Original Article

Investigation of *MKRN3* mutation in patients with familial central precocious puberty

**Short Title:** *MKRN3* mutation in patients with familial central precocious puberty.

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**What is already known on this topic?**

Since 2013, the underlying aetiology of some cases of familial idiopathic central precocious puberty (iCPP) has been elucidated.

However, the number of studies done in this area in Turkey is insufficient.

**What this study adds?**

This study showed a low rate of *MKRN3* mutation in cases of familial iCPP in Turkey.

A case study of a patient with *MKRN3* mutation highlights the importance of an accurate family history.

**Abstract**

**Objective:** Little is known about the genetic cause of idiopathic central precocious puberty (CPP). The aim of this clinical study was to determine the rate of *MKRN3* mutation in cases of familial idiopathic central precocious puberty.

**Methods:** Potential sequence variations in the maternally imprinted *MKRN3* gene were evaluated in 19 participants from 10 families using next-generation sequencing (NGS) analysis.

**Results:** In the whole group, the novel heterozygous mutation NM_005664.3:c.630_650delinsGCTGGGC (p.P211Lfs*16) was detected only in one male patient with a paternal history of precocious puberty; no mutation was detected in the other patients. The father of the patient with
MKRN3 mutation also had a history of precocious puberty and had the same mutation. This is a novel variant, and it was identified as a pathogenic variant by in silico analysis.

**Conclusions:** In conclusion, MKRN3 mutation was detected in only one (5.3%) of 19 individuals from 10 families with familial CPP. No MKRN3 mutation was detected in siblings with CPP. Given the fact that MKRN3 mutation was detected in only one patient with a paternal history of precocious puberty in our study, it is clear that an accurate family history is important, as it may reveal paternal inheritance of familial iCPP due to a mutation in MKRN3.

**Key Words:** MKRN3 mutation, familial central precocious puberty, genetic analysis

**Introduction**

Central precocious puberty (CPP) is defined as the development of secondary sex characteristics before 8 years of age in girls and 9 years of age in boys due to early activation of the hypothalamic–pituitary–gonadal (HPG) axis (1, 2). Owing to recent advances in genetics, the underlying aetiology has been revealed in some cases of idiopathic CPP (iCPP). Gain-of-function mutations in the KISS1 and KISSR1 genes and loss-of-function mutations in the makorin ring finger protein 3 (MKRN3) gene were shown to result in CPP (3, 4, 5).

The MKRN3 gene encodes the makorin ring finger 3 protein, which exerts an inhibitory effect on gonadotropin releasing hormone (GnRH) neurons. It has been proposed that the HPG axis is reactivated by loss-of-function mutations in the MKRN3 gene (6). It was reported that the frequency of MKRN3 was higher in cases with familial idiopathic CPP (iCPP) compared with sporadic cases (7, 8). However, the frequency can vary according to ethnicity (9). The aim of this clinical study was to determine the rate of MKRN3 mutation in cases with familial iCPP.

**Materials and Methods**

The study included siblings diagnosed with iCPP and iCPP cases with a positive family history who presented to the Endocrinology Outpatient Clinic of Dr Sami Ulus Obstetrics and Gynaecology, Children’s Health and Disease Training and Research Hospital. All parents gave written informed consent before participation. The study was approved by the Ethics Committee of the Zekai Tahir Burak Maternity Teaching Hospital, Ankara, Turkey. Children included with at least one first or second degree relative with documented iCPP. Other cases were excluded from the study.

The diagnosis of precocious puberty was made based on the following criteria. The Tanner and Marshall criteria (10, 11) were used for puberty staging. Girls who had at least Tanner stage 2 breast development and stage 2 pubarche before 8 years of age were assessed as cases of early puberty. Boys who had at least Tanner stage 2 testicular volume >4 mL or stage 2 pubarche before 9 years of age were assessed as cases of early puberty.

Luteinising hormone (LH), follicle-stimulating hormone (FSH), and 17β-oestradiol (E2) were measured using a morning blood sample in girls. A basal serum LH level ≥0.83 mIU/mL (if it was consistent with other findings) was accepted as activation of the hypothalamic–pituitary–gonadal (HPG) axis (12). Cases with a basal LH level <0.83 mIU/mL underwent the standard stimulation test of 100 µg GnRH (Ferring Pharmaceuticals, Inc., Parsippany, NJ, USA) by intravenous injection between 8:00 and 8:30 AM to assess early puberty, and blood samples were taken at 0, 40, 60, 90, and 120 min to measure serum LH and FSH levels. Peak LH ≥3.3 mIU/mL was accepted as the diagnostic criterion for activation of the HPG axis in girls (13). LH, FSH, and testosterone were measured using a morning blood sample in boys. A basal serum LH level ≥0.83 mIU/mL (if it was consistent with other findings) was accepted as activation of the HPG axis in boys (12). Cases with a basal LH level <0.3 mIU/mL underwent the standard stimulation test described above. Peak LH ≥4.1 mIU/mL was accepted as the diagnostic criterion for activation of the HPG axis (13).
Congenital adrenal hyperplasia was excluded by 17-OH progesterone (17-OHP) <1.5 ng/mL at measurements performed early in the morning and/or peak 17-OHP <10 ng/mL in the ACTH stimulation test. Cranial magnetic resonance imaging (MRI) was performed to exclude any organic lesion in all cases diagnosed with CPP.

Standing height was measured to the nearest 0.1 cm with a Harpenden fixed stadiometer, and body weight was measured on a SECA balance scale to the nearest 0.1 kg. Height and weight SDS were calculated using national data of Turkey for the same age and sex (www.ceddcozum.com) (14). Adult height prediction is calculated by dividing the height by the decimal fraction by using the following measure; table for predicting adult stature (15).

LH, FSH, and E2 levels were measured with an immunochemiluminometric (ICMA) assay using an Advia Centaur immunoanalyser (Bayer Diagnostics, Tarrytown, NY, USA). Bone age (BA) was assessed according to the Tanner and Whitehouse atlas (1549).

Genetic Analysis

Genomic DNA was isolated from EDTA blood sample by Magnesia DNA isolation Kit (Anatolia Geneworks, Istanbul, Turkey). Sequencing study was done by NGS technology and it was performed using the MiSeq next generation sequencing platform (Illumina Inc., San Diego, CA, USA). All coding exons of MKRN3 and their flanking regions were amplified using polymerase chain reaction (PCR) primers, designed with PRIMER – Primer Designer v.2.0 software (Scientific and Educational Software program). Amplicon libraries were prepared with the NexteraXT kit (Illumina Inc.). Sequences were aligned to the hg19 genome with MiSeq Reporter software (Illumina Inc.). Detection of variants was performed with IGV 2.3 (Board Institute) software. In silico analysis, database search and literature evaluations were done by Varsome, Polyphen2, HGMD-Public, PubMed, Google search, Clinvar, EXAC and 1,000 Genome studies.

Results

The study included 19 patients with CPP from 10 families. In the familial CPP group, there were 17 girls and 2 boys (one boy with a paternal history of precocious puberty) from 10 families. Clinical, anthropometric, and biochemical data of the included patients and their parents are displayed in Table 1 and 2. Among the 17 girls with familial idiopathic CPP, the mean age at the onset of secondary sex characteristics was 6.5 ± 1.5 years, and the mean age at treatment onset was 7.2 ± 1.4 years. In this group, the mean bone age was 8.7 ± 2.0 years, and the BA:CA ratio was 1.2 ± 0.1. The 17-OHP level was normal in all cases with pubarche. Therefore, none of the patients needed an ACTH test. Cranial MRI was normal in all cases.

Among the whole group, a novel heterozygous mutation, MKRN3:NM_005664.3:c.630_650delinsGCTGGGC (p.P211Lfs*16), was detected in only one boy with a paternal history of precocious puberty. Flow charts for CPP with children are shown on Figure 1. Mutation image of MKRN3 gene was shown on Figure 2. MKRN3 gene analysis was performed only in his father. We did not have the opportunity to study the genotype in his remaining family members. The patient with MKRN3 mutation presented with facial hair growth at 11 years and 7 months of age. Facial hair growth had appeared 1.5 years earlier. The family history assessment revealed that facial hair growth had appeared at the same age in his father. The patient had a brother who had no such symptoms, and who was found to be pre-pubertal in the examination performed at 10 years of age. The patient’s physical examination yielded the following findings: height, 156.5 cm (1.27 SD); body weight, 44.6 kg (0.3 SD); testicular volumes, 15 ml in both testicles; stage 5 pubarche; and axillary hair growth. The heights of mother and father were 167 (0.6 SDS) and 159 cm (-2.4 SDS), respectively. The patient’s target height was estimated as 169.5 cm (-1 SDS) and predicted height 168.8 cm (-1.1 SDS), respectively. Routine biochemistry tests and complete blood count were normal; the hormone test results were as follows: LH, 5.4 mIU/mL; FSH, 13.7 mIU/mL; total testosterone: 393.3 ng/dL; 17OHP: 0.6 ng/mL; and dehydroepiandosterone sulphate, 60.4 mcg/dL.
Bone age was compatible with norms for 14 years of age. The same mutation was also detected in his father (Figure 3).

This mutation is a frame shift variant and causing production of a truncated protein with 226 amino acids while normal protein has 507 aa. This variant most probably causes loss of function. Mutation taster predicts this variant as a disease-causing mutation.

Discussion

MKRN3, which encodes the makorin ring finger protein 3, is an intronless gene located on chromosome 15q11.2 in the Prader–Willi syndrome critical region (16). The imprinted MKRN3 gene is expressed only in the paternal allele, and it affects both sexes equally, in contrast to female preponderance in iCPP cases (16). The presence of a history of paternal precocious puberty, shorter final height, and detection of MRKN3 gene mutation confirm paternal inheritance. The MRKN3 protein, a product of this gene, includes two copies of a C3H motif in the N-terminal, a novel Cys–His configuration, a C3HC4 RING zinc finger, and a final C3H motif (6). A novel frameshift mutation (between C3H motifs in the N-terminal) in the imprinted MKRN3 gene was identified in one male case and his affected father. In silico analysis revealed that this genetic analysis revealed a pathogenic variant not previously reported.

In their study, Abreu et al. (5) revealed a loss-of-function mutation in the MKRN3 gene associated with familial iCPP. This work led to an investigation of the mechanism underlying familial iCPP, which has been important for a better understanding of the timing of puberty in humans. Since 2013, MKRN3 mutation has been the most frequently identified genetic cause of iCPP. In another study, the authors screened 40 individuals with familial iCPP from 15 families for MKRN3 mutations, and reported identifying MRKN3 mutation in 15 individuals from 5 families (37.5%) (5). In another study, MKRN3 mutation was detected in 13 of 20 cases (66%) with familial iCPP, and in only one of 18 cases with sporadic iCPP (7). In a study of 20 boys with iCPP from 17 families, Bessa et al. (17) detected MRKN3 mutation in 8 boys from 5 families. The authors emphasised the importance of investigating boys with MKRN3 mutation and a history of paternal precocious puberty. In a recent study reported from Turkey, Şimşek et al. (18) reported that two heterozygous frameshift mutations were identified in the MKRN3 gene in two probands with familial iCPP and in 7 patients with iCPP, as well as 11 unaffected family members. We investigated 19 individuals from 10 families with iCPP and found one novel frameshift (5.3%) mutation. Şimşek et al. (18) reported that, considering the imprinted pattern of inheritance, the phenotype skipped one generation in one family because the proband’s father and paternal uncle had inherited the mutated allele from their mothers. They also showed that in another family, because the proband’s father and affected paternal cousin’s father had inherited the mutated allele from the paternal grandfather, the phenotype was present in the second and third generations. A paternal aunt in the latter family also had iCPP, but her children were asymptomatic carriers of the same mutation. As those authors suggested, and as the history of our patient with MKRN3 mutation highlights, an accurate family history is extremely important, as it can reveal the paternal inheritance of familial iCPP due to a mutation in MKRN3. Physicians should consider this type of inheritance in patients with iCPP to promote MKRN3 genetic analysis, thereby providing an additional tool for the diagnosis and treatment of children with iCPP.

In boys, there may be delay in recognising indicators of precocious puberty compared with those (thelarche, menarche) in girls (5, 8, 9, 19, 20). The findings of precocious puberty were not recognised by the family in our MKRN3 mutation case, and he presented at the hospital in a late pubertal stage when he began to shave his facial hair. In the literature, the mean age at onset of puberty was reported as 8.2 years in 13 boys with MKRN3 mutation (5, 17, 19). Given that age at onset of puberty is approximately 6 years of age in girls with MKRN3 mutation (5, 16, 20), the acceleration is clearly less remarkable in males. In addition, the time from the onset of pubertal symptoms to diagnosis is longer in boys (5, 21). Furthermore, it has been reported that puberty can be successfully suppressed by GnRH agonist treatment in cases with MKRN3 mutation and that menarche and other pubertal indicators show a normal course following treatment (5, 7, 22).
Study limitations, the number of patients was small, and a wide range of criteria were used to diagnose CPP. In conclusion, MKRN3 mutation was detected in only one (5.3%) of 19 individuals from 10 families with familial CPP. No MKRN3 mutation was detected in siblings with CPP. Given the fact that the MKRN3 mutation was detected in only one patient with a paternal history of precocious puberty in our study, the importance of an accurate family history, which can reveal the paternal inheritance of familial iCPP due to a mutation in MKRN3, must be emphasised. Therefore, physicians should consider this type of inheritance in patients with iCPP to promote MKRN3 genetic analysis, thus providing an additional tool for the diagnosis and treatment of children with iCPP.

Ethics

The study was approved by the Ethics Committee of the Zekai Tahir Burak Maternity Teaching Hospital, Ankara, Turkey (46/2015), and was conducted according to the principles of the Declaration of Helsinki.

Informed Consent: Written consent was obtained from all subjects and their parents before the study.

Financial Disclosure: The authors declare that this study received no financial support.

References


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The father had a history of precocious puberty. &: MKRN3-mutation-positive patient;
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Table 2. Anthropometric characteristics of patients’ parents, target and predicted height of patients

*The father had a history of precocious puberty. &: MKRN3-mutation-positive patient
Children with CPP included in the study

- Sporadic cases excluded
- Children included with at least one first or second degree relative with documented iCPP
  - 18 children from 9 families
  - 1 child from a family with history of precocious puberty

No detected mutation in 18 children from 9 families
Mutation detected the child from a family with history of precocious puberty

Figure 1. Flow chart of the study recruitment.
Figure 2. Mutation image of *MKRN3* gene of the patient with IGV2.3 software (NM_005664.3:c.630_650delins GCTGGGC (p.P211Lfs*16)) and Varsome software image
Figure 3. Pedigree of family with MKRN3 mutation. Squares indicate male family members, circles indicate female family members, black symbols indicate clinically affected family members. The boy presented with acne, beard.

Father’s height (cm)/SD: 159/-2.4
Mother’s height (cm)/SD: 167/0.6

At admission
Chronological age: 11.6 years
Bone age: 14 years
Height: 156.5 cm
Height SD: 1.27