Genetic Variations of the *KISS1R* Gene in Korean Girls with Central Precocious Puberty

Yeon Joung Oh,[1]* Young-Jun Rhie,[2]*
Hyo-Kyoung Nam,[2] Hye Ryun Kim,[2]
and Kee-Hyoung Lee[2]

[1]Department of Pediatrics, Hallym University College
of Medicine, Seoul, Korea; [2]Department of
Pediatrics, Korea University College of Medicine,
Seoul, Korea

*Yeon Joung Oh and Young-Jun Rhie contributed
equally to this work.

The timing of puberty onset varies greatly among individuals, and much of this variation is modulated by genetic factors. This study aimed to identify the kisspeptin receptor (*KISS1R*) gene variations and to investigate the associations between these variations and central precocious puberty (CPP). Korean girls with CPP (n = 194) and their healthy controls (n = 99) were included in this study. The entire coding region and the exon-intron boundaries (exon 1 through 5) of the *KISS1R* gene were directly sequenced. Seven polymorphisms were identified in the *KISS1R* gene. A missense change c.1091T>A, and an intron variant c.738+64G>T showed significantly higher allele frequencies in CPP patients than in controls (c.1091T>A: 30.7% vs. 22.2%, *P* = 0.031; c.738+64G>T: 45.6% vs. 35.9%, *P* = 0.023). The missense variant (c.1091T>A) was a nonsynonymous polymorphism that induces amino acid substitution of p.Leu364His. The haplotype CAGTGTC was detected more frequently in the CPP group (*P* = 0.042). The sequence variants of the *KISS1R* gene can be inducible factors in the development of CPP. The association between sequence variants and CPP should be validated by further evidence obtained from larger samples of children with CPP.

Keywords: *KISS1R* Gene; Precocious Puberty; Central; Polymorphism; Timing of Puberty

INTRODUCTION

Human puberty is initiated by an increase in the amplitude and frequency of pulses of gonadotropin-releasing hormone (GnRH) after a quiescent period during childhood. The resurgence of pulsatile GnRH release from specialized hypothalamic neurons induces the secretion of gonadotropin by the pituitary gland, which leads to gonadal maturation and the attainment of reproductive capacity (1). Pubertal onset occurs across a wide range of ages in normal, healthy adolescents and is influenced by complex interactions among genetic, nutritional, environmental, and socioeconomic factors (2). The genetic factors influence pubertal timing in 50%–80% of the general population (3).

Precocious puberty is defined as the appearance of secondary sexual characteristics in girls younger than 8 years old and in boys younger than 9 years old. Central precocious puberty (CPP) is caused by the premature activation of the hypothalamic-pituitary-gonadal axis. CPP occurs more frequently in girls than in boys, and most of these cases are considered idiopathic (4-6). Numerous studies have suggested that genetic factors play important roles in CPP. Familial CPP can occur in up to 27.5% of cases and segregation analysis has suggested autosomal dominant transmission with incomplete sex-dependent penetrance

(7). Recently, mutations in *MKRN3*, the gene encoding makorin RING-finger protein 3, have been shown to cause familial CPP (8). However, the genetic determinants of CPP are largely unknown.

Over the last decade, the kisspeptin/kisspeptin receptor (*KISS1R*) signaling complex has emerged as a fundamental regulator of puberty and reproductive function. *KISS1R*, one of the G protein-coupled receptors, is a 398 amino acid protein going through the membrane seven times and is activated by its ligand, kisspeptin. The receptor is expressed widely in the brain, pituitary gland, and placenta (9). In 2003, two research groups independently reported inactivating mutations of the *KISS1R* gene in patients with idiopathic hypogonadotropic hypogonadism (IHH) (10,11). These studies confirmed the critical role played by the kisspeptin/*KISS1R* system in hypothalamic-pituitary-gonadal axis activation. Navarro et al. (12) showed that binding to the G protein-coupled receptor in hypothalamic GnRH neurons enables kisspeptin to act as a powerful elicitor of GnRH-dependent luteinizing hormone (LH) secretion. In our previous study (13), we demonstrated that serum kisspeptin levels were significantly higher in girls with CPP than in controls. The *KISS1* gene encoding kisspeptin and the *KISS1R* gene have recently been implicated in the pathogenesis of sexual precocity in humans. Activating mutations in *KISS1* and *KISS1R* were detected as genet-

ic causes of CPP (14,15). Accordingly, it is assumed that genetic variations in the *KISS1R* gene can lead to higher or lower risk of CPP. However, few mutations in the *KISS1R* gene that cause CPP have been identified in previous studies, with the exception of the study mentioned above.

The aims of our study were to evaluate the occurrence of genetic variations, including mutations and single-nucleotide polymorphisms (SNPs), of the *KISS1R* gene in 194 girls with CPP and to investigate the correlation between *KISS1R* gene variations and CPP in Korean girls.

MATERIALS AND METHODS

Subjects

Korean girls (n = 194) with idiopathic CPP were recruited from our outpatient clinic at Korea University Ansan Hospital, Korea between August 2012 and July 2013. The girls were diagnosed with idiopathic CPP if the following criteria were met: 1) objective breast budding appearing before the age of 8 years, 2) advanced bone age (BA) at least 1 year ahead of the chronological age (CA), 3) significantly higher peak LH values compared with the cutoff value of 5 mIU/mL according to a GnRH stimulation test conducted prior to the age of 9 years, and 4) a non-pathological brain magnetic resonance imaging (MRI). All the subjects showed normal brain MRI. BA was determined by a single observer according to the Greulich and Pyle method, and sexual maturity rating (SMR) was assessed by one pediatric endocrinologist according to the Tanner staging system.

The control group consisted of 99 healthy Korean girls who were recruited as volunteers on the basis of a freewill questionnaire concerning their breast development after the age of 9 years.

Genetic analysis

Genomic DNA was extracted from peripheral leukocytes of the study subjects using a DNA isolation kit (QIAamp DNA Blood Maxi prep kit; Qiagen, Valencia, CA, USA). The entire coding region and the exon-intron boundaries (exon 1 through 5) of the *KISS1R* gene (GenBank accession No. NM_032551.4) were amplified by polymerase chain reaction (PCR) with 12 pairs of specific primers (Table 1). The PCR conditions were as follows: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C–65°C for 30 seconds, initial extension at 72°C for 30–60 seconds, and final extension at 72°C for 10 minutes. The PCR products were purified using a MultiScreen384-PCR Filter Plate (Milipore, Billerica, MA, USA). The purified products were then sequenced using a BigDye Terminator Cycle Sequencing Kit and an ABI 3730xl automated sequencer (Applied Biosystems, Foster City, CA, USA). The sequencing primers were the same as those used for the PCR amplification. Mutational analyses were performed

Table 1. Primers used in the analysis of the *KISS1R* gene

Exons		Primer sequences (5´ to 3´)
Exon 1	Forward	CAGGAAACAGCTATGACCGTGGGCAGGGGAGGGAGT
	Reverse	TGTAAAACGACGGCCAGTACAGAGCCGGGTCCGAGA
Exon 2	Forward	CAGGAAACAGCTATGACCCTGGTCACTCGGACCAAGG
	Reverse	TGTAAAACGACGGCCAGTCGTCCCCACGTACGATCC
Exon 3	Forward	CAGGAAACAGCTATGACCGTATGTGCCTGAGTGTTCG
	Reverse	TGTAAAACGACGGCCAGTCTAGGCAGGCAGAGCTATT
Exon 4	Forward	CAGGAAACAGCTATGACCCAGGGTGGCTGGGTGAAC
	Reverse	TGTAAAACGACGGCCAGTCTGGACCCCTTGGGCTGT
Exon 5A	Forward	CAGGAAACAGCTATGACCGACAGCCCAAGGGGTCCA
	Reverse	TGTAAAACGACGGCCAGTCGTTGTCCTCCCCCAGGA
Exon 5B	Forward	CAGGAAACAGCTATGACCGCTGAACCCGCTGCTCTAC
	Reverse	TGTAAAACGACGGCCAGTGTCTGGGTGACAGAATGAGACA

KISS1R = kisspeptin receptor.

using Phred, Phrap, Consed, and Polyphred 5.04 software (University of Washington, Seattle, WA, USA).

Hormone assays

The GnRH stimulation test was conducted to evaluate pubertal status in all patients. Basal serum samples were obtained prior to GnRH (Relefact® LH-RH 0.1 mg; Aventis Pharma, Frankfurt, Germany) injection, and post-stimulation samples were acquired 30, 60, and 90 minutes after injection for measurements of LH and follicle-stimulating hormone (FSH) levels. The hormone levels were measured using a chemiluminescent microparticle immunoassay (Abbott Laboratories, Abbott Park, IL, USA).

Haplotype construction and statistical analysis

The allele frequencies were compared between the patient and control groups. Deviations from the Hardy-Weinberg equilibrium were tested by a comparison of observed and expected genotype frequencies. When the significant difference of allele frequencies between the patient and the control groups was found for each polymorphism, the clinical characteristics and results of the hormonal study were also compared between patients harboring a certain polymorphism and those lacking that polymorphism. Haploview 4.2 was used to calculate linkage disequilibrium (LD) (16) and standardized LD coefficient (D´) was plotted. The IBM SPSS 20.0 Network version program (IBM, Armonk, NY, USA) was used to perform statistical analyses, with $P < 0.05$ as the minimal level of statistical significance. Data were expressed as the means ± standard deviation (SD) or the standard deviation score (SDS). Fisher's exact test was used for data analysis. The odds ratios for SNPs were calculated with respective 95% confidence intervals (CIs).

Ethics statement

This study was approved by the Institutional Review Board of Korea University Ansan Hospital, Ansan, Korea (IRB number AS12131), and written informed consent was obtained from all subjects and their parents.

RESULTS

Clinical and hormonal characteristics of the study population

In the patient group, the mean age at diagnosis was 8.08 ± 0.77 years. The BA at diagnosis was 9.87 ± 0.98 years, and the mean difference from the CA was 1.79 ± 0.74 years. The mean height and body mass index (BMI) SDSs were 1.25 ± 0.88 and 0.55 ± 1.01, respectively. According to the results of the GnRH stimulation test, the basal and peak LH levels were 0.62 ± 0.70 IU/L and 13.80 ± 11.71 IU/L, respectively. The basal and peak FSH levels were 3.13 ± 3.54 IU/L and 15.12 ± 6.95 IU/L, respectively. The peak LH/FSH ratio was 1.02 ± 0.82. The mean estradiol level was 6.55 ± 5.34 pg/mL, and the mean SMR stage at diagnosis was 2.55 ± 0.82 for breast development.

In the control group, the mean CA and BA were 10.03 ± 0.58 and 10.55 ± 1.43 years. The mean BA advancement was 0.42 ± 1.13 years. The mean height and BMI SDSs were 0.65 ± 1.18 and 0.70 ± 1.11, respectively. Baseline characteristics and the results

Table 2. Baseline characteristics and results of hormone assays in the study population

Auxological parameters	Patients (n = 194)	Control (n = 99)	P value
Age, yr	8.08 ± 0.77	10.03 ± 0.58	< 0.001
Height, SDS	1.25 ± 0.88	0.65 ± 1.18	< 0.001
Weight, SDS	1.40 ± 1.25	1.17 ± 1.65	0.090
BMI, SDS	0.55 ± 1.01	0.70 ± 1.11	0.350
MPH, SDS	−0.25 ± 0.75	−0.08 ± 0.81	0.560
BA, yr	9.87 ± 0.98	10.55 ± 1.43	0.010
BA advancement, yr	1.79 ± 0.74	0.42 ± 1.13	< 0.001
SMR, stage	2.55 ± 0.82		
Basal LH, IU/L	0.62 ± 0.70		
Peak LH, IU/L	13.80 ± 11.71		
Basal FSH, IU/L	3.13 ± 3.54		
Peak FSH, IU/L	15.12 ± 6.95		
Peak/basal LH	50.91 ± 51.92		
Peak LH/FSH	1.02 ± 0.82		
Estradiol, pg/mL	6.55 ± 5.34		

Data are shown as the mean ± standard deviation (SD).
SDS = standard deviation score, BMI = body mass index, MPH = mid parental height, BA = bone age, SMR = sexual maturity rating, LH = luteinizing hormone, FSH = follicle-stimulating hormone.

of hormone assays in the study population are shown in Table 2.

Identified SNPs in *KISS1R* gene analysis

Concerning the genetic analysis, seven SNPs were detected in the *KISS1R* gene (Table 3). One of these polymorphisms, c.*120C>G, was previously identified in Chinese girls with CPP (17) and was located in 3´UTR. Moreover, the c.24A>G was a synonymous polymorphism and was previously found in CPP girls (18,19). A missense variant c.1091T>A was previously reported in Korean girls with CPP (20) as well as in the study of another population (18). It was a nonsynonymous SNP that induces amino acid substitution of p.Leu364His. We assessed this variant using three in silico prediction algorithms, Polymorphism Phenotyping-2 (Poly-Phen-2, http://genetics.bwh.harvard.edu/pph2/), Sorting Intolerant from Tolerance (SIFT, http://sift.jcvi.org/) and MutationTaster (http://www.mutationtaster.org/), and it was considered as a benign polymorphism in all three in silico analyses. The c.-123C>T was located in 5´UTR and the other three SNPs- c.245-34G>A, c.505+42T>G, and c.738+64G>T– were intron variants. Six of seven polymorphisms except one intron variant (c.505+42T>G) were identified in both the patient and control groups, and the results fitted the expectation by Hardy-Weinberg equilibrium. Although one intron variant (c.505+42T>G) was detected only in the CPP group, it did not induce an amino change and did not appear to affect the activity of the *KISS1R*. Clinical and laboratory characteristics in CPP patients with SNP are shown

Table 3. Polymorphisms of the *KISS1R* gene identified by sequencing (n = 293)

No.	Polymorphism	Location	mRNA position	dbSNP ID	MAF in samples	Note
1	c.-123C > T	Exon 1	39	rs3810423	0.061	5´UTR
2	c.24A > G	Exon 1	185	rs10407968	0.137	Synonymous
3	c.245-34G > A	Intron 1	-	rs117902808	0.177	-
4	c.505+42T > G	Intron 3	-	rs543723928	0.002	-
5	c.738+64G > T	Intron 4	-	rs350131	0.423	-
6	c.1091T > A	Exon 5	1,252	rs350132	0.278	p.Leu364His
7	c.*120C > G	Exon 5	1,478	rs3746146	0.063	3´UTR

The positions of the polymorphisms are defined according to NM_032551.4.
KISS1R = kisspeptin receptor, MAF = minor allele frequency, UTR = untranslated region.

Table 4. Clinical and laboratory characteristics in CPP patients with each polymorphism

No.	SNP		Time of diagnosis (yr)		SMR	LH (mIU/mL)		FSH (mIU/mL)		Peak LH/FSH ratio
			CA	BA		Basal	Peak	Basal	Peak	
1	c.-123C > T	-	7.8	9.2	2.5	0.5	11.8	2.6	16.8	0.77
2	c.24A/G	Synonymous	7.9	9.6	2.4	0.4	10.8	2.8	15.4	0.77
3	c.245-34G > A	-	8.1	9.8	2.8	0.8	15.4	3.7	15.9	1.07
4	c.505+42T > G	-	8.1	9.8	2.7	0.6	13.9	3.0	15.4	1.01
5	c.738+64G > T	-	8.2	9.8	2.5	0.7	14.5	3.0	15.3	1.07
6	c.1091T > A	p.Leu364His	7.9	9.5	2.4	0.3	11.9	2.3	13.9	0.94
7	c.*120C > G	-	8.1	9.7	2.5	0.6	13.7	3.0	15.5	0.99

The positions of the polymorphisms are defined according to NM_032551.4. Data are shown as the mean value.
CPP = central precocious puberty, SNP = single-nucleotide polymorphism, LH = luteinizing hormone, FSH = follicle-stimulating hormone, CA = chronological age, BA = bone age, SMR = sexual maturity rating.

Table 5. Allele and genotype frequencies of the *KISS1R* polymorphisms

SNP	Group	Allele frequency				P value	Genotype frequency						P value
		1		2			11		12		22		
c.-123C>T	CPP	363	0.936	25	0.064	0.672	170	0.876	23	0.119	1	0.005	0.753*
C = 1, T = 2	Control	187	0.944	11	0.056		88	0.889	11	0.111	0	0.000	
c.24A>G	CPP	332	0.856	56	0.144	0.441	141	0.727	50	0.256	3	0.017	0.450*
A = 1, G = 2	Control	174	0.879	24	0.121		76	0.768	22	0.222	1	0.010	
c.245-34G>A	CPP	322	0.830	66	0.170	0.513	132	0.680	58	0.299	4	0.021	0.559*
G = 1, A = 2	Control	160	0.808	38	0.192		64	0.647	32	0.323	3	0.030	
c.505+42T>G	CPP	387	0.997	1	0.003	1.000	193	0.995	1	0.005	0	0.000	1.000*
T = 1, G = 2	Control	198	1.000	0	0.000		99	1.000	0	0.000	0	0.000	
c.738+64G>T	CPP	211	0.544	177	0.456	0.023	60	0.309	91	0.469	43	0.222	0.006†
T = 1, G = 2	Control	127	0.641	71	0.359		37	0.374	53	0.535	9	0.091	
c.1091T>A	CPP	269	0.693	119	0.307	0.031	91	0.469	87	0.449	16	0.082	0.026*
A = 1, T = 2	Control	154	0.778	44	0.222		60	0.606	34	0.343	5	0.051	
c.*120C>G	CPP	362	0.933	26	0.067	0.590	169	0.871	24	0.124	1	0.005	0.661*
C = 1, G = 2	Control	187	0.944	11	0.056		88	0.889	11	0.111	0	0.000	

KISS1R = kisspeptin receptor, SNP = single-nucleotide polymorphism, CPP = central precocious puberty.
*P value for dominant model; †P value for recessive model.

in Table 4. There were no specific clinical characteristics in the patient with each SNP.

Allele counts and frequencies in the two groups are shown in Table 5. With Fisher's exact test, the associations between the polymorphisms and the two phenotype groups were investigated. Among the polymorphisms, two SNPs, c.738+64G>T and c.1091T>A, were detected significantly more frequently allele frequencies in the CPP group (c.738+64G>T, P = 0.023; c.1091T>A, P = 0.031). Using the T allele of the intron variant c.738+64G>T as the reference, the odds ratio of the G allele for CPP was 1.501 (95% CI, 1.055–2.135). Also, using the A allele of the missense variant c.1091T>A as the reference, the odds ratio of the T allele for CPP was 1.548 (95% CI, 1.039–2.306). No differences were found between the two groups in the frequencies of the other five polymorphisms.

Clinical significance of c.1091T>A (p.Leu364His) and c.738+64G>T in CPP patients

Based on the finding that two SNPs, c.1091T>A and c.738+64G>T, were detected more frequently in the patient group, we compared the clinical characteristics and hormone values between the two subgroups of these polymorphisms (c.1091T>A: the subgroup with AA and the subgroup with A/T or T/T, c.738+64G>T: the subgroup with TT or T/G and the subgroup with G/G). The subgroups of each polymorphism were classified according to the results of genotype frequencies (dominant model for c.1091T>A, and recessive model for c.738+64G>T). Basal and peak hormone levels, the LH/FSH ratio, and the auxological parameters at diagnosis—including CA, height, weight, BMI, mid parental height (MPH), SMR, and BA advancement—did not differ between the two subgroups of each polymorphisms in CPP patients. Data was not shown.
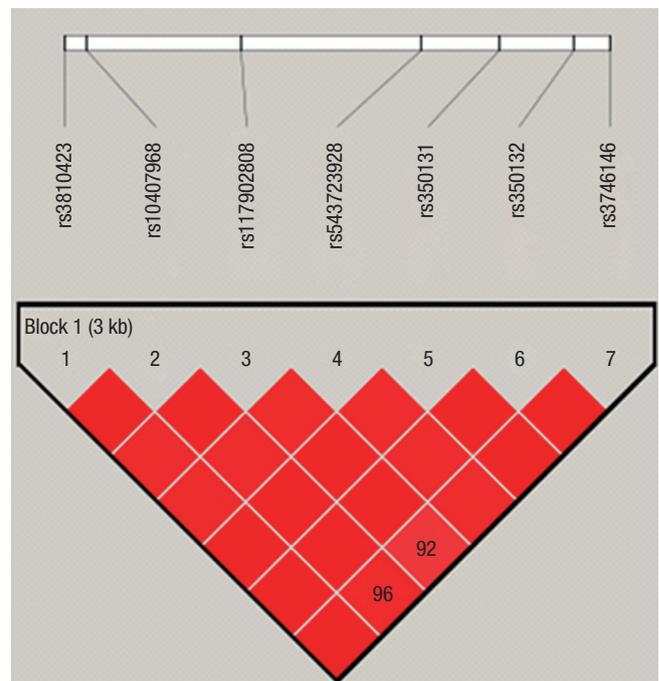

Fig. 1. LD plot for 7 SNPs in the *KISS1R* gene. The D´ values that correspond to each SNP pair are expressed as a percentage and shown within the respective square. Squares without numbers represent D´ values of 1.0, indicative of complete LD. The 7 SNPs constitute one haplotype block (Block 1).
LD = linkage disequilibrium, *KISS1R* = kisspeptin receptor, D´ = linkage disequilibrium coefficient, SNP = single-nucleotide polymorphism.

LD and haplotype analysis

LD was computed between every two SNPs to further analyze the haplotype structure. Fig. 1 shows the LD plot constructed using the 7 SNPs. The magnitude of LD between each SNP was extremely high, with pair-wise D´ values that were ≥ 0.92. Five haplotypes were constructed based on the LD plot and the results are shown in Table 6. Haplotypes with frequency less than 1% are not listed. The haplotype CAGTGTC was detected statis-

Table 6. Results of the haplotype analysis

Haplotypes*	CPP (n = 194)		Control (n = 99)		*P* value
	Counts	Frequencies	Counts	Frequencies	
CAATTAC	64	0.165	38	0.323	0.415
CAGTGTC	117	0.302	44	0.222	0.042
CAGTTAC	147	0.379	89	0.450	0.099
CGGTGAC	30	0.077	13	0.066	0.609
TGGTGAG	25	0.064	11	0.056	0.672

CPP = central precocious puberty, SNP = single-nucleotide polymorphism.
*Haplotypes are designated according to the sequence of each SNP position as follows: rs3810423, rs10407968, rs117902808, rs543723928, rs350131, rs350132, rs3746146.

tically significantly more frequently in the CPP group with a moderate *P* value (0.042). The other four haplotypes showed no differences in frequencies between the patients and control groups.

DISCUSSION

In this study, we evaluated a population of 194 girls with CPP for *KISS1R* gene mutations and polymorphisms, and we identified seven SNPs. Six polymorphisms except the intron variant c.505+42T>G were detected in both the CPP and control groups. Two polymorphisms, c.1091T>A and c.738+64G>T, were found to be statistically correlated to CPP. The missense variant (c.1091T>A) was a nonsynonymous polymorphism that induces amino acid substitution of p.Leu364His. The haplotype CAGTGTC was also significantly associated with CPP patients.

The pulsatile GnRH secretion mediated through the kisspeptin/*KISS1R* system appears to be a key event at the onset and during progression of puberty (11,21). The timing of puberty varies greatly among individuals and races, and much of this variation is due to genetic factors. The genetic modulation of puberty is assumed to arise from the additive effect of multiple genes (22). Many researchers have tried to ascertain a molecular mechanism of kisspeptin/*KISS1R* system associated with development of CPP. Silveira et al. (15) by investigating 83 children with CPP reported two novel *KISS1* missense mutations in 3 unrelated children with CPP. Moreover, Ko et al. (23) identified one polymorphism (p.P110T) relevant to CPP in *KISS1* gene in 101 Koreans girls with CPP and the polymorphism was suggested to exert a protective effect. In our previous study, we demonstrated that three *KISS1* polymorphisms were statistically correlated with CPP, but could not find any clinical importance of these SNPs (24). Recently, it was found that the *KISS1R* gene is relevant to CPP pathogenesis (14). An autosomal dominant missense mutation, Arg386Pro, in *KISS1R*, leading to prolonged activation of intracellular pathways in response to kisspeptin, has been suggested to cause CPP in an adopted girl. Since then, no other CPP cases with activating *KISS1R* mutations have been reported. On the other hand, a few *KISS1R* polymorphisms have been identified in CPP patients. Some researchers have found A/G synony-

mous SNP (c.24A>G) in girls with CPP, but it did not seem to have a pathogenic role in patients (18,19). The polymorphism c.*120C>G has been reported in Chinese girls with CPP and the SNP was not statistically correlated with CPP (17). In the same study, the missense mutation p.P196H in the *KISS1R* was also identified in one patient, although functional study was not performed (17). Moreover, a missense variant c.1091T>A has been identified in studies of Korean girls with CPP and other ethnic group (18,20). However, the researchers were unable to determine any disease associations with the SNP.

In the present study, the seven polymorphisms were detected via the sequencing of the *KISS1R* gene. According to the calculated statistical results, a missense variant c.1091T>A exhibited a statistical correlation with the phenotype (*P* = 0.031). This polymorphism induced amino acid substitution of p.Leu364His. This SNP was previously reported in a study of Korean CPP patients in 2011 (20) and the allele frequency in the study was 30.3%. This result was slightly different to that in our study, in which the allele frequency was calculated as 27.8%. Since both study subjects had same ethnic background, the reason of this discrepancy in the allele frequency is unclear. One possible reason is a different sample size. In the previous study, the results showed a similar allele frequency in patients (30.3%) and controls (30.8%) (20). However, in our study, the allele frequencies were significantly higher in the patients (30.7%) than in the controls (22.2%), although the polymorphism c.1091T>A was identified in both the patients and the controls. Our finding suggests that the missense variant c.1091T>A might affect the pathogenesis of CPP. In order to evaluate the clinical significance of p.Leu364His, we compared the clinical characteristics and hormone values between the subgroups with or without this SNP in the patient group. However, no significant differences were observed between the two subgroups.

The intron variant c.738+64G>T also exhibited a statistical correlation with CPP patients (*P* = 0.023). It was located in the fourth intron with G to T substitution at nucleotide +64 and did not result in any amino acid change. This SNP was not introduced in previous studies concerning CPP. Although introns are generally known as non-protein-coding sections in genes, it can produce genetic and phenotypical variety by regulating or facilitating the transposition of exons (25). Therefore, it can be hypothesized that the polymorphism c.738+64G>T may affect the gene expression of *KISS1R* relevant to the pathogenesis of CPP in Korean girls. In the comparison of clinical parameters and hormonal results, no significant differences were observed between the subgroups with or without this SNP in the patient group. More evidence will be necessary to confirm the association between intron variant in the *KISS1R* and CPP.

In the current study, we have also identified a synonymous SNP (c.24A>G) residing in exon 1 of the *KISS1R* gene. This SNP was not correlated with CPP in our study. Since this variant in-

duced a synonymous change, it did not appear to affect the activity of the *KISS1R*. This polymorphism was previously identified by others in a population of male patients with IHH, although no association with this disorder was found (26,27). It was also detected in CPP patients in previous studies, but it did not seem to have a pathogenic effect on CPP (18,19). These findings were in accord with our results. However, this polymorphism has not been recorded in a large population of Chinese girls (24 girls randomly selected from 272 girls with CPP) (17). The discordance in data between Korean and Chinese populations is partly due to different scale of typed samples. Also, the genetic background appeared to differ significantly, even though Koreans and Chinese are both Asian.

We identified one LD block that consisted of seven SNPs. The value of LD (D´) between each SNP was very high and five haplotype were constructed using the LD block. The haplotype CAGTGTC was detected more frequently in the CPP group (30.2%) than in the control group (22.2%) ($P = 0.042$). Our findings suggest that the haplotype CAGTGTC contributes to pathogenesis of CPP, although the P value of 0.042 was not sufficiently significant.

In conclusion, we have attempted to identify sequence variations of the *KISS1R* gene associated with CPP in Korean girls, and have noted some possibilities that the sequence variants within the *KISS1R* gene might be inducible factors in the pathogenesis of CPP. A possible limitation of the present study was that the sample scale was relatively small, and that P values were not very significant. Therefore, larger scale studies are necessary in order to clarify the association between the sequence variations of *KISS1R* and CPP.

DISCLOSURE

The authors have no potential conflicts of interest to disclose.

AUTHOR CONTRIBUTION

Research conception and design: Oh YJ, Rhie YJ, Lee KH. Performing the experiments: Oh YJ, Rhie YJ, Nam HK. Data acquisition: Nam HK, Rhie YJ, Kim HR. Data analysis and interpretation: Oh YJ, Rhie YJ, Lee KH. Statistical analysis: Oh YJ. Drafting of the manuscript: Oh YJ, Rhie YJ. Critical revision of the manuscript: Lee KH. Receiving grant: Rhie YJ, Lee KH. Approval of final manuscript: all authors.

ORCID

Yeon Joung Oh http://orcid.org/0000-0002-1664-8480
Young-Jun Rhie http://orcid.org/0000-0002-1250-6469
Hyo-Kyoung Nam http://orcid.org/0000-0003-1512-2062
Hye Ryun Kim http://orcid.org/0000-0002-6199-3654
Kee-Hyoung Lee http://orcid.org/0000-0002-4319-9019

REFERENCES

1. Tena-Sempere M. The roles of kisspeptins and G protein-coupled receptor-54 in pubertal development. *Curr Opin Pediatr* 2006; 18: 442-7.
2. Delemarre-van de Waal HA. Secular trend of timing of puberty. *Endocr Dev* 2005; 8: 1-14.
3. Gajdos ZK, Henderson KD, Hirschhorn JN, Palmert MR. Genetic determinants of pubertal timing in the general population. *Mol Cell Endocrinol* 2010; 324: 21-9.
4. Teilmann G, Pedersen CB, Jensen TK, Skakkebaek NE, Juul A. Prevalence and incidence of precocious pubertal development in Denmark: an epidemiologic study based on national registries. *Pediatrics* 2005; 116: 1323-8.
5. Brito VN, Latronico AC, Arnhold IJ, Mendonça BB. Update on the etiology, diagnosis and therapeutic management of sexual precocity. *Arq Bras Endocrinol Metabol* 2008; 52: 18-31.
6. Kakarla N, Bradshaw KD. Disorders of pubertal development: precocious puberty. *Semin Reprod Med* 2003; 21: 339-51.
7. de Vries L, Kauschansky A, Shohat M, Phillip M. Familial central precocious puberty suggests autosomal dominant inheritance. *J Clin Endocrinol Metab* 2004; 89: 1794-800.
8. Abreu AP, Dauber A, Macedo DB, Noel SD, Brito VN, Gill JC, Cukier P, Thompson IR, Navarro VM, Gagliardi PC, et al. Central precocious puberty caused by mutations in the imprinted gene MKRN3. *N Engl J Med* 2013; 368: 2467-75.
9. Muir AI, Chamberlain L, Elshourbagy NA, Michalovich D, Moore DJ, Calamari A, Szekeres PG, Sarau HM, Chambers JK, Murdock P, et al. AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1. *J Biol Chem* 2001; 276: 28969-75.
10. 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.
11. Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG, et al. The GPR54 gene as a regulator of puberty. *N Engl J Med* 2003; 349: 1614-27.
12. Navarro VM, Castellano JM, García-Galiano D, Tena-Sempere M. Neuroendocrine factors in the initiation of puberty: the emergent role of kisspeptin. *Rev Endocr Metab Disord* 2007; 8: 11-20.
13. Rhie YJ, Lee KH, Eun SH, Choi BM, Chae HW, Kwon AR, Lee WJ, Kim JH, Kim HS. Serum kisspeptin levels in Korean girls with central precocious puberty. *J Korean Med Sci* 2011; 26: 927-31.
14. Teles MG, Bianco SD, Brito VN, Trarbach EB, Kuohung W, Xu S, Seminara SB, Mendonca BB, Kaiser UB, Latronico AC. A GPR54-activating mutation in a patient with central precocious puberty. *N Engl J Med* 2008; 358: 709-15.
15. Silveira LG, Noel SD, Silveira-Neto AP, Abreu AP, Brito VN, Santos MG, Bianco SD, Kuohung W, Xu S, Gryngarten M, et al. Mutations of the KISS1 gene in disorders of puberty. *J Clin Endocrinol Metab* 2010; 95: 2276-80.
16. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; 21: 263-5.
17. Luan X, Yu H, Wei X, Zhou Y, Wang W, Li P, Gan X, Wei D, Xiao J. GPR54 polymorphisms in Chinese girls with central precocious puberty. *Neuroendocrinology* 2007; 86: 77-83.
18. Krstevska-Konstantinova M, Jovanovska J, Tasic VB, Montenegro LR, Ben-

eduzzi D, Silveira LF, Gucev ZS. Mutational analysis of KISS1 and KISS1R in idiopathic central precocious puberty. *J Pediatr Endocrinol Metab* 2014; 27: 199-201.

19. Leka-Emiri S, Louizou E, Kambouris M, Chrousos G, De Roux N, Kanaka-Gantenbein C. Absence of GPR54 and TACR3 mutations in sporadic cases of idiopathic central precocious puberty. *Horm Res Paediatr* 2014; 81: 177-81.

20. Ko JM, Lee HS, Lee HS, Hwang JS. Genetic variations of GNRH1, GNRHR and GPR54 genes in Korean girls with central precocious puberty. *J Korean Soc Pediatr Endocrinol* 2011; 16: 38-45.

21. Semple RK, Achermann JC, Ellery J, Farooqi IS, Karet FE, Stanhope RG, O'Rahilly S, Aparicio SA. Two novel missense mutations in g protein-coupled receptor 54 in a patient with hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2005; 90: 1849-55.

22. Palmert MR, Boepple PA. Variation in the timing of puberty: clinical spectrum and genetic investigation. *J Clin Endocrinol Metab* 2001; 86: 2364-8.

23. Ko JM, Lee HS, Hwang JS. KISS1 gene analysis in Korean girls with central precocious puberty: a polymorphism, p.P110T, suggested to exert a protective effect. *Endocr J* 2010; 57: 701-9.

24. Rhie YJ, Lee KH, Ko JM, Lee WJ, Kim JH, Kim HS. KISS1 gene polymorphisms in Korean girls with central precocious puberty. *J Korean Med Sci* 2014; 29: 1120-5.

25. Sapp G. Exons, introns, and talking genes: the science behind the human genome project (book). *Libr J* 1991; 116: 114-8.

26. Tenenbaum-Rakover Y, Commenges-Ducos M, Iovane A, Aumas C, Admoni O, de Roux N. Neuroendocrine phenotype analysis in five patients with isolated hypogonadotropic hypogonadism due to a L102P inactivating mutation of GPR54. *J Clin Endocrinol Metab* 2007; 92: 1137-44.

27. Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, Serin A, Mungan NO, Cook JR, Ozbek MN, et al. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for neurokinin B in the central control of reproduction. *Nat Genet* 2009; 41: 354-8.