Central Precocious Puberty in a Girl and Early Puberty in Her Brother Caused by a Novel Mutation in the MKRN3 Gene

Nikolaos Settas, Catherine Dacou-Voutetakis, Maria Karantza, Christina Kanaka-Gantenbein, George P. Chrousos, and Antonis Voutetakis

Division of Endocrinology, Metabolism, and Diabetes, First Department of Pediatrics, Medical School, National and Kapodistrian University of Athens, “Aghia Sophia” Children’s Hospital, GR-11527 Athens, Greece

Context: Central precocious puberty (CPP), defined as the development of secondary sex characteristics prior to age 8 years in girls and 9 years in boys, results from the premature activation of the hypothalamic-pituitary-gonadal axis. Mutations in the imprinted gene MKRN3 have been recently implicated in familial cases of CPP.

Objective: The objective of the study was to uncover the genetic cause of CPP in a family with two affected siblings.

Design and participants: The entire coding region of the paternally expressed MKRN3 gene was sequenced in two siblings, a girl with CPP and her brother with early puberty, their parents, and their grandparents.

Results: A novel heterozygous missense variant in the MKRN3 gene (p.C340G) was detected in the two affected siblings, their unaffected father, and the paternal grandmother. As expected, the mutated allele followed an imprinted mode of inheritance within the affected family. In silico analysis predicts the mutation as possibly damaging in all five software packages used. Furthermore, structural alignment of the ab initio native and mutant MKRN3 models predicts that the p.C340G mutation leads to significant structural perturbations in the 3-dimensional structure of the C3HC4 really interesting new gene motif of the protein, further emphasizing the functional implications of the novel MKRN3 alteration.

Conclusions: We report a novel MKRN3 mutation (p.C340G) in a girl with CPP and her brother with early puberty. MKRN3 alterations should be suspected in all cases with familial CPP or early puberty, especially if male patients are also involved or the precocious puberty trend does not follow the usually observed mother-to-daughter inheritance. (J Clin Endocrinol Metab 99: E647–E651, 2014)
tiation of puberty: the product of the makorin ring finger protein 3 (MKRN3) gene. In the present study, we report two siblings with CPP associated with a novel mutation of the MKRN3 gene.

Patients and Methods

Two siblings of Greek origin, a girl and a boy, were examined at age 7.1 years [bone age 10.5 y, height 128 cm (SD score 1.3, target height 160 ± 4.5 cm)] and 9.2 years [bone age 10 y, height 130.5 cm (SD score 1, target height 173 ± 4.5 cm)], respectively. At presentation, the girl showed breast development Tanner stage 3 and pubic hair Tanner stage 1 (breast development first noticed at age 6 y), and the boy had testes size 4 mL and pubic hair Tanner stage 1. Neither sibling was obese: body mass index in the girl was 18.9 kg/m² (75th percentile) and in the boy 17.5 kg/m² (25th to 50th percentile). Hormonal data indicated the diagnosis of CPP (Table 1) (5). Expected prepubertal levels for basal and peak levels of LH both in boys and girls are less than 0.15 and 5 IU/L, respectively. The values of thyroid hormones and adrenal steroids were within normal limits for both siblings. Brain magnetic resonance imaging did not disclose any abnormalities in either child.

Due to the familial nature of the disorder, a genetic cause for the CPP was suspected (6). We focused our study on the MKRN3 gene including the extended family. The study was approved by the Ethics Committee of the “Aghia Sophia” Children’s Hospital. For the genetic analyses, assent was obtained from the children, and informed consent was obtained from the parents of the two siblings and the adult family members.

DNA sequencing and mutational analysis of genomic DNA

Genomic DNA was isolated using the Maxwell 16 Instrument (Promega). Specific amplification of the intronless MKRN3 gene was performed in two overlapping fragments by PCR using the following pairs of primers: GAGATGCAACTTCCCCCAG, TCTCCTCCAGGAACACCTCC and TTAGTGTGTC-CAGGGCAGC, CAGAAGCACTGCTCAACAGC. The PCR products were purified (ExoSAP-IT reagent; Affimetrix-USB Products) and sequenced bidirectionally (ABI 3500; Applied Biosystems), using the same primers as for the PCR. Reference sequence was obtained from Ensembl (ENST00000314520).

Nonsynonymous single-nucleotide polymorphism (SNP) in silico analysis

For the prediction of the pathogenic nature of the nonsynonymous SNP substitution found and its evolutionary conservation, an in silico analysis was performed using the following software packages: Mutation taster (http://www.mutationtaster.org/); SNPs&GO (http://snps.biofold.org/snps-and-go/snps-and-go.html); PolyPhen-2 (Polymorphism Phenotyping version 2; http://genetics.bwh.harvard.edu/pph2/); Sorting Intolerant From Tolerant (SIFT Human Protein; http://sift.jcvi.org/www/SIFT_enst_submit.html); and CONsensus DELetious score of missense SNVs (Condel) (http://bg.upf.edu/condel/home).

Ab initio modeling

I-Tasser was used for the ab initio modeling of the native and the mutated MKRN3 deduced amino acid sequences of the novel p.C340G and the previously reported p.R365S (http://zhanglab.ccmb.med.umich.edu/I-TASSER) (4, 7–9). Predicted models were evaluated using http://modbase.compbio.ucsf.edu/modeval/ for discrete optimized protein energy and root mean square distance (RMSD) score (used to measure average distance between the backbones of the superimposed proteins), whereas the TM score (used to assess topological similarity) was calculated using TM-Align (10–12). The chosen 3-dimensional structures of the native and the mutated structures were further analyzed using PyMOL molecular graphics system (DeLano Scientific; http://pymol.sourceforge.net/).

Results

Pertinent clinical and hormonal data are depicted in Table 1.
DNA sequencing disclosed a novel heterozygous missense variant in the intronless MKRN3 gene (c.1018T>G, TGT>GGT, p.C340G) in the two affected siblings, their father, and the paternal grandmother. All other family members who participated in our study (mother and both maternal grandparents) carried nonmutated alleles (Figure 1, A and B).

**Nonsynonymous SNP in silico analysis**

Software packages used for the in silico analysis predicted the pathogenicity of the p.C340G alteration of MKRN3 as follows (prediction in parentheses): PolyPhen-2 (damaging), Mutation Taster (disease causing), SIFT (damaging), SNPs&GO (disease), and Condel (deleterious).

**Ab initio Modeling**

For the novel p.C340G, the TM score of the alignment was 0.86795 and the RMSD value was 2.81, indicating potential pathogenicity for the identified missense mutation. RMSD values greater than 0.15 are considered as an indicator for significant structural perturbations, which could have functional implications for the protein (13). The 3-dimensional effect of the p.C340G mutation (located in the C3HC4 really interesting new gene (RING) motif of the MKRN3 protein) was defined using ab initio modeling. As shown in Figure 1, C and D, the alignment of the ab initio native and mutant models reveals that the mutation structurally disrupts the tertiary protein structure. For the previously described p.R365S, the TM score was 0.87352 and the RMSD value was 3.13 (comparison with native and p.C340G in Figure 1, E–G) (4).

**Discussion**

Idiopathic central precocious puberty is a rather common endocrine problem, especially in girls, with a percentage as high as 27.5% being familial (6). Nevertheless, in extremely few cases has the underlying molecular defect been disclosed. Specifically, CPP has been linked to mutations in three genes: KISS1, KISS1R, and MKRN3 (1, 3, 4). The latter was recently reported by Abreu et al (4) in an elegant whole-exome sequencing study that uncovered three frameshift mutations and one missense mutation (p.R365S) that was expected to be “probably damaging” on the basis of PolyPhen2 prediction software. Our study in two siblings (a girl with CPP and a boy with early puberty) revealed a novel missense MKRN3 mutation in both patients, their father, and the paternal grandmother (Figure 1, A and B). The mutation (c.1018T>G, TGT>GGT, p.C340G) resides in the C3HC4 RING motif of MKRN3, which is responsible for ubiquitin ligase ac-

![Figure 1](https://academic.oup.com/jcem/article-abstract/99/4/E647/2537732/649)
activity. The p.C340G variant described herein is predicted to disrupt the protein function by all five software packages used (see Patients and Methods and Results for details). Moreover, the structural alignment of the ab initio native and mutant MKRN3 models predicts that the C340G mutation leads to significant structural perturbations in the 3-dimensional structure of the C3HC4 RING motif, further supporting the functional implications of the novel MKRN3 mutation (Figure 1, C–D, and comparison with previously described p.R365S in Figure 1, E–G) (13). Nevertheless, specific functional relevance of the p.C340G mutation depends on further in vitro studies.

It has been demonstrated that there is a striking reduction in MKRN3 levels immediately before puberty in the arcuate nucleus of mice (4). Hence, it is possible that the mutation herein described leads to MKRN3 deficiency, mimicking, albeit in an untimely manner, the waning inhibitory milieu that normally leads to GnRH pulsatile secretion and therefore premature initiation of puberty.

MKRN3 is located on chromosome 15q11.2, in the Prader-Willi syndrome (PWS) critical region, and is unmethylated on the paternal but methylated on the maternal allele (14). Due to maternal imprinting, only the paternal allele is expressed, and therefore, CPP can be expected only if the mutated (or deleted) MKRN3 allele comes from one’s father, through maternal uniparental disomy, or from chromosomal translocations (15, 16). In accordance with the expected mode of inheritance, our two heterozygous patients with CPP received the mutated gene from their father. Nevertheless, their father did not enter puberty prematurely. In fact, he recalls as “entering puberty late with respect to his classmates.” Our study revealed that the father inherited the mutated MKRN3 allele from his mother and was therefore expected to be an asymptomatic carrier (Figure 1B).

Since MKRN3 mutations cause CPP, one would expect that the deletion of the entire gene, as in the case of PWS (del15q11–13), would exert the same effect. However, PWS patients are usually characterized by incomplete, delayed, or disturbed pubertal development, which has been attributed to hypothalamic dysfunction. Therefore, PWS is an inadequate model for MKRN3 deletions. Nevertheless, it must be underlined that there have been rare reports of CPP in PWS patients that can be attributed to the variability in the deletion spectrum observed within PWS patients (17–18). Interestingly, in a patient with a paternal deletion of the MKRN3, MAGEL2, and NDN genes, CPP was documented (19).

In general, studies of familial cases of idiopathic CPP aiming at investigating the mode of inheritance have suggested an autosomal dominant transmission with incomplete, gender-dependent penetrance (6). Indeed, proven CPP-causing mutations in the KISS1 and KISS1R genes are inherited as a dominant trait. Moreover, CPP caused by MKRN3 mutations follows an imprinted mode of inheritance and seems to have an equal gender distribution (4). Nevertheless, although the number of reported MKRN3 cases in the study by Abreu et al (4) is small, it must be noted that the MKRN3 alterations seem to affect girls more severely than boys: the median age at the onset of puberty was −2.25 years in girls and only −0.9 years in boys with respect to the lower age limit for normal onset of puberty (ie, 8 and 9 y, respectively). This seems to hold true in our two patients: the girl entered puberty at a relatively younger age than her brother with respect to what is normally expected for each gender. Her breast development was first noticed by her parents as early as 6 years, whereas puberty initiation in the boy could be designated as early (and not precocious) at evaluation (age 9.2 y). Therefore, gender dimorphism, which characterizes the physiological pubertal process, is also manifested in the MKRN3 defect cases (20).

Conclusions

The study of unique natural prototypes and deviations has helped disentangle and understand complex physiological processes. The study of patients with idiopathic familial central precocious puberty recently uncovered the role of MKRN3 in puberty initiation. Herein we report a novel MKRN3 mutation (p.C340G) in two siblings, a girl with CPP and a boy with early puberty, further expanding the mutational spectrum and confirming the imprinted mode of inheritance. MKRN3 mutations affect both genders equally but seem to have a greater effect on girls with respect to the timing of puberty. MKRN3 alterations should be suspected in all familial CPP and early puberty cases, especially if male patients are also involved or the precocious puberty trend does not follow the usually observed mother-to-daughter inheritance.

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Address all correspondence and requests for reprints to: Dr Antonis Voutetakis, Division of Endocrinology, Metabolism, and Diabetes, First Department of Pediatrics, Medical School, National and Kapodistrian University of Athens, “Aghia Sophia” Children’s Hospital, Thivon and Papadiamantopoulou Street, Goudi, GR-11527, Athens, Greece. E-mail: voutetakis@yahoo.com.

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References