Genetic Variations of *GNRH1*, *GNRHR* and *GPR54* Genes in Korean Girls with Central Precocious Puberty

Jung Min Ko, M.D.[1], Hae Sang Lee, M.D.[2], Hyo Sung Lee, M.D.[2] and Jin Soon Hwang, M.D., Ph.D.[2]

Departments of Medical Genetics[1] and Pediatrics[2], Ajou University School of Medicine, Suwon, Korea

Purpose: Central precocious puberty (CPP) is defined as any sign of secondary sexual maturation appears at an age lower than two standard deviations of the mean for the average age. This process is driven by activation of hypothalamic gonadotropin releasing hormone (GnRH) secretion. Many genes expressed in the hypothalamus have been identified to play an important role in the onset and the progression of puberty. In this study, the *GNRH1*, its receptor (*GNRHR*), and kisspeptin receptor (*GPR54*) genes were scanned to investigate sequence alterations and their distribution in Korean girls with CPP.

Methods: One hundred and one Korean girls with CPP were recruited as the case group and 51 normal Korean women as the control group. The DNAs were extracted and amplified by polymerase chain reaction (PCR), and the products were sequenced directly. Statistical analyses were performed, and *P* values of < 0.05 were considered significant.

Results: Four polymorphisms were identified; however, no pathological mutation was found. Two of the polymorphisms were previously reported, c.47G > C in *GNRH1*, and c.1091T > A in *GPR54*. However, the other two (c.196C > T in *GNRH1* and c.546T > C in *GNRHR*) were novel. There was no polymorphism that was significantly associated with early onset or rapid progression of puberty.

Conclusion: Although the size of our study population was relatively small, simple genetic variations in *GNRH1*, *GNRHR*, and *GPR54* genes are not likely to be a substantial factor directly associated with the onset and progression of puberty. **(J Korean Soc Pediatr Endocrinol 2011;16:38-45)**

Key Words: Sexual precocity; *GNRH1* gene; *GNRHR* gene; *GPR54* gene; Polymorphism

Introduction

Puberty is a complex, coordinated biological process with multiple levels of regulation. It is initiated by the secretion of the gonadotropin-releasing hormone (GnRH) from specialized hypothalamic neurons that stimulate hormonal cascades and gonadal activation[1)]. The development of the pulsatile release of GnRH secretion mediated by the kisspeptin activation of the G-protein coupled receptor-54 (GPR54) appears to be a central event at the onset and during the progression of puberty[2-4)]. The function of GnRH neurons is controlled by a large number of inhibitory, stimulatory and permissive factors. Imbalance of this functional network may result in the alteration of the timing of this process[5, 6)].

Several mutations have been identified in an increasing number of genes that influence puberty. There have been at least 12 genes implicated in isolated hypogonadotrophic hypogonadism (IHH). Although their precise function in reproduction remains unclear, mutations in *GNRHR*, *GNRH1*, *KISS1*, *GPR54*, *TAC3* and *TACR3* genes can cause normosmic IHH, and mutations in the *KAL1*, *FGFR1*, *FGF8*, *CHD7*, *PROK2*, and *PROKR2* gene are related to Kallmann syndrome[7)].

Among these genes, *GNRH1* and its receptor (*GNRHR*) genes are pivotal regulators of the reproductive endocrine axis, and abnormalities of their function have significant impact on human physiology and pubertal development[8)]. The *GNRH1* gene is located on chromosome 8p21.2. It spans about 5 kb and contains 3 exons. It encodes the GnRH1 precursor, which contains 92 amino acids, and it is subsequently

Received: 13 April, 2011, Revised: 22 April, 2011
Accepted: 25 April, 2011
Address for correspondence: Jin Soon Hwang, M.D., Ph.D.
Department of Pediatrics, Ajou University School of Medicine,
Woncheon-dong, Yeongtong-gu, Suwon 443-721, Korea
Tel: +82.31-219-5166, Fax: +82.31-219-5169
E-mail: pedhwang@ajou.ac.kr

processed to GnRH1, an active decapeptide. The *GNRHR* gene is located on chromosome 4q13.2. Its genomic sequence covers about 19 kb and it includes 3 exons. *GNRHR* inactivating mutations were the first genetic alterations recognized as the monogenic cause of normosmic IHH in 1997, and several mutations have been identified in *GNRHR* to date[7, 9]. In 2009, *GNRH1* inactivating mutations have been reported as a new and a rare genetic cause of normosmic IHH[10, 11].

Although central precocious puberty (CPP) is the other extreme of pubertal development in contrast to IHH, the premature activation of GnRH secretion leading to CPP remains undefined. Recently, activating mutations in *KISS1* and *GPR54* genes were identified as causes of CPP in certain patients[12, 13]. However, these mutations are the only described genetic causes of CPP to date.

In this study, the occurrence and the distribution of sequence variations were evaluated, including mutations and single-nucleotide polymorphisms (SNPs), of the *GNRH1*, *GNRHR*, and *GPR54* genes in a Korean female cohort including 101 girls with CPP. The clinical association of sequence variations was studied.

Materials and Methods

1. Patients

Two groups of subjects were involved in this study. The patient group consisted of 101 Korean girls with idiopathic central precocious puberty (CPP) recruited from the Ajou University Hospital in Suwon, Korea. CPP was diagnosed in accordance with the following criteria: (1) objective breast budding appeared before the age of eight years, (2) advanced bone age more than one year above the chronological age, and (3) pubertal luteinizing hormone (LH) peak values (cut-off value; $\geq$ 5 mIU/mL) and the LH/follicle stimulating hormone (FSH) ratios above 1.00 with GnRH stimulation performed prior to nine years of age. The mean age of breast budding and menarche in Korean girls is 11.3 $\pm$ 1.3 years and 12.3$\pm$1.2 years, respectively[14]. Then, the age of 8 years proposed in our inclusion criteria of CPP is -2.54 SD away from the mean age of breast budding in Korean girls. The reference value for the LH/FSH ratio in response to GnRH stimulation is 0.89 $\pm$ 0.49 in Korean pubertal girls[15]. Girls with CPP that had an identified etiology, such as a brain tumor or cranial irradiation, were excluded. Bone age was measured via the method developed by Greulich and Pyle[16], and the sexual maturity rating (SMR) according to the Tanner staging system was assessed by one pediatric endocrinologist.

The control group consisted of 51 independent healthy Korean women more than 20 years of age that were recruited as volunteers on the basis of a questionnaire concerning their breast development after the age of 10 years, and menarche after 12 years of age. The mean age of menarche in the study population was 14.2 $\pm$ 1.3 years, and their height SDS was -0.42 $\pm$ 0.89. There are some limitations that this group was not composed by age - matched controls, and the mean age of menarche in this group was later reference data from the Korean cohort study[14]. However, considering that there is a downward secular trend in the menarche timing in Korean girls, the difference could be thought to be smaller than the presented data.

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

2. *GNRH1*, *GNRHR*, and *GPR54* genetic analysis

Genomic DNA was isolated from the peripheral blood leukocytes of the study subjects using a DNA isolation kit (QIAGEN; GmbH, Helden, Germany). All coding exons and the intronic flanking regions of the *GNRH1*, *GNRHR*, and *GPR54* genes were polymerase chain reaction (PCR) amplified with four pairs of specific primers (Table 1). For the *GNRH1* and *GNRHR* genes, the amplifications were conducted over 35 cycles, and each cycle consisted of denaturation at 94℃ for 30 sec, annealing at 60℃ for 30 sec, and extension at 72℃ for 30 sec. Additional extension at 72℃ for 10 min after the last amplification cycle was performed. For the *GPR54* gene, the amplifications were conducted over 35 cycles, and each cycle consisted of denaturation at 94℃ for 30 sec, annealing at 63℃ (exon 1, 3, 4, 5) or 68℃ (exon 2) for 30 sec, and extension at 72℃ for 30 sec. Additional extension at 72℃ for 10 min after last amplification cycle was carried out. PCR was performed in a reaction volume of 20 μL containing 100 ng of genomic DNA template, 1 μM of each primer, 10 mM of each dNTP, 25 mM $MgCl_2$, 100 mM KCl, 20 mM Tris-

Table 1. Primers used in the analysis of the *GNRH1*, *GNRHR*, and *GPR54* genes

	Forward (5' to 3')	Reverse (5' to 3')
GNRH1 gene		
Exon 1a	GCAGGAAAGATTTCAATGTCC	GATTTAGCCCTTGGGCTGTC
Exon 1b	CCATCTTCTGCAGGGTTAGTG	GCCTTATCTCACCTGGAGCA
Exon 2	CCCCACTCTCCACAATTTTT	CAGGAATGTAAGCCCCACAG
Exon 3	CAAACCCAATTTATCATGTCTCC	ACATGGAGGGCTCCCTTTG
GNRHR gene		
Exon1	CAGGGACAAAATTTGACATACG	CTGACTTCCAGAACCCAAGC
Exon2	GGCTAGCAGAGTACCAAAGAG	TGCCACTCTGTTTTTGACATTG
Exon3	TCCTTTTTGTCCACTTTGGTTT	TCCCAGATGGAGAGATCCA
GPR54 gene		
Exon 1	GGGCGGCCGGGAGGAGGA	CCGGGACGGCAGCAGGTG
Exon 2	AGGCTGAATGTTTGAGAT	AGGGAGATGCACTCAGAAG
Exon 3	CAGGCTCCCAACCGCGCAG	CGTGTCCGCCTTCTCCCGTG
Exon 4	GGTCTTCATCCTGGCTTGTGGC	CCAGCCTTGCTGTCCTCCCACC
Exon 5	TTCGTCTAACCACCTTCAC	TAACAGAAGAATAGCCGCTG

HCl (pH 8.3), and 1 U of Taq DNA polymerase (Takara Bio Inc., Shiga, Japan). After amplification, the PCR mixtures were separated on 2.0% agarose gels with ethidium bromide to confirm the size and purity of the PCR products.

Subsequently, DNA sequencing reactions were conducted using the same primer pairs and a BigDye Terminator V3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer's instructions. The sequencing reaction mixtures were electrophoresed and analyzed using an ABI3130xl Genetic Analyzer (Applied Biosystems) and Sequencing Analysis version 5.2 software.

3. Hormonal studies

The GnRH stimulation test was performed to evaluate the pubertal status in all patients. Basal serum samples were obtained prior to GnRH injection, and post-stimulation samples were acquired 30, 45, 60, and 90 min after injection for measurements of LH and FSH levels. The hormonal levels were measured via immunoradiometric assays (BioSource, Nivelles, Belgium). LH and FSH levels and the LH/FSH ratio were compared to previously documented age-related reference values[17].

4. Statistical analysis

The allele frequency of each polymorphism was compared between the patient and control group. We also compared the frequencies of the genotype combination resulting from noted polymorphisms between the two groups. Deviations from Hardy-Weinberg equilibrium were also evaluated by the comparison of the observed and expected genotype frequencies.

The SPSS for Windows (version 12.0, SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis, and the data were expressed as the means ± SD or the SDS. Fisher's exact test and the Mann-Whitney *U* test were used for the data analysis, and *P* values of < 0.05 were considered statistically significant.

Results

1. Clinical characteristics and the results of the GnRH stimulation test in patients with CPP

In the patient group, the mean age at diagnosis was 7.9 ± 1.0 years, and breast budding was first observed at a mean age of 7.1 ± 1.1 years. The bone age at diagnosis was 10.2 ± 1.4 years, and the mean discrepancy with the chronological age was 2.3 ± 0.4 years. The mean SMR stage at diagnosis was 2.5 ± 0.7 for breast development and 1.1 ± 0.5 for pubic hair development. Height and weight at diagnosis was 1.2 ± 1.0 and 1.0 ± 0.8 SDS respectively, and the patients' heights were higher than their mid-parental height (-0.5 ± 1.0), considered as the genetic target.

According to the results of the GnRH stimulation test, the basal and the peak LH values were 2.2 ± 0.9 mIU/mL (reference range; 0.01-0.21 mIU/mL) and 17.6 ± 14.5 mIU/mL, respectively. The basal (reference range; 0.50 --2.41 mIU/mL) and peak FSH values were 2.7 ± 1.5 mIU/mL and 12.5 ±

4.8 mIU/mL, respectively, and the peak/basal LH ratio and peak LH/FSH ratio were 9.8 ± 10.9 and 1.5 ± 1.1, respectively.

The baseline clinical characteristics and results of the GnRH stimulation tests in the patient group are summarized in Table 2.

2. Identified polymorphisms in the *GNRH1*, *GNRHR*, and *GPR54* genetic analysis

Direct sequencing of the *GNRH1*, *GNRHR* and *GPR54* genes revealed four polymorphisms as shown in Fig. 1 and listed in Table 3. However, no pathological mutation was identified in any of the three genes. Among the four polymorphisms detected in this study, two have been previously reported; however, the other two were novel polymorphisms. All of four polymorphisms were identified in both the patient and control groups, and the results fit Hardy-Weinberg equilibrium expectations.

For *GNRH1*, two of the SNPs caused amino acid changes. The known SNP, c.47G>C, has been repeatedly reported in Caucasians, and is nonsynonymous, resulting in the substitution of p.Trp16Ser[18, 19]. The other SNP, c.196C>T, was a novel sequence alteration and induced substitution of p.His66Tyr.

Table 2. Baseline clinical characteristics and results of GnRH stimulation test in patients with CPP

Auxological parameters	Patients (n = 101)
CA at breast budding (yr)	7.4 ± 1.1
CA at diagnosis (yr)	7.9 ± 1.0
BA - CA at diagnosis (yr)	2.9 ± 0.4
Weight at diagnosis	0.96 ± 0.84
Height at diagnosis	1.22 ± 0.95
BMI at diagnosis	0.58 ± 0.85
MPH	-0.50 ± 1.00
Height - MPH	1.72 ± 0.92
Birth weight	-0.40 ± 1.00
Breast SMR (stage)	2.5 ± 0.7
Pubic hair SMR (stage)	1.1 ± 0.5
Basal LH (mIU/mL)	2.2 ± 0.9
Peak LH (mIU/mL)	17.6 ± 14.5
Basal FSH (mIU/mL)	2.7 ± 1.5
Peak FSH (mIU/mL)	12.5 ± 4.8
Peak / basal LH ratio	9.8 ± 10.9
Peak LH / FSH ratio	1.5 ± 1.1

Values are presented as mean ± SD.
Abbreviations: CA, chronological age; BA, bone age; BA-CA, bone age advancement; MPH, mid-parental height; SMR, sexual maturity rating; LH, luteinizing hormone; FSH, follicle stimulating hormone.

For *GNRHR1*, one novel SNP, c.546T>C was found. Since this variant induced a synonymous change (p.His182His), it did not appear to affect the activity of the GNRH receptor. For *GPR54*, the only detected SNP, c.1091T>A, was a known sequence alteration, and it resulted in the substitution of p.Leu364His.

Allele counts and frequencies in the two groups are shown in Table 4. Using Fisher's exact test, the associations between the polymorphisms and the two groups were evaluated. However, the distribution of each allele and genotype of *GNRH1*, *GNRHR* and *GPR54* did not show any statistical differences between the patients and the controls.

Considering the four polymorphisms in the *GNRH1*, *GNRHR* and *GPR54* genes together, 19 combinations were constructed, and their relationship with CPP was investigated (Table 5). However, none of them exhibited significant correlations with the groups.

3. Clinical significance of detected polymorphisms in patients with CPP

Although there was no difference in allele and genotype

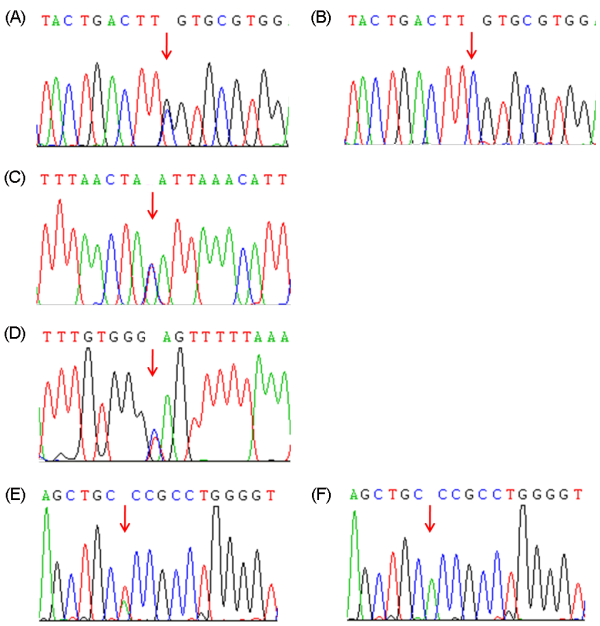

Fig. 1. Partial sequences of the *GNRH1*, *GNRHR*, and *GPR54* genes show the polymorphisms detected in the present study. *GNRH1*; (A) a heterozygote of c.47G>C, (B) a homozygote of c.47G>C, (C) a heterozygote of c.196C>T. *GNRHR*; (D) a heterozygote of c.546T>C. *GPR54*; (E) a heterozygote of c.1091T>A, (F) a homozygote of c.1091T>A. The positions of the polymorphisms are defined according to NM_00825.3 for *GNRH1*, NM_000406.2 for *GNRHR*, and NM_032551.4 for the *GPR54* gene.

Table 3. The *GNRH1*, *GNRHR*, and *GPR54* gene polymorphisms identified by sequencing (n = 152)

No.	mRNA position*	Allele	Location	dbSNP ID	Frequency in sequenced samples	AA position	Note
GNRH1 gene							
1	47	G/C	Exon 1b	rs6185	0.536 / 0.464	16	p.Trp16Ser
2	196	C/T	Exon 1b	-	0.980 / 0.020	66	Novel, p.His66Tyr
GNRHR gene							
1	546	T/C	Exon 2	-	0.928 / 0.072	182	novel, synonymous
GPR54 gene							
1	1091	T/A	Exon 5	rs350132	0.303 / 0.697	364	p.Leu364His

Abbreviations: AA, amino acid.
*The positions of the polymorphisms are defined according to NM_00825.3 for GNRH1, NM_000406.2 for GNRHR, and NM_032551.4 for GPR54 gene.

Table 4. Allele and genotype frequencies of the GNRH1, GNRHR, and GPR54 polymorphisms from 101 patients and 51 controls

Polymorphism	Allele frequency			Genotype frequency			*P* value*
	Group	1	2	11	12	22	Patient-Control
GNRH1 gene							
c.47G>C	Patient	115 0.569	87 0.431	27 0.267	61 0.604	13 0.129	0.116
(G = 1; C = 2)	Control	48 0.471	54 0.529	8 0.157	32 0.627	11 0.216	
c.196C>T	Patient	200 0.990	2 0.010	99 0.980	2 0.020	0 0.000	0.185
(C = 1; T = 2)	Control	98 0.961	4 0.039	47 0.922	4 0.078	0 0.000	
GNRHR gene							
c.546T>C	Patient	188 0.931	14 0069	87 0.861	14 0.139	0 0.000	0.625
(T = 1; C = 2)	Control	94 0.922	8 0.078	43 0.843	8 0.157	0 0.000	
GPR54 gene							
c.1091T>A	Patient	60 0.300	140 0.700	13 0.129	34 0.337	53 0.525	0.896
(T = 1; A = 2)	Control	32 0.308	72 0.692	6 0.115	20 0.385	26 0.500	

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

Table 5. Frequency distribution of the genotype combinations resulting from the four SNPs considered in patients (n = 100) and controls (n = 49)

GNRH1 c.47G>C	*GNRH1* c.196C>T	*GNRHR* c.546T>C	GPR54 c.1091T>A	Tota n (%)	Patients n (%)	Controls n (%)
GC	CC	TT	AA	36 (24.1)	23 (23.0)	13 (26.5)
GC	CC	TT	TA	27 (18.1)	19 (19.0)	8 (16.3)
CC	CC	TT	AA	13 (8.7)	10 (10.0)	3 (6.1)
GG	CC	TT	TA	12 (8.1)	8 (8.0)	4 (8.2)
GG	CC	TT	AA	11 (7.4)	*10 (10.0)	*1 (2.0)
GC	CC	TT	TT	10 (6.7)	5 (5.0)	5 (10.2)
GC	CC	TC	AA	10 (6.7)	8 (8.0)	2 (4.1)
GG	CC	TT	TT	7 (4.7)	6 (6.0)	1 (2.0)
CC	CC	TT	TA	6 (4.0)	†2 (2.0)	†4 (8.2)
GC	CC	TC	TA	4 (2.7)	2 (2.0)	2 (4.1)
GG	CC	TC	TA	2 (1.3)	2 (2.0)	0
GG	CC	TC	AA	2 (1.3)	1 (1.0)	1 (2.0)
GC	CT	TT	TA	2 (1.3)	1 (1.0)	1 (2.0)
CC	CT	TT	AA	2 (1.3)	0	2 (4.1)
GC	CT	TT	AA	1 (0.7)	1 (1.0)	0
GC	CC	TC	TT	1 (0.7)	1 (1.0)	0
GC	CT	TC	AA	1 (0.7)	0	1 (2.0)
CC	CC	TT	TT	1 (0.7)	1 (1.0)	0
CC	CC	TC	TA	1 (0.7)	0	1 (2.0)

*P = 0.102 and †P = 0.091 by Fisher's exact test.

frequency between the patients with CPP and controls, we compared the clinical characteristics and hormone values between the allele subgroups and the genotype subgroups (the subgroup without each of the SNPs, and the homozygous or heterozygous subgroups with each of the SNPs) in the patients with CPP. For each SNP, the basal and GnRH-stimulated hormone levels, the LH/FSH ratio, and the auxological parameters at diagnosis including height, weight, BMI, MPH, birth weight, SMR, and bone age advancement, did not differ between the allele and genotype subgroups in the patients with CPP (data not shown).

Discussion

CPP results from premature activation of the hypothalamic GnRH-secreting neurons. It mimics physiological pubertal development, leading to the development of secondary sexual characteristics at younger ages, acceleration in linear growth and advanced bone age resulting in premature epiphyseal closure and short stature[20]. CPP is considered to be an idiopathic disorder. The fact that family members share a similar age of pubertal onset and a similar pattern of pubertal development suggests that genetic factors may play a role in the pathogenesis of the pubertal process[7]. Researchers are currently studying molecular mechanisms that trigger pubertal onset and modulate the hormonal cascades inherent to puberty.

The genetic modulation of puberty likely is due to the additive effect of multiple genes. Although some genes have been identified that are associated with abnormal pubertal development, most of the genetic alterations of these genes result in IHH. The genes associated with IHH are also considered as candidate genes for mutations causing precocious puberty. However, only a few mutations in two genes, *KISS1* and *GPR54*, have been confirmed as rare causes of CPP to date[10, 11].

The *GNRH1*, *GNRHR*, and *GPR54* genes were examined for genetic variants among a Korean female cohort with and without CPP. Four polymorphisms were detected by sequencing of the three genes, and the frequency of each polymorphism was calculated and compared between the patient and control groups. Each polymorphism was also genotyped. According to the calculated statistical results, no polymorphism was found to be statistically significant.

For the *GHRH1* gene, c.47G>C (p.Trp16Ser) found in the present study is a known polymorphism reported in various ethnic groups[18, 19]. In a previous study performed in a Caucasian population, the allele frequency of c.47G and C in the control groups was 0.737 and 0.263 respectively[18]. However, in this study performed with Korean controls, the allele frequency of c.47G and C was 0.471 and 0.529 respectively. The distribution of this polymorphism was different between our Korean controls and the Caucasian controls ($P < 0.001$). However, this polymorphism is very common, and there is no specific evidence that it could affect clinical phenotypes such as the timing of puberty, and the risk of polycystic ovary syndrome or breast cancer[18, 19, 21]. In addition, the *GNRH1* gene has a novel variation, c.196C>T (p.His66Tyr). This sequence alteration was a novel one with the allele frequency of 0.010 and 0.039 in controls and patients respectively. Although this variation was not commonly identified, it was observed in both patients and controls and was thought to be an infrequent polymorphism rather than a mutation. The translated product of *GNRH1* is a 92-amino acid preprohormone; the linear decapeptide end-product as the active form of GnRH is synthesized in the preoptic anterior hypothalamus[22]. Considering that the sites of the two found SNPs are not contained by the active form of GnRH, these SNPs detected in the *GNRH* gene, in this study, are not likely to seriously influence the bioactivity of GnRH.

For the *GNRHR* gene, a synonymous SNP, c.546T>C, was analyzed for the first time in this study and showed an allele frequency of 0.078 and 0.069 in controls and patients respectively. The other SNPs in the coding region of the *GNRHR* gene reported in the database were not observed in the present study. However, there was no evidence that this SNP was associated with the CPP characteristics.

With regard to the *GPR54* gene, a common SNP, c.1091T>A (p.Leu364His), was analyzed and the results showed a similar allele frequency in patients (0.700) and controls (0.692) in accord with the data previously presented by other researchers[4, 23]. In addition, the allele frequency of this SNP has been reported as 0.698-0.770; this is not different from the studies performed in Caucasians[4, 23]. However, this SNP was not found in a previous Chinese study[24]; on the other hand, other five SNPs in the *GPR54* gene were detected. The genetic background of these two ethnic groups appeared to differ significantly, even though Koreans and Chinese are both

Asians. When the genotype combinations of these three genes were considered together, no significant difference was found between the patients and controls.

Puberty begins when the hypothalamic GnRH neurons secrete GnRH in a pulsatile manner. Pulsatile GnRH causes pituitary gonadotropins to release LH and FSH. LH induces the production of androstenedione in ovarian thecal cells, whereas FSH causes the aromatase enzyme in follicular cells to synthesize estradiol. The increase in serum estradiol causes breast tissue to enlarge and become palpable[25]. At the time of breast budding, the LH peak amplitude increases by approximately 10-fold and the FSH peak amplitude doubles. As puberty progresses, the LH pulse amplitude increases markedly, up to 20 - 40 fold from prepubertal levels during sleep[26, 27]. Only girls with GnRH-stimulated LH peak values in excess of 5 mIU/mL were diagnosed with CPP. In addition, the basal LH elevation, analyzed by new assay methods, has also been confirmed as another reliable method for the diagnosis of CPP[28]. In order to evaluate the clinical significance of each of the SNPs found, we compared the clinical characteristics and hormone values between the subgroups with or without this SNP in the patient group. However, there was no evidence that the SNPs were associated with the clinical and hormone findings including data from GnRH stimulation conditions.

The results of this study suggest that polymorphisms of the *GNRH1*, *GNRHR*, and *GPR54* gene are not likely to be significant factors associated with puberty in Koreans girls. However, the sample of population studied was relatively small and further investigations should be performed to confirm these findings.

한 글 요 약

한국인 중추성 성조숙증 여아에서 *GNRH1*, *GNRHR* 및 *GPR54* 유전자의 다형성 분석

아주대학교 의과대학 의학유전학과[1], 소아과학교실[2]

고정민[1] · 이해상[2] · 이효성[2] · 황진순[2]

목 적 : 중추성 성조숙증이란, 이차 성징의 징후가 동성에서의 평균 연령에 비해 2 표준편차 이상 어린 나이에 확인되는 경우로 정의된다. 중추성 성조숙증의 진행은 뇌하수체에서의 성선자극호르몬 방출호르몬(GnRH) 분비의 활성화에 의해 주도된다. 뇌하수체에서 발현되는 많은 유전자들이 사춘기의 시작과 진행에 중요한 역할을 하는 것으로 알려지고 있다. 본 연구에서는 한국인 중추성 성조숙증 여아를 대상으로, 이들 유전자 중에 GnRH와 이의 수용체를 암호화하는 *GNRH1* 및 *GNRHR* 유전자, kisspeptin의 수용체를 암호화하는 *GPR54* 유전자의 변이와 이의 임상적 의의를 확인하고자 하였다.

방 법 : 101명의 한국인 중추성 성조숙증 여아들이 환자군으로 모집되었으며, 대조군은 51명의 정상 사춘기 발달 과정을 거친 건강한 한국인 성인 여자들로 구성되었다. 환자군 및 대조군 모두를 대상으로 말초 혈액 백혈구에서 DNA를 추출하였으며, 각 유전자마다 특정 시발체를 사용하여 PCR한 후 그 산물을 이용하여 직접염기서열분석을 진행하였다. 환자군과 대조군 사이의 특정 염기서열 변이의 빈도 차이 및 환자군 내에서 특정 염기서열 보유 유무에 따른 여러 임상 변수의 차이를 통계 처리하여 분석하였다.

결 과 : *GNRH1*, *GNRHR* 및 *GPR54* 유전자에서 네 종류의 다형성이 확인되었다. 이중에 *GNRH1* 유전자의 c.47G>C와 *GPR54* 유전자의c.1091T>A 변이는 이미 보고되어 있는 다형성이었으나, *GNRH1* 유전자의 c.196C>T와 *GNRHR* 유전자의 c.546T>C 변이는 과거 보고된 적이 없는 새로운 변이였다. 그러나 발견된 다형성들의 빈도는 환자군과 대조군에서 차이를 보이지 않았으며, 환자군 내에서도 특정 다형성의 유무에 따른 임상적 변수들의 차이를 확인할 수 없었다.

결 론 : 비록 본 연구의 대상 인원이 비교적 적기는 하지만, *GNRH1*, *GNRHR* 및 *GPR54* 유전자의 단순 다형성들은 사춘기의 시작 및 진행 과정에 직접적으로 크게 영향을 미치지는 않는 것으로 생각된다.

References

1) Wildt L, Marshall G, Knobil E. Experimental induction of puberty in the infantile female rhesus monkey. Science 1980; 207:1373-5.
2) de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc Natl Acad Sci U S A 2003;100:10972-6.
3) Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK, et al. The GPR54 gene as a regulator of puberty. N Engl J Med 2003;349:1614-27.
4) Semple RK, Achermann JC, Ellery J, Farooqi IS, Karet FE, Stanhope RG, et al. Two novel missense mutations in g protein-coupled receptor 54 in a patient with hypogonadotropic hypogonadism. J Clin Endocrinol Metab 2005;90:1849-55.

5) Parent AS, Matagne V, Bourguignon JP. Control of puberty by excitatory amino acid neurotransmitters and its clinical implications. Endocrine 2005;28:281-6.
6) Mitsushima D, Hei DL, Terasawa E. gamma-Aminobutyric acid is an inhibitory neurotransmitter restricting the release of luteinizing hormone-releasing hormone before the onset of puberty. Proc Natl Acad Sci USA 1994;91:395-9.
7) Silveira LF, Trarbach EB, Latronico AC. Genetics basis for GnRH-dependent pubertal disorders in humans. Mol Cell Endocrinol 2010;324:30-8.
8) Achermann JC, Ozisik G, Meeks JJ, Jameson JL. Genetic causes of human reproductive disease. J Clin Endocrinol Metab 2002;87:2447-54.
9) de Roux N, Young J, Misrahi M, Genet R, Chanson P, Schaison G, et al. A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. N Engl J Med 1997;337:1597-602.
10) Chan YM, de Guillebon A, Lang-Muritano M, Plummer L, Cerrato F, Tsiaras S, et al. GNRH1 mutations in patients with idiopathic hypogonadotropic hypogonadism. Proc Natl Acad Sci USA 2009;106:11703-8.
11) Bouligand J, Ghervan C, Tello JA, Brailly-Tabard S, Salenave S, Chanson P, et al. Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. N Engl J Med 2009;360: 2742-8.
12) Teles MG, Bianco SD, Brito VN, Trarbach EB, Kuohung W, Xu S, et al. A GPR54-activating mutation in a patient with central precocious puberty. N Engl J Med 2008;358:709-15.
13) Silveira LG, Noel SD, Silveira-Neto AP, Abreu AP, Brito VN, Santos MG, et al. Mutations of the KISS1 gene in disorders of puberty. J Clin Endocrinol Metab 2010;95:2276-80.
14) Park MJ, Lee IS, Shin EK, Joung H, Cho SI. The timing of sexual maturation and secular trends of menarchial age in Korean adolescents. Korean J Pediatr 2006;49:610-6.
15) Choi JY, Kang HJ, Cho WK, Cho KS, Park SH, Hahn SH, et al. Analysis of gonadotropin-releasing hormone (GnRH) test results in girls with precocious puberty. Korean J Pediatr 2009;52:1377-82.
16) Greulich WW, Pyle SI. Radiologic atlas of skeletal development of the hand and wrist. 2nd ed. Palo Alto, CA: Stanford University Press; 1959.
17) Nicholson JF, Pesce MA. Laboratory medicine, drug therapy, and reference tables. In: Behrman RE, Kliegman RM, Jenson HB, Jenson HB, editors. Nelson textbook of pediatrics. 17th ed. Philadelphia: Saunders; 2004.
18) Valkenburg O, Uitterlinden AG, Piersma D, Hofman A, Themmen AP, de Jong FH, et al. Genetic polymorphisms of GnRH and gonadotrophic hormone receptors affect the phenotype of polycystic ovary syndrome. Hum Reprod 2009; 24:2014-22.
19) Canzian F, Kaaks R, Cox DG, Henderson KD, Henderson BE, Berg C, et al. Genetic polymorphisms of the GNRH1 and GNRHR genes and risk of breast cancer in the National Cancer Institute Breast and Prostate Cancer Cohort Consortium (BPC3). BMC Cancer 2009;9:257.
20) Kakarla N, Bradshaw KD. Disorders of pubertal development: precocious puberty. Semin Reprod Med 2003;21:339-51.
21) Sedlmeyer IL, Pearce CL, Trueman JA, Butler JL, Bersaglieri T, Read AP, et al. Determination of sequence variation and haplotype structure for the gonadotropin-releasing hormone (GnRH) and GnRH receptor genes: investigation of role in pubertal timing. J Clin Endocrinol Metab 2005;90:1091-9.
22) Campbell RE, Gaidamaka G, Han SK, Herbison AE. Dendrodendritic bundling and shared synapses between gonadotropin-releasing hormone neurons. Proc Natl Acad Sci USA 2009;106:10835-40.
23) Lanfranco F, Gromoll J, von Eckardstein S, Herding EM, Nieschlag E, Simoni M. Role of sequence variations of the GnRH receptor and G protein-coupled receptor 54 gene in male idiopathic hypogonadotropic hypogonadism. Eur J Endocrinol 2005;153:845-52.
24) Luan X, Zhou Y, Wang W, Yu H, Li P, Gan X, et al. Association study of the polymorphisms in the KISS1 gene with central precocious puberty in Chinese girls. Eur J Endocrinol 2007;157:113-8.
25) DiVall SA, Radovick S. Endocrinology of female puberty. Curr Opin Endocrinol Diabetes Obes 2009;16:1-4.
26) Apter D, Bützow TL, Laughlin GA, Yen SS. Gonadotropin-releasing hormone pulse generator activity during pubertal transition in girls: pulsatile and diurnal patterns of circulating gonadotropins. J Clin Endocrinol Metab 1993;76:940-9.
27) Mitamura R, Yano K, Suzuki N, Ito Y, Makita Y, Okuno A. Diurnal rhythms of luteinizing hormone, follicle-stimulating hormone, testosterone, and estradiol secretion before the onset of female puberty in short children. J Clin Endocrinol Metab 2000;85:1074-80.
28) Resende EA, Lara BH, Reis JD, Ferreira BP, Pereira GA, Borges MF. Assessment of basal and gonadotropin-releasing hormone-stimulated gonadotropins by immunochemiluminometric and immunofluorometric assays in normal children. J Clin Endocrinol Metab 2007;92:1424-9.