New Causes of Central Precocious Puberty: The Role of Genetic Factors

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Key Words

Central precocious puberty · Genetics ·
Gonadotropin-releasing hormone · Kisspeptin · MKRN3

Abstract

A pivotal event in the onset of puberty in humans is the re-emergence of the pulsatile release of the gonadotropin-releasing hormone (GnRH) from hypothalamic neurons. Pathways governing GnRH ontogeny and physiology have been discovered by studying animal models and humans with reproductive disorders. Recent human studies implicated the activation of kisspeptin and its cognate receptor (KISS1/KISS1R) and the inactivation of MKRN3 in the premature re-activation of GnRH secretion, causing central precocious puberty (CPP). *MKRN3*, an imprinted gene located on the long arm of chromosome 15, encodes makorin ring finger protein 3, which is involved in ubiquitination and cell signaling. The MKRN3 protein is derived only from RNA transcribed from the paternally inherited copy of the gene due to maternal imprinting. Currently, *MKRN3* defects represent the most frequent known genetic cause of familial CPP. In this review, we explored the clinical, hormonal and genetic aspects of children with sporadic or familial CPP caused by mutations in the kisspeptin and MKRN3 systems, essential genetic factors for pubertal timing.

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Introduction

The onset of puberty is first detected as an increase in the amplitude and frequency of gonadotropin-releasing hormone (GnRH) pulses after a quiescent period during childhood. The reemergence of pulsatile GnRH secretion leads to increases in the secretion of the gonadotropins and of the luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by the anterior pituitary gland and to the consequent activation of the gonadal function [1].

Pubertal timing is influenced by complex interactions of genetic, nutritional, environmental and socioeconomic factors [2, 3]. Compelling evidence supporting genetic effects on pubertal timing has mainly been provided by population-based studies [4]. The role of genetic factors is also illustrated by the strong correlation between age of menarche in mothers and daughters, among members of an ethnic group, and by a greater concordance of pubertal timing between monozygotic as compared to dizygotic twins [5–7]. In addition, the pubertal pattern may be influenced by familial trends, such that families with a member with precocious or delayed puberty have a higher probability of having another affected member [8].

Central precocious puberty (CPP), also known as gonadotropin-dependent precocious puberty, is clinically defined by the development of secondary sexual characteristics before the age of 8 years in girls and 9 years in

boys [9]. It results from the premature reactivation of the hypothalamic-pituitary-gonadal axis with pubertal levels of gonadotropins and consequent gonadal stimulation [10, 11]. Affected children have premature and progressive sexual development associated with an acceleration in linear growth and advancement in bone age. No hypothalamic tumors or lesions are detected in the great majority of CPP patients, suggesting a different underlying mechanism, such as genetic, epigenetic and/or environmental factors [9, 10].

It is noteworthy that CPP has a striking predominance in girls. The estimated incidence of CPP in American girls is 1:5,000 to 1:10,000 [9]. Among Danish girls, the prevalence of CPP was 1:500 based on national registries over a 9-year period [12]. Precocious puberty can result in short stature and psychological and behavioral disorders in untreated patients [10]. Accumulating evidence suggests an association between early timing of puberty and adverse health outcomes in later life [13]. Early age at menarche has been associated with increased risks of obesity, hypertension, type 2 diabetes, ischemic heart disease, stroke, cancer and cardiovascular mortality [14–17].

In this review, we explored the recent human genetic findings described in patients with CPP that have shed light on the mechanisms involved in premature GnRH secretion.

Familial CPP

Familial CPP is usually defined by the existence of more than 1 affected member either in the proband generation or in the pedigree [8]. A proportion of children presenting with precocious or early puberty may have a positive family history. In an elegant observational study of children with early puberty, de Vries et al. [8] described 43 (42 girls and 1 boy) out of 156 children with idiopathic CPP who met the criteria for familial CPP (27.5%). Segregation analysis suggested an autosomal dominant inheritance pattern with incomplete sex-dependent penetrance. Among the children with a family history of early puberty, a parent was affected in 47.8% and a first-degree relative in 93.5% of cases. Interestingly, precocious puberty was inherited maternally in 21 families and paternally in 10 families. In only 4 families, precocious puberty was inherited both maternally and paternally. Comparison between the familial and sporadic cases revealed a significantly lower maternal age at menarche (mean: 11.47 ± 1.96 vs. 12.66 ± 1.18 years) and a more advanced puberty in the familial CPP group [8].

Rare Gain-of-Function Mutations in *KISS1* and *KISS1R*

In the last decade, the kisspeptin system was revealed as a major gatekeeper of puberty onset [18, 19]. Several recessive loss-of-function mutations in *KISS1R*, a G-protein-coupled receptor, previously known as GPR54, were described in patients with congenital isolated hypogonadotropic hypogonadism [20]. In 2008, the kisspeptin system was also implicated in the pathogenesis of CPP in humans. Teles et al. [21] identified the first heterozygous activating mutation in the *KISS1R* (p.Arg386Pro) in an adopted Brazilian girl with CPP. This patient had thelarche from birth that progressed at 7 years of age and was associated with accelerated growth velocity, advanced bone age (11 years) and borderline pubertal GnRH-stimulated LH peak levels. In vitro studies revealed that the p.Arg386Pro mutation in *KISS1R* led to prolonged activation of intracellular signaling pathways in response to kisspeptin, resulting in a significant reduction in the desensitization rate [22]. Later, Silveira et al. [23] described a rare heterozygous missense mutation in *KISS1* (p.Pro74Ser) in a boy with very early idiopathic CPP (1 year of age). He had very high basal LH and testosterone levels in the absence of brain lesions or tumors. His mother and maternal grandmother, who had normal pubertal development, also carried the p.Pro74Ser mutation in the heterozygous state, suggesting incomplete sex-dependent penetrance. The p.Pro74Ser mutation showed significantly greater capacity to stimulate signal transduction compared to the wild type after incubation with human serum in vitro, reproducing in vivo potential conditions. These findings suggested that the *KISS1* variant might be more resistant to degradation, leading to greater kisspeptin bioavailability [23]. Although these case reports expanded the genotype-phenotype correlations for the kisspeptin pathways, no other CPP cases with gain-of-function mutations in *KISS1R* or *KISS1* have been reported, suggesting that these genetic abnormalities are very rare. Other candidate genes implicated in the regulatory mechanism of GnRH secretion have also been screened (*GABRA1*, *NPY-Y1R*, *LIN28B*, *TAC3*, *TACR3*, *TTF1* and *EAP1*), but no pathogenic variants were identified until recently [24–28].

Loss-of-Function Mutations in *MKRN3*

In 2013, applying whole exome sequencing, Abreu et al. [29] were able to identify 4 loss-of-function mutations in a single gene, *MKRN3*, in 5 out of 15 families (33%)

Table 1. *MKRN3* mutations described in children with typical clinical and hormonal CPP

Family No. (origin)	Patient No.	<i>MKRN3</i> mutation		Initial clinical manifestation (age, years)	Diagnosis			LH, IU/l		Basal FSH, IU/l	E2, pg/ml	T, ng/dl
		DNA	protein		age, years	breast/genital development Tanner stage	bone age, years	basal	peak after LHRH			
1 (USA)	I	637delC	Arg213Glyfs*73	Thelarche (5.7)	6.5	2	7.7	0.8	14	3.8	25	–
	II	637delC	Arg213Glyfs*73	Gonadarche (8.0)	8.7	3	11	2.9	20	2.5	–	78
	III	637delC	Arg213Glyfs*73	Thelarche (6.5)	6.7	2	7.8	1.1	17	4.5	<15	–
2 (Brazil)	I	1171_1172insA	Tyr391*	Thelarche (6.2)	7.0	3	7.0	<0.6	36	1.1	13	–
	II	1171_1172insA	Tyr391*	Thelarche (5.7)	6.0	3	6.0	<0.6	37	1.1	11	–
3 (Brazil)	I	475_476insC	Ala162Glyfs*14	Gonadarche and pubarche (5.9)	8.1	3	10	1.2	6.7	1.5	–	116
	II	475_476insC	Ala162Glyfs*14	Gonadarche and pubarche unknown	9.7	3	9.7	1.6	11	0.8	–	548
4 (USA)	I	475_476insC	Ala162Glyfs*14	Gonadarche and pubarche (8.5)	8.8	3	11	4.1	–	3.1	–	216
	II	475_476insC	Ala162Glyfs*14	Thelarche (5.0)	6.5	2	8.3	–	7.4	13	13	–
5 (Brazil)	I	482delC	Pro161Argfs*10	Thelarche and pubarche (6.4)	7.8	3	11	1.0	12	5.9	16	–
6 (Brazil)	I	482_483insC	Pro161Argfs*16	Thelarche (5.4)	6.1	3	8.8	1.3	–	5	36	–
7 (Brazil)	I	482_483insC	Pro161Argfs*16	Thelarche (6.0)	7.9	4	11	3.4	–	10	80	–
8 (Brazil)	I	482delC	Pro161Argfs*10	Thelarche (3.0) and pubarche (6.0)	6.7	3	8.8	1.8	62	4.9	60	–
9 (Brazil)	I	675_676insA	Gln226Thrfs*6	Thelarche (4.0)	6.8	4	12	6.1	–	4.7	62	–
10 (Brazil)	I	766_767delA	Glu256Glyfs*36	Thelarche and pubarche (6.0)	6.6	3	7.8	1.6	–	4.6	20	–
11 (Brazil)	I	482_483insC	Pro161Argfs*16	Thelarche (6.0) and pubarche (6.3)	6.9	3	9.3	2.7	17	6.5	47	–
12 (Belgium)	I	1095G>T	Arg365Ser	Gonadarche and pubarche unknown	9.7	3	12	2.0	19	4.4	–	67
	II	1095G>T	Arg365Ser	Thelarche (6.2)	6.4	2	9.4 ^a	<0.1	7.3	7.0	49	–
	III	1095G>T	Arg365Ser	Thelarche and pubarche (5.4)	5.7	2	8.5	0.5	12	3.6	<15	–
13 (Brazil)	I	1249T>A	Phe417Ile	Thelarche (6.0)	7.1	3	8.8	0.3	7.5	4.4	44	–
14 (Greece)	I	1018T>G	Cys340Gly	Thelarche (6.0)	7.1	3	10	1.3	39	3.8	23	–
	II	1018T>G	Cys340Gly	Gonadarche unknown	9.2	2	10	0.7	14	1.3	–	41

Gonadarche or testicular enlargement, breast/genital development Tanner stage and bone age assessed at the time of diagnosis [29, 31, 32]. E2 = Estradiol; T = testosterone.

^a Bone age at 7.4 years.

with CPP. From a total of 40 members studied, 15 patients from both sexes (8 girls and 7 boys) with CPP carried *MKRN3* mutations. *MKRN3* is an imprinted gene located on the long arm of chromosome 15q11.2 in the Prader-Willi syndrome critical region and encodes the makorin ring finger protein 3. The 5 affected families were origi-

nated from North America, Brazil and Belgium, indicating that the *MKRN3* mutations in patients with CPP were not restricted to a specific ethnic group (table 1). Their genotypes included 3 *MKRN3* frameshift mutations predicted to encode truncated proteins and 1 missense mutation (p.Arg365Ser) predicted to disrupt protein function.

Fig. 1. Schematic illustration of MKRN3 domains and the mutations identified in patients with CPP. The protein has 5 zinc finger domains: 3 C3H motifs, 1 C3HC4 RING motif with presumed ubiquitin ligase activity and 1 MKRN-specific Cys-His domain (green square). The numbers correspond to the amino acid positions in the protein. Frameshift mutations are shown in red, the nonsense mutation in black and the 3 missense mutations in green.

All affected individuals inherited their *MKRN3* mutations from their fathers, demonstrating a perfect segregation with the mode of inheritance expected for a maternal imprinted gene.

Later, Settas et al. [30] reported a novel heterozygous *MKRN3* mutation (p.Cys340Gly) in 2 Greek siblings, 1 girl with CPP and 1 boy with early puberty, further expanding the *MKRN3* mutational spectrum. In accordance with the maternal imprinted pattern of *MKRN3*, these 2 patients with CPP also received the mutated gene from their asymptomatic father. The p.Cys340Gly mutation was located in the C3HC4 RING motif of *MKRN3* and it was predicted to disrupt the protein function in distinct *in silico* prediction programs.

More recently, Macedo et al. [31] analyzed 215 unrelated children (207 girls and 8 boys) with idiopathic CPP from 3 different university medical centers. No family history of premature sexual development was reported in the great majority of these patients (213 cases). Automatic sequencing of *MKRN3* in this sporadic CPP cohort revealed 5 novel heterozygous mutations in 8 unrelated girls, including 4 frameshift variants and 1 missense variant (p.Phe417Ile). Segregation analysis demonstrated that the mutant allele was paternally inherited in all studied families. This finding confirmed a previous suspicion that the familial nature of CPP is frequently underrecognized [31]. Indeed, most children with CPP present for medical con-

sultation accompanied by their mothers, and their paternal family history is often unknown or unavailable. Another limitation to the detection of familial CPP cases is to determine the precise onset of puberty in boys and in their fathers, since testicular enlargement is not as obvious as breast development and menarche in girls [32].

Currently, 10 different loss-of-function mutations of *MKRN3* have been described in 22 patients with CPP from 14 multiplex families, including 6 frameshift defects, 3 missense mutations and 1 nonsense mutation (table 1). Interestingly, the frameshift mutations were largely located in the amino-terminal region of the protein which is codified by a rich poly C site, suggesting that this area can be a potential hot spot (fig. 1). These recent findings indicated that *MKRN3* loss-of-function mutations are a cause of familial CPP in humans, establishing a clear and strong genetic component of this reproductive condition.

Clinical Phenotype of Patients with *MKRN3* Mutations

Patients with CPP due to *MKRN3* mutations had typical clinical and hormonal features of premature activation of the reproductive axis, including early pubertal signs, such as breast, testis and pubic hair development, accelerated linear growth, advanced bone age and elevat-

ed basal and/or GnRH-stimulated LH levels. Only 3 patients (2 girls and 1 boy) with loss-of-function mutations in *MKRN3* had mild nonspecific syndromic features, including 2 related patients with esotropia and 1 girl with high-arched palate, dental abnormalities, clinodactyly and hyperlordosis [29, 31]. Notably, no major signs of Prader-Willi syndrome were detected in any patient with CPP caused by *MKRN3* defects.

The age of pubertal onset in girls with CPP due to *MKRN3* mutations ranged from 3.0 to 6.5 years (mean: 5.9 years; median: 6.0 years) [29–31], whereas in boys the age of pubertal onset or at diagnosis ranged from 5.9 to 9.7 years (mean: 8.5; median: 8.8 years) [29, 30]. There is no explanation for the variation in the timing of precocity in patients with CPP due to *MKRN3* mutations. Notably, no correlation was identified among patients who carried truncating mutations in comparison with those with missense mutations, suggesting that this age variability is not caused by the severity of the *MKRN3* identified defects.

The mean ages of pubertal onset of patients with *MKRN3* defects are in contrast with those reported in the 2 patients with gain-of-function mutations involving the kisspeptin system, who started pubertal development in the 1st year of life [21, 23]. It must be noted that *MKRN3* alterations seem to affect girls more severely than boys, since the mean age of pubertal onset was more anticipated in girls than in boys [30]. Therefore, gender dimorphism, which characterizes the physiological pubertal process, is also manifested in the *MKRN3* affected cases.

No significant difference was observed in the median age of pubertal onset between patients with and without *MKRN3* mutations [31]. Given the median age (6 years) of pubertal onset of affected girls with *MKRN3* mutations, it was speculated that the prepubertal inhibitory tonus on GnRH secretion apparently took place normally, but it was lost prematurely in patients with *MKRN3* mutations. This clinical observation suggests that *MKRN3* may not be crucial for GnRH suppression after the mini-puberty of early infancy, but that its downregulation plays a relevant role for the reemergence of GnRH pulses in the pubertal onset [31].

Simultaneous development of breast and pubic hair, as the initial clinical manifestations, was observed in 50% of the patients with CPP due to *MKRN3* loss-of-function mutations [31]. Advanced bone age was found in all cases [29–31]. Median basal and GnRH-stimulated LH levels were 1.3 U/l (ranging from 0.1 to 6.1 U/l) and 14 U/l (ranging from 6.7 to 62.5 U/l), respectively [29–31]. Interestingly, significantly higher basal FSH levels in patients with *MKRN3* mutations (median: 4.9 U/l, ranging from 4.4 to

10 U/l) compared to those without *MKRN3* mutations (median: 3.6 U/l, ranging from 1.0 to 9.8 U/l) were demonstrated in one study [31]. No definitive explanation for this difference was provided so far. If *MKRN3* could play distinct roles in the release of the 2 pituitary gonadotropins, LH and FSH, remains unknown. The possibility of divergent neuroendocrine phenotypes was previously noted in patients with nonsyndromic hypogonadotropic hypogonadism caused by *TAC3/TACR3* mutations. Pulsatile GnRH administration increased the α -subunit pulsatile frequency and reduced the FSH/LH ratio in these hypogonadic cases [33]. This gonadotropin axis dysfunction was related to low GnRH pulsatile frequency [33].

It is well known that depot GnRH agonists are the first-line treatment in progressive CPP [10]. Adequate clinical and hormonal control was reported in 6 of 8 patients with CPP due to *MKRN3* mutations chronically treated with depot GnRH agonists [31]. Menarche occurred at an appropriate age in patients with continued follow-up. In addition, the predicted genetic final height was reached in the few reported patients with CPP due to *MKRN3* who were adequately treated with GnRH analogs [31]. The long-term follow-up of patients with *MKRN3* defects will certainly be very interesting to establish their risk of metabolic disorders and estrogen-dependent tumor development during later life.

***MKRN3* Expression and Protein Organization**

Human *MKRN3* is an intronless gene located on chromosome 15q11.2. The maternal allele of this gene is silenced and only the paternal allele is expressed (monoallelic expression). The maternal methylation is established promptly after fertilization. Interestingly, *MKRN3* can be reactivated in male gametogenesis for the next generation. Therefore, *MKRN3* is a classic example of a methylated (inactivated) gene in female gametogenesis and of an unmethylated gene in male gametogenesis, being transmitted as an active gene in the sperm [34]. Hershko et al. [35], as part of an effort to generate a mouse model for the Prader-Willi and Angelman syndromes, demonstrated that the murine *mkrn3* gene (or *Zfp127*) is also differentially methylated on the maternal allele. Jong et al. [36] showed that *Zpf127* is expressed in the brain, heart and kidney of mice.

The precise mechanism by which *MKRN3* deficiency leads to an early reactivation of pulsatile GnRH secretion remains to be elucidated. Abreu et al. [29] demonstrated higher *Mkkn3* mRNA levels in the arcuate nucleus of male and female mice at young ages, with a prominent reduc-

tion immediately before puberty, suggesting that the *Mkx3* mRNA expression pattern correlates with an inhibitory input in puberty initiation in these animals.

Proteins of the MAKORIN family have a highly distinctive zinc finger motif composition with 2 or 3 copies of a C3H motif in the N-terminal portion of the protein followed by a novel Cys-His configuration, a C3HC4 RING zinc finger and a final C3H motif [37]. C3H zinc finger motifs have been implicated in RNA binding, while the RING zinc finger motif is found in most E3 ubiquitin ligases and is responsible for the ubiquitin ligase activity [38]. The widespread species conservation of the MAKORIN protein family points to vital cellular roles, with abundant expression in the developing brain, including the arcuate nucleus, where there is a group of genes whose expression is relevant to puberty [39]. Ubiquitination can also be a signal for transduction, cell cycle regulation, differentiation and morphogenesis, and other nonproteolytic fates [29].

The dramatic reduction in *MKRN3* expression at the onset of puberty in mice in conjunction with the identification of loss-of-function mutations in humans with CPP suggested that *MKRN3* might control puberty by potentially repressing downstream targets instead of activating the GnRH neuronal network [40]. Interestingly, *MKRN3* is a member of the zinc finger family that can play a role as a transcriptional repressor of the human pubertal process. Indeed, this inhibitory mechanism appears to be a core component of the neuroendocrine circuitry that regulates the timing of puberty in primates [41].

Recently, the structural alignment of the native and mutant *MKRN3* models predicted that the *MKRN3* variant (p.Cys340Gly), identified in a Greek family with CPP, leads to significant structural perturbations in the 3-dimensional structure of the C3HC4 RING motif, supporting the functional implications of this *MKRN3* missense mutation [30].

Potential Imprinting Defects in CPP

The Prader-Willi syndrome is a human imprinting disorder resulting from the disturbance of imprinted-gene expression within human chromosome 15 [42]. This genetic condition appears to be a contiguous gene syndrome caused by the loss of at least 2 of a number of genes expressed exclusively from the paternal allele, including *MKRN3*, *MAGEL2*, *NDN*, *SNRPN* and several snoRNAs [42], but it is not yet known which specific genes in this region are associated with this syndrome. Deletion of

chromosome 15q11–q13, which encompasses *MKRN3*, contributes to the Prader-Willi syndrome. Analyses of patients with the Prader-Willi syndrome and balanced translocations have implicated the *SNURF-SNRPN* locus, telomeric to *MKRN3* [43]. Particularly, a girl with a paternal deletion in *MKRN3*, *MAGEL2* and *NDN* genes, who had few features of the Prader-Willi syndrome, had signs of puberty at the age of 7 years and 6 months with advanced bone age, leading to a diagnosis of CPP [43]. Considering that *MKRN3* loss-of-function mutations cause CPP, the deletion of these genes is likely the underlying mechanism of the early puberty in this patient.

Interestingly, genes located on the long arm of chromosome 15 have been involved in human pubertal development based on clinical and genetic studies. It is well known that patients with the Prader-Willi syndrome typically have incomplete sexual development but, paradoxically, some begin to exhibit signs of puberty much earlier than expected [44]. Few cases of children with the Prader-Willi syndrome and precocious puberty have been reported [44]. Recently, it was investigated if CPP could arise from loss of *MKRN3* expression by the paternal allele due to a de novo deletion, maternal uniparental disomy or an imprinting defect, mechanisms recognized in the pathogenesis of the Prader-Willi syndrome [31]. Using methylation-specific multiplex ligation-dependent probe amplification of chromosome 15q11, Macedo et al. [31] detected no abnormalities in the *MKRN3* copy number as well as in the methylation pattern of the 15q11 locus in 52 patients with familial and sporadic CPP and normal *MKRN3* sequence, suggesting that epigenetic defects in this chromosome region are probably rare mechanisms in this disorder.

Distinct chromosomal abnormalities have been associated with complex syndromic phenotypes, including CPP, such as 1p36 deletion [45], 1q11.23 microdeletion (Williams-Beuren syndrome) [46], 9p deletion [47], and maternal uniparental disomy of chromosomes 7 (Silver-Russell syndrome) and 14 (Temple syndrome) [34, 48]. Interestingly, chromosome 7 and 14 maternal uniparental disomies are genomic imprinting disorders like the Prader-Willi syndrome. It is unknown if the impairment of one single gene located in these affected chromosome regions could explain the premature sexual development phenotype of syndromic patients, similarly to the recent description of *MKRN3* on chromosome 15 [43]. Potentially, human genomic sequence and global methylation analyses in patients with familial or sporadic CPP could establish new genetic and epigenetic alterations in the pathogenesis of this reproductive pediatric disorder.

Conclusions and Perspectives

The study of unique natural prototypes and deviations has helped disentangle and understand complex physiological processes. The study of patients with idiopathic familial CPP recently uncovered the role of *MKRN3* in puberty initiation. Currently, *MKRN3* defects represent the most frequent known genetic causes

of familial CPP, and their analysis may provide an additional tool for the diagnosis of familial CPP cases. How *MKRN3*, an example of a neurobiological brake, interacts with other major players of puberty, such as kisspeptin, GnRH, leptin, neurokinin and a host of neurotransmitters (excitatory and inhibitory) will certainly continue to captivate scientists interested in the reproductive axis [39].

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