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High Frequency of *MKRN3* Mutations in Male Central Precocious Puberty Previously Classified as Idiopathic

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Abstract

Background/Aims—Recently, loss-of-function mutations in the *MKRN3* gene have been implicated in the etiology of familial central precocious puberty (CPP) in both sexes. We aimed to analyze the frequency of *MKRN3* mutations in boys with CPP and to compare the clinical and hormonal features of boys with and without *MKRN3* mutations.

Methods—This was a retrospective review of clinical, hormonal and genetic features of 20 male patients with idiopathic CPP evaluated at an academic medical center. The entire coding regions of *MKRN3*, *KISS1* and *KISS1R* genes were sequenced.

Results—We studied 20 boys from 17 families with CPP. All of them had normal brain magnetic resonance imaging. Eight boys from 5 families harbored four distinct heterozygous MKRN3 mutations predicted to be deleterious for protein function, p.Ala162Glyfs * 14, p.Arg213Glyfs * 73, p.Arg- 328Cys and p.Arg365Ser. One boy carried a previously described KISS1-activating mutation (p.Pro74Ser). The frequency of *MKRN3* mutations among these boys with idiopathic CPP was significantly higher than previously reported female data (40 vs. 6.4%, respectively, $p < 0.001$). Boys with *MKRN3* mutations had typical clinical and hormonal features of CPP. Notably, they had later pubertal onset than boys without MKRN3 abnormalities (median age 8.2 vs. 7.0 years, respectively, $p = 0.033$).

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Disclosure Statement

The authors have nothing to disclose.

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Conclusion—We demonstrated a high frequency of *MKRN3* mutations in boys with CPP, previously classified as idiopathic, suggesting the importance of genetic analysis in this group. The boys with CPP due to *MKRN3* mutations had classical features of CPP, but with puberty initiation at a borderline age.

Keywords

MKRN3 gene; Male precocious puberty; Gonadotropin-releasing hormone; Genetics

Introduction

Normal human puberty is triggered by the pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus to stimulate the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary, which leads to the consequent activation of gonadal function [1]. During the childhood years, neurons secreting GnRH are subjected to persistent transsynaptic inhibition by mechanisms that are not completely understood [2]. The initiation of puberty requires loss of inhibitory inputs and gain in excitatory inputs to the complex GnRH neuronal network [2, 3]. The beginning of puberty in boys is marked by testicular enlargement, assessed as testicular volume >4 ml or testicular length >2.5 cm (genital Tanner stage 2) [1]. A mean age at male pubertal onset of 11.6 years has been described in past decades, but more recent studies have suggested that boys are entering puberty earlier [4–6].

Sexual precocity in boys is defined by the development of secondary sexual characteristics before the age of 9 years [7]. The most common mechanism of progressive precocious puberty is early activation of the hypothalamic-pituitary-gonadal axis (central precocious puberty, CPP) [1]. Diagnostic confirmation of CPP relies on the demonstration of pubertal basal and/or GnRH-stimulated LH levels [1, 8]. CPP may result from a central nervous system (CNS) lesion, such as hypothalamic hamartoma, astrocytoma, arachnoid cyst or hydrocephalus [1, 9]. When no cause is identified, it is called idiopathic CPP [1]. Interestingly, idiopathic CPP is almost 10 times less frequent in boys than in girls [10, 11]. Among boys with CPP, a higher prevalence of CNS lesions has been demonstrated (40–90%), indicating the need for brain magnetic resonance imaging (MRI) in all boys with CPP [1, 9, 11, 12]. However, a recent study suggested that the number of cases of male idiopathic CPP is increasing over time [13].

The factors that regulate the onset of puberty remain a mystery. Certainly, genetic, nutritional, environmental, socioeconomic and epigenetic factors are important regulators of the initiation of puberty [2, 14–16]. The role of genetic factors in pubertal timing is illustrated by the occurrence of familial CPP, which is defined by the presence of more than one affected member in a family [16]. Currently, loss-of-function mutations in the makorin ring finger protein 3 *(MKRN3)* gene are the most frequent known genetic cause of familial CPP, affecting both sexes [17–19]. Other than mutations in *MKRN3*, only one other genetic defect in association with male idiopathic CPP has been reported, in a boy with a gain-of-function mutation in *KISS1* [20].

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Limited research has been conducted in boys with CPP in the absence of CNS abnormalities. To date, little clinical and hormonal data from males with CPP or early puberty caused by *MKRN3* mutations have been reported [17, 21–23]. The aims of the present study were to describe the clinical, hormonal and genetic features of boys with CPP due to *MKRN3* mutations, to compare their phenotypes with that of boys with idiopathic CPP, and to assess the frequency of *MKRN3* mutations in boys with idiopathic CPP.

Patients and Methods

A total of 254 consecutive patients (234 girls and 20 boys) with CPP were referred for clinical and/or genetic evaluation to the Endocrinology Unit at São Paulo University from 1998 to 2015. In all of them, CNS abnormalities were excluded by MRI. Boys with idiopathic CPP had different origins (8 Brazilians, 3 Greeks, 3 Turkish, 3 Argentineans, 2 Americans and 1 Belgian). Nine of them (9/20, 45%) had first-degree relatives with a history of premature sexual development. Patients 10 and 11 were brothers from a Brazilian family, and patients 15, 16 and 17 were also brothers from an Argentinean family (table 1). Idiopathic CPP in boys was diagnosed based on the presence of genital Tanner stage 2 (testicular volume >4 ml or length >2.5 cm) before the age of 9 years, pubertal basal and/or GnRH-stimulated LH levels, and normal brain MRI [8, 24–26]. Six male cases (patients 9 to 14, table 1) have been previously reported [17, 20]. This study was approved by the local ethics committee, and written informed consent was obtained from the parents of the children.

Hormone Assays

Serum LH, FSH and testosterone concentrations were measured by ultrasensitive assays (immunofluorometric assay – IFMA, immunochemiluminometric assay – ICMA or electrochemiluminometric assay – ECLIA) with good correlation among them. The interassay and intraassay coefficients of variation were 5% or less for all assays. For IFMA, serum LH and FSH concentrations were determined by commercial, solid phase, two-site fluoroimmunometric assays (FIA; AutoDELFIA hLH Spec and AutoDELFIA hFSH; Wallac Oy, Turku, Finland), and serum testosterone concentrations were measured by commercial solid-phase FIAs (AutoDELFIA Testosterone; Wallac Oy). Functional sensitivity was set at 0.6 IU/l for LH, 1.0 IU/l for FSH and 14 ng/dl for testosterone. For ICMA, LH and FSH were measured using an Immulite 1000 automated system and commercial kits (Diagnostic Products Corp., Medlab, Los Angeles, Calif., USA). Sensitivity was set at 0.1 IU/l for both LH and FSH and 19 ng/dl for testosterone [26]. Finally, for ECLIA, commercial kits for LH (Ref. 11732234) and FSH (Ref. 11775863) (Roche Diagnostics GmbH, Mannheim, Germany) were used and assessed in the modular Cobas e601 analyzer. The functional sensitivities of both LH and FSH assays were 0.1 U/l, according to the second WHO IS 80/552 for LH and the second IRP 78/549 for FSH. For total testosterone, functional sensitivity was set at 12 ng/dl. For the acute GnRH stimulation test, serum LH was measured at –15, 0, 15, 30, 45 and 60 min after i.v. administration of 100 μg of GnRH. Basal LH levels >0.6 U/l (IFMA) or 0.2 U/l (ICMA and ECLIA) were considered as pubertal levels, and a GnRH-stimulated LH peak >9.6 U/l (IFMA) or 5.0 U/l (ICMA and ECLIA) was considered as a pubertal response [8, 24–26]. In only 1 case (patient 9, table 1), was the hormonal

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profile measured by radioimmunoassay (RIA), and a GnRH-stimulated LH peak >25 U/l was considered as a pubertal response [20]. Basal testosterone levels higher than 19 ng/dl (IFMA), 14 ng/dl (ICMA and ECLIA) or 30 ng/dl (RIA) were considered as pubertal values.

Genetic Analyses

Genomic DNA was extracted from peripheral blood leukocytes from all patients using standard procedures. The single exon of *MKRN3* (GenBank accession No. NC_000015.9) was amplified by PCR followed by automated sequencing of the products in all 20 boys [27]. The boys without mutations in the coding sequence of *MKRN3* were screened for abnormalities in the promoter region of *MKRN3*. A 1,000-bp region (−750 to +350 in relation to the transcriptional start site) of the *MKRN3* gene, including putative transcription factor motifs (PEA3, SRE, SRF, C/EBP, AP2, testis-R), was amplified by PCR followed by automated sequencing.

Additionally, all patients were screened for mutations in *KISS1* and *KISS1R* genes [20, 28]. The promoter region and the three exons of the *KISS1* gene (GenBank accession No. NC_000001.10) were amplified by PCR followed by automated sequencing of the products [20]. The entire coding region and the exon-intron boundaries of *KISS1R* (GenBank accession number NC_000019.9) were also amplified by PCR and sequenced on an automated sequencer [28].

Two databases (1000 Genomes and NHLBI EVS) were used to exclude common variants (minor allele frequency >1%) [29, 30]. Computational algorithms (PolyPhen-2, SIFT, Panther and MutationTaster) were used to predict the pathogenicity of the variants [31, 32].

Statistical Analyses

Data are presented as median and range. The nonparametric Mann-Whitney U test was used to compare clinical and hormonal data between patients with and without *MKRN3* mutations.

A previously reported female cohort assembled by São Paulo University composed of 234 girls from 221 families with idiopathic CPP was used for statistical comparison between boys and girls [17, 27]. *MKRN3* mutations were identified in 15 girls (11 of them were unrelated) in this large cohort. The χ^2 test was applied to analyze the association between categorical variables, considering both the entire group and only index cases. Statistical analyses were performed using the software Sigmastat for Windows 3.5, and statistical significance was set at $p < 0.05$.

Results

The clinical and hormonal features of the 20 boys with idiopathic CPP are described in table 1. The median age at pubertal onset in these boys was 7.5 years (ranging from 0.9 to 8.6 years). Testicular enlargement and pubarche were reported as the first signs of puberty in 45% of the boys. At the time of first evaluation (median age 8.7 years), genital Tanner stage 3 was observed in 50% of the boys. The median Δbone age – chronological age was 1.7 years (ranging from 0 to 7.9 years). The median body mass index (BMI) Z-score was 1.2

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(ranging from 0.1 to 4.0). All boys had pubertal basal LH levels (median 1.4 IU/l, ranging from 0.7 to 6.7 IU/l). The median GnRH-stimulated LH peak was 17.7 IU/l (ranging from 6.7 to 33.4 IU/l). The median testosterone level was 104 ng/dl (ranging from 19 to 548 ng/dl). One patient (patient 17, table 1) had prepubertal testosterone level despite pubertal basal LH, and he was excluded from the testosterone level analysis.

Genetic Findings

Four *MKRN3* mutations were detected in 8 boys (patients 10 to 17, table 1) from 5 families with CPP. Three mutations have been reported previously, including 2 frameshift (p.Ala162Glyfs * 14 and p.Arg213Glyfs * 73) and one missense (p.Arg365Ser) mutations [17]. Automated sequencing of *MKRN3* revealed one novel missense variant (p.Arg328Cys) in the Argentinean family, comprised of 3 brothers with CPP. All four *MKRN3* variants were not identified in population database (1000 Genomes and NHLBI EVS) and were predicted to be ‘damaging’ or ‘disease causing’ by four different in silico programs, suggesting deleterious effect. A KISS1-activating mutation (p.Pro74Ser) was previously identified in one boy with sporadic CPP (patient 9, table 1) [20]. The other 11 boys did not have any detectable rare coding variants (minor allele frequency <1%) in *MKRN3*, *KISS1* or *KISS1R*. The study of the promoter regions of *MKRN3* and *KISS1* revealed no rare variants in this group of boys.

Clinical Features of CPP Boys with MKRN3 Defects

The age at puberty initiation in the 8 boys with *MKRN3* mutations ranged from 5.9 to 8.6 years (median 8.2 years). Four of them exhibited testicular enlargement and pubarche as the first signs of puberty by medical records, whereas the remaining 4 patients initially presented with only testicular enlargement. At the time of first evaluation, their ages ranged from 8.1 to 9.8 years (median 8.7 years). The Δbone age – chronological age ranged from 0 to 2.7 years (median 1.7 years). The median BMI Z-score was 1.2 in this group, ranging from 0.1 to 2.2. Median basal and GnRH-stimulated LH levels were 1.5 IU/l (ranging from 0.7 to 4.1 IU/l) and 10.9 IU/l (ranging from 6.7 to 20 IU/l), respectively. The median testosterone level was 200 ng/dl (ranging from 67 to 548 ng/dl). Except for the significantly later pubertal onset in the boys with *MKRN3* mutations (median 8.2 years, ranging from 5.9 to 8.6 years) compared to those without *MKRN3* mutations (median 7.0 years, ranging from 0.9 to 8.0 years), there were no other differences between their clinical and hormonal features (table 2 ; fig. 1).

Frequency of MKRN3 Mutations in Boys with Idiopathic CPP

Considering the entire group, the frequency of *MKRN3* mutations among the 20 boys with idiopathic CPP was significantly higher than the female data [8 of 20 boys (40%) vs. 15 of 234 girls (6.4%), $p < 0.001$, by χ^2 test]. When only index cases were considered in this analysis, 3 boys of 17 families (17.6%) versus 11 girls of 221 families (5%) carried *MKRN3* mutations ($p = 0.05$). Of note, 2 boys (patients 12 and 14, table 1) with *MKRN3* mutations belonged to two families whose index cases were females, and they were not included in the latter analysis.

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Discussion

This study reveals the importance of genetic analysis in boys with idiopathic CPP, especially in those with a family history of premature sexual development. Since the discovery that MKRN3 deficiency causes familial CPP, 5 other boys with CPP or early puberty due to mutations in this gene have been described [21–23, 33, 34]. To date, there are 10 unrelated families that include boys presenting with CPP or early puberty caused by MKRN3 defects (table 3). *MKRN3*, an imprinted gene located on the long arm of chromosome 15 (Prader-Willi critical region), encodes makorin ring finger protein 3, which is the first factor with an inhibitory effect on GnRH secretion. The MKRN3 protein is derived only from RNA transcribed from the paternally inherited copy of the gene due to maternal imprinting. Segregation analysis of the families with CPP due to MKRN3 defects clearly demonstrated an autosomal dominant inheritance with complete penetrance [18].

Herein, *MKRN3* mutations were detected in 5 of 17 families (29.4%) with male idiopathic CPP cases. Four different heterozygous loss-of-function MKRN3 mutations were identified in 8 boys from 5 families. Two of these mutations were frameshift mutations (p.Ala162-Glyfs * 14 and p.Arg213Glyfs * 73), resulting in premature stop codons. The other two were missense variants (p. Arg328Cys and p.Arg365Ser), located in the C3HC4 RING motif of MKRN3, a putative ubiquitin ligase protein domain. Both missense mutations were predicted to be deleterious to protein function. Interestingly, Settas et al. [23] reported that two MKRN3 missense mutations (p.Cys340Gly and p.Arg365Ser), both residing in the C3HC4 RING motif, disrupt the three-dimensional structure of the protein, emphasizing the functional effects of these defects. The p.Arg328Cys mutation is also located in the same MKRN3 domain, suggesting a similar consequence to protein function. All current MKRN3 loss-of-function mutations identified in CPP patients are illustrated in figure 2.

We demonstrated that boys with CPP due to *MKRN3* mutations started pubertal development at a borderline early age (median 8.2 years) when considering the lower limit age of 9 years for normal male puberty onset. A similar chronological age at puberty onset in association with *MKRN3* mutations was reported in other recent studies [21, 23, 34]. In contrast, a median age at puberty onset of 6.0 years has been described in girls with CPP caused by *MKRN3* mutations [18, 35]. Therefore, MKRN3 deficiency has a smaller impact on puberty onset in boys, with affected boys manifesting CPP at an older age than girls. Nevertheless, it must be noted that the determination of age at pubertal onset in boys is a challenge since testicular enlargement is not as obvious as thelarche and menarche in girls [10]. The borderline early age at pubertal onset in these boys with *MKRN3* mutations can compromise the precise identification of puberty by parents and general pediatrics, and therefore leading to an underestimated incidence of CPP in this group. Commonly, male patients remember only late events of puberty, such as the age at initiation of full facial shaving and the age at voice change [16, 34, 36, 37].

The median age at puberty onset in CPP boys with *MKRN3* mutations also differs strikingly from that described in boys with other causes of premature pubertal development. Boys with CPP due to hypothalamic hamartoma classically manifested physical signs of puberty before 2 years of age [38]. Moreover, the one boy with a gain-of-function mutation in the *KISS1*

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gene had very early pubertal onset (1.0 year) [20]. In addition, testotoxicosis or familial male-limited precocious puberty, a gonadotropin-independent cause of precocious puberty that exclusively affects boys, usually occurs before 4 years of age [39, 40].

The basal and GnRH-stimulated LH levels were similar in boys with and without *MKRN3* mutations. The serum testosterone levels in boys with MKRN3 abnormalities ranged from 67 to 548 ng/dl. Notably, 1 boy who carried the p.Arg328Cys mutation (patient 17, table 1) had a prepubertal testosterone level at the first medical evaluation. This was likely because he was at a very early stage of pubertal development at the time of assessment. Treatment was initiated at genital Tanner stage 2, with testes of 4–5 ml, basal LH 0.7 IU/l and basal FSH 1.2 IU/l. At this stage, testicular enlargement is mainly due to Sertoli cell proliferation, and there is very little Leydig cell activity (FSH predominates over LH), explaining the low testosterone level in this case.

The observation that *MKRN3* mutations represented a frequent cause of male CPP led us to hypothesize that boys were more likely than girls to carry *MKRN3* mutations. Indeed, the prevalence of *MKRN3* mutations in boys with unknown cause of CPP (8 of 20 boys, 40%) was significantly higher compared to a large cohort of CPP girls (15 of 234 girls, 6.4%) ($p < 0.001$). However, when only index cases were considered, a higher prevalence of *MKRN3* mutations in male patients (17.6%) compared to female patients (5.0%) was found with a borderline difference ($p = 0.05$). These findings might indicate that male CPP without CNS abnormalities has a greater probability of a genetic etiology.

We do not yet understand why boys with idiopathic CPP may have a higher prevalence of *MKRN3* mutations than girls. Although the mechanism by which the loss of MKRN3 results in early puberty initiation is still not fully understood, the expression pattern of *Mkrn3* in the hypothalamus of mice and the ubiquitin ligase protein structure suggest that MKRN3 is inhibiting GnRH secretion during childhood [17, 19]. The similar expression pattern of *Mkrn3* in the arcuate nucleus of male and female mice suggests that Mkrn3 inhibitory tonus is present in both sexes but does not entirely rule out a sex-specific action of MKRN3. It is well known that girls undergo puberty initiation at an earlier age than boys and have a higher incidence of CPP [4, 11, 41]. Conversely, boys have a higher incidence of delayed puberty [41]. Based on these observations, we can speculate that the inhibition of GnRH during childhood is weaker in girls than in boys, and that as a result girls are more prone to the consequences of disruption of the ‘brake’ restraining puberty initiation.

MKRN3 loss-of-function mutations have been associated with normal CNS MRI in both sexes [17, 27, 33, 34]. In affected boys, the later puberty onset also makes organic, structural causes less likely. These recent observations could modify the clinical decision-making for performing a CNS MRI in this group. In particular, in boys with familial CPP, the genetic study of the *MKRN3* gene could precede the brain MRI. In these cases, MRI should be postponed (nonmutated cases) or completely avoided (mutated cases). Furthermore, this genetic study is less costly than a brain MRI, an imaging exam that frequently requires anesthesia in children [42].

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Clearly, recent human studies support that MKRN3 is a strong component of puberty regulation in both sexes [17–19, 43]. Currently, *MKRN3* mutations represent a prevalent cause of familial CPP. In conclusion, this study suggested a higher prevalence of *MKRN3* mutations in boys than in girls with idiopathic CPP. Remarkably, the affected boys had classical features of CPP with pubertal onset at a borderline age.

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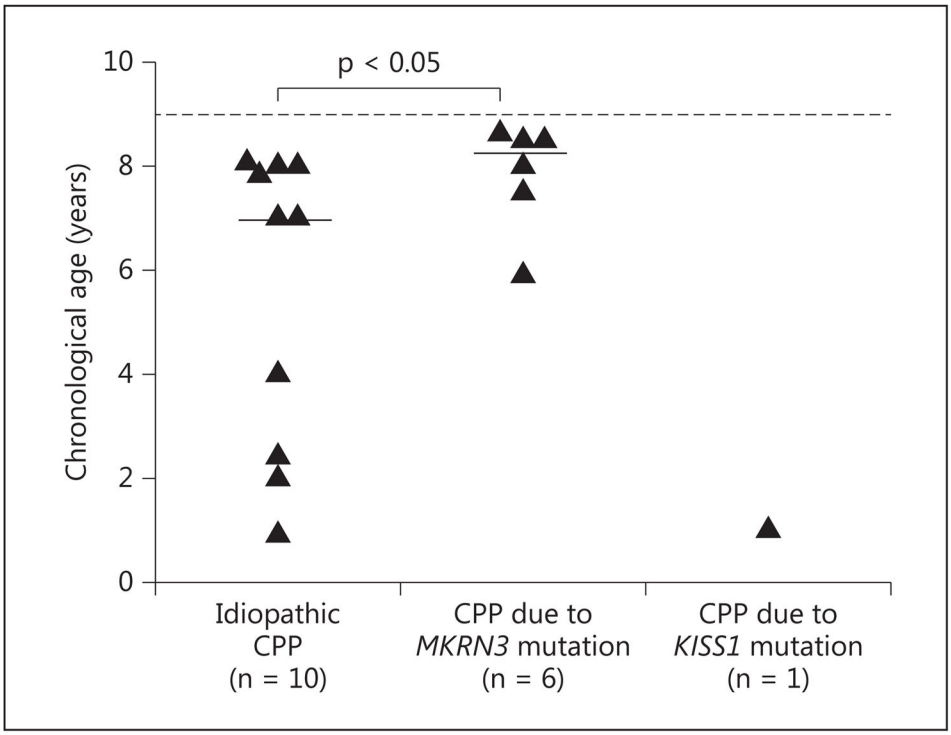

Fig. 1.

Age at puberty onset in boys with idiopathic and genetic CPP. This information was not clear in 3 of 20 cases, which were excluded from the figure. The dashed line indicates the lower age limit for normal male puberty onset (9.0 years). The one boy with a *KISS1* mutation started puberty at 1.0 year of age. The short horizontal lines indicate the median age at puberty onset (7.0 years in the boys with idiopathic CPP and 8.2 years in those with *MKRN3* mutations). The boys with *MKRN3* mutations had a significantly later pubertal onset compared to those with idiopathic CPP. p value as assessed with Mann-Whitney U test.

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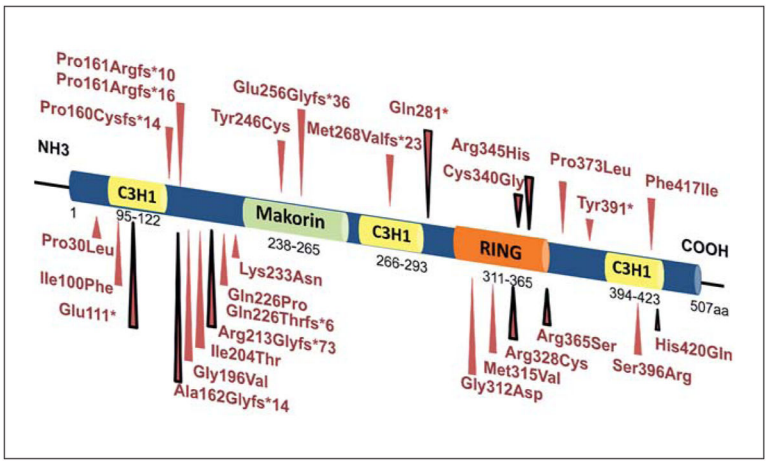

Fig. 2.

Schematic protein structure of MKRN3 and locations of loss-of-function mutations identified in patients with CPP. The three C3H zinc finger motifs are shown in yellow, the C3HC4 RING finger motif in orange, and the MKRN-specific Cys-His domain in green. The numbers correspond to the amino acid positions in the protein. Red arrows indicate the location of all described MKRN3 mutations in patients with CPP; black-red arrows indicate the mutations identified in boys with CPP.

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Table 1

Clinical, hormonal and genetic features of 20 boys from 17 families with idiopathic CPP

Family No.	Patient No.	Origin	Initial clinical manifestation (age, years)	Time of diagnosis			BA years	LH, IU/l		Basal FSH, IU/l	T, ng/dl	Hormone assay	Familial case	Genetic analysis
				age years	genital Tanner stage	BMI Z-score		basal	after GnRH					
I	1	Turkey	Penis enlargement and pubarche (2.4)	3.4	2	4.0	5	6.7	n.a.	5.9	42	ICMA	No	–
II	2	Turkey	Penis enlargement (0.9)	1.4	2	n.a.	3	1.2	23.7	5.1	297	ICMA	No	–
III	3	Turkey	Penis enlargement and pubarche (4.0)	4.2	2	2.0	5	6.0	n.a.	3.8	160	ECLIA	No	–
IV	4	Brazil	Testicular enlargement and pubarche (8.0)	10.1	3	0.2	13	0.9	8.4	2.5	68	IFMA	No	–
V	5	Brazil	Pubarche (7.0) and testicular enlargement (9.0)	9.8	2	n.a.	n.a.	0.7	18.0	1.0	75.7	IFMA	No	–
VI	6	Brazil	Testicular enlargement and pubarche (8.0)	10.4	3	0.9	13	2.5	n.a.	1.3	163	IFMA	No	–
VII	7	Brazil	Testicular enlargement (7.0) and pubarche (9.0)	8.7	2	0.3	8	1.2	33.4	2.5	19	IFMA	No	–
VIII	8	Brazil	Pubarche (2.0), testicular and penis enlargement (3.0)	5.1	4	1.0	13	1.1	n.a.	1.1	92	IFMA	No	–
IX	9	Brazil	Pubarche and penis enlargement (1.0)	1.4	2	n.a.	3	11.5	47.2	8.3	600	RIA	No	KISS1(p.Pro74Ser)
X	10	Brazil	Testicular enlargement and pubarche (5.9)	8.1	3	0.9	10	1.2	6.7	1.5	116	ICMA	Yes	MKRN3 (p.Ala162Glyfs*14)
	11		Testicular enlargement and pubarche (unknown)	9.7	3	1.4	9.7	1.6	10.9	0.8	548	ICMA		
XI	12	Belgium	Testicular enlargement and pubarche (unknown)	9.7	3	1.2	12	2.0	19.5	4.4	67	ICMA	Yes	MKRN3 (p.Arg365Ser)
XII	13	USA	Testicular enlargement and pubarche (8.5)	8.8	3	n.a.	11.5	4.1	n.a.	3.1	216	ICMA	Yes	MKRN3 (p.Ala162Glyfs*14)
XIII	14	USA	Testicular enlargement (8.0)	8.7	3	2.2	11	2.9	20.0	2.5	78	ICMA	Yes	MKRN3 (p.Arg213Glyfs*73)
XIV	15	Argentina	Testicular enlargement (7.5)	8.4	3	1.18	10	1.1	10.2	1.5	466	ICMA	Yes	MKRN3 (p.Arg328Cys)
	16		Testicular enlargement (8.5)	9.8	3	0.19	11	1.4	n.a.	2.4	200	ICMA		
	17		Testicular enlargement (8.6)	8.6	2	0.14	9	0.7	n.a.	1.2	<10	ICMA		
XV	18	Greece	Pubarche and testicular enlargement (unknown)	9.5	3	2.4	12	4.7	21.5	5.5	40.9	ECLIA	No	–
XVI	19	Greece	Pubarche and testicular enlargement (7.8)	8.8	2	1.7	10.5	1.2	14.2	1.9	27.7	ECLIA	Yes	–

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Family No.	Patient No.	Origin	Initial clinical manifestation (age, years)	Time of diagnosis			BA years	LH, IU/l		Basal FSH, IU/l	T, ng/dl	Hormone assay	Familial case	Genetic analysis
				age years	genital Tanner stage	BMI Z-score		basal	after GnRH					
XVII	20	Greece	Pubarche and testicular enlargement (8.0)	10	4	1.7	13	1.5	17.5	0.7	243	ECLIA	No	–

BA = Bone age; T = testosterone; n.a. = not available.

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Table 2

Comparison between clinical and hormonal features of boys with and without *MKRN3* mutations

	***MKRN3* mutations (n = 8)**	**Idiopathic CPP (n = 11)**	**p value**
Age at pubertal onset, years	8.2 (5.9 – 8.6)	7.0 (0.9 – 8.0)	0.033
Age at first evaluation, years	8.7 (8.1 – 9.8)	8.7 (1.4 – 10.4)	0.817
BMI, Z-score	1.2 (0.14 – 2.2)	1.7 (0.2 – 4.0)	0.314
Bone age advancement, years	1.7 (0 – 2.7)	1.7 (0 – 7.9)	0.383
Basal LH, IU/l	1.5 (0.7 – 4.1)	1.2 (0.7 – 6.7)	1.000
LH after GnRH, IU/l	10.9 (6.7 – 20)	18 (8.4 – 33.4)	0.268
Basal FSH, IU/l	1.9 (0.8 – 4.4)	2.5 (0.7 – 5.9)	0.649
Testosterone, ng/dl	200 (67 – 548)	75.7 (19 – 297)	0.103

Data are presented as median (range). The boy with a *MKRN3* mutation and testosterone level <10 ng/dl (patient 17, table 1) was excluded from the testosterone level analysis.

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Table 3

MKRN3 mutations identified in boys with CPP

Family No.	Origin	MKRN3 mutation		Location of MKRN3 mutation in the protein	Affected boys, n	Age at pubertal onset, years	Reference
		DNA	protein				
1	Brazil	475_476insC	Ala162Glyfs*14	N-terminal	2	5.9 Unknown	17
2	Belgium	1095G>T	Arg365Ser	C-terminal (C3HC4 RING motif)	1	Unknown	17
3	USA	475_476insC	Ala162Glyfs*14	N-terminal	1	8.5	17
4	USA	637delC	Arg213Glyfs*73	N-terminal	1	8.0	17
5	Greece	1018T>G	Cys340Gly	C-terminal (C3HC4 RING motif)	1	Unknown	23
6	Germany	331G>T	Glu111*	N-terminal (C3H motif)	1	~9.0	21
7	Israel	1260T>G	His420Gln	C-terminal (C3H motif)	1	Unknown	22
8	Denmark	1034G>A	Arg345His	C-terminal (C3HC4 RING motif)	1	Unknown	34
9	Korea	841C>T	Gln281*	MKRN-specific Cys-His domain	1	Unknown	33
10	Argentina	982C>T	Arg328Cys	C-terminal (C3HC4 RING motif)	3	7.5 8.5 8.6	Current study

Boys from families 1, 2, 3, 4 and 10 were included in the current study. In family 10, father's DNA was not available for segregation analysis. He reported early puberty. In the other 9 families, the affected boys inherited the mutations from their fathers, as expected for the pattern of inheritance.