Association of aromatase $(TTTA)_n$ repeat polymorphisms with central precocious puberty in girls

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- Abstract-

Objective: Precocious puberty is characterized by early activation of the pituitary gonadal axis. Estrogen is the final key factor to start onset of puberty. The cytochrome P450 19A1 (CYP19A1) gene encodes an aromatase that is responsible for the conversion of androgens to estrogen, which is key step in estrogen biosynthesis. The aim of this study is to identify CYP19A1 gene mutations or polymorphisms in girls with central precocious puberty (CPP).

Methods: We evaluated the frequency allelic variants of the CYP19A1 exons and the tetranucleotide tandem repeat (TTTA)n in intron 4 in 203 idiopathic central precocious puberty (CPP) girls and 101 normal healthy women.

Results: The genotype analysis of the CYP19A1 $(TTTA)_n$ polymorphism revealed six different alleles ranging from 7 to 13 repeats. Among the 6 different repeat allele detected in this study, $(TTTA)_{13}$ repeat allele was only detected in patients group and the carriers of allele $(TTTA)_{13}$ was significantly associated with a increased risk of CPP (OR=1.509, 95% CI=1.425-1.598, P=0.033). The carriers of the $(TTTA)_{13}$ repeat allele were significantly younger age at pubertal onset and had higher levels of estrogen than non-carriers of $(TTTA)_{13}$ repeat allele. Although nine polymorphisms were detected in exons of CYP19A1 gene, no clinical significance was observed.

Conclusion: In this study, the carriers of higher repeat $(TTTA)_{13}$ polymorphism in intron 4 of the CYP19A1 gene had higher level of estrogen in this study. The carrying the $(TTTA)_{13}$ repeat allele may have a higher risk of developing CPP.

Key words: Aromatase, TTTA repeats, precocious puberty

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I. Introduction

Central Precocious puberty (CPP) is characterized by an increased levels of hypothalamic gonadotropin releasing hormone (GnRH), which leads the growth acceleration and the development of secondary sexual characteristics before 8 years of age in girls and 9 years of age in boys (Carel and Leger, 2008). Estrogen plays a key factor of pubertal development and has an important role of the secretion of GnRH. Estradiol decreases gonadotropin secretion at low concentrations but causes positive feedback at higher values (Bourguignon and Juul, 2012). In animal studies, the rise in the number of *Kiss1*neurones at the hypothalamus depends on the levels of estrogen (Clarkson et al., 2009; Smith, 2009). So, estrogen might operate as crucial modulators of pubertal onset through estrogen-kisspeptin positive feedback relationship.

Aromatase, an enzyme that converts androgens to estrogens is a significant role in estrogen biosynthesis. Aromatase is encoded by the CYP19A1 gene, which is located on chromosome 15q21.2. CYP19A1 spans 123kb with a coding region of nine exons (about 30kb, exon II to X) and a large 5'-untranslated region of 93kb with alternative tissue-specific promoters (Meinhardt and Mullis, 2002). Aromatase is expressed in a wide range of tissues, including ovarian granulose cells, luteal corpus, bone, adipose tissue, breast, brain and testis (Simpson et al., 1994). Aromatase polymorphisms were shown to affect risks for various diseases, such as breast cancer, endometrial cancer, and osteoporosis, whereas local aromatase activity has been associated with adenomyosis and leiomyomas(Ishihara et al., 2003; Tofteng et al., 2004; Talbott et al., 2008; Setiawan et al., 2009). Also, the tetranucleotide tandem repeat (TTTA)n in intron 4 of CYP19A1 has been analyzed for possible associations between the repeat number and estrogen biosynthesis (Hammoud et al., 2010; Ferraldeschi et al., 2012). These studies support the role of CYP19A1 polymorphisms in altered gene function and subsequent effects on circulating estrogen levels. However, there are no reports on the relationship between the CYP19A1 gene polymorphisms and precocious puberty.

We hypothesized that CYP19A1 gene polymorphisms might be associated with pubertal onset though estrogen pathway. Therefore, the aim of this study was to evaluate the association between CYP19A1 gene polymorphisms and CPP in girls.

II. Subjects and Methods

A. Subjects

Subjects in the final analysis included 203 girls who were diagnosed with idiopathic CPP in the Ajou University Hospital between March 2010 and December 2012. This study was a single center, prospective study. CPP was diagnosed in girls with objective breast budding appearing before the age of 8 years, advanced bone age, and a pubertal response on the GnRHstimulation test. Advanced bone age was defined as more than 1 year above chronological age. A pubertal response on the GnRH stimulation test was defined as a peak LH ≥5 IU/L on immunoradiometric assay (IRMA) and a peak LH level 2 to 3 times more than the basal level (Neely et al., 1995). Precocious puberty with an identified etiology, such as brain tumor or cranial irradiation, was excluded. All patients had normal MRI studies of the hypothalamic-pituitary area. Plasma thyroxin and thyroid stimulating hormone concentrations were measured to exclude hypothyroidism. Pubertal status (Tanner stage for breast development) was assessed and documented by one pediatric endocrinologist. Patients were categorized based on their Tanner stage (Tanner II-V). Bone age was determinedusing an X-ray of the left hand according to Greulich and Pyle(Greulich and Pyle, 1959). BMI was calculated as weight/height^2, and the standard deviation scores (SDS) were calculated based on the 2007 Korean National Growth Charts(Moon et al., 2008). All subjects were performed GnRH stimulation test. Serum LH and FSH were determined after the intravenous bolus administration of GnRH (100μg Relefact, Sanofi-Aventis, Frankfurt am Main, Germany). Serum LH and FSH levels were measured by immunoradiometric assay with a detection limit of 0.2 IU/L and 0.1 IU/L, respectively (BioSource, Nivelles, Belgium). E_2 levels were determined on radioimmunoassay with a detection limit of 5pg/mL (RIA; Coat-A-Count, Diagnostic Products, Los Angeles, CA, USA). The control population consisted of 101 Korean healthy women with normal pubertal development, who were recruited as volunteers through announcement on the basis of freewill questionnaire concerning their breast development after the age of 10 years and menarche after 12 years of age. The median age in the control group was 31 years (range 23-42 years) and median menarcheal age was 13.1 years (range 12.5-15 years). Height and weight of all control subjects were within the normal range of recently published Korean reference (Moon et al., 2008). The protocol was

approved by the Institutional Review Board of the Ajou University Hospital, and written informed consent was obtained from all the subjects or their parents at the beginning of the study.

B. Genetic analysis

Genomic DNA was isolated from the peripheral blood leukocytes of the study population using a DNA isolation kit (QIAGEN; GmbH, Helden, Germany). Each exons of the CYP19A1 gene were amplified using the primers as shown in Table 1. PCR reactions were performed in 50 μl volumes using 100ng of genomic DNA template, 1.0 μM of each primer, 30 mMKCl, 10 mMTris-HCl pH 9.0, 1.5 mM $MgCl_2$, 250 μM of each desoxynucleotide triphosphate, and 1.5 unit of Taq DNA polymerase (Takara Bio Inc.; Shiga, Japan). The PCR was performed with an initial denaturing step performed at 95 °C for 3 min, then 30 cycles beginning with 95 °C for 1 min, followed by an annealing step for 1 min, an elongation step for 2 min and a final extension step at 72 °C for 10 min.

The DNA region containing the polymorphic $(TTTA)_n$ repeat is located in intron 4 of the CYP19A1 gene. PCR amplification was performed using primers 5'-AGTGCATCGGTATGCATGAG -3 (forward) and 5'-GAGGCTGAGGTGGGAGAATC-3 (reverse). The reaction was carried out in mixture of 10 μl containing 20 ng genomic DNA, 50 mM KCL, 10mM Tris HCL (pH 8.3), 1.5 mM $MgCl_2$, 250 μM of each desoxynucleotide triphosphate, 2 pM of each primer, and 1 unit of Taq DNA polymerase (Takara Bio Inc.; Shiga, Japan). The PCR was performed with an initial denaturing step performed at 95°C for 3 min, then 30 cycles beginning with 95°C for 1 min, followed by an annealing step for 1 min, an elongation step for 2 min and a final extension step at 72°C for 10 min. After amplification, the PCR mixtures were separated on 1.5% agarose gels with ethidium bromide to confirm the site and purity of the PCR products.

Subsequently, DNA sequencing reactions were performed using the same primer pairs, and the BigDye Terminator V3.1 Cycle Sequencing kit (Applied Biosystems; Foster City, CA) was used in accordance to the manufacturer's instructions. Electrophoresis and analysis of sequencing reaction mixtures were achieved with an ABI3130xl Genetic Analyzer (Applied Biosystems) and Sequencing analysis v.5.2.

C. Statistical analysis

Statistical analyses of differences in genotype and allele frequencies were compared between patients group and control group using the chi-square test. Deviations from the Hardy-Weinberg equilibrium were also evaluated via the comparison of observed and expected genotype frequencies. When significant difference of allele frequencies between the patient and the control groups was noted for each polymorphism, the clinical characteristics and results of the hormonal study were compared between patients harboring a certain polymorphism and those lacking that polymorphisms. Mann-Whitney U test were used for the analysis of the data. Statistical analysis was performed using SPSS version 18.0 (SAS Institute, Chicago, USA). Statistical significance was defined as $P< 0.05$. Results are described as mean ± SD unless otherwise stated.

III. Results

A. CYP19A1 gene analysis

The clinical and laboratory characteristics of the subjects are shown in Table 2. The genotype in the patients and control group were distributed in accordance with the Hardy-Weinberg equilibrium (p>0.05). Direct sequencing of the CYP19A1 gene detected nine polymorphisms. Among the 9 polymorphisms detected in this study, 4 have been previously reported, while the other 5 are novel single nucleotide polymorphisms (SNPs). Allele counts and frequencies are shown in Table 3. The allele frequency differed significantly in the non-synonymous polymorphism 51529112 *A/G* (p. M82V) between patients and controls, while the other 8 SNPs showed no significant difference. M82V polymorphism was observed in lower frequency in the patients compared with the control group. But, we compared the clinical characteristics and hormone values between the two subgroups within the patients group (the subgroup with 51529112 *A/A* and 515291192 *A/G*) and there were no significant differences in the auxological parameters, LH, FSH, and E_2 levels between the two subgroups (data not shown).

B. Relationship between CYP19A1 $(TTTA)_n$alleles and CPP

The genotype analysis of the CYP19A1 $(TTTA)_n$ polymorphism revealed six different alleles ranging from 7 to 13 repeats (Table 4). The frequency distribution of TTTA repeat alleles did not differ from that previously reported in other population (Ribeiro et al., 2006; Xita et al., 2010; Lazaros et al., 2013). The bimodal distribution of the TTTA repeat alleles, showing two major peaks at 7 (50.9%) and 11 (36.8%) repeats. Among the 6 different repeat allele detected in this study, $(TTTA)_{13}$ repeat allele was only detected in patients group and the all carriers were heterozygous for the $(TTTA)_{13}$ repeat allele. Also, the carriers of allele $(TTTA)_{13}$ was significantly associated with a increased risk of CPP (OR=1.509, 95% CI=1.425-1.598, P=0.033). The effect of $(TTTA)_{13}$ repeat allele on CPP was examined by comparing carriers with no carriers of $(TTTA)_{13}$ repeat allele. The carriers of the $(TTTA)_{13}$ repeat allele were significantly younger age at pubertal onset than non-carriers of

$(TTTA)_{13}$repeat allele (Table 5). Under GnRH stimulation tests, the carriers with $(TTTA)_{13}$ repeat allele presented significantly higher basal serum E_2,peak stimulated E_2 and LH levels compared to non-carriers of $(TTTA)_{13}$ repeat allele.

Also, $(TTTA)_7$ repeat allele was found to be a lower allele frequency in patients group compared with control group (47.6% versus 56.9%, P<0.001). Although no statistical significant difference in hormonal parameters was observed between the carriers and non-carriers of $(TTTA)_7$ repeat allele, the carriers of (TTTA)7 repeat allele showed a tendency to have a higher basal LH, E2, peak LH, and peak E2 levels than non-carriers (Table 6).

Table 1. Oligonucleotide primers used for PCR

Exon	Primer
Exon 2F	5’-GGCTTCCTTGTTTTGACTTG-3’
Exon 2R	5’-TCCCAAGTCCTCATTTGCTA-3’
Exon 3F	5’-TCGTGATTCACAGATATACATCACAT-3’
Exon 3R	5’-GCGTTAGAAACAAAGACATCAAGA-3’
Exon 4F	5’-GCTAAGAGAGCTACCTCCTAGTCAA-3’
Exon 4R	5’-AAAAAGGCACATTCATAGACAAAA-3’
Exon 5F	5’-TCTCCTTCCGTTCATTCATTC-3’
Exon 5R	5’-GCAATGTAGAAAATGGCATGTG-3’
Exon 6F	5’-CTGAGCTTAGAACCCCCAGA-3’
Exon 6R	5’-AATCAACAGCTCCCTTGTGG-3’
Exon 7F	5’-TCATAAGCACCAATGTTTTCAA-3’
Exon 7R	5’-GGGCTATTTGGATTGGGATT-3’
Exon 8F	5’-CAGTCGGCCTAATCGTTCAT-3’
Exon 8R	5’-TCAAATGTGACAGAATGTCTTGC-3’
Exon 9F	5’-TGTGGCCCAAGCATAACATA-3’
Exon 9R	5’-GGTGGCAGAGGGAATGAGTA-3’
Exon 10F	5’-TGGAGGGCATTGTAGCTGAT-3’
Exon 10R	5’-ATGGGCCACTGAGTGTTCA-3’

Table 2. Clinical and laboratory characteristics of the subjects

Characteristics	Total (n=203)
Age at onset of puberty (year)	7.7 (7.2-7.9)
Age at diagnosis of CPP (year)	8.3 (7.7-8.7)
Height SDS	0.90 ± 0.78
Weight SDS	0.74 ± 0.78
BMI SDS	0.42 ± 0.87
BA-CA at diagnosis (year)	2.0 ± 0.6
Basal LH (IU/L)	1.2 (0.9-1.7)
Basal FSH (IU/L)	2.6 (1.7-3.2)
Basal E2 (pg/mL)	7.1(5.1-10.0)
Peak LH (IU/L)	8.9 (6.2-14.6)
Peak FSH (IU/L)	12.3 (10.8-17.7)
Peak E2 (pg/mL)	8.5(6.2-11.5)
Peak LH/FSH ratio	0.72 (0.55-1.23)

* Age at onset of puberty, Age at diagnosis of CPP, biochemical value (LH, FSH,E2), and peak LH/FSH ratio are described as median with interquartile range. Other data are means ± standard deviation.

*The standard deviation scores (SDS) were calculated based on the 2007 Korean National Growth Charts.

Table 3. Allele frequencies of the CYP19A1 polymorphisms form 203 patients and 101 controls

No.	Location	dbSNP	Polymorphism	Group	Allele frequency 1		Allele frequency 2		*P-value
1	Exon2	rs2236722	51534995 T/C T=1; C=2	Patient Control	391 194	0.963 0.955	15 8	0.047 0.045	0.871
2	Exon3	Novel, p. M82V	51529112 A/G A=1; G=2	Patient Control	195 115	0.480 0.569	211 87	0.520 0.431	0.039
3	Exon3	Novel, p. G74G	51529142 C/T C=1; T=2	Patient Control	406 201	1.000 0.995	0 1	0.000 0.005	0.332
4	Exon5	rs35900050	51514673 C/A C=1; A=2	Patient Control	406 201	1.000 0.995	0 1	0.000 0.005	0.332
5	Exon6	Novel, p. T242T	51500759 C/G C=1; G=2	Patient Control	405 202	0.997 1.000	1 0	0.003 0.000	0.668
6	Exon7	rs700519	51507968 C/T C=1; T=2	Patient Control	336 167	0.827 0.826	70 35	0.173 0.174	0.979
7	Exon9	rs2304461	51504556 C/T C=1; T=2	Patient Control	402 202	0.990 1.000	4 0	0.010 0.000	0.157
8	Exon9	Novel, p. W378L	51514663 G/T G=1; T=2	Patient Control	406 201	1.000 0.995	0 1	0.000 0.005	0.332
9	Exon10	Novel, p. V378I	51503112 G/A G=1; A=2	Patient Control	405 202	0.997 1.000	1 0	0.003 0.000	0.668

*Comparison of the allele frequencies between the patient group and the control group

The positions of the polymorphisms were defined according to contig NT_010194.17.

Table 4. CYP19A1 intron 4 (TTTA)n repeat allele frequencies in central precocious puberty

Repeat number	Number of alleles (%)		P value	OR(95% CI)
	Controls	Patients		
$(TTTA)_7$	115 (56.9%)	193 (47.6%)	<0.001	0.528(0.369-0.751)
$(TTTA)_8$	1 (0.5%)	2 (0.4%)	1.000	0.995(0.090-11.039)
$(TTTA)_{10}$	1 (0.5%)	5 (1.2%)	0.669	2.506(0.291-21.596)
$(TTTA)_{11}$	70 (34.7%)	154 (37.9%)	0.475	1.152(0.810-1.639)
$(TTTA)_{12}$	15 (7.4%)	43 (10.6%)	0.242	1.477(0.799-2.728)
$(TTTA)_{13}$	0 (0.0%)	9 (2.3%)	0.033	1.509(1.425-1.598)

Table 5. Clinical significance of CYP19A1 $(TTTA)_{13}$ genotypes in patients with CPP

Characteristics	$(TTTA)_{13}$ carriers	$(TTTA)_{13}$ non carriers	P value
Age at onset of puberty (year)	6.8 ± 2.3	7.5 ± 0.8	0.025
Height SDS	1.01 ± 0.80	0.89 ± 0.78	0.672
Weight SDS	0.86 ± 1.00	0.73 ± 0.77	0.704
BMI SDS	0.42 ± 0.88	0.42 ± 0.87	0.992
Bone age (year)	9.5 ± 1.8	10.1 ± 0.1	0.125
Tanner stage (Breast)	2.3 ± 0.7	2.3 ± 0.5	0.897
BA-CA at diagnosis (year)	1.9 ± 0.8	2.0 ± 0.6	0.858
Basal LH (mIU/mL)	1.93 ± 1.89	1.45 ± 0.83	0.123
BasalFSH (mIU/mL)	3.24 ± 1.15	2.64 ± 1.24	0.163
Basal E2 (pg/mL)	14.33 ± 20.95	8.90 ± 5.61	0.022
Peak LH (mIU/mL)	22.34 ± 33.92	12.58 ± 11.91	0.027
Peak FSH (mIU/mL)	17.22 ± 4.25	14.23 ± 5.18	0.072
Peak E2 (pg/mL)	14.77 ± 20.78	9.50 ± 5.65	0.028
Peak LH/FSH ratio	1.15 ± 1.66	1.06 ± 1.63	0.872

* Data are means ± standard deviation.

Table 6. Clinical significance of CYP19A1 $(TTTA)_7$ genotypes in patients with CPP

Characteristics	$(TTTA)_7$ carriers (n=144)	$(TTTA)_7$ non carriers (n=59)	P value
Age at onset of puberty (year)	7.5 ± 0.9	7.4 ± 1.1	0.458
Height SDS	0.91 ± 0.80	0.85 ± 0.79	0.617
Weight SDS	0.67 ± 0.78	0.89 ± 0.76	0.069
BMI SDS	0.32 ± 0.88	0.67 ± 0.79	0.010
Bone age (year)	10.1 ± 1.1	10.0 ± 1.1	0.920
Tanner stage (Breast)	2.3 ± 0.5	2.3 ± 0.5	0.469
BA-CA at diagnosis (year)	2.0 ± 0.6	2.0 ± 0.7	0.997
Basal LH (mIU/mL)	1.41 ± 0.87	1.62 ± 0.96	0.151
Basal FSH (mIU/mL)	2.68 ± 1.27	2.63 ± 1.20	0.778
Basal E2 (pg/mL)	8.67 ± 5.34	10.03 ± 9.86	0.235
Peak LH (mIU/mL)	12.53 ± 12.35	14.17 ± 14.54	0.416
Peak FSH (mIU/mL)	14.55 ± 5.38	13.91 ± 4.64	0.425
Peak E2 (pg/mL)	9.19 ± 5.34	11.18 ± 9.83	0.146
Peak LH/FSH ratio	0.94 ± 0.83	1.37 ± 2.72	0.237

* Data are means ± standard deviation.

IV. Discussion

In this study, we showed significantly higher frequency of the allele with $(TTTA)_{13}$ in the CYP19A1 gene in CPP patients compared to control subjects. The results indicated that individuals carrying the $(TTTA)_{13}$ repeat allele may have a higher risk of developing CPP. Although nine polymorphisms were detected in CYP19A1 gene, no clinical significance was observed.

Several previous studies reported that the number of $(TTTA)_n$ repeats in CYP19A1 gene may influence the levels of E_2 and estrogen biosynthesis. Berstein et al. found that genotypes with higher numbers of $(TTTA)_n$ repeats enhanced aromatase activity and they suggested that the longest alleles of $(TTTA)_n$ may predispose to the induction of higher rate of local estrogen biosynthesis (Berstein et al., 2004). Gennari et al. (Gennari et al., 2004) reported that men with higher number of $(TTTA)_n$repeats had higher estrogen levels when compared with men a lower number of $(TTTA)_n$ repeats. Also, Haiman el al. (Haiman et al., 2000) found that postmenopausal women with the $(TTTA)_7$ repeat allele had significantly lower estrogen sulfate than women with non-carriers. In contrast to these studies, Dick et al. (Dick et al., 2005) reported that the free estradiol level was higher in $(TTTA)_7$ repeat allele present group compared with the $(TTTA)_7$ allele absent group. In a recent study, Xita et al. (Xita et al., 2010) demonstrated that the carriers of short $(TTTA)_7$ allele had earlier menarche than girls with other genotypes. The differences in our results may be explained by the differences in ethnicity and sample size. The timing of pubertal onset have found to vary by race and ethnicity (Wu et al., 2002). Although previous researches showed inconsistent results, several studies support an association between long $(TTTA)_n$ repeat allele and increased estrogen level (Haiman et al., 2000; Berstein et al., 2001; Berstein et al., 2004; Gennari et al., 2004). Our results showed that the carriers of long $(TTTA)_{13}$ repeat allele had higher estradiol and LH level than non-carrier group. Compared to non-carrier, the pubertal onset was earlier in the $(TTTA)_{13}$ repeat allele. Also, the $(TTTA)_7$ repeat allele was lower frequency in CPP patients group compared with control group. Although there was no significant differences in laboratory characteristics between the carrier of $(TTTA)_7$ repeat allele and non-carrier, the non-carrier of $(TTTA)_7$ repeat allele demonstrated higher LH and E_2 levels than the girls with seven (TTTA) repeats. Our results may indicated that the higher repeat of $(TTTA)_n$ polymorphism up-regulated the estrogen pathway, which resulted in elevated LH levels and

was associated with pubertal onset at earlier age than the low repeat of $(TTTA)_n$ polymorphism. The CYP19A1 gene encodes aromatase, a key steroidogenic enzyme that catalyzes the conversion of androgen to estrogen (Simpson et al., 1994). So, we postulated that the variation in CYP19A1 gene might affect gene activity and be associated with pubertal onset or pubertal disease such as precocious puberty. Puberty begins with the maturation and activation of the hypothalamic-pituitary-gonadal (HPG) axis (Carel and Leger, 2008). Since 2003, Kiss1 gene and their receptor, G-protein coupled receptor-54 (GPR54) were revealed as a critical component of the HPG axis and are necessary for pubertal onset (de Roux et al., 2003; Seminara et al., 2003). However, the mechanisms responsible for activation of the HPG axis remain unclear. Recent studies showed that estrogen is a key regulator of kisspeptin expression in animal model (Clarkson et al., 2009; Takase et al., 2009; Mayer et al., 2010). Clarkson et al. (Clarkson, 2013) reported that kisspeptin peptide expression in the rostral hypothalamus was regulated by estradiol and estradiol increased kisspeptin biosynthesis during pubertal development. They suggested that GnRH neuron activation should precede changes in circulating estrogens and kisspeptin and GPR54 could not be the trigger factor for pubertal onset, although kisspeptin and GPR54 play important roles in pubertal progression. In another study by Takase et al.(Takase et al., 2009), kisspeptin neurons mature in response to positive-feedback action of estrogen on kiss1 expression in female rats. GnRH surge is occured by estradiol thorough up-regulation of the expression of *Kiss1* mRNA in the anteroventral periventricular nucleus (AVPV) (Smith et al., 2006). These studies supported that the change of estrogen biosynthesis could lead to precocious puberty.

This study had several limitations. First, the main limitation of this study stem from its small control subjects than patients and the control group didn't match age to the study subjects. Second, the age at menarche was reported by recall method. However, we excluded the subject who could not remember correct age at menarche. Also, previous study reported that age at menarche by recall during middle-age was correlated highly with the original childhood data (Must et al., 2002). Third, the carriers of $(TTTA)_{13}$ polymorphism was relatively small than other non-carrier in spite of statistically significant results. So, further research is needed to evaluate $(TTTA)_n$ polymorphism of more patients with precocious puberty and healthy girls with normal puberty.

V. Conclusion

In conclusion, the carriers of higher repeat $(TTTA)_{13}$ polymorphism in intron 4 of the CYP19A1 gene had higher level of estrogen in this study. We suggest that the $(TTTA)_n$ repeat polymorphism may affect CYP19A1 gene activity and the increased circulating estrogen levels may affect the pubertal onset and maturation in girls. To our best knowledge, this is the first study to detect significant associations of $(TTTA)_n$ repeat polymorphism of CYP19A1 gene with precocious puberty.

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-국문요약-

성조숙증 여아에서 아로마타제 유전자 (CYP19A1) 의 다형성 연구

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목적: 중추성성조숙증이란, 이차성징의징후가동성에서의평균연령에비해 2 표준편차이상어린나이에확인되는경우로정의된다. 본연구에서는한국인중추성성조숙증여아를대상으로 아로마타제유전자의변이와이의임상적의의를확인하고자 하였다.

방법: 2010년부터 2012년까지 아주대 병원 소아청소년과에 내원하여특발성 중추성성조숙증으로 진단받은 203명의여아들이환자군으로모집되었으며, 대조군은 101명의정상사춘기발달과정을거친건강한한국인성인여자들을 대상으로 아로마타제 유전자의 엑손과인트론에 대한 유전자 분석을 시행하였다.

결과: 203명의 성조숙증환아와 101명의 정상 대조군의아로마타제 유전자 분석을 시행하였다. 유전자 분석에 엑손 부위에서 9개의 유전자 변이가 발견되었으나, 임상적으로 의미있는 변이는 발견하지 못하였다. 그러나, 본 연구에서 인트론 4에 위치한 (TTTA) 염기서열의 반복 변이의 수가 환자군과 대조군 사이에 차이가 있음을발견하였다. $(TTTA)_{13}$ 반복서열 변이는 성조숙증환아에서만 발견되었고 황체화호르몬과에스트로젠의 농도도 $(TTTA)_{13}$ 반복서열을 보이는 환자군에서 통계적으로 유의하게 높은 것을 알 수 있었다.

결론: 본 연구에서성조숙증 발현에 아로마타제 유전자의 변이가 영향을 미칠 수도 있음을 알 수 있었고, 정확한 아로마타제 유전자의 작용을 알기 위해서는 향후 좀더 많은 환자를 대상으로 하는 연구가 필요할 것으로 생각된다.

핵심어: 성조숙증, 아로마타제 유전자, 에스트로젠