Research Article

Gene Polymorphisms Associated with Central Precocious Puberty and Hormone Levels in Chinese Girls

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Central precocious puberty (CPP) is associated with adverse health outcomes in females; however, CPP pathogenesis remains unclear. In this study, we investigated the association of 20 single nucleotide polymorphisms (SNPs) in eight genes with CPP risk and hormone levels. A case-control study on 247 and 243 girls with and without CPP, respectively, was conducted at Kunming Children’s Hospital, China, from September 2019 to August 2020. The genotype of the SNPs and their haplotypes were identified. Additionally, the effects of the polymorphisms on hormone levels were investigated. Three variants (rs10159082, rs7538038, and rs5780218) in KISS1 and two variants (rs7895833 and rs3758391) in SIRT1 were related to an increased CPP risk (odds ratio (OR) = 1.524, 1.507, 1.409, 1.348, and 1.737; 95% confidence interval (CI) = 1.176–1.974, 1.152–1.970, 1.089–1.824, 1.023–1.777, and 1.242–2.430, respectively). Rs3740051 in SIRT1 and rs1544410 in VDR reduced CPP risk (OR = 0.689, 0.464; 95% CI = 0.511–0.928, 0.232–0.925, respectively). Rs1544410, rs7975232, and rs731236 in VDR were negatively correlated with peak follicle-stimulating hormone (FSH; β = −2.181; P = 0.045), basal FSH (β = −0.391; P = 0.010), and insulin-like growth factor (β = −0.360; P = 0.041) levels, respectively. KISS1, SIRT1, and VDR variants were associated with CPP susceptibility, and VDR SNPs influenced hormonal levels in Chinese females with CPP. In particular, VDR polymorphism rs1544410 was associated with both CPP risk and GnRH-stimulated peak FSH levels. Further functional research and large-scale genetic studies of these loci and genes are required to confirm our findings.

1. Introduction

Precocious puberty is a global phenomenon that predominantly occurs in females; it is defined as puberty that starts before the age of nine in males and before the age of eight in females [1]. In the United States, breast development (Tanner stage ≥2) is observed in 18.3%, 30.9%, and 42.9% of eight-year-old white, Hispanic, and black non-Hispanic females, respectively [2]. In Europe, approximately 5% of females exhibit the onset of breast development before the age of eight [3]. In China, 2.9% of females from six different cities exhibit breast development before the age of eight [4]. However, another recent study from Shanghai primary school in China revealed that 17.2% of females aged 6-7 presented with breast development at Tanner stage ≥2, and 19.0% were diagnosed with precocious puberty [5]. Therefore, precocious puberty has attracted considerable attention owing to its high incidence and adverse effects on health outcomes, including obesity, ischemic heart disease, type 2 diabetes, hypertension, estrogen-dependent cancer [6, 7], and behavioral and mental disorders [8]. Understanding the underlying pathogenesis of this disorder is crucial for effective diagnosis and treatment.
Activation of the hypothalamic–pituitary–gonadal (HPG) axis is a hallmark of pubertal onset. The gonadotropin-releasing hormone (GnRH) secreted from the hypothalamus stimulates the pituitary gland to release gonadotropins such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH). In turn, gonadotropins stimulate gonadal maturation and the synthesis of sex steroids [9]. Premature activation of the HPG axis leads to central precocious puberty (CPP), the most common type of precocious puberty. However, the pathogenesis of CPP is not completely clear. Pubertal onset is modulated by a complex combination of genetic, environmental, nutritional, and socioeconomic factors [10], with genetic factors contributing to 50–80% of the variation in pubertal timing in the general population [11]. Genome-wide association studies have identified hundreds of genetic loci that impact pubertal timing in several ethnic groups, as well as numerous loci variations involved in precocious puberty [12]. Moreover, Vries et al. [13] used segregation analysis and reported that autosomal dominant inheritance with incomplete, sex-dependent penetrance was responsible for familial CPP, which accounts for 27.5% of CPP. Thus, genetic factors may play a major role in the pathogenesis of CPP.

Previous genetic association studies have identified several single nucleotide polymorphisms (SNPs) in KISS1, ERα, ERβ, LIN28B, TMEM38B, and vitamin D receptor (VDR) genes related to precocious puberty or their clinical features [14–22]. In this study, we analyzed the relationship between human CPP risk and carefully selected SNPs according to the following criteria: (a) SNPs previously associated with precocious puberty or their clinical features and (b) SNPs previously associated with metabolic disorders. The SIRT1 gene was selected because it plays an important role in metabolic regulation, which may influence pubertal onset [23]. Moreover, SIRT1 SNPs have been linked with metabolic diseases such as obesity and type 2 diabetes [24–26], and animal studies have demonstrated that the SIRT1 protein advances puberty through the regulation of KISS1 expression, which stimulates GnRH secretion [27]. Thus, we hypothesized that SIRT1 variants may be associated with human CPP risk. Further, the association between the aforementioned SNPs and the hormonal levels in Chinese females with CPP has not been well studied before. Therefore, we conducted a genetic association study to explore the relationships of the selected SNPs with CPP risk and hormonal levels in Chinese females.

2. Materials and Methods

2.1. Study Subjects. A total of 243 healthy control females and 247 females with CPP were recruited for this study at Kunming Children’s Hospital, Kunming City, Yunnan Province, China, from September 2019 to August 2020. According to the 2018 guidelines of the Chinese Medical Association [28], the CPP inclusion criteria were as follows: females exhibiting secondary sex characteristics before the age of eight or menarche before the age of 10; accelerated linear growth and bone age (BA) that exceeds the chronological age (CA) by more than one year; enlarged ovaries and uterus, with at least one ovarian follicle greater than 4 mm in diameter; HPG axis function characterized by peak LH > 3.1–5.0 IU/L and a peak LH/FSH ratio > 0.60 according to a GnRH stimulation test. The CPP exclusion criteria were as follows: cases diagnosed with peripheral precocious puberty or precocious puberty caused by an organic lesion, brain tumor, McCune–Albright syndrome, congenital adrenal hyperplasia, hypothyroidism, etc. Healthy females matched to the age of subjects with CPP were selected as control subjects and exhibited no secondary sexual characteristics, abnormal growth and development, or chronic wasting diseases.

Clinical data including age, height, weight, body mass index (BMI), BA, BA advancement (i.e., BA minus CA), level of estradiol (E2), insulin-like growth factor (IGF-1), basal FSH, basal LH, peak LH, and peak FSH, and the peak LH/FSH ratio were collected and evaluated by a trained pediatric endocrinologist. BA was established from a left-hand radiograph. The GnRH stimulation test was conducted by measuring LH and FSH levels in blood samples taken at 0, 30, 60, and 90 min after intravenous injection of gonadotropin. The levels of LH, FSH, E2, and IGF-1 were detected by chemiluminescence with a Diagnostics Roche cobas e602 analyzer. Informed consent was obtained from parents or guardians of all participants. This study was approved by the ethics committee of Kunming Children’s Hospital [No. 20190717003].

2.2. DNA Extraction. Ethylenediaminetetraacetic acid anticoagulant-treated whole blood was collected and transferred to a microcentrifuge tube and maintained at −80°C prior to its use for DNA extraction. Purified genomic DNA was extracted from whole-blood leukocytes using the QIAamp DNA blood mini kit (Qiagen, CA, USA) and stored at −20°C before genotyping.

2.3. SNP Selection and Genotyping. Twenty SNPs (rs10159082, rs5780218, rs7538038, rs7759938, rs314280, rs364663, rs221634, rs2234693, rs4452860, rs7861820, rs7895833, rs3758391, rs3740051, rs33957861, rs731236, rs7975232, rs1544410, rs2228570, rs1256049, and rs3761170) located in eight genes (KISS1, LIN28B, ERα, ERβ, TMEM38B, SIRT1, VDR, and PLCB1) associated with precocious puberty, its clinical features, or metabolic disorders were screened as candidate loci according to previous reports [14–22, 24–26]. The minor allele frequency of the selected SNP loci in the Chinese population is more than 0.05 and the polymorphisms do not exhibit complete linkage disequilibrium (LD) (r² < 1).

SNPs were genotyped using multiple polymerase chain reaction (PCR) and the Snapshot genotyping method based on the Gene Amp PCR system 9600 (Applied Biosystems, Inc., USA) and ABI 3730XL sequencing platform (Thermo Fisher Scientific, Inc., USA). PCR analysis of the selected SNPs was performed using primers that were prepared using the primer data obtained from NCBI (https://www.ncbi.nlm.nih.gov). Primer details are shown in Table S1.
2.4. Statistical Analysis. Clinical data are presented as the mean ± SD or the standard deviation score (SDS). Means of two groups were compared using Student’s t-test. PLINK version 1.07 was used to perform all genetic analyses [29]. The Hardy–Weinberg equilibrium for each SNP was estimated according to the χ² test in the control group. The genotype distribution was then calculated. The genotype of each SNP was assessed using logistic regression analysis with the additive model, and the odds ratio (OR) was calculated with a 95% confidence interval (CI). Linear regression under the additive model was employed to assess the relationships between SNPs and the peak LH, peak FSH, LH/FSH ratio, base LH, base FSH, E2, and IGF-1, adjusting for age and BMI in CPP cases. PLINK and Haploview 4.2 were used to analyze the haplotype blocks of the loci [30], and the pairwise LD was calculated for SNPs within 500 kb. A two-sided P value of less than 0.05 was considered statistically significant for all analyses.

3. Results

3.1. Clinical and Hormonal Parameters of the Study Population. Clinical parameters including age at diagnosis, height, weight, BMI, height SDS, weight SDS, and BMI SDS in the control and CPP groups are presented in Table 1. BA, BA advancement, and hormone parameters—including E2, IGF-1, IGF-1 SDS, basal LH and FSH, peak LH and FSH levels, and peak LH/FSH ratio—of the study population are also shown in Table 1.

3.2. Single Nucleotide Polymorphisms Associated with CPP Risk. As summarized in Tables 2, 20 SNP loci were assessed in this study. Genotyping of the selected SNPs had a 100% success rate and the Hardy–Weinberg equilibrium of all SNPs was more than 0.05 in the control samples. To evaluate the correlation between candidate loci in KISS1, LIN28B, ERα, ERβ, TMEM38B, SIRT1, VDR, and PLCB1 genes and CPP risk, the alleles of the selected SNPs were analyzed in both the CPP and the control samples. As shown in Table 3, seven variants in KISS1, SIRT1, and VDR were found to be related to CPP. Three variants (rs10159082, rs7538038, and rs5780218) in KISS1 and two variants (rs7895833 and rs3758391) in SIRT1 showed a significant association with increased CPP risk (OR, 1.524, 1.507, 1.409, 1.348, and 1.737; 95% CI, 1.176–1.974, 1.152–1.970, 1.089–1.824, 1.023–1.777, and 1.242–2.430, respectively). Moreover, rs3740051 in SIRT1 and rs1544410 in VDR were inversely correlated with CPP (OR, 0.689, 0.464; 95% CI, 0.511–0.928, 0.232–0.925, respectively).

3.3. Haplotype Analysis in the Study Population. The LD coefficient (D’) was calculated to represent LD, where a D’ value of ≥0.8 indicated that the related SNPs formed one block. Figure 1 shows the LD plot. Haplotype analysis was performed for SNPs in KISS1, SIRT1, and VDR associated with CPP; the results are shown in Table 4. Haplotype CGdelA in the KISS1 gene was associated with an increased risk of CPP (P = 0.003), whereas haplotype AAA in KISS1 was associated with a decreased risk of CPP (P = 0.007). Interestingly, haplotype ACAC in SIRT1 significantly increased CPP risk (P = 0.0007), whereas haplotype GTGC in SIRT1 reduced CPP risk (P = 0.014). Haplotype ACAC had a lower P value (P = 0.0007) than that of the individual SNPs (0.0013 < 3P < 0.0340), indicating that these SNPs are in LD with a more significantly associated variant; however, we could not confirm whether this difference in P values was significant.

3.4. Single Nucleotide Polymorphisms Associated with Hormonal Levels. To investigate the effect of hereditary factors on hormonal levels, we performed a linear regression analysis for all SNPs under the additive model, adjusting for age and BMI. As shown in Table 5, rs1544410, rs7975232, and rs731236 in VDR were negatively correlated with the peak FSH (β = −2.181; P = 0.045), basal FSH (β = −0.391; P = 0.010), and IGF-1 (β = −50.360; P = 0.041P = 0.041) levels.

4. Discussion

In the current study, we explored the association of selected SNPs with CPP risk and hormonal levels in Chinese girls. We found seven variations in KISS1, SIRT1, and VDR associated with CPP risk. Three SNPs in VDR showed a significant correlation with peak FSH, basal FSH, and IGF-1 levels in CPP groups. To our knowledge, this is the first study to report an association between SIRT1 polymorphisms and CPP risk in Chinese females. VDR polymorphisms were also shown to affect hormonal levels in Chinese females with CPP for the first time, which gave rise to the possibility that rs1544410 polymorphism in VDR had the protective effect on CPP risk by affecting GnRH-stimulated peak FSH level.
Kisspeptin, encoded by KISS1, is regarded as a fundamental gatekeeper of pubertal onset through strong stimulation of the GnRH secretion [31]. For example, the administration of kisspeptin to immature animals induced advanced activation of the HPG axis and puberty development [32]. Moreover, humans with gain-of-function mutations in KISS1, which is expressed on GnRH neurons, showed earlier pubertal onset [33]. These studies imply that the KISS1 gene contributes to the pathogenesis of precocious puberty. In our study, we found that rs10159082, rs7538038, and rs5780218 in KISS1 were significantly associated with an increased risk of CPP. Additionally, our haplotype analysis indicated that haplotypes CGdelA and AAA in KISS1 had a close relationship with the CPP risk. Rs5780218, located in the promoter region of KISS1, may also affect KISS1 transcription [34]. Our results are consistent with the findings reported by Li et al. [14], who showed that rs5780218 plays a role in CPP risk in Chinese females. A large-scale candidate-gene association study also revealed a significant correlation between the rs7538038 variant and the age at menarche [15], and that rs10159082 was in LD

### Table 2: Primary information of selected single nucleotide polymorphisms.

| SNP       | Chromosome | Position | Gene     | Minor/major allele | Genotypes | HWE   |
|-----------|------------|----------|----------|--------------------|-----------|-------|
| rs10159082| chr1       | 204191322| KISS1    | C/A                | 32/111/100| 0.890 |
| rs7538038 | chr1       | 204191898| KISS1    | G/A                | 26/109/108| 1.000 |
| rs5780218 | chr1       | 204196482| KISS1    | delA/A             | 35/126/82 | 0.286 |
| rs7759938 | chr6       | 104931079| LIN28B   | C/T                | 24/95/124 | 0.356 |
| rs314280  | chr6       | 104952962| LIN28B   | A/G                | 24/95/124 | 0.356 |
| rs364663  | chr6       | 104995314| LIN28B   | T/A                | 25/96/122 | 0.361 |
| rs221634  | chr6       | 105080213| LIN28B   | A/T                | 57/113/73 | 0.305 |
| rs2234693 | chr6       | 151842200| ERα      | C/T                | 42/118/83 | 1.000 |
| rs4452860 | chr9       | 106163108| TMEM38B  | A/G                | 51/112/80 | 0.301 |
| rs7861820 | chr10      | 106174393| TMEM38B  | T/C                | 11/95/137 | 0.379 |
| rs7895833 | chr10      | 6786299   | SIRT1    | A/G                | 18/98/127 | 1.000 |
| rs3758391 | chr10      | 67883584  | SIRT1    | C/T                | 5/61/177  | 1.000 |
| rs3740051 | chr10      | 67884201  | SIRT1    | T/C                | 5/53/185  | 0.570 |
| rs7957232 | chr12      | 47844974  | VDR      | A/C                | 21/100/122| 1.000 |
| rs1544410 | chr12      | 47846052  | VDR      | T/C                | 0/26/217  | 1.000 |
| rs2228570 | chr12      | 47791192  | VDR      | A/G                | 49/125/69 | 0.608 |
| rs1256049 | chr14      | 64257333  | ERβ      | T/C                | 28/109/106| 1.000 |
| rs3761170 | chr20      | 8737066   | PLCB1    | A/G                | 3/48/192  | 1.000 |

HWE: Hardy–Weinberg equilibrium, which was estimated for each SNP using the $\chi^2$ test using individuals in the control group.

### Table 3: Association analysis between the selected single nucleotide polymorphisms and central precocious puberty risk.

| Gene  | SNP       | Minor/major allele | MAF      | Control | OR      | 95% CI    | P     |
|-------|-----------|--------------------|----------|---------|---------|-----------|-------|
| KISS1 | rs10159082| C/A                | 0.462    | 0.360   | 1.524   | 1.176–1.974| 0.001 |
| KISS1 | rs7538038 | G/A                | 0.423    | 0.331   | 1.507   | 1.152–1.924| 0.003 |
| KISS1 | rs5780218 | delA/A             | 0.486    | 0.403   | 1.409   | 1.089–1.824| 0.009 |
| LIN28B| rs7759938 | C/T                | 0.249    | 0.294   | 0.812   | 0.620–1.063| 0.130 |
| LIN28B| rs314280  | A/G                | 0.261    | 0.294   | 0.861   | 0.659–1.124| 0.271 |
| LIN28B| rs364663  | T/A                | 0.265    | 0.300   | 0.854   | 0.654–1.114| 0.243 |
| LIN28B| rs221634  | A/T                | 0.431    | 0.467   | 0.867   | 0.675–1.113| 0.263 |
| ERL   | rs2234693 | C/T                | 0.379    | 0.416   | 0.850   | 0.654–1.105| 0.225 |
| TMEM38B| rs4452860| G/A                | 0.498    | 0.440   | 1.242   | 0.973–1.584| 0.082 |
| TMEM38B| rs7861820| T/C                | 0.213    | 0.241   | 0.848   | 0.626–1.149| 0.286 |
| SIRT1 | rs795833  | A/G                | 0.338    | 0.276   | 1.348   | 1.023–1.777| 0.034 |
| SIRT1 | rs3758391 | C/T                | 0.227    | 0.146   | 1.737   | 1.242–2.430| 0.001 |
| SIRT1 | rs3740051 | G/A                | 0.207    | 0.274   | 0.689   | 0.511–0.928| 0.014 |
| SIRT1 | rs33957861| T/C                | 0.113    | 0.130   | 0.855   | 0.580–1.262| 0.431 |
| VDR   | rs731236  | G/A                | 0.030    | 0.047   | 0.618   | 0.315–1.216| 0.164 |
| VDR   | rs7975232 | A/C                | 0.283    | 0.292   | 0.958   | 0.726–1.264| 0.761 |
| VDR   | rs1544410 | T/C                | 0.026    | 0.054   | 0.464   | 0.232–0.925| 0.029 |
| VDR   | rs2228570 | A/G                | 0.492    | 0.459   | 1.157   | 0.889–1.505| 0.278 |
| ERβ   | rs1256049 | T/C                | 0.346    | 0.340   | 1.030   | 0.791–1.340| 0.827 |
| PLCB1 | rs3761170 | A/G                | 0.089    | 0.111   | 0.782   | 0.514–1.190| 0.252 |

SNP: single nucleotide polymorphisms; MAF: minor allele frequency; CI: confidence interval; OR: odds ratio. P value was obtained from the additive model. Significant P values (<0.05) are highlighted in bold.
with rs7538038 \((r^2 = 0.58)\). These two variants in the KISS1 gene are located in its intron region; however, there is no available literature on their function. Intron variants do not alter the protein sequence of the gene but may impact gene expression or be in LD with another variant that does. Our results revealed that the three variants of KISS1 (rs5780218, rs7538038, and rs10159082) were in LD with each other; accordingly, these associations are not independent. Further functional analysis is required to identify the association between intron variants in KISS1 and CPP risk.

Pubertal timing is driven by not only genetic factors but also nutritional and metabolic factors \[23\]. Changes in the nutritional or metabolic conditions of an individual can eventually impact the secretion of GnRH \[35\]. SIRT1, which acts as a key cellular metabolic sensor, can contribute to puberty by controlling the expression of the KISS1 gene \[36\]. That is, when puberty begins, SIRT1 is separated from the KISS1 promoter, increasing KISS1 transcription. Under conditions of energy excess, such as nutritional excess or early-onset obesity, SIRT1 is prematurely evicted from the promoter of KISS1, which promotes KISS1 expression, causing early puberty \[27\]. However, few studies have focused on the effect of variants in the SIRT1 gene on CPP risk. Our findings showed that rs3758391 and rs7895833 in SIRT1 could increase CPP risk, whereas rs3740051 in SIRT1 exhibited a protective effect on CPP risk. Moreover, haplotype analysis of these loci indicated that haplotypes GTGC and ACAC in SIRT1 were correlated with the CPP risk. Rs3758391 in SIRT1 is located at the p53-binding site, which is in the distal promoter required for human SIRT1 transcription. Moreover, the C variation of rs3758391 disrupts the mirror-image symmetry of the p53-binding sequence, affecting SIRT1 mRNA expression \[37\]. Tang et al. \[38\] reported that rs3758391 variation was significantly related to the occipital cortex SIRT1 expression in humans and that the C allele of rs3758391 was associated with lower SIRT1 expression. In addition, Cruz et al. \[26\] observed that the minor C allele of rs3758391 conferred a higher risk of type 2 diabetes in the

![Figure 1: Linkage disequilibrium (LD) plot for VDR, SIRT1, and KISS1 genes. LD coefficient values \((D')\) corresponding to each SNP pair are expressed as a percentage and shown within the respective square. Three SNPs in VDR, four SNPs in SIRT1, and three SNPs in KISS1 comprised one block (Block 1, Block 2, and Block 3, respectively).](image)

### Table 4: Haplotype analysis in the study population.

| Gene | SNP | Haplotype | Freq. | Distribution freq. | Risk | P  |
|------|-----|-----------|-------|--------------------|------|----|
|      |     | Case      | Control |                    |      |    |
|      |     |           |        |                    |      |    |
| VDR  | rs731236-rs7975232-rs1544410 | ACC  | 0.712 | 0.717 | 0.708 | +  | 0.762 |
|      |     | AAC  | 0.241 | 0.249 | 0.232 | +  | 0.546 |
|      |     | GAT  | 0.032 | 0.022 | 0.041 |  | 0.091 |
| SIRT1| rs7895833-rs3758391-rs3740051-rs33957861 | ATAT | 0.116 | 0.108 | 0.124 |  | 0.443 |
|      |     | GTGC | 0.240 | 0.206 | 0.274 |  | 0.014 |
|      |     | ACAC | 0.184 | 0.226 | 0.141 | +  | <0.001 |
|      |     | GTAC | 0.445 | 0.449 | 0.440 | +  | 0.776 |
| KISS1| rs10159082-rs7538038-rs5780218 | CG delA | 0.339 | 0.383 | 0.293 | +  | 0.003 |
|      |     | AG delA | 0.013 | 0.007 | 0.019 |  | 0.090 |
|      |     | CA delA | 0.034 | 0.040 | 0.029 | +  | 0.379 |
|      |     | AA delA | 0.059 | 0.056 | 0.062 |  | 0.711 |
|      |     | CGA  | 0.025 | 0.031 | 0.019 | +  | 0.229 |
|      |     | CAA  | 0.013 | 0.008 | 0.019 |  | 0.137 |
|      |     | AAA  | 0.516 | 0.473 | 0.559 |  | 0.007 |

*P value was obtained from the \(\chi^2\) test via Haploview. Significant \(P\) values \((<0.05)\) are highlighted in bold. +: increased risk; -: decreased risk.*
In mice [39]. In humans, the SNP: singlenucleotide polymorphisms; LH: luteinizing hormone; FSH: follicle-stimulating hormone; E2: estradiol; IGF-1: insulin-like growth factor.

Linear regression under the additive model was used to assess the associations between SNPs and peak LH, peak FSH, peak LH/FSH ratio, basal LH, basal FSH, E2, and IGF-1, adjusting for age and body mass index in CPP groups. Significant P values (<0.05) are highlighted in bold.

Mexican population. Rs7895833 in SIRT1 is located 21 kb upstream of SIRT1, which may lie within the promoter region, where it could influence SIRT1 expression [25]. The SIRT1 rs3740051 variant is also located in the promoter region. Resveratrol, an activator of SIRT1, increases energy expenditure in mice [39]. In humans, the G allele of SIRT1 rs3740051 is related to higher levels of energy expenditure [39]. These results suggest that the G allele at rs3740051 could increase the activity of SIRT1. In addition, SIRT1 gene polymorphisms rs3758391, rs7895833, and rs3740051 may influence SIRT1 protein activity and expression. Animal studies have reported that decreased SIRT1 levels are associated with advanced puberty, whereas increased SIRT1 levels are associated with delayed puberty [27]. Considering this study, SIRT1 polymorphisms may affect KISS1 transcription by influencing SIRT1 protein activity and expression, thereby causing susceptibility to CPP.

VDR is a member of the steroid hormone receptor family that serves as a transcriptional activator of many target genes. The four variants rs731236, rs7975232, rs1544410, and rs2228570 in the VDR gene are commonly related to metabolic disorders, such as diabetes, metabolic syndrome (MetS), and polycystic ovary syndrome (PCOS) [40, 41].

VDR is also crucial for female reproduction [42]. However, literature on the association between VDR polymorphisms and CPP is scarce. Santos et al. [18] reported a relationship between VDR polymorphism and precocious pubarche in the studied population. In our study, we found that the mutant allele of rs1544410 in VDR was associated with a reduced risk of CPP. rs1544410, which is located in the 3′-UTR, may be responsible for VDR mRNA stability and expression of VDR [43]. Zhao et al. [40] found rs1544410 to be negatively correlated with the risk of metabolic syndrome in the Northern Chinese population. Sahin et al. [44] showed that the A allele of rs1544410 was associated with a decreased risk of type 1 diabetes. Zhong et al. [45] suggested that the minor A allele of rs1544410 may be a susceptibility marker of diabetic nephropathy in type 2 diabetes in the Chinese population. Collectively, these studies imply that rs1544410 in VDR may be a genetic marker for endocrine disorders. This supports our findings on the role of rs1544410 polymorphism in CPP.

Interesting findings emerged from our analysis of the association between the selected SNPs and hormonal parameters. Specifically, we found a negative correlation between VDR rs1544410 polymorphism and peak FSH levels. FSH is essential for female reproductive maturation and, when bound to its G protein-coupled receptor (FSHR), regulates folliculogenesis, oocyte selection, and the synthesis of sex steroid hormones in the ovary [46]. With the onset of puberty, FSH stimulates the ovaries to produce the estradiol responsible for breast tissue growth, which is one of the earliest clinical signs of puberty. Hagen et al. [47] found that puberty occurred later in healthy females with the FSHR-29AA (reduced FSHR expression) genotype than in individuals with FSHR-29GG/GA. This strongly suggested that FSH action impacts pubertal timing in females. In addition, VDR mRNA is expressed in the human pituitary gland, and VDR could play a role in controlling the expression of human pituitary hormone genes, including FSH and LH [48]. Taken together, it appears that VDR rs1544410 decreases CPP risk by reducing the GnRH-stimulated peak
FSH level. However, further research is required to determine the precise molecular mechanism. Additionally, rs1544410 was negatively correlated with the peak LH level in the CPP groups; however, the correlation was not statistically significant ($P = 0.10$). This lack of significance might be attributed to the relatively small number of subjects.

Our study revealed the negative and nonsignificant association between two variants (rs7975232 and rs731236) in the VDR gene and CPP risk; however, we also found a negative and significant correlation between rs7975232 and rs731236 and the level of basal FSH and IGF-1, respectively. The rs7975232 locus, located in the 3′-UTR region, regulates VDR expression by affecting mRNA stability, as does rs731236 [49]. As previously mentioned, VDR may influence FSH secretion. Therefore, rs7975232 polymorphism affects the level of basal FSH. IGF-1, which modulates the expression of the KISS1 gene, plays an important role in pubertal onset [50]. A significant increase in serum IGF-1 close to the onset of puberty can induce prepubertal GnRH secretion and advance the timing of puberty [51]. IGF-binding protein 3 (IGFBP-3) mainly transports IGFs in the plasma. Lower IGFBP-3 levels shorten the half-life of IGF-1 in the circulation, resulting in lower IGF-1 levels [52]. VDR acts as a transcription factor for the IGFBP-3 gene and modulates the expression of IGFBP-3 mRNA [52, 53], which indicates that VDR influences the IGF-1 level by regulating IGFBP-3 expression. Taken together, these findings indicate that the VDR rs731236 polymorphisms that modulate IGFBP-3 mRNA expression reduce the IGF-1 level, and therefore exhibit a protective effect on earlier pubertal onset.

This case-control study investigated the association of selected SNPs with CPP risk and hormonal levels. However, there are several limitations to our study. First, our study lacks measurement on hormonal levels for control group. Therefore, for SNPs associated with both CPP risk and hormonal levels within CPP cases, the results are not enough for addressing whether or not the association with CPP is caused by its association with hormonal levels. Second, the number of loci investigated was not sufficient to fully reveal the relationship between genetic factors and CPP susceptibility. To confirm our findings, further genetic studies with more susceptibility variants and better design are required.

5. Conclusion

We identified associations between the gene polymorphisms rs10159082, rs7538038, rs7580218, rs3758391, rs7895833, rs3740051, and rs1544410 in KISS1, SIRT1, and VDR and CPP risk. This is the first study to report the effect of SIRT1 polymorphisms on CPP risk in Chinese girls. Further, this study was the first to report that rs1544410, rs7975232, and rs731236 in VDR affect hormonal levels in Chinese girls with CPP. In particular, rs1544410 in VDR was associated with both CPP risk and GnRH-stimulated peak FSH levels. This study provides potential genetic biomarkers for assessing CPP risk in Chinese girls. As several of the SNPs identified in our study have not previously been reported, they need to be validated in a large-scale study.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors’ Contributions

Yunwei Li and Na Tao contributed equally to this work and share first authorship.

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Supplementary Materials

Table S1. Primers details. STROBE-checklist-v4-combined. (Supplementary Materials)

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