

불임여성에서 NAT2, GSTM1, CYP1A1 유전자 다형성과 자궁내막증의 상관관계에 관한 연구

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Association between Endometriosis and Polymorphisms of *N-acetyl Transferase 2 (NAT2)*, *Glutathione S-transferase M1 (GSTM1)* and *Cytochrome P450 (CYP) 1A1* Genes in Korean Infertile Patients

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Objective: To investigate the association between endometriosis and polymorphisms of *N-acetyl transferase 2 (NAT2)*, *glutathione S-transferase M1 (GSTM1)*, and *cytochrome P450 (CYP) 1A1* genes in Korean infertile patients.

Materials and Methods: A total of 303 infertile patients who had undertaken diagnostic laparoscopy during January, 2001 through December, 2003 at Samsung Cheil Hospital enrolled in this study. The patients were grouped according to laparoscopic findings: minimal to mild endometriosis (group I: n=147), moderate to severe endometriosis (group II: n=57), normal pelvic cavity (n=99). Peripheral blood was obtained and genomic DNA was extracted. The genotypes of each genes were analyzed using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). For *NAT2*, RFLP was used to detect the wild type (wt) and mutant (mt) alleles, enabling classification into slow (mt/mt) or fast (wt/wt or wt/mt) acetylation genotypes. For *GSTM1*, PCR was used to distinguish active (+/- or +/+) from null (-/-) genotypes. For *CYP1A1*, *MspI* digestion was used to detect the wild type (A1A1), heterozygote (A1A2) or mutant (A2A2) genotypes.

Result: The genotype frequencies of *NAT2* slow acetylator was 12.8%, 10.9%, 12.8% in group I, group II and control, respectively. The genotype frequencies of *GSTM1* null mutation was 55.3%, 41.8%, 53.2% in group I, group II and control, respectively. The genotype frequencies of *CYP1A1 MspI* polymorphism was 16.3%, 9.1%, 18.1% in group I, group II and control, respectively. No significant difference was observed between endometriosis and normal controls in the genotype frequencies of the *NAT2*, *GSTM1*, *CYP1A1 MspI* polymorphism.

Conclusion: The *NAT2*, *GSTM1*, *CYP1A1* gene polymorphism may not be associated with the

susceptibility of endometriosis in Korean women.

Key Words: Endometriosis, Polymorphism, *NAT2*, *GSTM1*, *CYP1A1*

(stroma) (gland) . *CYP1A1* 15q22-q24
 , 가 (*MspI* *Ile-Val*)
 , *MspI* 가
¹⁰
 .
 Glutathione S-transferase (GST) isoenzyme
 가 (familial study) (twin *GSTM1* *GSTT1* , II
 study) , . *GSTM1* 1p13
 , (null allele)
 . *GSTM1*
 가 .
 , 가 ^{11,12}
 N-acetyl transferase (NAT) II
 , *NAT1* *NAT2* 가 polymorphic gene
 . *NAT2*
 가
¹⁻⁵
 Rier ⁶ Yang ⁷
 , 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin)
 8p22 wild-type
 , *NAT2* allele (*4) 가 fast acetylators ,
 variant alleles (*5, *6, *7) 가
 slow acetylators .¹³ Slow acetylator
¹⁴ 가 , fast acetylator
¹⁵ 가 .
 NAT2,
⁸
 procarcinogen , estradiol *GSTM1*, *CYP1A1*
 가 , proinflammatory growth
 factor , remodeling enzyme
 가
 가 .⁹ Arylhydrocarbon receptor (AhR)
 1.
 aryl hydrocarbon receptor nuclear translocator (ARNT) 2001 1 2003 12
 (303
) 가
⁹
 .
 가
 Cyochrome P450 (CYP) 56
 , I
 CYP1A1, CYP1A2, CYP1B1 revised American Fertility Society (1985)

Table 1. Sequences of oligonucleotide primers and PCR conditions for *NAT2*, *GSTM1* and *CYP1A1*

		Sequences (5' 3')	Anneling temp ()	Products sizes (bp)
<i>NAT2</i>	Forward	GCT GGG TCT GGA AGC TCC TC	62	547
	Reverse	TTG GGT GAT ACA TAC ACA AGG G		
<i>GSTM1</i>	Foreward	CTG CCC TAC TTG ATT GAT GG	64	218
	Reverse	CTG GAT TGT AGC AGA TCA TGC		
<i>GYP1A1</i>	Forward	CAG TGA AGA GGT GTA GCC GCT	62	340
	Reverse	TAG GGA GTC TTG TCT CAT GCC T		

가 .

2. Genomic DNA

Blood Kit (QIAGEN Inc, Chatsworth, CA, USA) genomic DNA

QIAamp genomic DNA 4 -20

PCR

*Bam*HI gel UV

(Figure 1). *GSTM1* (+/+ +/ -) null (-/-) mutation

CYP1A1 *Msp*I genotype

3. (Polymerase chain reaction)

NAT2, *GSTM1* *CYP1A1* polymorphism polymorphism loci primer

SPSS version 10.0 Chi-square test

PCR (Table 1). PCR total 20 µl

10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.5 units of *Taq* DNA polymerase (Roche Diagnostics GmbH, Mannheim, Germany), 50~100 ng genomic DNA primer

10 pmol . PCR

denaturation 94 2

40 35 cycles

94 40 , 58 , 60 , 62 1 ,

72 1 cycle

extension 10

4.

NAT2 polymorphism wild 12.8%

allele (*4) mutant allele (*5, *6, *7)

PCR

*Kpn*I, *Dde*I, *Taq*I 2% agarose genotype PCR band null (Figure 2).

*Msp*I PCR genotype

5.

SPSS version 10.0 Chi-square test

. *p* 0.05

(Mean ± STD) 24~42 31.59±3.38 31.78±3.54 (Mean ± STD) 25~40

NAT2 , (group I) slow acetylator 12.8% , moderate-severe (group II) 10.9%, (Table 2).

GSTM1 ,

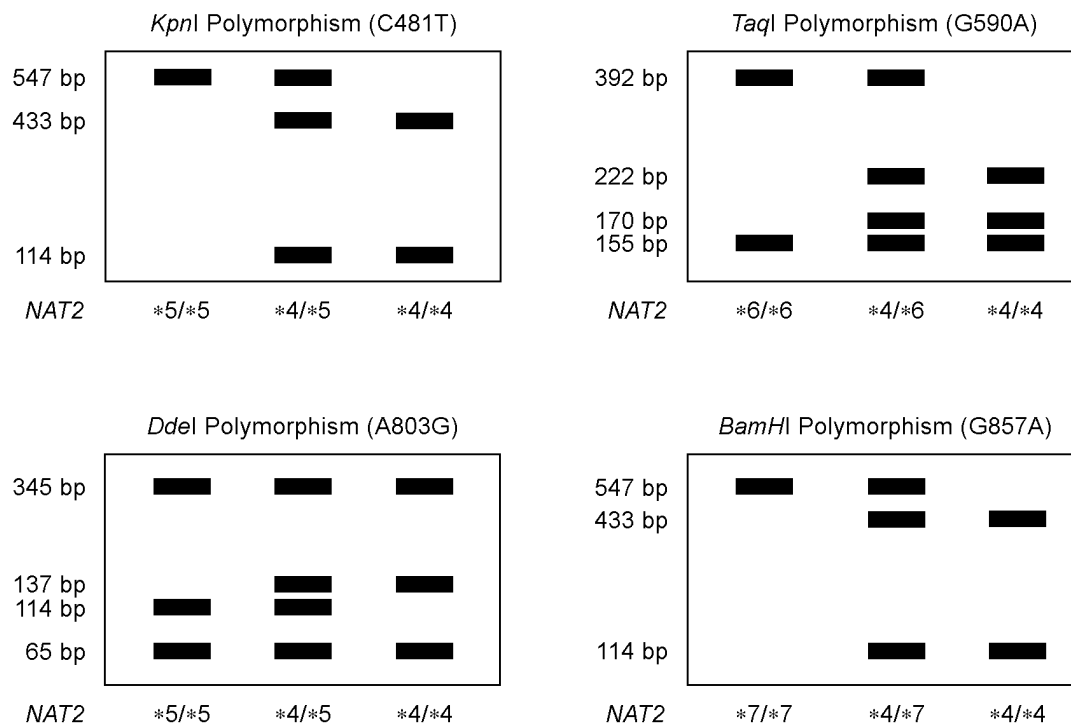


Figure 1. Genotype determination by restriction fragment length polymorphism analysis of the *NAT2* gene PCR products. Following PCR amplification, separate digestions of each PCR product were carried out with the restriction enzymes *KpnI*, *DdeI*, *TaqI* and *BamHI* to detect the substitutions C481T, A803G, G590A, and G857A, respectively. The sizes of the digested products for each restriction enzyme which allow the individual's genotype to be determined are shown diagrammatically (quoted from Nakago *et al.*, Mol Hum Reprod, 2001).

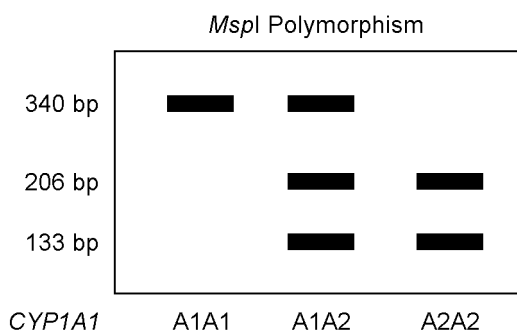


Figure 2. Genotype determination by restriction enzyme digestion of the *CYP1A1* gene PCR products. Following PCR amplification, digestions of each PCR product were carried out with the restriction enzymes *MspI*. A1A1, wild genotype; A1A2, heterozygous genotype; A2A2, homozygous genotype for polymorphism.

가 (Table 2).
CYP1A1
CYP1A1 MspI mutant
 group I 16.3%, group II 9.1%,
 18.1% 가
 (Table 2).

GSTM1 group I 55.3%,
 group II 41.8%, 53.2%

CYP1A1 Hadfield 16 가

Table 2. Frequencies of observed *NAT2*, *GSTM1* and *CYP1A1* genotypes between endometriosis patients and controls

Gene	Polymorphisms	Control	Group I ^a	Group II ^a	p value
<i>NAT2</i>	Slow ^b (mt/mt)	12.8% (12/94)	12.8% (18/141)	10.9% (6/55)	p>0.05
	Fast ^b (wt/mt, wt/wt)	87.2% (82/94)	87.2% (123/141)	89.1% (49/55)	p>0.05
<i>GSTM1</i>	Null (-/-)	53.2% (50/94)	55.3% (78/141)	41.8% (23/55)	p>0.05
	Active (+/+, +/-)	46.8% (44/94)	44.7% (63/141)	58.2% (32/55)	p>0.05
<i>CYP1A1</i>	Mutant (mt/mt)	18.1% (17/94)	16.3% (23/141)	9.1% (5/55)	p>0.05
	Wild (wt/wt, wt/mt)	81.9% (77/94)	83.7% (118/141)	90.9% (50/55)	p>0.05

^a Group I, minimal-mild endometriosis; Group II, moderate-severe endometriosis (rAFS, 1985)

^b Slow, only two variant alleles; Fast, presence of at least one wild-type (*4) allele

21%, 18%, 24% *CYP1A1* *MspI* polymorphism 가 . *GSTM1* 49.2%, 61.5% 가 *CYP1A1* *MspI* polymorphism 가 가 . II, 55.3%, 41.8%, 53.2% 가 group I, 17 가 . *NAT2* Baranova ¹⁸ (wt/wt) 45.1%, slow 34.3% 가 . acetylators 60.0% 38.9% *CYP1A1* mutant type (mt/mt, wt/mt) group I, II, 16.3%, 9.1%, 18.1% 가 3, 4 48.3% 가 *GSTM1* Baranova ¹⁸ 3, 4 *GSTM1* fast acetylator 가 57.4% (45.8%) minimal-mild 33.3% (75.6%) moderate-severe (79.3%) 32.3% *GSTM1* 가 21 , 16 *GSTM1* 가 , Hadfield 45%, slow acetylator 12.2%, 10.2% 52%, 45% Baxer ¹⁹ 가 group I, II, 가 . *GSTM1* 가 가 . 47.6%, 48.9% 17 , *GSTM1* 33.3%, 3, 4 56.9% *GSTM1* 가 ,

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- 가
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