

MKRN3 Mutations in Central Precocious Puberty: A Systematic Review and Meta-Analysis

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MKRN3 mutations represent the most common genetic cause of central precocious puberty (CPP) but associations between genotype and clinical features have not been extensively explored. This systematic review and meta-analysis investigated genotype-phenotype associations and prevalence of *MKRN3* mutations in CPP. The search was conducted in seven electronic databases (Cochrane, EMBASE, LILACS, LIVIVO, PubMed, Scopus, and Web of Science) for articles published until 4 September 2018. Studies evaluating *MKRN3* mutations in patients with CPP were considered eligible. A total of 22 studies, studying 880 subjects with CPP, fulfilled the inclusion criteria. Eighty-nine subjects (76 girls) were identified as harboring *MKRN3* mutations. Girls, compared with boys, exhibited earlier age at pubertal onset (median, 6.0 years; range, 3.0 to 7.0 vs 8.5 years; range, 5.9 to 9.0; $P < 0.001$), and higher basal FSH levels (median, 4.3 IU/L; range, 0.7 to 13.94 IU/L vs 2.45 IU/L; range, 0.8 to 13.70 IU/L; $P = 0.003$), and bone age advancement (Δ BA; median, 2.3 years; range, -0.9 to 5.2 vs 1.2 years; range, 0.0 to 2.3; $P = 0.01$). Additional dysmorphisms were uncommon. A total of 14 studies evaluating 857 patients were included for quantitative analysis, with a pooled overall mutation prevalence of 9.0% (95% CI, 0.04 to 0.15). Subgroup analysis showed that prevalence estimates were higher in males, familial cases, and in non-Asian countries. In conclusion, *MKRN3* mutations are associated with nonsyndromic CPP and manifest in a sex-dimorphic manner, with girls being affected earlier. They represent a common cause of CPP in western countries, especially in boys and familial cases.

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Precocious puberty is traditionally defined by the onset of progressive pubertal development before the age of 8 years in girls and 9 years in boys. Central precocious puberty (CPP), also known as gonadotropin-dependent precocious puberty, is a condition resulting from the early

Abbreviations: BA, bone age; CPP, central precocious puberty; E3, ubiquitin-protein ligase; *MKRN3*, makorin ring finger protein 3; PWS, Prader-Willi syndrome.

reactivation of the hypothalamic-pituitary-gonadal axis [1, 2]. In the absence of central nervous system abnormalities, CPP is classified as idiopathic, and it predominantly affects girls. Idiopathic CPP is familial in as many as 27.5% of the cases, emphasizing the genetic origin of this disorder [3].

The involvement of the makorin ring finger protein 3 (*MKRN3*) gene in the pathogenesis of CPP was first described by Abreu *et al.* in 2013 after evaluating patients with familial CPP using whole-exome sequencing [4]. Subsequently, mutations in *MKRN3* have been described in multiple patients with CPP from different countries [5–16].

MKRN3, previously known as *ZNF127*, is an intronless and imprinted gene located on chromosome 15q11-q13, in the Prader-Willi syndrome (PWS) region [17]. Because of maternal imprinting, only the paternal allele is expressed. Therefore, CPP can be expected only if the mutated allele is paternally inherited [2, 4, 18]. It encodes a protein belonging to the Makorin family of zinc finger proteins with proposed ubiquitin-protein ligase (E3) activity, based on its structure and on activity of the other members of the makorin family. The findings of loss of function mutations in *MKRN3* associated with the pattern of the hypothalamic *Mkfn3* mRNA expression in mice suggest that *MKRN3* acts to inhibit puberty initiation [4, 18], though its exact mechanism of action remains to be elucidated.

Currently, *MKRN3* mutations are recognized as the main genetic cause of CPP and appear to be particularly common in familial cases (up to 33% to 46%) [7, 18], although no mutations were described in the subgroup of Korean girls with familial CPP [12]. In the sporadic form of CPP, the prevalence was 3.9% in a large cohort of Brazilian girls [5], however it was variable in other reports, ranging from 0% to 20% in small cohorts of Italian and Bulgarian girls, respectively [19, 20]. Thus, the exact prevalence of mutations in patients with CPP and possible geographical differences worldwide has not been explored.

Patients carrying *MKRN3* mutations exhibit typical clinical and biochemical features of premature reactivation of the reproductive axis. Few studies compared data of patients with and without mutations, and higher levels of basal FSH and an earlier age at pubertal onset had been described among girls with mutations [5, 7], whereas affected boys exhibited later pubertal onset in comparison with those with CPP and no mutations [15]. Thus, it is still not known whether their phenotype differs from those without mutations. Therefore, we performed a systematic review and meta-analysis aiming to identify and synthesize data on *MKRN3* mutations in CPP, to investigate genotype-phenotype associations, and to estimate the worldwide prevalence of *MKRN3* mutations in patients with idiopathic CPP.

1. Methods

The methodology of this systematic review was based on the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses [21]. The protocol was registered at the international prospective register of systematic reviews with the number CRD42018095144 (<http://www.crd.york.ac.uk/PROSPERO>).

A. Eligibility Criteria

Observational human studies and clinical trials evaluating *MKRN3* mutations in patients with idiopathic CPP were considered eligible. Idiopathic CPP was defined by progressive pubertal development before the age of 8 years in girls and 9 years in boys, pubertal basal and/or GnRH-stimulated LH levels, advancement of BA as assessed by the Greulich and Pyle method, and absence of central nervous system abnormalities on MRI.

Exclusion criteria were defined as follows: (i) review articles, letters, conference abstracts, personal opinions, and book chapters; (ii) articles that were not in the Latin (Roman) alphabet; (iii) studies that had duplicate data; and (iv) studies investigating single nucleotide polymorphisms in patients without a confirmed diagnosis of CPP.

B. Search Strategy and Study Selection

A systematic electronic search was conducted in seven databases with neither language nor year restriction (Cochrane, EMBASE, LILACS, LIVIVO PubMed, Scopus, and Web of Science). The grey literature [22] search was performed using Google Scholar and ProQuest. At the end of the search phases, the bibliographies of selected articles were manually screened for the identification of additional eligible studies. The details of search strategy are important to the comprehension of this study [23].

Two independent authors (L.P.V. and C.G.M.) conducted the study selection. First, titles and abstracts were evaluated and publications that did not fulfill the inclusion criteria were discarded. In the second phase, full texts of selected articles were reviewed independently by the same authors (L.P.V. and C.G.M.) to confirm their eligibility. Any disagreement between reviewers was resolved by consensus. A third author (I.P.D.T) was consulted if disagreements between the two initial evaluators were not resolved by consensus.

C. Data Extraction

The following data were extracted from each study: first author, year and country of publication, study design, sample size, number of patients with *MKRN3* mutations, family history of CPP, and sequencing technique. In addition, clinical variables of interest including sex, age at pubertal onset, Tanner stage, BA, hormonal parameters at diagnosis, type of *MKRN3* mutation, and information regarding treatment were extracted for individual patients. Data and genetic analyses from adult parents with a diagnosis of CPP based on past medical history were not included.

D. Risk of Bias in Individual Studies

Critical appraisal of included studies was performed independently by two authors (L.P.V. and C.G.M.) according to a checklist developed by Joanna Briggs Institute for Studies Reporting Prevalence Data [24]. Disagreements in the assessment of the risk of bias were resolved by discussion, with the involvement of a third reviewer author if necessary.

E. Statistical Analysis

We pooled the predefined clinical and biochemical features that were more frequently reported in the selected studies (age at pubertal onset and at diagnosis, Tanner stage, BA, basal and poststimulated LH, basal FSH, estradiol, testosterone, and type of *MKRN3* mutation).

Possible differences between data of girls and boys with *MKRN3* mutations, and of Asian girls and girls from western countries, were analyzed using the nonparametric Mann-Whitney test because data did not follow a normal distribution. Statistical significance was defined as $P < 0.05$. Analyses were performed using SPSS, version 20.

In studies eligible for analysis of prevalence, the number of patients screened positive for *MKRN3* mutations (numerator) and the total number of CPP patients screened (denominator) were used to estimate prevalence. Meta-analysis was performed using StataCorp Software, version 13 (College Station, TX) with the “metaprop” command; a random effect model was used to estimate the pooled prevalence of *MKRN3* mutations and the 95% CIs. Potential sources of heterogeneity were examined by performing a series of pre-specified subgroup analyses for the following variables: sex, family history of CPP, and geographical distribution (Asian and non-Asian countries). Heterogeneity was assessed using the I^2 statistic, where $\leq 25\%$, 50% , and $\geq 75\%$ indicated low, moderate, and high heterogeneity, respectively.

2. Results

A. Study Selection

The initial search through the electronic databases was conducted on 6 February 2018 and resulted in a total of 161 studies after removing duplicates. Four other articles were identified through updated search on 4 September 2018. No additional study was included from the references of the retrieved articles or from grey literature. After a comprehensive evaluation of titles and abstracts, 31 articles were considered for the second phase, in which the full texts were reviewed. Finally, 22 studies complied with the inclusion criteria. A flow chart detailing the selection process is shown in [Fig. 1](#).

B. Study Characteristics

The main study characteristics are summarized elsewhere [23]. All studies were published between 2013 and 2018 in the English language. Of them, 7 were case reports, 2 were case series, 11 were cross-sectional, and 2 studies were classified as cross-sectional and translational. The 14 studies included in the quantitative synthesis (meta-analysis) of prevalence comprised a total of 857 patients with idiopathic CPP screened for *MKRN3* mutations. In the study of Bessa *et al.* [15], data of some patients had been previously published [4] and therefore were counted only once for the analysis of prevalence.

Regarding risk of bias by Joanna Briggs Institute Critical Appraisal Checklist for Studies Reporting Prevalence Data, 2 studies were classified as carrying moderate risk of bias [25, 26] and 20 were classified as with low risk of bias [4–16, 19, 20, 23, 27–31].

C. Demographic and Phenotypic Characteristics Associated With *MKRN3* Mutations

MKRN3 defects were described in 89 patients (76 girls and 13 boys) with CPP from 17 countries: Argentina [15], Belgium [4], Brazil [4, 5, 15, 16, 31], Bulgaria [20], Cyprus [25, 26], Denmark [28], France [7], Germany [9], Greece [6], Israel [8], Italy [7, 10, 19, 27], Japan [14], Korea [12], Spain [13], Taiwan [29], Turkey [11, 30], and United States of America [4]. Eighty-eight patients harbored mutations in the coding sequence and one exhibited a deletion in the promoter region of the gene.

Patients with *MKRN3* mutations presented with signs and symptoms of early reactivation of the hypothalamic-pituitary-gonadal axis, represented by precocious development of sexual characteristics, BA advancement, and pubertal levels of basal or poststimulated LH. The detailed clinical and hormonal profiles of patients with *MKRN3* mutations identified are shown elsewhere [23].

In the pooled analysis, the median age at pubertal onset of girls with *MKRN3* mutations ($n = 76$) was 6.0 years (range, 3.0 to 7.8). Median basal and poststimulated LH levels were 1.27 IU/L (range, 0.1 to 6.1) and 22.0 IU/L (range, 4.0 to 95.9), respectively. Asian girls were significantly older at diagnosis (8.0 years; range, 6.8 to 10.3 years) in comparison with girls from western countries (6.7 years; range, 3.6 to 8.4; $P < 0.001$) and tended to be older at pubertal onset (6.8 years; range, 5.0 to 7.8 years *vs* 6.0 years; range, 3.0 to 7.5 years; $P = 0.072$). In addition, Asian girls presented with lower basal FSH levels than western girls (3.0 IU/L; range, 0.8 to 7.64 IU/L *vs* 4.5 IU/L; range, 0.7 to 13.94 IU/L, respectively; $P = 0.042$), as shown in [Table 1](#).

The median age at pubertal onset of boys ($n = 13$) was 8.5 years (range, 5.9 to 9.0). In comparison with boys, girls exhibited a significantly earlier age at pubertal onset (2.0 years below the sex-specific lower age limit *vs* 0.5 years below this limit in boys, $P = 0.001$), higher basal FSH levels (median, 4.3 IU/L; range, 0.7 to 13.94 IU/L *vs* 2.45 IU/L; range, 0.8 to 13.70 IU/L in boys; $P = 0.003$), and greater Δ BA (median, 2.3 years; range, -0.9 to 5.2 years *vs* 1.2 years; range, 0.0 to 2.3 years, respectively; $P = 0.01$) ([Table 2](#)).

There was no difference among the age at pubertal onset, Δ BA, and hormonal data when comparing patients harboring different subtypes of *MKRN3* mutations, according to their predicted severity. However, patients with missense mutations were older at diagnosis than

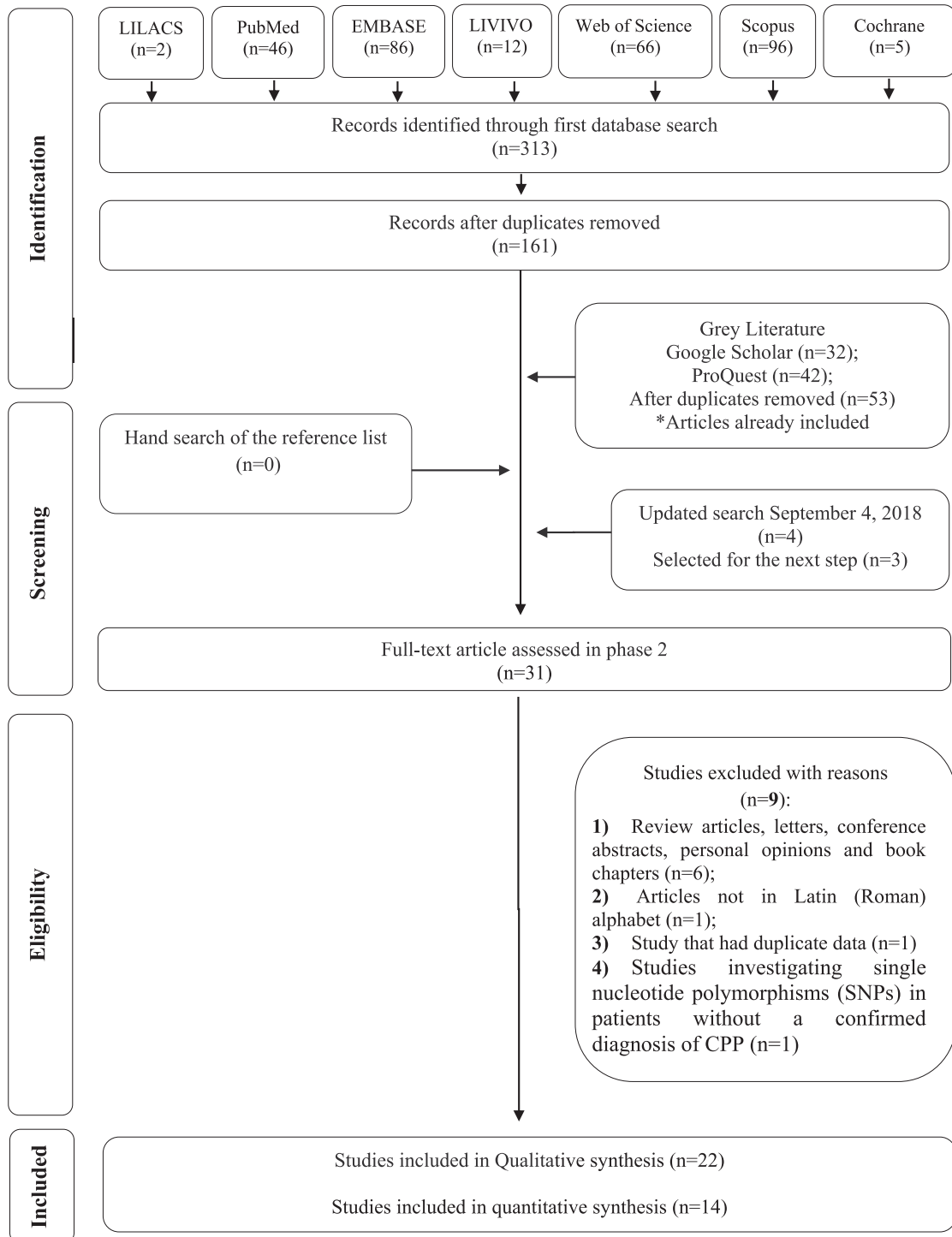


Figure 1. Flow chart for identifying eligible studies.

patients with mutations that encode premature stop codons (nonsense and frameshift) and a promoter deletion (7.72 years; range, 3.6-10.3 *vs* 6.75 years; range, 3.8 to 13.2, respectively). This finding may suggest a slower rate of puberty progression associated with mutation subtype (Table 3). Notably, the majority of mutations found in Asian girls were missense [9 of 11 (82%)] whereas in girls from western countries missense mutations were found only in 21 of 65 (32%) of the patients ($P = 0.002$).

Table 1. Pooled Comparative Analysis of Clinical and Biochemical Data of Girls From Western Countries (n = 65) and Girls From Asian Countries (n = 11)

Clinical and Biochemical Data	Western Girls, Median (Range)	Asian Girls, Median (Range)	P Value
Age at pubertal onset, y (64/6)	6.0 (3.0–7.5)	6.8 (5.0–7.80)	0.072
Age at diagnosis, y (61/11)	6.7 (3.6–8.4)	8.0 (6.8–10.3)	<0.001 ^a
ΔBA-CA, y (58/11)	2.3 (–0.9 to 5.2)	2.1 (1.2–3.0)	0.928
Basal FSH, IU/L (58/11)	4.5 (0.7–13.94)	3.0 (0.8–7.64)	0.042 ^a
Basal LH, IU/L (55/11)	1.1 (0.1–6.1)	1.6 (0.36–3.74)	0.454
Poststimulated LH, IU/L (54/9)	22.1 (4.9–95.9)	11.6 (5.6–52.5)	0.321
Estradiol, pg/mL (52/2)	25.5 (2.0–80.0)	38.5 (29.0–48.0)	0.507

In the first column, numbers in parentheses refer to the number of patients from each group in which the information was available for comparative analysis.

Abbreviation: ΔBA-CA, difference between bone age and chronological age.

^a*P* < 0.05, using the Mann-Whitney *U* test for comparison between the groups.

Additional dysmorphisms were rare and include esotropia detected in two affected siblings [18], and clinodactyly and lumbar hyperlordosis in two unrelated girls, one of whom also exhibited a high-arched palate and dental abnormalities [5, 10]. Premature ovarian failure was reported by Grandone *et al.* [10] in the mutated grandmother of the index case, although it could be an incidental finding.

There were few studies comparing data of patients with and without *MKRN3* mutations. Macedo *et al.* [5] described higher levels of basal FSH in girls with *MKRN3* mutations compared with nonmutated girls (median, 4.9 IU/L; ranging from 4.4 to 10 IU/L vs 3.6 IU/L; range, 1.0 to 9.8 IU/L; *P* = 0.016). Simon *et al.* [7] found that girls with *MKRN3* mutations were younger at puberty onset than nonmutated ones [median age, 6.0 years (range, 5.4 to 6.0) vs 7.0 years (range, 6.0 to 7.0), *P* = 0.01], but no additional differences regarding hormonal levels, uterus length, Tanner Stage, and ΔBA were observed between the groups. Data of male patients are limited because of the small number of reported boys with *MKRN3* mutations (n = 13). Data from Bessa *et al.* [15] suggested a later pubertal onset in mutated boys (median age, 8.2 years vs 7.0 years in boys with CPP without *MKRN3* mutations; *P* = 0.033), although no differences in hormonal status were identified between the groups.

Younger carriers of *MKRN3* mutations identified by family screening before the development of pubertal signs have also been described [7, 11], and their clinical follow-up are still not known. Stecchini *et al.* reported a case of a girl identified as a carrier of *MKRN3* mutation at the age of 4 because of a positive family history, who was diagnosed with CPP at the age of 6.7 years [16].

Table 2. Pooled Comparative Analysis of Clinical and Biochemical Data of Girls (n = 76) and Boys (n = 13) With *MKRN3* Mutations

Clinical and Biochemical Data	Girls, Median (Range)	Boys, Median (Range)	P Value
Age at pubertal onset, y (70/8)	6.0 (3.0–7.8)	8.5 (5.9–9.00)	<0.001 ^a
Δ Age at pubertal onset (70/8)	2.0 (0.2–5.0)	0.5 (0.0–3.9)	0.001 ^a
Age at diagnosis, y (72/12)	6.8 (3.6–10.3)	9.45 (8.1–13.2)	<0.001 ^a
ΔBA-CA, y (69/11)	2.3 (–0.9–5.2)	1.2 (0.0–2.3)	0.01 ^a
Basal FSH, UI/L (69/12)	4.3 (0.7–13.94)	2.45 (0.8–13.70)	0.003 ^a
Basal LH, UI/L (66/12)	1.27 (0.1–6.1)	1.35 (0.7–5.41)	0.239
Poststimulated LH, UI/L (63/7)	22.0 (4.9–95.9)	13.9 (6.7–20.00)	0.089

In the first column, numbers between parentheses refer to the number of patients from each group in which the information was available for comparative analysis; Δ age at pubertal onset express the difference between the sex-specific lower age limit for pubertal development and the age at onset of puberty.

^a*P* < 0.05, using the Mann-Whitney *U* test for comparison between the groups.

Table 3. Pooled Comparative Analysis of Clinical and Biochemical Data of Patients With *MKRN3* Mutations According to Subtype of Mutation

Clinical and Biochemical Data	Severe Mutations ^a (n = 52) Median (Range)	Missense Mutations (n = 37), Median (Range)	P Value
Age at pubertal onset, y (51/27)	6.0 (3.0–9.0)	6.0 (3.5–8.5)	0.645
Age at diagnosis, y (48/36)	6.75 (3.8–13.2)	7.72 (3.6–10.3)	0.028
ΔBA-CA (44/36)	2.0 (–0.9 to 5.2)	2.5 (0.2–3.7)	0.166
Basal FSH, UI/L (47/34)	4.0 (0.7–13.94)	3.85 (0.8–8.9)	0.497
Basal LH, UI/L (44/34)	1.3 (0.1–6.1)	1.29 (0.2–3.74)	0.341
Poststimulated LH, UI/L (40/30)	20.0 (4.9–62.5)	19.75 (5.6–95.9)	0.506
Estradiol, pg/mL (34/20) ^b	27.85 (5.0–80.0)	25.5 (2.0–61.0)	0.502
T, ng/mL (5/5) ^c	216.0 (78.0–548.0)	198.8 (41.0–466.0)	0.347

In the first column, numbers in parentheses refer to the number of patients from each group in which the information was available for comparative analysis.

Abbreviation: T, testosterone.

^aSevere mutations encompassed frameshift mutations (n = 43), nonsense mutations (n = 8) and promoter deletions (n = 1).

^bEstradiol comparative analysis was performed between girls.

^cT comparative analysis was performed between boys; *P* < 0.05, using the Mann-Whitney *U* test for comparison between the groups.

Ultimately, information regarding treatment was available only for 54 patients and all were treated with GnRH analog. A satisfactory therapeutic response was described in all but two girls who were noncompliant with treatment. One Turkish girl whose parents refused treatment had menarche at the age of 8.9 years [11]. However, menarche seemed to occur in an appropriate age after an adequate treatment adherence with GnRH analog. In addition, the predicted final height appeared to be within the target height in the few cases in which this information was available [5].

D. *MKRN3* Mutations

To date, 39 inactivating mutations in the coding sequence of *MKRN3* have been described, including 4 nonsense, 13 frameshift, and 22 missense mutations (Fig. 2).

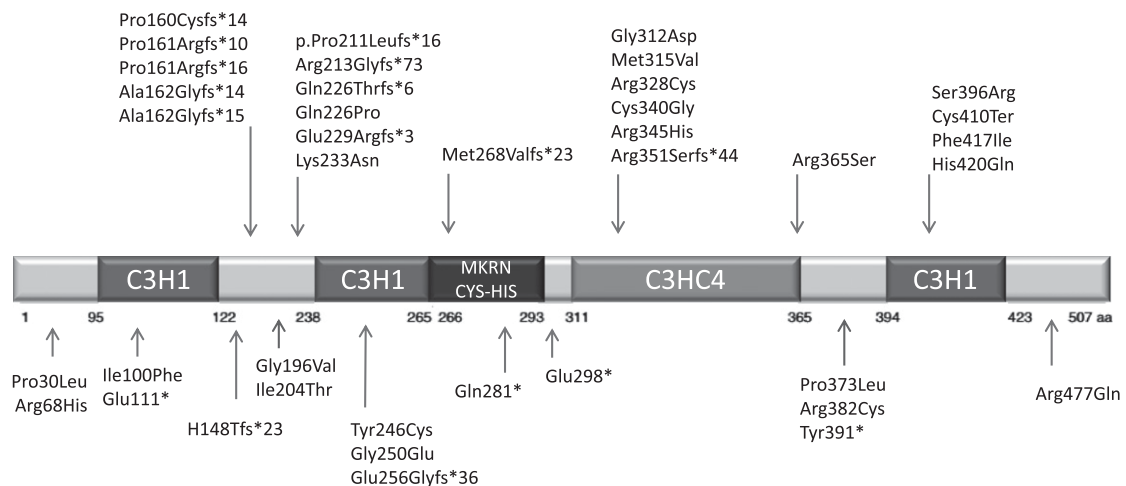


Figure 2. Structure of the *MKRN3* human protein showing the location of the mutations in coding sequence identified to date. Notably, 36% (14 of 39) of mutations are located in the loop structure between 2 C3H1 zinc finger motifs in the aminoterminal region of the protein and these are almost all frameshift mutations, thereby affecting protein structure. The C3HC4 is the second domain where mutations tend to cluster; this area has mostly missense mutations.

Frameshift variants are predicted to result in the translation of truncated proteins by creation of premature stop codons. Notably, 5 frameshift mutations, identified in almost one-third of the patients ($n = 27$), are located in a cytosine-rich region between nucleotides 476 and 482, confirming this site as a hotspot.

Most of the missense mutations were predicted to be pathogenic by *in silico* analysis. Of these, six are located within the C3HC4 ring zinc finger domain of the protein (p.Gly312Asp, p.Met315Val, p.Arg328Cys, p.Cys340Gly, p.Arg345His, p.Arg356Ser) and five within a C3H zinc finger motif (p.Tyr246Cys, p.Gly250Glu, p.Cys410Ter, p.Phe417Ile, p.His420Gln). These domains are related to the E3 ubiquitin-ligase activity and RNA-binding, respectively, and seem to be essential for protein function. Six missense variants were predicted to be benign or neutral by *in silico* analysis (p.Ile100Phe, p.Ile204Thr, p.Gln226Pro, p.Lys233Asn, and p.Ser396Arg), which were detected in Korean girls, and p.Arg477Gln, detected in a single Taiwanese girl [12, 29]. However, the lack of *in vitro* analysis of the missense mutations may be a possible limitation of interpretation.

In addition to defects in the coding sequence, a heterozygous 4-nucleotide deletion (c.-150_147delTCAG) in the promoter region of *MKRN3* was recently described in 1 of 110 patients with CPP without mutations in the coding sequence. This deletion is predicted to cause the loss of a binding site to a putative transcriptional factor for *MKRN3* expression. Using an *in vitro* luciferase reporter assay, the authors showed a substantial reduction of *MKRN3* promoter activity in cells transfected with a plasmid encoding the deletion [32].

Other genetic or epigenetic mechanisms that could disrupt the expression of an imprinted gene, such as copy number variations or methylation abnormalities, of the 15q11 locus were not detected in 52 patients evaluated by methylation-specific multiplex ligation-dependent probe amplification [5]. In addition, partial or complete *MKRN3* deletions were not found in 32 CPP patients without *MKRN3* mutations investigated through a quantitative PCR gene dosage assay [7].

E. Prevalence of *MKRN3* Mutations in Idiopathic CPP

MKRN3 mutations have been described in patients with familial and sporadic CPP from multiple countries and may account for a substantial proportion of familial cases (33% to 46%) [4, 7]. However, *MKRN3* mutations were not identified in a subgroup of Korean girls with familial CPP [12], and a lower frequency of mutations in familial cases was also described in other series (8.7% and 5.3%, respectively) [27, 30]. In addition, the frequency of *MKRN3* mutations appears to be higher among males (40% in one report) [15].

The pooled overall prevalence of *MKRN3* mutations in patients with idiopathic CPP was 9.0% (95% CI, 0.04 to 0.15; I^2 , 80.91%; $P = 0.00$; Fig. 3). Interestingly, the pooled prevalence of *MKRN3* mutations was much lower in patients from Asian countries (2.0%; 95% CI, 0.01 to 0.04; I^2 , 0.0%) than in those from non-Asian countries (11.0%; 95% CI, 0.04 to 0.21; I^2 , 81.31%) (Fig. 4).

Considering the variation in the prevalence of *MKRN3* mutations according to the geographical distribution and the lack of information regarding family history in some reports, studies from Asian countries were not included in the subanalysis by family history. The pooled prevalence of *MKRN3* mutations in patients with familial CPP was 19.0% (95% CI, 0.05 to 0.36; I^2 , 69.16%). Considering the sporadic cases, the estimated pooled prevalence was 2.0% (95% CI, 0.01 to 0.04; I^2 , 0%) (Fig. 5).

Afterward, in sex-based subanalysis, the pooled prevalence of *MKRN3* mutations in males (22.0%; 95% CI 0.0 to 0.62; I^2 , 70.19) was higher than in females (7.0%; 95% CI, 0.03 to 0.13; I^2 , 77.73%) (Fig. 6).

3. Discussion

This systematic review and meta-analysis investigated data of patients with CPP caused by *MKRN3* mutations, and, to our knowledge, it represents the most comprehensive review of the reported data published to date. In addition, it provides qualitative and quantitative analysis of published data, expanding the knowledge on the epidemiology of *MKRN3* mutations.

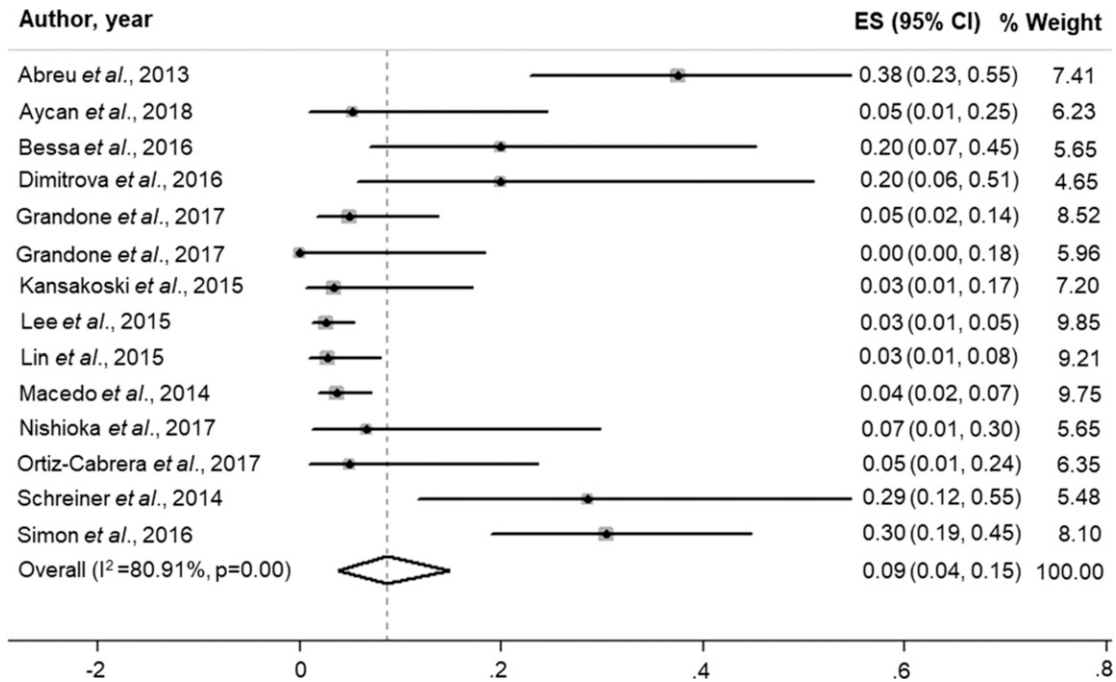


Figure 3. Pooled overall prevalence of *MKRN3* mutations in patients with central precocious puberty (9.0%; 95% CI, 0.04 to 0.15; I^2 , 80.91%; $P = 0.00$). ES, prevalence estimate.

There was no specific clinical or laboratory finding that identified a patient with CPP as a carrier of a *MKRN3* mutation. In one report, higher basal FSH levels were the only parameter found to differ between girls with and without *MKRN3* mutations [5]. In another study, lower age at puberty onset was recorded in the subgroup of girls with mutations in *MKRN3* compared with girls without mutations [7]. On the other hand, later age at pubertal onset is described in mutated boys compared with those without mutations [15].

Additional dysmorphisms were rare (described in only four patients worldwide) and major criteria for PWS were not described in any patient, reinforcing the hypothesis that *MKRN3* mutations are associated with the phenotype of nonsyndromic CPP. Curiously, premature ovarian failure was described in the grandmother of an Italian girl identified as a carrier of *MKRN3* gene mutation. Although it could be an incidental finding, gonadal dysfunction has been described in patients with PWS [33, 34], and long-term follow-up of mutated girls is necessary to evaluate a possible relationship between *MKRN3* mutations and premature ovarian failure.

The onset of physiologic puberty in girls occurs 1 to 2 years earlier than in boys, which is consistent with sexual dimorphism of the major drivers of puberty [35]. The pooled analysis of available data showed that mutations in *MKRN3* appear to affect pubertal onset in a sex-dimorphic manner, with girls entering puberty at a younger age than boys (median age, 2 years *vs* 0.5 years, below their sex-specific lower age limit, respectively; $P = 0.001$). In addition, girls with CPP exhibited higher basal FSH levels and greater Δ BA in comparison with boys. These differences do not reflect a more advanced stage of puberty at the time of diagnosis in girls than in boys; rather, the proportion of subjects with Tanner stages 3 and 4 was higher in boys than in girls (76% *vs* 58%, respectively). Moreover, FSH predominance is routinely seen during minipuberty [36] and female puberty [37], whether it is precocious or normally timed. A marked sex difference in circulating *MKRN3* levels was also reported, with prepubertal boys displaying a 50% lower mean serum *MKRN3* level compared with prepubertal girls [38]. Of note, serum levels of *MKRN3* negatively correlated with circulating FSH and LH in prepubertal girls [39], albeit no substantial correlation were observed between *MKRN3* and gonadotropin levels in boys. The mechanisms underlying this sex

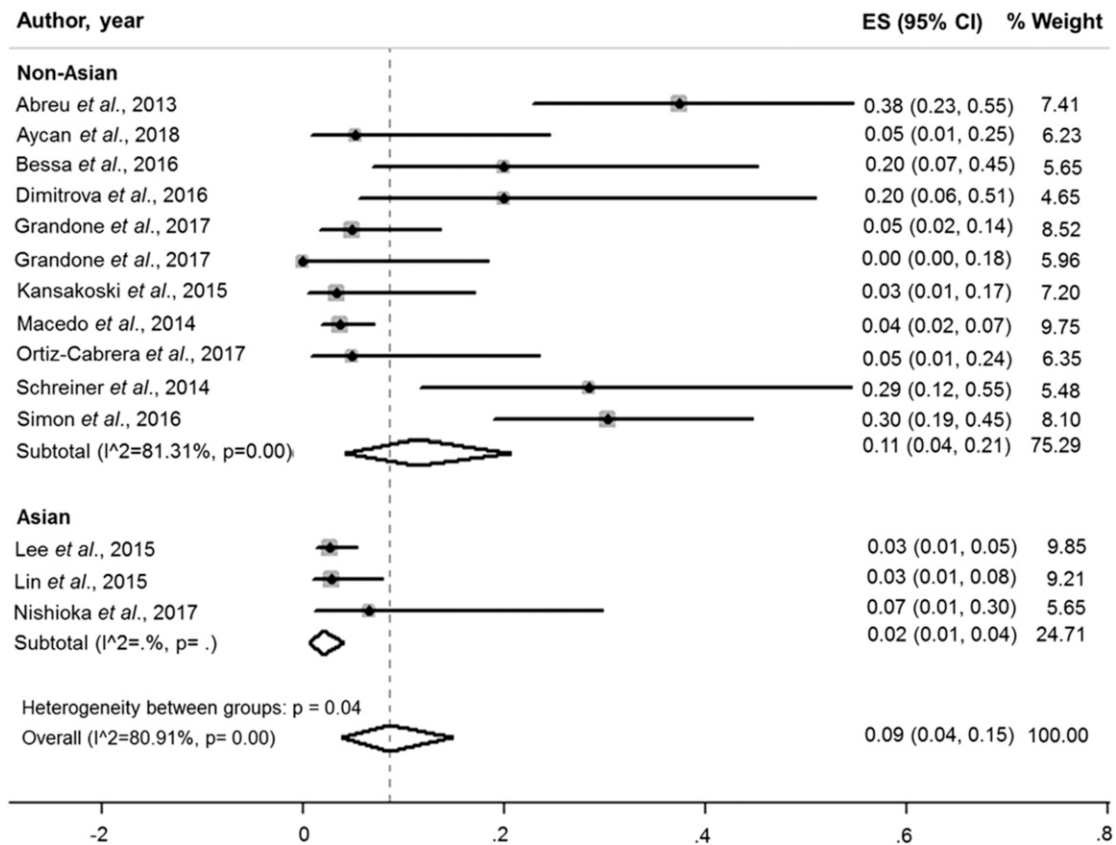


Figure 4. Pooled prevalence of *MKRN3* mutations among central precocious puberty patients from Asian (2.0%; 95% CI, 0.01 to 0.04; I^2 , 0.0%) and non-Asian countries (11.0%; 95% CI, 0.04 to 0.21; I^2 , 81.31%).

difference are not yet understood, and a sex-specific action of *MKRN3* on signaling pathways with a role on GnRH secretion cannot be ruled out. However, more studies are needed to clarify a putative sexually dimorphic action of *MKRN3*.

Girls from Asian countries with *MKRN3* mutations exhibited a tendency of older age at pubertal onset than girls from western countries (median, 6.8 years *vs* 6.0 years), older age at diagnosis (median, 8.0 years *vs* 6.7 years), and lower FSH levels (median, 3.0 IU/L *vs* 4.5 IU/L). It is well known that pubertal timing is influenced by genetic, metabolic, nutritional, and ethnic factors. In previous reports, menarche in Asian girls occurred later than in girls from western countries (at an average age of 14.5 years) [40]. However, two recent Asian studies reported that the mean age at menarche of Chinese and Korean girls declined to 12.6 and 12.7 years, respectively, throughout the past decade [41, 42]. These recent findings are similar to European and American studies, in which the reported mean age at menarche was near 12.5 years [43, 44]. The differences in clinical presentation of Asian girls with *MKRN3* mutations from that of girls from western countries may have been influenced by the difference observed in mutation subtypes, ethnicity, and environmental factors, such as diet and exposure to endocrine disruptors. In particular, higher body mass index and body adiposity are associated with earlier onset of puberty in girls [35, 45]. Unfortunately, it was not possible to analyze differences in body mass index between the groups because this information was absent in most of the reports. Additionally, interactions of metabolic and environmental factors that could potentially coregulate *MKRN3* signaling pathways are still under investigation. Other possibility may be attributed to selection bias. For example, selection criteria for *MKRN3* testing may have been more comprehensive for Asian studies compared with western studies.

Regarding treatment-related outcomes, patients with CPP caused by *MKRN3* mutations seem to be satisfactorily treated with GnRH analogs, although information regarding

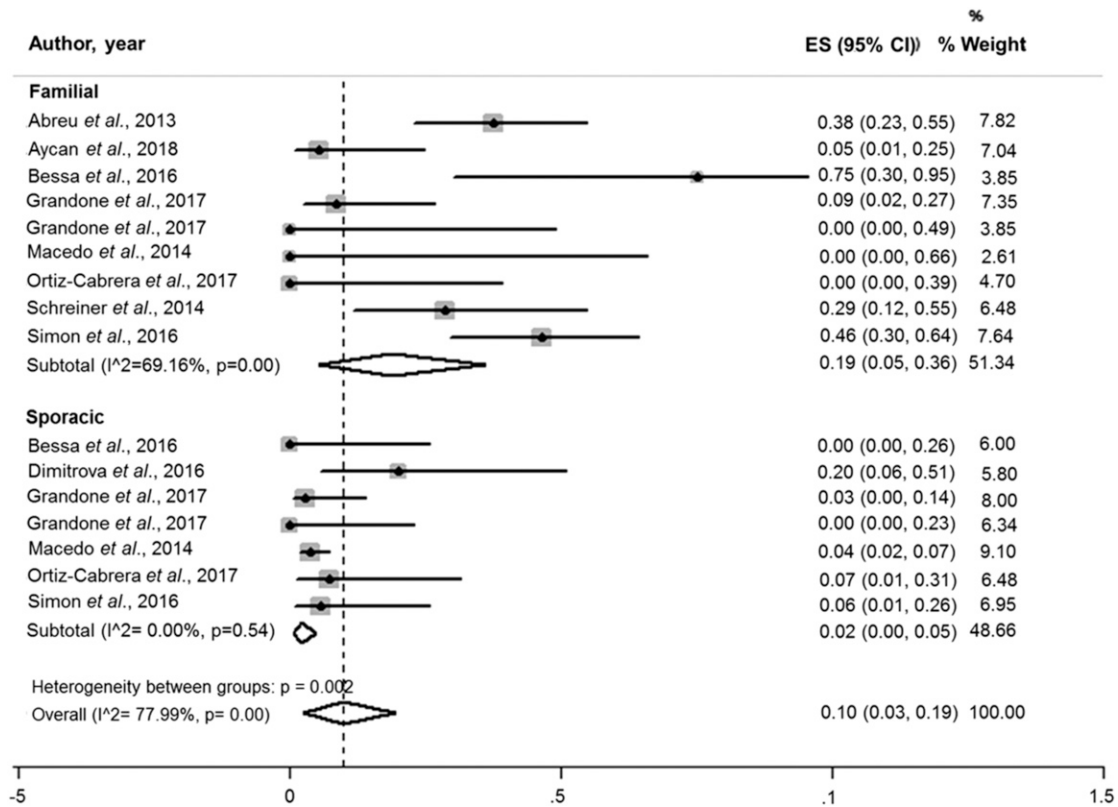


Figure 5. Pooled prevalence of *MKRN3* mutations according to family history. The pooled prevalence of *MKRN3* mutations in patients with familial CPP was 19.0% (95% CI, 0.05 to 0.36; I^2 , 69.16%). Considering the sporadic cases, the pooled estimates for prevalence was 2.0% (95% CI, 0.01 to 0.04; I^2 , 0%).

response to treatment was absent in almost one-third of the cases [4, 6, 9, 10, 12, 13, 15, 29]. The predicted final height appears to be preserved; however, the limited number of patients in whom this information was available precludes a definite conclusion about the potential of achieving target height [5, 12].

Early pubertal timing and menarche have been associated with an increased risk of obesity, type 2 diabetes, cardiovascular disease, and estrogen-dependent cancers [2, 46, 47]. It is still not known if *MKRN3* mutations are associated with a higher risk for adverse health outcomes in later life in comparison with patients without mutations. Long-term clinical follow-up of these patients is necessary to clarify this issue.

MKRN3 is expressed ubiquitously in human tissues. The human protein has 507 amino acids and contains a typical C3HC4 RING zinc finger motif and several C3H zinc finger motifs with predicted E3 ubiquitin-ligase activity and RNA-binding activity, respectively [18]. To date, 39 inactivating mutations in the coding sequence (including nonsense, missense, and frameshift mutations) and 1 promotor deletion have been described in 89 patients worldwide. The fact that more girls ($n = 76$) than boys ($n = 13$) have been identified to harbor such mutations may reflect the well-known higher prevalence of precocious puberty in girls than in boys [2]. Recent familial CPP studies have demonstrated that affected males were retrospectively identified based on medical history and genetic analysis and perhaps this prevalence might reflect an under diagnosis of CPP in the male group [7, 11]. Another explanation could be the relative deficit of boys among the offspring of men with *MKRN3* mutations, as observed by Simon *et al.*, who speculated that Y-bearing sperm cells carrying *MKRN3* mutations may be defective [7]. Accordingly, in this systematic review, the offspring of 41 *MKRN3* mutated men consists of 68 (71%) girls and 28 (29%) boys, supporting this hypothesis. However, confirmation in a larger group of men with *MKRN3* mutations is required for a definitive conclusion.

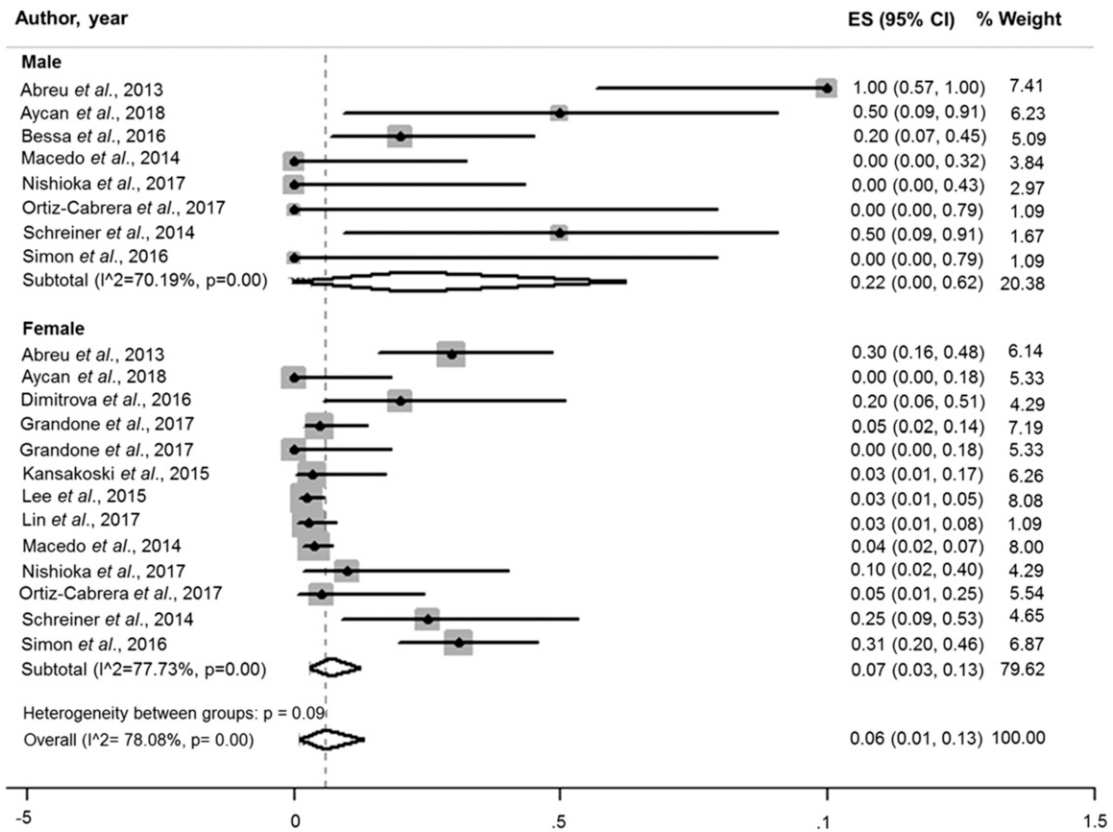


Figure 6. Pooled prevalence of *MKRN3* mutations according sex. The prevalence of *MKRN3* mutations in males was 22.0% (95% CI, 0.0 to 0.62; I^2 , 70.19) and in females 7.0% (95% CI, 0.03 to 0.13; I^2 , 77.73%).

Mutations are distributed over the entire coding region, but tend to cluster in the loop structure between two C3H zinc finger motifs in the aminoterminal region of the protein. Notably, the poly-C region between nucleotides 476 and 482 seems to be a mutation hotspot, comprising 5 of the common frameshift mutations identified in almost one-third of the patients. In particular, the p.Ala162Glyfs15* mutation was identified in 8 families from France and Italy [7], suggesting a possible founder effect among individuals from those countries.

The C3HC4 RING zinc finger motif, which is responsible for the ubiquitin-ligase activity, was recognized as another region where mutations, especially missense mutations, tend to cluster. This may reflect the location of functionally important amino acid residues within the protein and suggests that variants in this domain are more likely to cause deleterious effects on protein function. It is notable that six of the missense mutations were predicted to be benign according to *in silico* analysis [12, 29]. However, considering the typical phenotype of the girls harboring these missense variants, it is reasonable to assume their role in the pathogenesis of CPP, but complementary functional studies would strengthen this association.

Abnormalities in the regulatory region of *MKRN3* may be rarely implicated in the pathogenesis of CPP because a deletion in the promotor region has been recently identified in one girl [31]. Other genetic or epigenetic mechanisms that could disrupt the expression of imprinted genes, such as deletions, uniparental disomy or alterations in DNA methylation markers within the imprinted cluster have not been detected so far, indicating these abnormalities may represent very rare mechanisms in this disorder, although they have not been widely investigated to date [5, 7].

Currently, *MKRN3* mutations represent the main genetic cause of CPP. The prevalence is quite variable, ranging from 0 in a small sample from Italy [19] to 46% considering

the subgroup of patients with familial CPP reported by Simon *et al.* [7]. In the current meta-analysis of 14 studies including 857 patients with CPP, the pooled overall prevalence of *MKRN3* mutations was 9.0% (95% CI, 0.04 to 0.15). Interestingly, our data set demonstrates geographic predominance of *MKRN3* mutations in patients from western countries (prevalence estimates of 11%; 95% CI, 0.04 to 0.21) and a lower frequency of mutations in patients from Asian countries (2%; 95% CI, 0.01 to 0.04), suggesting genetic variation according to ethnic background. In addition, it is noteworthy that specific single nucleotide polymorphisms near the paternal allele of *MKRN3* have also been associated with earlier age at menarche in large populational dataset studies, although those articles were not included in our meta-analysis because they did not fulfill the inclusion criteria [48, 49].

Our meta-analysis also supports the previous observation that the frequency of *MKRN3* mutations is higher among boys with CPP than in girls (pooled prevalence of 22%; 95% CI, 0.00 to 0.62 *vs* 7%; 95% CI, 0.03 to 0.13, respectively). Considering that *MKRN3* is located on an autosomal chromosome, the reasons underlying the predominance of mutations in males are still not understood. Of note, all mutated boys exhibited a positive family history of precocious puberty; these data highlight the importance of evaluating boys, especially those with familial CPP, for *MKRN3* mutations.

The prevalence of mutations in familial cases was variable among different studies. Our meta-analysis showed a pooled prevalence of 19.0% (95% CI, 0.05 to 0.36) in patients with familial CPP from western countries, with a moderate level of heterogeneity (I^2 , 69.16%). Potential sources of variation could be due to different criteria of selection of familial cases and small sample sizes in some reports. In addition, considering the imprinted pattern of inheritance in which a generation can be skipped, the familial nature may be underrecognized if not carefully investigated, whereas the prevalence of *MKRN3* mutations in familial cases might have been underestimated in some series.

Our study also had some potential limitations. First, a meta-analysis of specific association between genotype and phenotype was not possible because of the limited number of studies comparing clinical data of patients with and without *MKRN3* mutations. Second, the number of affected boys identified so far is still small and information about pubertal onset was absent in some of them. Further limitations relate to the type of studies, with exclusively observational data, including case reports and case series, to the small sample size of some reports and to inconsistency of study designs. Thus, larger studies evaluating patients with idiopathic CPP and with comparative phenotypic analysis of individuals with and without mutations are needed to establish a more precise genotype-phenotype relationship.

In summary, our meta-analysis shows that *MKRN3* mutations are associated with the phenotype of nonsyndromic CPP. Girls are more severely affected than boys and exhibit an early age at pubertal onset, higher basal FSH levels, and greater Δ BA. Overall, the pooled estimated prevalence of *MKRN3* mutations in CPP was 9.0% (95% CI, 0.04 to 0.15), with variations according to sex, family history, and geographical distribution. Additional studies are needed to establish a precise genotype-phenotype relationship and long-term follow-up of patients harboring *MKRN3* mutations is necessary to clarify whether they exhibit higher risks of adverse health outcomes later in life.

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