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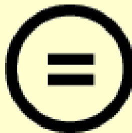

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의학박사 학위논문

Aryl hydrocarbon receptor repressor, glutathione-S-transferase M1, glutathione-S-transferase T1 유전자 다형성과 다낭성 난소증후군의 연관관계 규명

2019년 02월

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이 논문을 의학박사학위논문으로 제출함

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Abstract

Association between susceptibility to polycystic ovary syndrome and the genetic polymorphisms of aryl hydrocarbon receptor repressor, glutathione-S-transferase M1 and glutathione-S-transferase T1 genes

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Objective: It is well known that genetic polymorphism is also involved in the onset of polycystic ovary syndrome (PCOS), but currently there is no study about the polymorphism of detoxification enzymes in PCOS patients. We investigated the effect of genotype on clinical features of PCOS by comparing the distribution of aryl hydrocarbon receptor repressor (AhRR), glutathione-S-transferase M1 (GSTM1) and glutathione-S-transferase T1 (GSTT1) gene polymorphisms and phenotypes

Design: The study was a case-control study including 478 women with PCOS and 376 women without PCOS from the Seoul National University Hospital and the Seoul National University Hospital Healthcare System.

Materials and Methods: Genomic DNA was extracted from peripheral blood. Genotyping of the AhRR codon 185 was performed by real-time PCR analysis on an ABI Prism 7000 Sequence Detection System. GSTM1 and GSTT1 genotyping for gene deletions were carried out by multiplex PCR analysis.

Results: There was a trend for an association of C/G+G/G genotypes with the patients with PCOS (p=0.06). But there was no difference in the genotype distribution of GSTM1 or GSTT1. Nevertheless, analyzing AhRR and GSTT1 together, we found that patients with high-risk genotypes at both loci have increased in patients with PCOS compared with controls (p=0.03, OR=1.54, CI=1.04-2.29). When classification of PCOS patients according by phenotype, there were significant differences in the combined G-allele genotype and GSTT1 null genotypes between no hirsutism group and controls (p=0.02). The combined GSTM1/GSTT1 null genotypes were also a trend for an association with PCOS.

Conclusions: The AhRR codon 185, GSTM1 and GSTT1 mutant variants individually are not associated with the pathophysiology of PCOS. However, combined G allele of AhRR codon 185 and GSTT1 null genotype or combined GSTT1/GSTM1 null genotypes could be associated with pathophysiologic aberrance involved in PCOS.

Keywords: polycystic ovary syndrome, Endocrine disruptor, polymorphism, aryl hydrocarbon receptor repressor, glutathione-S-transferase
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I. Introduction

Polycystic ovary syndrome (PCOS) is one of the common endocrine disorders and affects from 2-27% of reproductive age women [1-3]. Three definitions are most commonly cited about PCOS, National Institute of Child Health and Human Development (NICHD) criteria (1990), the European Society for Human Reproduction and Embryology and American Society for Reproductive Medicine (ESHRE/ASRM or Rotterdam) criteria (2003) and Androgen Excess Society (AES) criteria (2006). All three definitions emphasized the importance of hyperandrogenism and ovulation disorders [4,5] and of these three, the Rotterdam criteria is the most widely used.
PCOS is also associated with several long-term health consequences such as infertility, hirsutism, diabetes, cardiovascular disease, and breast cancer. But the exact causes of PCOS are not fully explained. Some recent studies have explained that genetic factors play an important role in the etiology and pathogenesis of PCOS [6,7], some studies suggested that the environmental factors have been related to the pathogenesis and prevalence of PCOS [8-10].

Endocrine disruptors or endocrine-disrupting chemicals (EDCs) are known as ‘environmental hormones’ because they disrupt normal hormone synthesis, secretion, transport or function in the body and affect the endocrine system of the body and have negative effects [7,11-15]. Importantly, many EDCs show a non-monotonic dose-response curves, and low-dose exposure may influence certain endocrine system in the body [16]. EDCs have been reported to be associated with adipocyte formation or obesity, and these suggest the association

between EDCs and metabolic disorders [14,17]. Due to increase in precocious puberty, high expectations are being placed on the role of EDCs acting on female hormones [13]. The effects of endocrine disruptors on human body are related to the detoxification process, especially detoxification enzyme gene polymorphisms such as Aryl hydrocarbon receptor repressor (AhRR), glutathione-S-transferase M1 (GSTM1) and glutathione-S- transferase T1 (GSTT1) have been reported that it can be a major mediator between the endocrine disruptor and reproductive disease, and the association between endometriosis and the AhRR, GSTM1, and GSTT1 polymorphisms has been reported in Human Reproduction [18].

Glutathione S-transferase (GST) is a typical enzyme of phase II conjugation enzymes which are function to inactivate environmental toxins. GST is involved in the metabolism of environmental carcinogens such as the enzymes of the cytochrome p-450 family and have several isozymes which metabolize specific substrates or respond to duplicate substrates [19-21]. Eight classes of mammalian cytosolic GSTs are currently reported, such as alpha (A), mu (M), kappa (K), omega (O), pi (P), sigma (S), theta (T), and zeta (Z) [22].

The expression of GSTs enzymes is mainly influenced by the genetic polymorphisms of the GSTs genes. It is reported that the GST genotype differs according to race, and the relative risk for disease varies with race. A number of GSTs are expressed as polymorphisms, and the first two GSTs reported for polymorphisms were GSTT1 and GSTM1. These polymorphisms have been reported to vary by race, GSTM1 is homozygously deleted in about 50% and GSTT1 in 20% of caucasian individuals in central Europe [23]. Haase et al. reported that GSTM1 null

genotype accounted for about 50% in European, Japanese, and Caucasian populations and about 25% of Afro-Americans, whereas GSTT1 null genotype is common in Asian populations and are not well documented in Europeans and other ethnic groups [24]. And based on meta-analysis of case-control studies, it were reported that the GSTT1 null genotype increased significantly the risk of chronic myeloid leukemia in nine Caucasian, East Asian, and Indian populations, but not to East Asian people. These studies suggest that antioxidant status and degree of DNA damage of GSTM1 and GSTT1 genotypes vary depending on race [25]. According to studies for race-specific GST polymorphisms, the GSTT1 null genotype appeared 50-60% in Asians and 10-20% in Europeans and Americans and the GSTM1 null genotype accounted for 50-60% of Europeans, Japanese and Caucasian, while India and the United States were 25% respectively [24]. In the GST-related studies conducted on Koreans, the GSTT1 null genotype appeared 50-60% and GSTM1 null genotype accounted for 48-55% in Koreans [18,26,27]. GSTM1 and GSTT1 are known to be polymorphisms in humans and both of them have null alleles resulting from gene deletion [28]. The null genotypes of GSTM1 and GSTT1 are less likely to be able to remove activated carcinogens, resulting in a higher risk of cancer. Meta-analyses have revealed that the deletion of either GSTM1 or GSTT1 is associated with a significant increased risk of hypertension, coronary heart disease and several cancers [27,29-31], and also the deletions associated with reproductive disorders such as endometriosis or male infertility [18,32,33]. But currently there is no study about the polymorphisms of GSTM1 or GSTT1 in PCOS patients.

The aryl hydrocarbon receptor (AhR) is a nuclear receptor transcription factor present in the cytoplasm and the superfamily of

basic Helix-Loop-Helix/ Per-ARNT-Sim (bHLH/PAS) proteins [34,35]. When AhR bounds to dioxin ligands such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), they form complexes and move to the nucleus, where they combine with AhR nuclear translocator (ARNT) to form a dimer. Activation of these pathways may cause endocrine abnormality, immune-toxicity or carcinogenesis. AhR target genes encode for drug-metabolizing enzymes, such as cytochrome P450 (CYP) [34,35]. The AhR is ligand-dependent transcription factor localized to cytoplasm that regulates gene expression, immune response, cell cycle control, and cell differentiation [36-39].

The AhRR is a member of growing superfamily, is a bHLH/PAS protein, located in chromosome 5p15.3 [40] and regulates AhR signaling by repressing AhR-dependent gene expression. The N-terminal part of the AhRR protein has high structural similarities with AhR, by contrast, its C-terminal has no structural similarities with AhR [35,37]. AhRR acts as a suppressor AhR-dependent gene expression and modulate AhR-gene expression by competitive binding to ARNT [40]. There are three general forms in the AhRR gene; c.565C>G (Pro185Ala, rs2292596); c.1462G>A (Arg 473 Gln, rs769781351); and c.5303NA>T (NA, rs10078). The G allele polymorphism has caused by exchange of proline (Pro) 185 to alanine (Ala) 185 amino acid [41]. The G-allele of AhRR has been reported to be associated with a variety of diseases including endometriosis, male infertility, germ cell cancer, and oral cancer [41,42].

The expression of AhRR is high in testis, lung, ovary, spleen and pancreas in adults, whereas expression is low in all tissues in fetuses [43]. Also AhR and AhRR are all expressed in the female reproductive system and could change expression under specific pathologic conditions [44]. Reduced expression of AhRR has been observed in many types of

cancerous human tissue, including breast cancer, prostate cancer, hepatocellular carcinoma, and others [40,45,46], and also in reproductive abnormality such as the endometriosis [18,41]. But as far as we know, no previous reports on the expression status of AhRR in polycystic ovarian syndrome.

I compared the distribution of AhRR, GSTM1 and GSTT1 gene polymorphisms and phenotypes associated with the detoxification process of exogenous endocrine disruptors in PCOS and normal control women to determine the effect of genotype on clinical features of PCOS. The primary goal of the present study was to explore the association between AhRR polymorphism and the risk of PCOS in a Korean population and also tried to determine whether the genetic polymorphisms of GSTM1 and GSTT1 were associated with PCOS in the same population.

II. Materials and Methods

Subjects

There were recruited 478 PCOS patients using the Rotterdam criteria and 376 age-matched controls visited the Seoul National University Hospital Healthcare System Gangnam Center. Their ages ranged from 18–40 years in the PCOS patient group and control group and they all agreed to participate in the investigation of PCOS genetic predisposition.

The diagnosis of PCOS was based on the 2003 Rotterdam criteria. When a patient meets, PCOS was diagnosed at least two of the following must be present: 1) oligo-or anovulation, 2) hyperandrogenism (either clinical or biochemical), and 3) a polycystic ovary (PCO) as determined by ultrasonography. Oligomenorrhoea was defined as less than eight periods per year or cycles longer than 35 days, and amenorrhea was defined as no menstruation for more than 3 months without pregnancy. Clinical hyperandrogenism was defined by a modified Ferriman and Gallwey score (mF-G score) of six or greater [47] and biochemical hyperandrogenism was defined as total testosterone (T) >0.68 ng/ml, free T>1.72 pg/ml, and free androgen index (FAI) [48]. PCO was defined as the presence of 12 or more follicles measuring 2–9 mm in diameter, or an increased ovarian volume (exceeds 10 cm^3). The exclusion of other related disorders and the definition of oligo-ovulation, anovulation, and clinical and biochemical hyperandrogenism have been previously reported [49,50].

A total of 376 age-matched premenopausal women served as control subjects visited the Seoul National University Hospital Healthcare System Gangnam Center. They visited as an individual need for a check-up or a part of group check-up for work with no specific health problems,

and these women did not have any of the three items apply the same three diagnostic items of Rotterdam criteria. The Institutional Review Board of Seoul National University approved the study (IRB number: H-0501-140-008).

Clinical and Biochemical Measurements

Clinical factors, such as height, body weight, waist circumference (WC), and blood pressure (BP), were measured from all women in both groups. Body mass index (BMI) was calculated as body mass (kg) divided by the square of the body heigh (m^2), and WC was measured by a trained person with the measuring tape placed horizontally at the smallest diameter between the costal margin and the iliac crest while the participant gently exhaled. All subjects in both groups had taken no medications, such as oral contraceptives, lipid-lowering agents, and insulin sensitizers in the last 6 months. The subjects were evaluated for serum hormonal or metabolic parameters with the use of a previously described protocol [50].

Genomic DNA analysis

Blood was drawn from each participants who fasted overnight, and genomic DNA was extracted with the Wizard DNA Purification Kit (Promega, Madison, WI, USA).

The AhRR polymorphism was genotyped using real-time polymerase chain reaction (PCR) analysis on an ABI PRISM® 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The reaction mixture contained 10 μl of the 2x Taq-Man Universal PCR Master Mix and 25 ng of DNA. The PCR cycling conditions consisted of a 2-minute cycle at

50℃ and a 10-minute cycle at 95℃, followed by 40 cycles of 95℃ for 15 seconds and 60℃ for 1 minute. Distilled water was used as a negative PCR control in each amplification. Each 20 ml PCR reaction contained 10 pmol of forward primer 5’-AGACGGATGTTATGCACCAGA A-3’, 10 pmol of reverse primer 5’-AGAGGCAGCGATGTGTTATGG -3’, 4 pmol of C-allele probe 5’-(FAM)-TGGGCAGCCCCCCGCC-(TAM RA)-3’, 4 pmol of G-allele probe 5’-(VIC)-TGGGCAGGCCCCGCC-(TA MRA)-3’, 10 ml of 2X TaqMan Universal PCR Master Mix (Applied Biosystems) and 25 ng DNA. The PCR cycling conditions consisted of one 2 min cycle at 50 ℃ and one 10 min cycle at 95 ℃, followed by 40 cycles of 95 ℃ for 15 s and 60 ℃ for 1 min. Distilled water was used as a negative PCR control in each amplification. The PCR primers for the GSTM1 and the GSTT1 polymorphisms were 5’-GAACTCCCTGAAAAGCTAAAGC-3’ (forward), 5 ‘-GTTGGGCTCAAA TATACGGTGG-3’ (reverse) (Bioneer, Seoul, Korea), 5’-TTCCTTACTGG TCCTCACATCTC-3’ (forward), and 5’-TCACCGGATCATGGCCAGCA -3’ (reverse) (Bioneer) (Table 1).

The subjects assessed for serum hormonal and metabolic parameters with the use of a previously described protocol [18] (Figure 1).

Statistical analysis

Power calculations were performed with the QUANTO v.1.2.4 (log-additive model) software (http://hydra.usc.edu/gxe/). Based on the specified sample size (377 patients with PCOS and 386 controls), the power was calculated to detect an allelic odds ratio of 1.5 at an α value of 0.05.

Normal distributions were achieved using a natural logarithmic or square root transformation and Continuous parameters were compared

using Student's t-test. In the controls, the genotype distribution was examined for significant departures from the Hardy-Weinberg equilibrium using a chi square goodness-of-fit test. To evaluate the association between the patient-control status and each individual SNP, logistic regression analyses were used to calculate the odds ratios (ORs) and 95% confidence intervals (CIs). In all the analyses, the homozygote of wild-type allele was defined as the reference category. Differences in phenotypic variables and differences in including diagnostic criteria among PCOS patients were analyzed by 1-way ANOVA followed by the least significant difference test for post hoc comparisons. In the patients with PCOS, the multi-variate linear regression model was used for comparing the HOMA-IR, HOMA β cell, and 2h 75-g insulin levels according to each individual genotype. All the data analyses were performed using SPSS software (version 17.0; SPSS Inc. Chicago, IL, USA), and the significance was accepted for two-sided P-values < 0.05.

Table 1. PCR multiplex for genotyping GSTM1 and GSTT1

Genes	Primer sequences	Size
GSTM1	5’ -GAACTCCCTGAAAAGCTAAAGC-3’	219 bp
	5’ -GTTGGGCTCAAATATACGGTGG-3’	
β-globulin	5’ -CAACTTCATCCACGTTCACC-3’	268 bp
	5’ -GAAGAGCCAAGGACAGGTAC-3’	
GSTT1	5’ -TTCCTTACTGGTCCTCACATCTC-3’	400 bp
	5’ -TCACCGGATCATGGCCAGCA-3’	

*base pair=bp

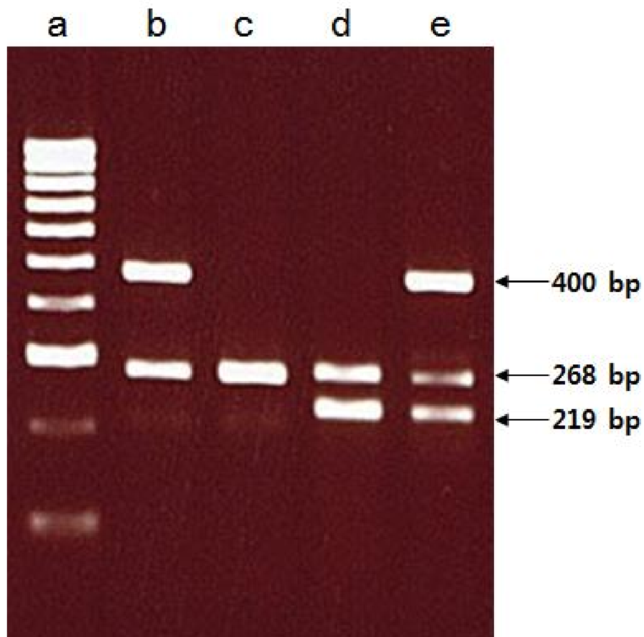

Figure 1. Agarose gel electrophoresis of the multiplex PCR for genotyping GSTM1 and GSTT1. β-globulin gene was used as an internal positive control. Line a: molecular marker (100 bp DNA ladder); line b:GSTM1 null/GSTT1 present; line c:GSTM1 null/GSTT1 null; line d: GSTM1 present/GSTT1 null; line e: GSTM1 present/GSTT1 present. *base pair=bp

III. Results

A total of 478 women with PCOS and 376 age-matched controls were recruited in the current study. Genotypic distributions of the codon 185 of AhRR genes were in Hardy-Weinberg equilibrium in both groups.

Analysis of AhRR genes polymorphism frequency

The genotype distribution of codon 185 polymorphism in the AhRR gene in two groups was as follows: CC/CG/GG rates were 37.2% /48.5% /14.2% in the PCOS group and CC/CG/GG rates were 43.6% /44.7% /11.7% in control group. There was a tendency to an association of C/G+G/G genotypes with PCOS patients (62.8% versus 56.4%, p=0.06, OR: 1.14, 95% CI: 0.91-1.43), which did not show statistical significance in two groups (Table 2).

Analysis of GSTM1 and GSTT1 polymorphism frequency

The proportion of null mutation at GSTT1 also tended to increase in patients with PCOS, but there was no statistically significant difference (52.3% versus 48.9%, p=0.33, OR: 1.14, 95% CI: 0.87-1.49). The proportion of null mutation at GSTM1 showed a slightly different pattern, which did not also reach statistical significance in both groups. In contrary to GSTT1 result, the proportion of null mutation at GSTM1 in PCOS patients was slightly decreased than in control group (47.3% versus 51.3%, p=0.24, OR: 0.85, 95% CI: 0.65-1.11) (Table 3).

Analyzing AhRR and GSTT1/GSTM1 genes polymorphism together

The combined G-allele genotype and GSTT1 null genotypes were

statistically significant higher in PCOS patients compared to controls (31.6% versus 27.4%, p=0.03, OR=1.54, CI=1.04-2.29) (Table 4). Analyzing AhRR and GSTM1 together, it was also tended to increase in patients with PCOS, but there was no statistically significant difference (28.9% versus 30.1%, p=0.51, OR: 1.14, 95% CI: 0.77-1.68) (Table 4).

Analyzing AhRR and GSTT1/GSTM1 genes polymorphism together according to diagnostic criteria or phenotypic variables

There was no statistically significant difference in polymorphism of genotype according to the diagnostic criteria in the classification based on PCOS diagnostic criteria (the 2003 ARSM/ESHRE consensus meeting guideline). But, classification of PCOS patients according to phenotype showed similar pattern to previous results. PCOS patients group was divided in to two groups according to the phenotype (H-core <6 and H-score ≥6). Then, the genotype distributions of codon 185 polymorphism in the AhRR gene, GSTT1 and GSTM1 were compared in the control group. By ANOVA analysis, there were significant differences in the combined G-allele genotype and GSTT1 null genotypes among three groups. Further analysis (Tukey) showed statistical differences between no hirsutism group and controls (p=0.02) (Table 6). There are no statistical differences in a single genotype distribution of AhRR, GSTT1 or GSTM1 in three groups.

Table 2. Distribution of codon 185 polymorphism in the AhRR gene in PCOS patients and controls

	PCOS (n=478)	Control (n=376)	*P value*	OR (95% CI)
AhRR gene polymorphism				
CC (%)	178 (37.3%)	164 (43.6%)		
CG (%)	232 (48.5%)	168 (44.7%)	0.10[b]	1.27 (0.95-1.70)
GG (%)	68 (14.2%)	44 (11.7%)	0.11[b]	1.42 (0.92-2.20)
CC (%)	178 (37.2%)	164 (43.6%)		
non-CC	300 (62.8%)	212 (56.4%)	0.06 [a]	1.30 (0.99-1.72)
C allele	410 (57.7%)	332 (61.0%)		
G allele	300 (42.3 %)	212 (39.0%)	0.25 [a]	1.14 (0.91-1.43)

[a] evaluated by chi-square test in comparison with the control group

[b] evaluated by logistic regression analysis in comparison with the control group

Table 3. Prevalence of homozygosity for the GSTM1 and GSTT1 null mutations in PCOS patients and controls

	PCOS (n=478)	Control (n=376)	*P value*	OR (95% CI)
GSTM1				
Present	252 (52.7%)	184 (48.7%)		0.85 (0.65-1.11)
Null	226 (47.3%)	193 (51.3%)	0.24[a]	
GSTT1				
Present	228 (47.7%)	192 (51.1%)		1.14 (0.87-1.49)
Null	250 (52.3%)	184 (48.9%)	0.33[a]	

[a] evaluated by chi-square test in comparison with the control group

Table 4. Risk of PCOS according to the number of possible high risk AhRR and GSTT1 genotypes

Number of high risk genotypes	AhRR	GSTT1	PCOS (n=478)	Control (n=376)	*P value*	OR (95% CI)
0	CC	Present	79 (16.5%)	83 (22.1%)		
1	CC CG/GG	Null Present	248 (51.9%)	190 (50.5%)	0.09 [a]	1.37 (0.96-1.97)
2	CG/GG	Null	151 (31.6%)	103 (27.4%)	0.03 [a]	1.54 (1.04-2.29)

[a] evaluated by logistic regression analysis in comparison with the control group

Table 5. Risk of PCOS according to the number of possible high risk AhRR and GSTM1 genotypes

Number of high risk genotypes	AhRR	GSTM1	PCOS (n=478)	Control (n=376)	P value	OR (95% CI)
0	CC	Present	90 (18.8%)	84 (22.3%)		
1	CC CG/GG	Null Present	250 (52.3%)	179 (47.6%)	0.14 [a]	1.30 (0.92-1.86)
2	CG/GG	Null	138 (28.9%)	113 (30.1%)	0.51 [a]	1.14 (0.77-1.68)

[a] evaluated by logistic regression analysis in comparison with the control group

Table 6. Distribution of codon 185 polymorphism in the AhRR gene and prevalence of homozygosity for the GSTM1 and GSTT1 null mutations in controls according to H-score

H-score<6 (n=155) vs. H-score≥6 (n=153) vs. control (n=376)	P value [a]	
AhRR	NS	
GSTT1	NS	
GSTM1	NS	
AhRR + GSTT1	0.02	H-score<6 vs. control*
AhRR + GSTM	NS	
CC vs non-CC	NS	
GSTT1+GSTM1	NS	

[a] evaluated by 1-way ANOVA in comparison with the control group

* post hoc tests run by Tukey's HSD

Ⅳ. Discussions

The goal of this study was to evaluate the association between AhRR polymorphism and the risk of PCOS and to determine whether the genetic polymorphisms of GSTM1 and GSTT1 are associated with susceptibility to PCOS in a Korean population. There was no significant difference in a single genotype distribution of AhRR, GSTT1 or GSTM1 in PCOS patients compared to controls. But, combined two risk factors, such as the non-CC genotypes/ GSTT1 null mutation have been associated with PCOS comparing to controls. There were no differences in polymorphism of genotype according to the diagnostic criteria in the classification based on PCOS diagnostic criteria. Only in PCOS groups without hirsutism showed significant statistical differences in the combined G-allele genotype and GSTT1 null genotypes comparing to controls.

In PCOS, one of the most common reproductive endocrine diseases of reproductive-age women, the association with endocrine disruptor has been reported. Takeuchi et al. have shown that Bisphenol A (BPA) concentrations were increased in women diagnosed with PCOS and testosterone levels were increased as BPA clearance decreased [51]. Also, Galazis et al. reported that the expression level of GSTM3 was increased compared with controls in PCOS omental fat and Glutathione S-transferase P (GSTP) protein expression was increased in the endometrial tissues of PCOS patients diagnosed with endometrial cancer [52,53]. Finally, it can be assumed that environmental factors are involved in the pathophysiology of PCOS. In recent years, Yang, et al. found increased expression of p,p′-dichlorodiphenyldichloro ethylene (p,p′-DDE) and polycyclic aromatic hydrocarbons in serum with PCOS [54].

Previous studies have demonstrated the associations between EDCs and PCOS but there are limitations with the small sample size and there is no study about the polymorphism of detoxification enzymes in PCOS patients. The AhR pathway is known to be critical for cellular events, especially for those caused by several environmental chemicals such as TCDD. Upon binding to TCDD, they form complexes and translocates into the nucleus, where they combine with ARNT to form a dimer. The AhR/ARNT dimer recognizes a DNA enhancer element sequence, designated xenobiotic responsive element (XRE), located in the promoter region of CYP1A1 gene, causing gene expression [54,55]. AhR is a ligand-dependent transcription factor present in ovarian tissues and AhR plays an important role in the regulation of ovarian follicular growth and secretion of hormones [56]. Kim at el. have shown that detoxification enzyme gene polymorphisms such as AhRR, GSTM1 and GSTT1 have a major mediator between the EDCs and endometriosis [18]. That study has shown that a single genetic polymorphism involving AhRR, GSTM1 or GSTT1 exhibits a marginal effect, but can confer a significant increase in the risk of endometriosis if the putative high-risk genotypes at the two loci are combined (P=0.015, OR:1.86, 95% CI: 1.13-3.06).

GST is the primary cellular defense mechanism against reactive oxygen species as phase II detoxification enzymes found mainly in the cytosol. Cytosolic GSTs of mammals were originally classified into Mu, Kappa, Alpha, Pi, accounting for 4% of the total cytosolic proteins [20,57]. Among these, GSTM1 and GSTT1 genes are mainly polymorphic in humans and the null genotypes causing by loss of function mutations are resulted in lack of enzyme activity [28,56].

GSTT1 and GSTM1 differ according to race, and the relative risk for disease varies with race. These polymorphisms have been reported to vary by race, GSTM1 is homozygously deleted in about 50% and GSTT1 in 20% of caucasian individuals in central Europe [23]. GSTM1 gene polymorphism in European, Japanese, and Caucasian populations accounted for about 50% of GSTM1 null genotype and about 25% of Afro-Americans genotype, whereas GSTT1 null genotypes are common in Asian populations [24]. The frequencies of the GSTM1 and GSTM1 null genotype in this study were similar to other studies in Koreans. The GSTM1-null genotype was found in 51.5% and the GSTT1-null genotype was found in 50.8% participants (Table 3).

Several studies related to the association between the GST isoenzyme family and environmental factors in diabetes mellitus (DM) have been reported in recently. In a recent report, there are findings the combined, both GSTM1/GSTT1 null genotypes are to be considered among the polymorphic genetic risk factors for type 2 DM. The null GSTM1 and GSTT1 alleles did not reveal an increased frequency but, the combined GSTM1/GSTT1 null genotypes were statistically significantly higher in DM patients compared to control subjects (p=0.0021, OR=0.313, CI=0.149-0.655) [58]. Previous studies did not show an association between AhRR polymorphism and some disease such as endometriosis [59] or lung cancer [60]. But some studies showed that the negative feedback effect of AhRR on the dioxin for the C (pro) allele is a significant association with reduced male fertility [61,62] and the GG allele of AhRR has a negative result on the sexual system.

As shown in table 2 and table 4, a single genetic polymorphism involving AhRR, single GSTM1 and GSTT1 mutant variants exhibit no

significantly associated with PCOS compared to controls. But the combined high-risk genotypes at the two loci, such as AhRR (G allele)/GSTT1 null genotype or GSTM1/GSTT1 null genotypes were significant higher in PCOS women compared to controls. Our results come in agreement with other studies in different endocrine diseases [11,18,48,58,63,64].

In conclusion, a single genetic polymorphism involving AhRR, GSTM1 and GSTT1 mutant variants individually are not associated with PCOS but combined the high-risk genotypes could be considered genetic risk factor in PCOS. Our data suggest that combined genetic polymorphisms in the several candidate genes might be involved in the pathogenesis of PCOS. In future studies, more accurate results might be obtained by calibrating these variables in PCOS patients and controls. Although the present study has these limitations, to the best of our knowledge this is the first reported study about the polymorphism of detoxification enzymes in PCOS patients.

V. References

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국문요약 (국문초록)

Aryl hydrocarbon receptor repressor glutathione-S-transferase M1, glutathione-S-transferase T1 유전자 다형성과 다낭성 난소증후군과의 연관관계 규명

목적: 다낭성 난소증후군의 발병에 유전적 소인 역시 관여함은 주지의 사실이나, 현재 다낭성 난소증후군 환자를 대상으로 detoxification 효소 유전자 다형성 양상을 본 연구는 전무한 실정이다. 이에 aryl hydrocarbon receptor repressor (AhRR), glutathione-S-transferase M1 (GSTM1), glutathione-S-transferase T1 (GSTT1) 효소 유전자 다형성 분포를 비교를 통해 유전형이 다낭성 난소증후군의 임상양상에 미치는 영향을 분석하고자 하였다.

연구방법: 다낭성 난소증후군 유전적 소인규명 연구 참여에 동의한 다낭성 난소증후군 환자군 478명과 정상대조군 376명의 여성을 대상으로 하였다. 환자의 말초혈액에서 채취한 혈액에서 유전체 DNA를 추출하였다. AhRR 유전자 codon 185의 유전형질분석은 실시간 중합효소 연쇄반응을 통해 수행되었으며 GSTM1과 GSTT1의 유전자 결손에 의한 유전형질분석은 대용량 중합효소 연쇄반응 분석을 통해 시행하였다.

결과: 다낭성 난소증후군 환자군에서 정상대조군에 비해 C/G+G/G의 유전자형에 대한 높은 연관성을 나타내었으나 (p=0.06), 두 군에서 GSTM1과 GSTT1의 유전자형 분포에 유의한 차이가 없었다. 반면, AhRR과 GSTT1의 유전자형을 함께 분석했을 때, 다낭성 난소증후군 환자군에서 정상대조군에 비해 고위험 유전자형이 유의하게 높게 관찰되었다 (p=0.03, OR=1.54, CI=1.04-2.29). 또한, 표현형에 따라 다낭성 난소증후군 환자를 분류했을 때, AhRR과 GSTT1의 고위험 유전자형 다모증이 없는 다낭성 난소증후군 환자군과 정상대조군에서 유의한 차이를 보였다 (p=0.02).

결론: The AhRR codon 185, GSTM1 그리고 GSTT1 변이 유전자형은 각각 개별적으로는 다낭성 난소증후군의 병태생리학에 유의한 연관성을 보이지 않았다. 그러나 AhRR codon 185의 G 대립유전자와 GSTT1의 결여 유전자형이, 또는 GSTT1과 GSTM1의 결여 유전자형이 같이 표현될 때 다낭성 난소증후군의 병태생리학적 이상과 연관될 수 있을 것으로 사료된다.

주요어: 다낭성 난소증후군, 외인성 내분비 교란화학물질, 유전자 다형성, aryl hydrocarbon receptor repressor, glutathione-S-transferase
학번: 2010-30544

감사의 글

박사과정을 진행하면서 논문이 잘 마무리될 수 있도록 많은 분들이 도움을 주셨습니다. 이글을 통해 감사의 인사를 드리고자 합니다.

학위과정을 진행하면서 부족한 저에게 의사로서 연구자로서 방향을 제시해 주시고 아낌없는 열정으로 지도해주신 최영민 교수님께 감사의 인사와 존경을 드립니다.

바쁘신 와중에도 기꺼이 논문 심사위원장을 맡아주신 김인규 교수님과 시간을 내어 심사해 주시고 좋은 연구가 될 수 있도록 충고와 조언을 해주셨던 전종관 교수님, 조영민 교수님, 그리고 김성훈 교수님께 감사의 말씀을 전합니다.

연구를 진행하고 학위논문을 준비하는 과정에 진심 어린 조언과 도움을 주신 김진주 교수님께 감사드립니다. 연구를 진행할 때 바쁘지만 도움을 준 연구실 동료들, 홍민아 선생님과 김종미 선생님 감사드립니다.

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