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Makorin RING finger protein 3 and central precocious puberty

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Abstract

Makorin RING finger protein 3 (MKRN3) is a key inhibitor of the hypothalamic–pituitary–gonadal axis. Loss-of-function mutations in *MKRN3* cause familial and sporadic central precocious puberty (CPP), while polymorphisms are associated with age at menarche. To date, 115 patients with CPP carrying *MKRN3* mutations have been described, harboring 48 different genetic variants. The prevalence of *MKRN3* mutations in genetically screened populations with CPP is estimated at 9.0%. Girls are more commonly and more seriously affected than boys. *MKRN3* is expressed in humans and rodents in the central nervous system. Circulating levels in humans and hypothalamic expression in rodents decrease during pubertal progression. Although some *MKRN3* regulators have been identified, the precise mechanism by which MKRN3 inhibits the hypothalamic–pituitary–gonadal axis remains elusive. The role of makorins in developmental physiology and organ differentiation and the role of maternal imprinting are discussed herein.

Keywords

Puberty; Makorin; Estradiol; GnRH; Kisspeptin; Development; Imprinting

Central precocious puberty

Puberty is a complex developmental process allowing organisms to acquire full sexual maturation and reproductive capacity. It is the result of a coordinated sequence of events controlled by genetic, neurochemical, metabolic, and environmental cues. Neural elements are activated within an intricate hypothalamic network, which culminate in GnRH neuron stimulation and hypothalamic–pituitary–gonadal (HPG) axis activation [1,2]. The HPG axis is active during various stages of human life: there is strong evidence for HPG axis activity during the final months of fetal development, with sustained and detectable activation for several months after birth (commonly referred to as minipuberty). Subsequently, the HPG axis remains physiologically quiescent during childhood.

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Note added in proof

While this work was under revision, Li et al. [33] described a mouse model lacking *Mkrn3*. These mice manifested an early onset of puberty, as indicated by timing of vaginal opening and first estrus in females and preputial separation in males, mirroring the central precocious phenotype seen in humans with loss-of-function *MKRN3* mutations.

Conflict of interest statement

Nothing declared.

Central precocious puberty (CPP) is the result of early reactivation of the HPG axis, revealed by the occurrence of the first signs of puberty more than 2–2.5 standard deviations earlier than the general population with similar genetic backgrounds. In Western countries, this definition roughly corresponds to the onset of pubertal changes before the age of 8 years in girls and 9 years in boys, although thresholds are likely changing in recent decades. Precocious activation of the HPG axis leads to sex steroid production and secretion by the gonads, with a remarkable impact on adult height, body proportions, and psychological health, as well as associations with adverse health outcomes in later life [3].

Table 1 summarizes the major causes of CPP and their relative frequency. Diencephalic lesions and environmental factors, historically evoked as the most common etiologies of CPP, have more recently given way to a wider landscape of etiologies, most notably the recent identification of causative genetic mutations in the imprinted *MKRN3* and delta-like noncanonical Notch ligand 1 (*DLK1*) genes in familial and sporadic forms of CPP [4,5].

Makorin RING finger protein family function and molecular properties

Makorin RING finger proteins contain a characteristic array of zinc finger motifs conserved in many eukaryotes. They typically contain several C3H zinc fingers, which suggest RNA binding properties, a Cys- and His-rich makorin motif, and a specific RING pairwise zinc finger domain [6] (Figure 1). The presence of a RING finger domain is characteristic of RING-class E3 ubiquitin protein ligases, which transfer ubiquitin from an E2 enzyme to a substrate protein, supporting the hypothesis that makorin proteins act in cell processes modulated by protein ubiquitination, such as protein degradation (by addition of polyubiquitin chains), protein–protein interactions, and other changes in protein stability (monoubiquitination or oligoubiquitination). A recent study showed that makorin RING finger protein 1 (MKRN1) participates in the ribosome-associated quality control complex, mediating the recognition of messenger RNA with prematurely polyadenylated tails to prevent the production of aberrant proteins, thereby maintaining proteome integrity [7]. Nevertheless, despite extensive predictions through the study of functional domains and some evidence from experimental studies, the exact function and the networking ecosystem of the makorins remain largely elusive.

MKRN1 is ubiquitously expressed in humans but appears to be more restricted to the gonads and central nervous system (CNS) in mice. *Mkrn1*-null mice are viable and fertile but are leaner and resistant to obesity under high-fat diet [8]. Makorin RING finger protein 2 (MKRN2) expression is prevalent in the gonads. In both human males and male rodents, *MKRN2* alterations are associated with infertility and sperm abnormalities. A detailed analysis of testicular tissues revealed that, among the different stages of spermatogenesis, spermiation is the most disrupted stage in *Mkrn2*-null mice [9,10]. Makorin RING finger protein 3 (MKRN3) has been widely implicated in pubertal onset (see the following section). Makorin RING finger protein 4 exists in the human genome, but it has been annotated as a pseudogene, and its function is currently undetermined.

In addition to mammals, makorins are present in a broad range of species, including invertebrates and plants, suggesting a highly conserved role during evolution. In *Drosophila*

melanogaster, the makorin ortholog *Mkrn1* regulates the timing of larval developmental and body size [11]. Embryonic patterning and gonadal commitment in *Drosophila* are tightly regulated by the interaction of the Mkrn1 ortholog with poly(A)-binding proteins [12]. In Caenorhabditis *elegans*, the makorin ortholog *Lep-2* regulates neuronal and sexual maturation by promoting *Lin28* degradation [13]. In rice and in peas, the makorin ortholog is expressed in a temporal and spatial manner, and its levels rise during imbibition and progressively decline after germination and after maximal somatic elongation [14].

Taken together, data regarding the different makorins suggest that a highly regulated temporal expression is a common characteristic in this class of proteins. A progressive decline in makorin expression occurs after maximal cell body elongation, organ differentiation, or neural development. All of these findings support a role for the makorin family in development.

Makorin RING finger protein 3

In humans, *MKRN3* is an intronless gene located in the Prader–Willi syndrome region (chromosomal location 15q11.2-q13). This genomic region is maternally imprinted and is expressed solely from the paternal allele. As with other makorins, *Mkrn3* transcript levels in several species are maximal during early developmental stages and progressively decrease over time [4,15]. Although ubiquitous in the CNS in rodents in early postnatal life, *Mkrn3* transcripts begin to be restricted more discretely in the hypothalamus in key areas important for the control of puberty, such as the arcuate nucleus and the anteroventral periventricular nucleus [16]. Moreover, *MKRN3* was recently shown to selectively repress the gene promoter activity of key GnRH secretagogues, such as *KISS1* and *TAC3* (Figure 2) [17].

Rat *Mkrn3* has recently been shown to be specifically inhibited by the micro-RNA miR-30 [15]. In the rat mediobasal hypothalamus, miR-30 levels increase as *Mkrn3* transcripts decline, and transfection studies showed that miR-30 directly targets a regulatory region in the *Mkrn3* 3’-untranslated region. Furthermore, central administration of a miR-30 blocker during the juvenile-to-puberty transition delayed puberty in rats and maintained hypothalamic *Mkrn3* expression [15]. The regulatory mechanism upstream of miR-30 controlling the temporal expression of this noncoding RNA molecule to drive *Mkrn3* suppression and consequently HPG onset remains to be elucidated.

In mice, *Mkrn3* is also abundantly expressed in the testis, especially in gametes in late postmeiotic spermatogenic stages, including in mature spermatozoa. As *Mkrn3* must remain active in the paternal lineage, it has been proposed that this expression could contribute to the setting of the paternal-specific gametic imprint at this locus [17].

In vitro protein–protein interaction analyses after mass spectrometry purification showed a wide range of proteins interacting with MKRN3, including those involved in RNA transport and metabolism and cell–cell adhesion, cell cycle regulators, and other zinc finger proteins [18]. Empirical computational approaches evaluating *MKRN3* codon changes within human databases and interspecies alignments confirm positive selection, especially within regions involved in ubiquitin ligase activity and in RNA binding [19]. A recent protein–protein

interaction study showed that MKRN3 may interact with targets involved in neural lineage specification, such as the neural pentraxin-1, a CNS-restricted extracellular matrix protein involved in synapse remodeling and synaptic macromolecule uptake [20].

MKRN3 mutations in patients with CPP

The first families with *MKRN3* mutations in association with CPP were described in 2013 [4]. Pedigrees belonging to five different families reported herein clearly showed a pattern of maternal imprinting. An example of a pedigree illustrating the paternal transmission of *MKRN3* and CPP is shown in Figure 3 [21]. From 2013 to 2019, a total of 115 patients carrying *MKRN3* mutations have been described in familial and sporadic cases of CPP (Table 2). A clear prevalence of affected females (approximately 6:1) is found in almost all published series.

Girls with *MKRN3* mutations had signs of pubertal onset at a median age of 6.0 years (range: 3.0–7.8), whereas boys manifest signs of pubertal onset at a significantly older age (8.5 years, range: 5.9–9.2). Girls with *MKRN3* mutations exhibited a more marked advance in first signs of pubertal development (i.e. at lower standard deviations with respect to female reference populations) than boys, indicating a sexually dimorphic effect of *MRKN3* on pubertal advance. Girls typically present with premature thelarche, whereas boys present with testicular enlargement. In addition, boys are more commonly diagnosed because of the presence of CPP in a first-degree relative, typically a sister, and the borderline age of onset of pubertal signs may in part explain the gender imbalance reported in observational studies. Girls with *MKRN3* mutations also had higher FSH levels at the time of diagnosis, and a greater difference was observed between chronological and bone age [22]. Patients with *MKRN3* mutations have not been reported to manifest clinical characteristics other than pubertal advance. Similar to CPP caused by other etiologies, *MKRN3*-related CPP responds to pharmacological inhibition of the HPG axis by GnRH analogs [23].

The age at the onset of first pubertal signs appears to vary based on the type of mutation; patients with more deleterious mutations (truncating or frameshift) develop signs of pubertal onset at a younger age than those with missense variants. At the mildest end of this spectrum are *MKRN3* polymorphisms, which were shown to predict age at menarche in a parent-of-origin–specific manner in large genome-wide association studies [24]. To date, a total of 48 mutations in the *MKRN3* gene (coding sequence or regulatory regions) have been described in association with CPP. Table 2 reports the individual *MKRN3* mutations, as well as the number of reported cases of each mutation and the relative frequency of variants in a reference population.

These include 4 nonsense, 13 frameshift, and 27 missense variants as well as 5 variants in the upstream promoter or regulatory regions. These genetic events do not appear to be scattered throughout the sequence, but clustered in specific protein domains, suggesting the importance of critical functional domains and the need to disrupt them to produce a phenotype (Figure 1). Indeed, an enrichment of missense mutations is found within the second zinc finger domain, the makorin-type cysteine-rich hinge region, and the RING finger domain (Figure 1). Methylation defects and whole-gene deletions have not yet been

reported in patients with CPP [25]. Overall, the prevalence of *MKRN3* mutations in patients with CPP has been estimated at 9.0% (95% confidence interval, 0.04 to 0.15) in a recent meta-analysis, with variations based on sex, family history, and geographical distribution [22].

It is worth noting that, among the aforementioned mutations, 11 of them have also been found in the Genome Aggregation Database, a public database. However, the finding of these variants in the general population does not necessarily rule out a causal role in CPP. First, the maternally imprinted nature of this gene implies that mutations will not lead to any clinical manifestations if inherited from the mother. The parent-of-origin allelic status is not reported in the Exome Aggregation Consortium database or Genome Aggregation Database. Second, it is possible that some presumed healthy controls included in these databases may have manifested some signs of early puberty during their growth and development that were undiagnosed or unreported.

The MKRN3 level has been measured in peripheral blood. Similar to the decline in Mkrn3 expression in the CNS in rodents, circulating MKRN3 levels decrease progressively in association with pubertal onset [26]. Serum MKRN3 levels were reported to be lower in patients with CPP than in matched controls [27]. These findings suggest that, regardless of the cause of CPP, a decline in serum MKRN3 levels appears to be a common pattern associated with HPG reactivation and pubertal onset. However, the source and physiological significance of peripheral MKRN3 in humans is not yet known [26].

In vitro studies conducted on mutant *MKRN3* variants show that truncated and some missense variants impair the repressive action on human *KISS1* and *TAC3* promoters and/or the MKRN3-dependent ubiquitination [16].

Evolutionary significance of MKRN3 imprinting on pubertal timing

The *MKRN3* gene is located in a maternally imprinted genomic region. This feature is not shared by other members of the makorin family. Interestingly, loss-of-function mutations in *DLK1*, another maternally imprinted gene, were recently identified as a cause of genetic CPP [5]. Genome-wide association studies showed an enrichment of menarche signals in several imprinted regions, including DLK1-WDR25, MKRN3-MAGEL2, and KCNK9 [24]. Recent theories on imprinting suggest that maternally expressed genes might promote early sexual maturation that is beneficial for the mother at the expense of the child's survival [28]. By contrast, paternally expressed genes confer a greater likelihood for a child to grow and survive. Indeed, *MKRN3* and *DLK1* genes are paternally expressed and act as inhibitors of pubertal development.

In addition, the timing of menarche has been associated with changes in birth size, body size, and body composition, with earlier age at menarche associated with low weight at birth but higher fat mass in childhood [29]. It is of interest that, taken as a whole, several maternally imprinted genes, such as *DLK1*, *IGF2*, *PEG1*, *PEG3*, and *RASGRF1*, have been found to promote growth, especially during embryo development [30]. *MKRN3* has not been demonstrated to promote growth directly as its function only seems to prevent the onset

of puberty. However, in various species, including humans, a relationship between puberty and growth exists, with the onset of puberty being a major determinant to influence adult height [31]. Hence, by preventing progression through puberty, *MKRN3* might indirectly have a permissive effect on growth.

Conclusion

Mutations in *MKRN3* have been identified in 115 patients with CPP and represent the most common genetic cause of CPP. Forty-eight variants have been described, either within the coding sequence or in regulatory regions. The genomic location of *MKRN3* and the more recently identified *DLK1* in maternally imprinted regions underscore the important role of genomic imprinting in the regulation of puberty initiation. The E3 ubiquitin ligase activity of MKRN3 suggests an inhibitory action on post-translational regulation of proteins. Although GnRH and its known activators, kisspeptin and neurokinin B, have been proposed as the most likely targets of Mkrn3 action [16], recent studies report additional potential targets, including neural pentraxin-1 and Lin28b, involved in neural differentiation and in regulation of age at menarche, respectively [20,32]. Moreover, *Mkrn3* has recently been shown to be specifically inhibited by miR-30, unveiling for the first time the role of hypothalamic miRNAs in regulating Mkrn3 expression [15]. Makorin proteins have been identified in a broad range of species, including invertebrates and plants. They have been demonstrated to decline progressively after body elongation, organ differentiation, and/or neural development, supporting the hypothesis of an important and conserved role in embryonic and postnatal development. Understanding the hypothalamic network surrounding the action of Mkrn3 will be a major challenge for future research to fully understand the regulation of puberty initiation.

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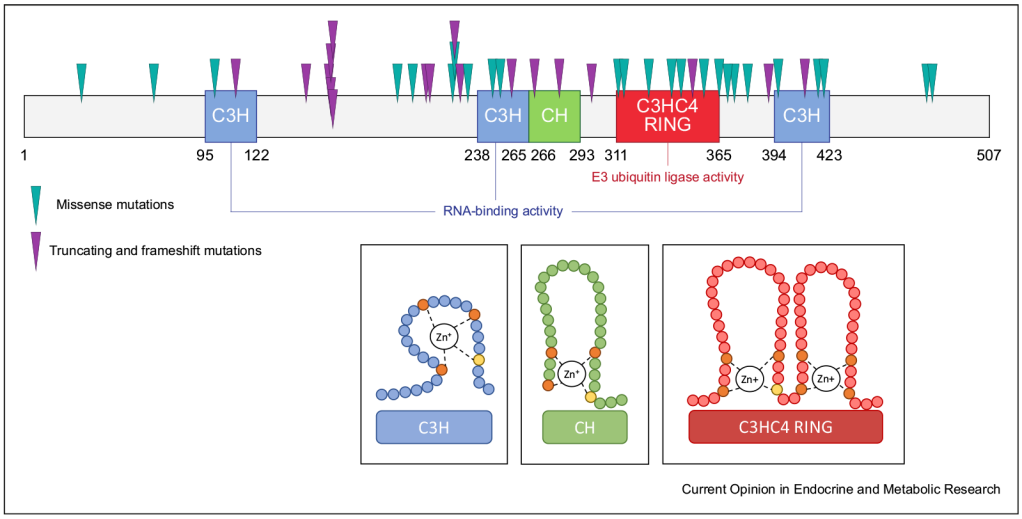

Figure 1. Schematic depiction of the makorin RING finger protein 3 (MKRN3) structure and location of mutations reported in patients with central precocious puberty.

Protein structure: numbers indicate amino acids from 1 to 507. The canonical zinc fingers (C3H) typical of RNA binding activity are depicted in blue, the makorin-type Cys–His (CH)–rich hinge region is depicted in green, and the RING finger domain (C3HC4) characteristic of E3 ubiquitin ligase activity is depicted in red. The locations of mutations in *MKRN3* identified in patients with central precocious puberty that result in amino acid substitutions are indicated by green arrows (missense variants) and those resulting in protein truncation or frameshift mutations are indicated by purple arrows (nonsense variants). The insets show two-dimensional details of the different MKRN3 domains.

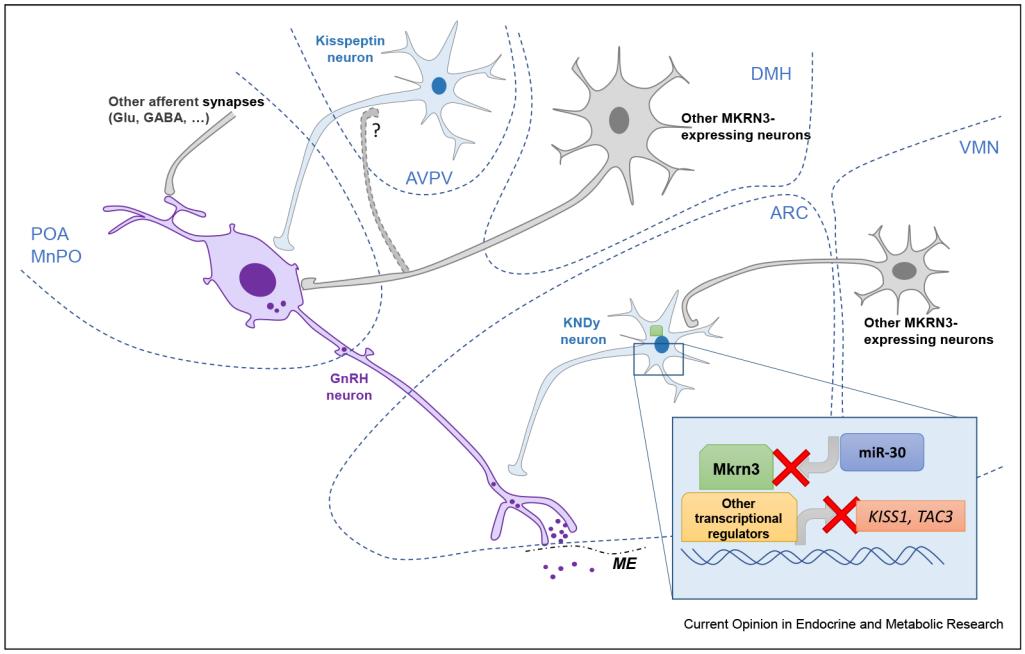

Figure 2. Schematic depiction of hypothalamic regions involved in MKRN3 action.

This figure summarizes the predominant sites of expression of MKRN3 within the hypothalamus. Putative regions and nuclei are encircled by dotted blue lines. The GnRH neuron is depicted in purple. Kisspeptin and KNDy neurons are shown in blue. Other putative MKRN3-expressing neurons are shown in gray. The inset shows the intracellular site of action of MKRN3 and the currently proposed mechanisms of action and regulation. The red X indicates inhibitory action. ARC, arcuate nucleus; AVPV, anteroventral periventricular nucleus; DMH, dorsomedial hypothalamic nucleus; ME, median eminence; MnPO, median preoptic nucleus; POA, preoptic area; VMN, ventromedial nucleus.

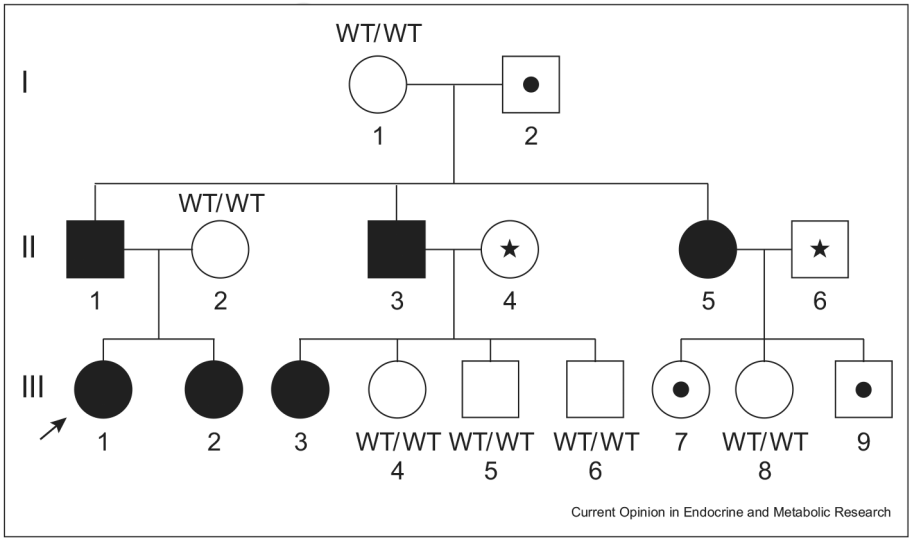

Figure 3. Pedigree showing a family with the M268Vfs*23 *MKRN3* mutation associated with central precocious puberty (CPP).

Squares indicate males, circles indicate females, black symbols indicate CPP-affected members, and symbols with a black dot inside indicate asymptomatic carriers. Symbols with a star inside indicate DNA sample unavailability. The arrow indicates the proband. Reprinted from the study by Simsek et al. [21] with permission. Please note that only individuals inheriting the mutation from the father are affected. By contrast, those inheriting the mutation from their mother are unaffected carriers.

Table 1

Etiologies of central precocious puberty and relative frequency of causes.

Etiology	Frequency
Genetic	
MKRN3 loss-of-function mutations (MIM #603856)	>>>
DLK1 loss-of-function mutations (MIM #176290)	>
KISS1 gain-of-function mutations	>
KISS1R (former *GPR54)* gain-of-function mutations (MIM #604161)	>
Syndromic	
Type 1 neurofibromatosis (MIM #162200)	>
Tuberous sclerosis (Bourneville disease, MIM #191100)	>
Temple syndrome (MIM #616222)	>
Silver-Russell syndrome (MIM #180860)	>
Prader–Willi syndrome (MIM #176270)	>
Williams-Beuren syndrome (MIM #194050)	>
Diencephalic tumors	
Hamartoma (hypothalamus)	>
Gliomas/oligodendrogliomas	>
Hydrocephaly	>
Other intracranial hypothalamic tumors (rare)	>
Environmental factors	
Adoption/early life social stressors	>>
Nutritional excess	>
Traumatic brain injury	>
Cranial irradiation	>
Prepubertal exposure to sex steroids	>
Prepubertal exposure to endocrine disruptors	>
Idiopathic	>>>

Frequency of etiology: >>> (>5%), >> (2–5%), and > (<2%).

Table 2

MKRN3 mutations identified in patients with CPP (updated through April 2020).

Nucleotide change	Protein change	No. of reported cases	Inheritance pattern	GnomAD	Reference
g.−865G>A	–	1	Sporadic	–	[34]
g.−166G>A	–	1	Sporadic	–	[34]
g.−886C>T	–	1	Sporadic	–	[34]
g.+13C>T	–	1	Sporadic	–	[34]
c.−150_−147delTCAG	–	1	Sporadic	–	[35]
c.−81C>T	–	1	Sporadic	–	[36]
c.89C>T	P30L	1	Sporadic	1.20 e–5	[37]
c.203G>A	R68H	1	Sporadic	1.20 e–5	[38]
c.298A>T	I100F	1	Sporadic	None	[39]
c.331G>T	E111*	2	Familial	None	[40]
c.441_441delG	H148Tfs*23	3	Familial	4.00 e–6	[21]
c.477_485del	P160Cfs*14	3	Familial	None	[41]
c.482delC	P161Rfs*10	3	Sporadic	None	[42]
c.482_483insC	P161Rfs*16	4	Familial/sporadic	None	[43,44]
c.475_476insC	A162Gfs*14	9	Familial/sporadic	None	[4,45]
c.482insC	A162Gfs*15	13	Familial	None	[37]
c. 587G>T	G196V	2	Sporadic	9.21 e–5	[39,46]
c.611T>C	I204T	1	Sporadic	3.99 e–6	[39]
c.630_650delins GCTGGGC	P211Lfs*16	2	Familial	None	[47]
c.637delC	R213Gfs*73	4	Familial/sporadic	None	[4,45]
c.673C>G	L225V	1	Sporadic	None	[48]
c.675_676insA	Q226Tfs*6	1	Sporadic	None	[43]
c.677A>C	Q226P	1	Sporadic	None	[39]
c.683_684insA	E229Rfs*3	2	Familial	None	[49]
c.699G>C	K233N	1	Sporadic	None	[39]
c.737A>G	Y246C	1	Sporadic	None	[37]
c.749G>A	G250E	1	Sporadic	None	[46]
c.766_767delA	E256Gfs*36	1	Sporadic	None	[43]

Nucleotide change	Protein change	No. of reported cases	Inheritance pattern	GnomAD	Reference
c.802–803del	M268Vfs*23	7	Familial	3.98 e–6	[21]
c.841C>T	Q281*	3	Familial	None	[39]
c.891A>T	E298*	3	Familial	None	[50]
c.934G>A	G312D	2	Familial	None	[51]
c.943A>G	M315V	2	Familial	None	[37]
c.982C>T	R328C	6	Familial/sporadic	3.98 e–6	[45,52]
c.1018T>G	C340G	2	Familial	None	[53]
c.1034G>A	R345H	1	Sporadic	2.12 e–5	[54]
c.1053_1056delACAG	R351Sfs*44	1	Sporadic	None	[42]
c.1071C>G	I357M	2	Familial	None	[48]
c.1095G>T	R365S	4	Familial	3.98e–6	[4,45]
c.1212C>G	S368C	1	Sporadic	3.98e–6	[55]
c.1118C>T	P373L	2	Familial	None	[37]
c.1138G>A	E380K	3	Familial/sporadic	None	[48]
c.1171_1172insA	Y391*	2	Familial	None	[4]
c.1188C>A	S396R	1	Sporadic	2.78 e–5	[39]
c.1229G>A	C410*	1	Sporadic	None	[52]
c.1249T>A	F417I	1	Familial	None	[37,43]
c.1259T>G	H420Q	5	Familial	None	[56]
c.1420T>A	L474M	1	?	None	[48]
c.1430G>A	R477Q	1	Sporadic	5.66 e–5	[46]

CPP, central precocious puberty; GnomAD, Genome Aggregation Database.

GnomAD: allele frequency from the GnomAD, a public database, https://gnomad.broadinstitute.org.