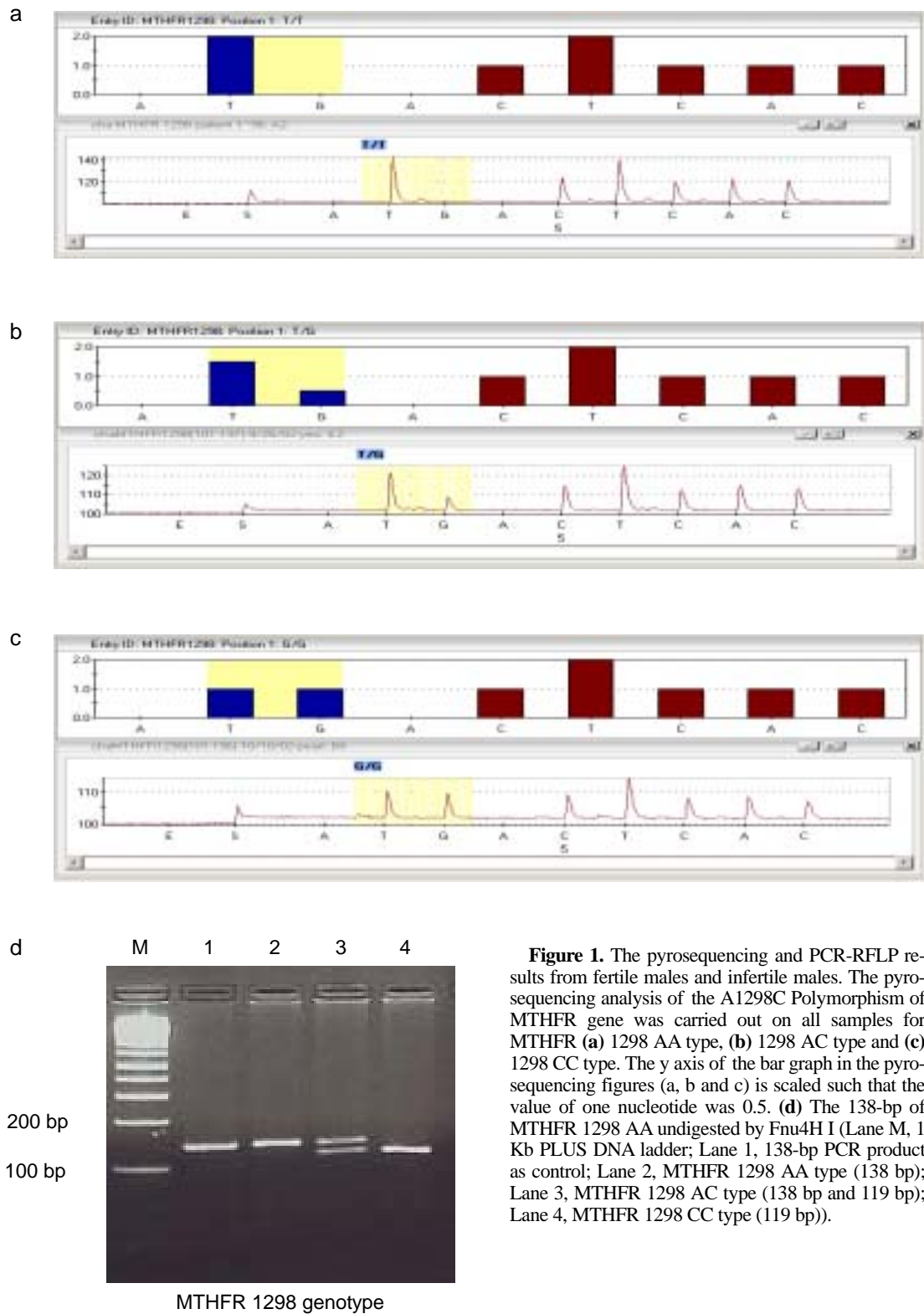
Key Words: Male infertility/ MTHFR/ Pyrosequencing

MTHFR (methylenetetrahydrofolatereductase) 36.3 .^{1,2} MTHFR 5,10-methylenete-
(gene) 11 exon (coding trahydrofolate 5-methyltetrahydrofolate
region) 1,980 bp 가 (chromosome) 1p- , (homocysteine)

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(methionine) B₁₂ .¹¹
 (remethylation)
 (carbon donor)가 5-
 methyltetrahydrofolate (reactive oxygen metabolite) H₂O₂ (hydrogen peroxide)
 (methyl donor)가 .³ MTHFR DNA
 (polymorphism) C677T 가 (damage) , DNA
 , MTHFR (male infertility) .¹²⁻¹⁴
 , 1298 exon 7 adenine cy- 가 , MTHFR
 tosine (substitution) . MTHFR A1298C A1298C
 glutamic acid (E) alanine (A)
 (enzyme activity) 1298
 .⁴ 가 . ,
 (homocysteine metabolism) MTHFR
 MTHFR MTHFR MTHFR 1298
 S-adenosylmethionine (SAM) S-adenosylhomocysteine
 (SAH) DNA (hy-
 pomethylation) .⁵ MTHFR
 5-methyltetrahydrofolate 1.
 2000 1 2002 8
 (hyperhomocysteinemia) 377 ,
 . 1 396
 (folate) dihydrofo-
 late MTHFR 33.3 (age range, 26~47)
 . 42.7 (age range, 26~72) .
 DNA (semen analysis) Kruger¹⁵
 (zinc) 가 가¹⁶ 가 (281
 (non-obstructive azoospermia), 26
 .⁶ (oligoasthenoteratozoospermia (OAT)),
 A1298C C677T (neural tube 59 (severe OAT), 11
 defect), (acute lymphocytic leu- remnants) ,
 kemia) .⁷⁻¹⁰ Y 가 (chromosome
 MTHFR 가 domain disorder and Yq deletion) ,
 . (unexplained
 MTHFR 677 N-terminal catalytic males) 가
 domain , MTHFR 1298 .
 C-terminal regulatory domain exon 7 2.
 1298 C- MTHFR
 (allosteric inhibitor) SAM Cytogenic (lymphocyte
 MTHFR (metaphase)가 (spread)



DNA

'sequence primer' 5'-AAA GAC TTC AAA GAC
ACT-3'

SRY 12 (Sequence
Tagged Sites (STSs)) (AZF-a region: sY 84, sY 86, AZF-
b region: sY 134, sY 138, MK5, AZF-c region: sY 152,
sY 147, sY 254, sY 255, sPGY1, sY 269, sY 158)

137† Y (pri-
mer) multi-PCR .^{17,18}

MTHFR 1298
(pyrosequencing)
DNA polymerase
(nucleotide)† (PPi; pyro-
phosphate)

ATP sulfurylase ATP
luciferase
(sequence) 1298
(genotype)
forward primer (5'-Biotin-TTT GGG
GAG CTG AAG GAC TA-3') reverse primer (5'-
TGG TTC TCC CGA GAG GTA AA-3')

(PCR) (MJ research thermal cycler, Waltham,
USA)

1298 141 bp†

(annealing temperature) 58
45 2% †
(agarose gel) ethidium bromide
(biotin)

(Restriction Fragment Length Polymorphism)
1298 forward primer (5'-GGG
AGG AGC TGA CCA GTG CAG-3') reverse primer
(5'-GGG GTC AGG CCA GGG GCA G-3')

(MJ research thermal cycler,
Waltham, USA) 138 bp
95 , 61 30
, 72 30
(extension) 35
. 1298 A C
Fnu4H I (New England Biolabs, Beverly,
MA, USA) 37 1
glutimic acid
alanine 119 bp 19 bp
. 4 3% †
ethidium bromide

3.
SAS (SAS Insti-
tute, Cary, NC) (Chi-square)
, (Fisher's exact)
ratio; OR) 95% (95 percent confidence
intervals, 95% CI) . p 0.05

Table 1. Distribution of the genotypes of MTHFR (methylenetetrahydrofolatereductase) A1298C in infertile and fertile males

	Fertile males (n=396)	Infertile males (n=377)	OR (95% CI)	P
1298AA	67.93% (n=269)	63.40% (n=239)		
1298AC	28.03% (n=111)	31.83% (n=120)	1.22 (0.89~1.66)	0.2166
1298CC	4.04% (n=16)	4.77% (n=18)	1.27 (0.63~2.54)	0.5056
AC + CC	32.07% (n=127)	36.60% (n=138)	1.22 (0.91~1.65)	0.1843
C allele	18.06% (n=143)	20.69% (n=156)	1.18 (0.92~1.52)	0.1899

OR = odds ratio; CI = confidence interval

가 . 1298 RFLP Figure
 1 .
 Table 1
 377 , (frequency) . 1298 A/C
 396 . (p=0.2166) C/C (p=0.5056)

Table 2. The frequency of MTHFR (methylenetetrahydrofolatereductase) A1298C genotype according to the subtype of infertile male group

	1298AA	1298AC	1298CC
Azoospermia (n=281)	61.21% (n=172)	33.10% (n=93)	5.69% (n=16)
OR (95% CI)	-	1.31 (0.94~1.83)	1.56 (0.76~3.21)
P	-	0.1139	0.2196
OAT* (n=26)	61.54% (n=16)	34.62% (n=9)	3.84% (n=1)
OR (95% CI)	-	1.36 (0.58~3.18)	1.05 (0.13~8.43)
P	-	0.4714	0.9628
severe OAT† (n=59)	74.58% (n=44)	23.73% (n=14)	1.69% (n=1)
OR (95% CI)	-	0.77 (0.41~1.46)	0.38 (0.05~2.95)
P	-	0.4256	0.3388
Remnant‡ (n=11)	63.64% (n=7)	36.36% (n=4)	-
OR (95% CI)	-	1.38 (0.40~4.82)	-
P	-	0.6077	-
Male control (n=396)	67.93% (n=269)	28.03% (n=111)	4.04% (n=16)

*Oligoasthenoteratozoospermia (sperm count $<20 \times 10^6$, progressive motility $<50\%$, normal morphology $<14\%$),
 †Severe oligoasthenoteratozoospermia (sperm count $<0.5 \times 10^6$, progressive motility $<10\%$, normal morphology $<4\%$),
 ‡Retractile testis, oligozoospermia (sperm count $<20 \times 10^6$), difficulty in ejaculation, OR = odds ratio; CI = confidence interval

Table 3. The frequency of MTHFR (methylenetetrahydrofolatereductase) A1298C in infertile males according to cytogenetic analysis and Yq deletion analysis

	1298AA	1298AC	1298CC
Unexplained males (n=262)	61.83% (n=162)	33.21% (n=87)	4.96% (n=13)
OR (95% CI)	-	1.30 (0.92~1.83)	1.35 (0.63~2.87)
P	-	0.1303	0.4369
Chromosome disorder and Yq deletion (n=115)	66.96% (n=77)	28.70% (n=33)	4.34% (n=5)
OR (95% CI)	-	1.04 (0.65~1.65)	1.09 (0.39~3.08)
P	-	0.8728	0.8681
Male control (n=396)	36.62% (n=145)	50.50% (n=200)	12.88% (n=51)

OR = odds ratio; CI = confidence interval

0.05). Table 1 A/C C/C (p> (A/ furylase, apyrase, luciferase)
C+C/C) C (allele) 가 가 ,
C allele (Single Nucleotide Polymorphism)
, 가 .¹⁹
가 (p>0.05). MTHFR 1298
Table 2 (Table 2)
가 (Table 3) .
, Table 1 1298 A/C C/C
remnant 가 , Table 2
MRHFR 1298 (, remnant) 1298
(p>0.05). A/C, C/C Table 3
Table 3 MTHFR 1298 가 cytogenic Y
Y 가 (Y
. 1298 A/C, C/C)
MTHFR 1298
(p>0.05). MTHFR 1298
MTHFR 1298 가
, 가
MTHFR (Cardiovascular 가 .⁸
disease),²⁰ (Down's syndrome)⁹ 1298 가,
(pediatric Stroke)²¹ 677 MTHFR 677
1298 가 가 가
. MTHFR . 677
CBS (cystathionine beta-synthase),
, ¹⁰ MTHFR 677 MS (methionine synthase)
(p=0.008)
, MTHFR 1298
. MTHFR 677 12984
MTHFR 1298 .
MTHFR 1298 .
, 가 (polymerase, ATP sul-

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