Outcomes of Patients with Central Precocious Puberty Due to Loss-of-Function Mutations in the *MKRN3* Gene after Treatment with Gonadotropin-Releasing Hormone Analog

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Keywords

Central precocious puberty · Gonadotropin-releasing hormone analog · *MKRN3* · Final height · Body composition · Gonadal function

Abstract

Introduction: Loss-of-function mutation of *MKRN3* represents the most frequent genetic cause of familial central precocious puberty (CPP). The outcomes of gonadotropin-releasing hormone analog (GnRHa) treatment in CPP patients with *MKRN3* defects are unknown. **Objective:** To describe the clinical and hormonal features of patients with CPP with or without *MKRN3* mutations after GnRHa treatment. Anthropometric, metabolic and reproductive parameters were evaluated. **Patients and Methods:** Twenty-nine female patients with CPP due to loss-of-function mutations in the *MKRN3* and 43 female patients with idiopathic CPP were included. Their medical records were retrospectively evaluated for clinical, laboratory, and imaging study, before, during,

and after GnRHa treatment. All patients with idiopathic CPP and 11 patients with CPP due to *MKRN3* defects reached final height (FH). **Results:** At the diagnosis, there were no significant differences between clinical and laboratory features of patients with CPP with or without *MKRN3* mutations. A high prevalence of overweight and obesity was observed in patients with CPP with or without *MKRN3* mutations (47.3 and 50%, respectively), followed by a significant reduction after GnRHa treatment. No significant differences in the values of mean FH and target height were found between the 2 CPP groups after GnRHa treatment. Menarche occurred at the expected age in patients with or without CPP due to *MKRN3* mutations (11.5 ± 1.3 and 12 ± 0.6 years, respectively). The prevalence of polycystic ovarian syndrome was 9.1% in patients with CPP due to *MKRN3* mutations and 5.9% in those with idiopathic CPP. **Conclusion:** Anthropometric, metabolic, and reproductive outcomes after GnRHa treatment were comparable in CPP patients, with or without *MKRN3* mutations, suggesting the absence of deleterious effects of *MKRN3* defects in young female adults' life.

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Introduction

Central precocious puberty (CPP) refers to the onset of pubertal signs before the age of 8 in girls and 9 in boys because of the early activation of the hypothalamic-pituitary-gonadal axis [1, 2]. The absence of organic abnormalities of the central nervous system or genetic causes classifies CPP as “idiopathic.” In contrast, several cerebral malformations and acquired insults can cause “organic” CPP [3]. CPP can also be classified as sporadic or familial forms [2, 3].

In the last decade, the genetic causes of CPP were identified in previously considered idiopathic CPP. Abreu et al. [4] identified loss-of-function mutations in *MKRN3* (makorin ring-finger 3 gene), in 33% of the families who had members with CPP. *MKRN3* is a maternally imprinted gene, located at chromosome 15q11.2 in the Prader-Willi syndrome critical region, and it encodes the makorin ring-finger protein 3 with a potential inhibitory effect on gonadotropin-releasing hormone (GnRH) secretion [4]. The reduction in *MKRN3* expression in arcuate nucleus at the onset of puberty in mice, together with the identification of loss-of-function mutations in humans with familial CPP, implies that *MKRN3* might control puberty by repressing downstream targets instead of activating the GnRH neuronal network [4, 5]. To date, loss-of-function mutations in *MKRN3* represent the most frequent genetic cause of familial CPP [6, 7]. In addition, a rare deletion in the regulatory region of *MKRN3* was also described in 1 patient with apparently sporadic CPP [8].

Since the 1980, long-acting GnRH analog (GnRHa) has been the first-line treatment for CPP of all etiologies [9, 10]. Several studies on long-term outcomes after discontinuation of GnRHa treatment in patients with idiopathic CPP have been reported [9, 11–15]. It has been demonstrated that GnRHa treatment preserved the genetic adult height potential in patients with idiopathic CPP especially if diagnosed before the age of 6 years [10]. Overweightness and obesity are common at the diagnosis of CPP, and GnRHa treatment has no impact on the body mass index (BMI). However, an increase of body fat mass percentage has been demonstrated during GnRHa treatment [16, 17]. It has been demonstrated that gonadal function is completely recovered in patients after discontinuation of GnRHa treatment, and reports on the prevalence of polycystic ovarian syndrome (PCOS), in both treated and untreated women, still remain controversial [15, 17]. However, there is no report on the long-term follow-up of patients with CPP due to *MKRN3* mutations after GnRHa treatment.

In this study, we described the initial clinical and laboratory features of 29 CPP patients with CPP due to loss-of-function mutations of *MKRN3*, as well the long-term follow-up features after GnRHa treatment of a subset of 11 female patients, emphasizing on anthropometric, metabolic, and reproductive outcomes during early adulthood. Furthermore, their data were compared with those from 43 patients with idiopathic CPP treated with GnRHa.

Patients and Methods

This is an observational, retrospective, and longitudinal study of a multicenter cohort of 72 female patients with CPP (29 patients with CPP due to *MKRN3* mutations and 43 with idiopathic CPP). Fifty-one female patients were diagnosed and treated at the Developmental Endocrinology Unit of the Endocrinology and Metabolism Division of the Sao Paulo University Medical School, between 1985 and 2019. The remaining 21 female patients with CPP due to *MKRN3* mutations were referred from other national (Ribeirao Preto Medical School, University of Sao Paulo; Universidade Estadual de Campinas, Faculdade de Ciências Médicas; private office Governador Valadares-MG; Universidade do Estado do Rio de Janeiro-RJ) and international Services (Division of Endocrinology, Diabetes, and Metabolism, Nemours Children’s Clinic, Jacksonville, FL, USA; Department of Pediatrics, University Hospital Gasthuisberg, University of Leuven, Leuven, Belgium; Hospital de Niños R. Gutiérrez, Buenos Aires, Argentina; Department of Pediatric Endocrinology, University of Health Sciences, Umraniye Training and Research Hospital, Adem Yavuz Caddesi, Elmalikent Mah. Yekta Sok., Istanbul, Turkey; Pediatric Endocrine and Diabetes Unit the Chaim Sheba Medical Center, Israel) for genetic study at the Laboratory of Hormones and Molecular Genetics LIM/42 of the Medical School of the University of São Paulo and they followed their directions.

The diagnosis of CPP was based on pubertal development before the age of 8 years, accelerated growth velocity, advanced bone maturation, and pubertal basal and/or GnRH-stimulated LH levels [1]. All patients were treated exclusively with GnRHa, with monthly (3.75 mg leuporelin) and/or quarterly dosage (11.25 mg leuporelin). Height was considered final if the growth rate was >0.5 cm/year during the preceding year and bone age (BA) was >15 years [13].

Serum LH (luteinizing hormone), FSH (follicle stimulating hormone), total testosterone, and estradiol levels were measured by radioimmunoassay till 1991, immunofluorometric assay (IFMA, AutoDELFIA, Turku, Finland) till 2012, and electroimmunoluminescence assays (ECLIA, cobas e601, Roche Diagnostics, Indianapolis, IN, USA) after 2012. Basal LH >0.6 IU/L (IFMA) or >0.3 IU/L (ECLIA) were considered as pubertal levels [18, 19]. Progesterone, DHEA-S (dehydroepiandrosterone sulfate), and insulin levels were assessed by ECLIA. Androstenedione was assessed by liquid chromatography and mass spectrometry. All assays presented intra- and inter-assay coefficients of variation <7%. Pubertal response was considered when GnRH/GnRHa-stimulated LH peak was >15 IU/L for girls and >25.5 IU/L for boys (radioimmunoassay), >6.9 IU/L for girls and >9.6 IU/L for boys

(IFMA), and >8 IU/L for both sexes (ECLIA) [18, 19]. BA was assessed using the Greulich and Pyle's method [20].

All patients underwent brain magnetic resonance imaging study, which included pre- and post-gadolinium-enhanced sagittal T1-weighted images, as well as coronal T1- and T2-weighted images, with 3-mm slices [21].

The GnRHa depot (3.75 or 11.25 mg leuporelin acetate) was administered subcutaneously or intramuscularly, every 28 or 84 days respectively. The clinical and laboratory parameters were monitored every 3 months to assess the regression or stabilization of secondary sexual characters, adequate growth velocity, and suppressed sexual steroids levels with basal and/or GnRH-stimulated LH levels at prepubertal range (basal LH <0.6 IU/L for IFMA and <0.2 IU/L for ECLIA; LH: 120 min after GnRHa <4 IU/L) [22]. BA was assessed yearly once. GnRHa treatment was discontinued when normal puberty could be expected (10–14 years) and, ideally, when normal height for chronological age (CA) and BA was reached. Target height (TH) was calculated from parents' height measurements and determined as follows: mid-parental height minus 6.5 cm for girls or plus 6.5 cm for boys. TH range was established as $TH \pm 8.5$ cm [23, 24].

The BMI (weight [kg]/square of height [m]) was calculated using documented anthropometric measurements. Among children and adolescents (<18 years), BMI percentiles were used to define overweightness (≥ 85 th) and obesity (≥ 95 th) [25]. For BMI calculation in adults, the index of body weight, according to the WHO criteria, was applied: underweight <18.5 kg/m², normal weight 18.5–24.9 kg/m², overweight 25.0–29.9 kg/m², and obesity >30 g/m² [26].

The assessment of body composition was done using multi-frequency bioimpedance analysis (In-Body 720, Biospace, Seoul, Korea); in this method, the body composition is analyzed by plethysmography multifrequency, in which body readings are made using 8 electrodes measuring the resistance in 5 specific frequencies and reactance in 3 specific frequencies.

Metabolic parameters were assessed by measuring fast glucose, insulin, HOMA-IR, total cholesterol and fractions (HDL and LDL), and triglycerides levels. Insulin resistance was considered to prevail at the value of HOMA-IR >3.80 [27]. Blood pressure was assessed in all patients and hypertension was defined with values of systolic blood pressure ≥ 140 mm Hg and diastolic blood pressure ≥ 90 mm Hg [25].

After the discontinuation of GnRHa treatment, resumption of menarche, menstrual cycle pattern, achievement of pregnancy, and birth of live fetus were documented in females. The reproductive function in females during adulthood was evaluated by the measurement of gonadotropins and estradiol at the follicular phase as well as uterine length, ovarian morphology through pelvic ultrasound at the follicular phase, and progesterone levels at the luteal phase. The polycystic ovarian morphology was defined by the presence of 12 or more follicles of 2–9 mm in diameter and/or an increased ovarian volume >10 mL (without a cyst or dominant follicle) in either ovary [28].

Signs of clinical hyperandrogenism, including acne (severity and localization) and presence and degree of hirsutism (scored by Ferriman-Gallwey scale), were also evaluated. Laboratory hyperandrogenemia was assessed by measuring DHEA-S, total testosterone, and androstenedione serum levels at the follicular phase. The diagnosis of PCOS was done according to Rotterdam's criteria [29].

Genetic Analysis

Genomic DNA was isolated from peripheral blood leukocytes of all patients and selected family members using standard procedures (salting-out method). The entire coding region (intronless gene) and the promoter region of *MKRN3* were amplified by polymerase chain reaction followed by automatic sequencing of the products using the Sanger method in all patients, according to previously published protocol [4, 8].

Statistical Analysis

Statistical analysis was performed using SigmaStat for Windows version 3.5, Systat Software, Inc. Data are presented as mean $\pm$ SDs or median and range. Comparisons among values of means of numerical variables were made using Student *t* test or Mann-Whitney U test, when appropriate. Categorical variables were compared using chi-square test. For statistical purposes, the percentages of overweight and obesity (categorical variables) in each group were grouped. Correlations between 2 variables were determined by Pearson's correlation coefficient analysis. Statistical significance was set at $p < 0.05$.

Results

Clinical and Hormonal Data at the Onset of Puberty

The mean CA of onset of puberty was 5.9 ± 1.2 years and 6.4 ± 1.3 years in patients with CPP due to *MKRN3* mutations and idiopathic CPP groups respectively ($p = 0.12$). In addition, the mean BA at the onset of puberty was 9 ± 1.6 years and 10.5 ± 1.3 years in patients with CPP due to *MKRN3* mutation and idiopathic CPP groups respectively ($p < 0.001$). Familial history of precocious puberty was positive in all patients with CPP due to *MKRN3* mutations and 25.6% of those with idiopathic CPP. The mean height SDS at the diagnosis was 1.4 ± 0.9 in patients with CPP due to *MKRN3* mutation and 1.7 ± 1.1 in patients with idiopathic CPP ($p = 0.34$). At the onset of puberty, the prevalence of overweightness and obesity was observed in 47.3% of girls with CPP due to *MKRN3* mutations and 50% of those with idiopathic CPP.

At the diagnosis, mean basal LH was at pubertal levels in 96% of patients with CPP due to *MKRN3* mutations and in 73.1% of patients with idiopathic CPP (1.4 ± 0.9 vs. 1.7 ± 1.7 IU, $p = 0.35$). The mean GnRH-stimulated LH peak was at the pubertal range in all patients from both groups, 24.9 ± 19.8 vs. 18.7 ± 11.2 IU in patients with CPP due to *MKRN3* mutations and idiopathic CPP groups, respectively, $p = 0.8$. Mean basal FSH and estradiol levels before GnRHa treatment did not differ between groups, $p = 0.8$ and 0.17 , respectively. Clinical and hormonal data at the diagnosis are summarized in the Table 1.

In the 29 patients with CPP due to *MKRN3* mutations, 13 distinct inactivating mutations in the coding region of

Table 1. Initial clinical and hormonal features of female patients with CPP due to *MKRN3* loss-of-function mutations and idiopathic form

	CPP due to <i>MKRN3</i> mutation	Idiopathic CPP	<i>p</i> value
Number	29	43	
CA at pubertal onset, years, mean $\pm$ SD	5.9 $\pm$ 1.2	6.4 $\pm$ 1.3	0.12
CA at first visit, years, mean $\pm$ SD	6.8 $\pm$ 0.9	8.1 $\pm$ 1.3	<0.001
Pubertal stage			
Breast development	B2–B5	B2–B4	
Pubic hair	PH1–PH3	PH1–PH3	–
Menarche	None	3/43	
Familial CPP history, %	100	11/43 (25.6)	NA
Initial BA, years, mean $\pm$ SD	9 $\pm$ 1.6	10.5 $\pm$ 1.3	<0.001
Δ BA – CA, years, mean $\pm$ SD	2.1 $\pm$ 1.4	2.3 $\pm$ 1.2	0.7
Height, cm, mean $\pm$ SD	127.8 $\pm$ 8.2	135.6 $\pm$ 8.1	0.02
Height, mean $\pm$ SD	1.4 $\pm$ 0.9	1.7 $\pm$ 1.1	0.34
BMI, kg/m ² *	16.8 (15.6–19.3)	17.9 (12.2–22.6)	0.14
Overweight/obesity, %	47.3 (26.3/21)	50 (26.2/23.8)	0.16
Basal LH, IU/L, mean $\pm$ SD	1.4 $\pm$ 0.9	1.7 $\pm$ 1.7	0.35
LH peak, IU/L, mean $\pm$ SD	24.9 $\pm$ 19.8	18.7 $\pm$ 11.2	0.18
Basal FSH, IU/L, mean $\pm$ SD	4.6 $\pm$ 1.9	4.4 $\pm$ 2.8	0.8
Basal estradiol, ng/mL, mean $\pm$ SD	35.4 $\pm$ 22.1	27.8 $\pm$ 19.4	0.17

* Median (interval).

CPP, central precocious puberty; CA, chronological age; BA, bone age; BMI, body mass index; NA, not applicable.

MKRN3, and 1 deletion in the promoter region were identified. Twenty-two patients (75.8%) had severe mutations (18 frameshifts, 3 nonsense mutations, and 1 deletion) and 7 patients had missense mutations.

During GnRHa treatment, all patients with CPP patients, with or without *MKRN3* mutation, reached adequate clinical and hormonal controls, according to clinical and laboratory criteria mentioned earlier in this manuscript (data not shown).

Anthropometric Data after GnRHa Treatment and at the Final Height

Final height (FH) was achieved in 11 patients with CPP due to *MKRN3* mutations and in all patients with idiopathic CPP. In these patients, the mean duration of GnRHa treatment was 2.9 $\pm$ 0.9 and 2.6 $\pm$ 1.1 years in patients with CPP due to *MKRN3* mutation and idiopathic CPP, respectively, (p = 0.55). In the patients with CPP due to *MKRN3* mutations, the mean CA and BA at the end of GnRHa treatment was 10.6 $\pm$ 1 and 12.5 $\pm$ 0.9 years, respectively. In the idiopathic CPP group, the mean CA and BA at the end of GnRHa treatment was 10.9 $\pm$ 0.5 and 12.3 $\pm$ 0.7 years respectively. The mean height SDS at the end of treatment was 1.2 $\pm$ 1.3; and there was prevalence of

overweight, or obesity was present in 36.2% of patients with CPP due to *MKRN3* mutations group. In patients with idiopathic CPP, the mean height SDS at the end of treatment was 1.3 $\pm$ 1.1; and there was prevalence of overweight or obesity was present in 47.5% of the patients. There were no statistically significant differences in these variables between groups (p > 0.05; Table 2). At the last visit, the mean CA of the patients with CPP due to *MKRN3* mutations and idiopathic CPP was 18.6 $\pm$ 4 and 16.6 $\pm$ 3.2 years respectively (p = 0.14). The mean post-treatment growth until reaching FH was 9.6 $\pm$ 3.6 and 10.1 $\pm$ 3.5 cm in patients with CPP due to *MKRN3* mutations and idiopathic CPP, respectively, (p = 0.6). The mean FH was 157.6 $\pm$ 5.1 and 161.3 $\pm$ 6.5 cm in patients with CPP due to *MKRN3* mutation and idiopathic CPP groups respectively (p = 0.09). All but 1 patient (patient 9 – Table 3) with CPP due to *MKRN3* mutation were within their TH range. The FH of 42/43 patients was within their TH range in the idiopathic CPP group, and no statistically significant difference was found between mean FH and TH. With regard to the severity of *MKRN3* mutations, 10 out of 11 patients harbored severe mutations (7 frameshifts, 2 nonsense mutations, and 1 deletion in the promoter region) and 1 patient had a missense *MKRN3* mutation (Table 3).

Table 2. Clinical and hormonal data of female patients with CPP due to *MKRN3* loss-of-function mutations and idiopathic form at the end of GnRHa treatment, and at final height

	CPP due to <i>MKRN3</i> mutations	Idiopathic CPP	<i>p</i> value
Number	11	43	
At end of treatment, mean ± SD			
CA, years	10.6±1	10.9±0.5	0.1
BA, years	12.5±0.9	12.3±0.7	0.43
Δ BA – CA, years	2±1.2	1.4±0.8	0.1
Overweight/obesity, %	36.2	47.5	0.16
BMI, kg/m ² *	19.8 (15.9–29.3)	20.7 (15.5–30)	0.4
Height, cm	148±5.4	151.2±5.6	0.12
Height	1.2±1.3	1.3±1.1	0.68
Duration of treatment, years	2.9±0.9	2.6±1.1	0.55
At final height, mean ± SD			
CA, years	18.6±4	16.5±3.4	0.14
Age at menarche, years	11.5±1.3	12±0.6	0.1
Interval (age at menarche – end of GnRHa treatment), years	0.9±0.7	1.1±0.5	0.27
Post-treatment growth, cm	9.6±3.7	10.2±3.7	0.6
Height, cm	157.6±5.4	161.4±6.6	0.09
Height	–0.7±0.9	–0.15±1.1	0.1
Target height, cm	159.2±5.6	159±6	0.94
Target height	–0.5±0.9	–0.5±1	0.94
BMI, kg/m ² *	21.4 (17.4–36.8)	22.7 (17.4–31)	0.6
Overweight/obesity, %	27.3	33.3	0.16
%fat mass, mean ± SD, <i>n</i>	35.4±16.7 (3)	31.6±6.7 (28)	0.43
Metabolic abnormalities			
Insulin resistance, <i>n</i>	1/7	4/35	0.8
High LDL, <i>n</i>	2/7	3/35	0.2
Total metabolic dysfunction, <i>n</i> (%)	3/7 (42.8)	6/35 (17.1)	0.25
Irregular menstrual cycles, %	18.2	18.1	1.0
Hormonal data**, mean ± SD, <i>n</i>			
LH, IU/L*	4.5 (0.8–19; 6)	3.1 (1.3–35.5; 34)	0.5
FSH, IU/LS	4.2±1.9 (6)	4.9±1.6 (34)	0.3
Estradiol, pg/mL*	37.7 (17–247; 6)	59 (14.6–391; 34)	0.4
DHEA-S, ng/mL	2,166±1,875 (4)	2,100±884 (34)	0.9
Androstenedione, ng/mL	1±0.4 (3)	1.4±0.7 (34)	0.4
Total testosterone, ng/dL*	39.5 (12–93; 4)	31 (12–79; 34)	0.8
PCOS, %	9.1	5.9	0.4

* Median (interval).

** Reference values: LH: 2.4–12.8 IU/L; FSH: 1.5–12.4 IU/L; Estradiol: <10–122 pg/mL; DHEA-S: 1,480–4,070 ng/mL; androstenedione: 0.25–2.2 ng/mL; total testosterone: <48 ng/dL.

CPP, central precocious puberty; GnRHa, gonadotropin-releasing hormone analog; CA, chronological age; BA, bone age; BMI, body mass index; PCOS, polycystic ovarian syndrome.

The prevalence of overweight or obesity at the FH in patients with CPP due to *MKRN3* mutation was 27.3% (3/11). In this group, only 3 patients underwent body composition analysis with a high mean percentage of body fat mass (35.4 ± 13.6% – reference value: 18–28%). In the idiopathic CPP group, the prevalence of over-

weight and obesity at the FH was 34.8% (15/43) and the mean percentage of body fat mass was 31.6 ± 6.6%. The prevalence of categorical BMI percentiles and BMI revealed a significant decrease in the percentage of overweight and obesity from the diagnosis to FH in both groups (*p* < 0.05; Fig. 1).

Table 3. Genetic and clinical data of 11 patients with CPP due to *MKRN3* mutations after GnRHa treatment

Patient	<i>MKRN3</i> mutations	CA at last visit, years	Post treatment growth, cm	TH, cm	TH, SD	BMI	FH, cm	FH, SD
1	p.Pro161Argfs*10	20.6	9.5	153	−1.5	36.8	158.5	−0.6
2	p.Gln226Thrfs*6	29.9	8	161.5	−0.1	28.4	165	0.4
3	p.Glu256Glyfs*36	16.5	9	156.5	−0.9	23.4	153	−1.5
4	c.-150_-147delTCAG	16.3	9.9	163.5	0.2	21.4	162	0
5	p.Pro161Argfs*16	18.9	8.5	160.5	−0.3	19.4	160.5	−0.28
6	p.Pro161Argfs*10	16	7.5	159.5	−0.45	23.2	160.5	−0.28
7	p.Phe417Ile	14.6	13.5	156.2	−1	17.9	162	0
8	p.Pro161Profs*16	15.7	1.5	152	−1.7	31.3	149	−2.2
9	p.Ala162Glyfs*15	18	11.5	170	1.3	18.1	159	−0.53
10	p.Tyr391*	20	10	NA	NA	20.1	148.5	−2.2
11	p.Tyr391*	18	16.5	NA	NA	NA	156	−1

CPP, central precocious puberty; GnRHa, gonadotropin-releasing hormone analog; CA, chronological age; TH, target height; FH, final height; BMI, body mass index; NA, not available.

Fig. 1. Representation of the prevalence of categorical BMI at the diagnosis, at the end of GnRHa treatment, and at the FH in patients with CPP due to *MKRN3* mutations and idiopathic CPP. CPP; central precocious puberty; GnRHa, gonadotropin-releasing hormone analog.

Correlations between Variables Influencing FH

In patients with CPP due to *MKRN3* mutation, there were positive and significant correlations between FH and height SDS at the diagnosis and the end of GnRHa treatment ($r = 0.8$, $p = 0.005$, and $r = 0.76$, $p = 0.0063$ respectively).

Regarding FH of the 43 girls with idiopathic CPP, there were a positive and significant correlations between FH SDS and height SDS at the diagnosis and the end of GnRHa treatment ($r = 0.734$, $p < 0.0001$ and $r = 0.79$, $p < 0.001$, respectively), as well as between FH and post-treat-

ment growth after GnRHa ($r = 0.5$, $p = 0.0006$), and FH and TH ($r = 0.44$, $p = 0.004$).

Metabolic and Reproductive Outcomes

The long-term follow-up of the metabolic and reproductive parameters was done for a subset of patients with CPP: 7 patients with CPP due to *MKRN3* mutations and 35 patients with idiopathic CPP. In patients with CPP due to *MKRN3* mutations, insulin resistance was identified in 1 patient and high LDL levels were identified in 2 patients with high BMI, indicating that 3 out of 7 patients had

metabolic abnormalities; hypertriglyceridemia was not identified in these patients. In the 35 patients with idiopathic CPP, insulin resistance was identified in 4 patients (11.4%), high LDL levels in 3 patients (8.6%), and hypertriglyceridemia in 1 patient (2.8%; Table 2). Therefore, metabolic abnormalities were present in 17.1% of patients with idiopathic CPP. These metabolic abnormalities occurred in patients with normal and high BMI. Diabetes and hypertension were not observed in this cohort (Table 2).

In patients with CPP due to *MKRN3* mutation and those with idiopathic CPP, the mean interval between the discontinuation of GnRHa treatment and the occurrence of spontaneous menarche was 0.9 ± 0.7 and 1.1 ± 0.5 years, respectively, $p = 0.27$. In addition, the mean CA of menarche was 11.5 ± 1.3 and 12 ± 0.6 years for patients with CPP due to *MKRN3* mutations and those with idiopathic CPP, respectively, $p = 0.1$. Abnormal menstrual cycles were reported by 2 out of 11 (18.2%) patients with CPP due to *MKRN3* mutation and by 6/33 (18.1%) patients with idiopathic CPP. Clinical and/or laboratory criteria for hyperandrogenism were positive for 4 patients with CPP due to *MKRN3* mutations and for 34 patients with idiopathic CPP (Table 2). One patient with CPP due to *MKRN3* mutation and 4 patients with idiopathic CPP had hyperandrogenism. One patient with CPP due to *MKRN3* mutation and 6 patients with idiopathic CPP fulfilled criteria for polycystic ovarian (ovarian volume >10 mL) in pelvic ultrasound. One patient with CPP due to *MKRN3* mutation and 2 with idiopathic CPP group were positive for PCOS criteria, which was in accordance with the Rotterdam PCOS criteria [29]. Basal progesterone levels were at an ovulatory range in 2 and 6 patients with CPP due to *MKRN3* mutations and idiopathic CPP respectively. One patient became pregnant and conceived a healthy offspring. Clinical and laboratory data at adulthood are summarized in Table 2.

Discussion

Long-acting GnRHAs are the treatment of choice for CPP, and outcomes after treatment of idiopathic CPP have been described in several studies [3, 10, 30]. In contrast, long-term follow-up of patients with CPP due to loss-of-function mutations of *MKRN3* after GnRHa withdrawal has not been reported so far [6, 31, 32]. In the present study, we have described the initial clinical and hormonal data of 29 female patients with CPP due to *MKRN3* mutations and long-term follow-up of a subset group (11

patients), taking into consideration anthropometric, metabolic, and reproductive features.

In this study, the mean CA of pubertal onset was similar in patients with CPP due to *MKRN3* mutations and in those with idiopathic CPP, confirming the data reported previously [31, 32]. In contrast with our data, Simon et al. [6] demonstrated that girls with CPP due to *MKRN3* mutation were younger at puberty onset than those without *MKRN3* mutation [6].

A systematic review and meta-analysis on *MKRN3* mutations concerning CPP, which was reported earlier, included 22 studies comprising 880 patients with CPP [32]; 89 (76 girls) of them had *MKRN3* defects [32]; in the pooled comparative analysis of clinical and biochemical data between girls and boys, the authors reported significant differences in clinical (age at pubertal onset and at diagnosis) and hormonal (basal FSH) parameters, which is related to sex-dimorphic characteristics. Conversely, we compared data between female patients with and without *MKRN3* mutations, and no clinical or laboratory parameter was indicative of harboring *MKRN3* defects. Regarding hormonal data, Macedo et al. [31] previously reported a higher level of mean basal FSH in girls with *MKRN3* mutations compared to those without *MKRN3* mutations.

Regarding family history, the meta-analysis showed a prevalence of *MKRN3* mutation of 19% in patients with familial CPP and 2% in patients with sporadic CPP [28]. In our cohort, the familial history of precocious puberty was positive in all patients with CPP due to *MKRN3* mutations and in 25.6% of patients with idiopathic CPP, suggesting that other genetic defects can be probably involved in the etiology of CPP. It must be noted that the high prevalence of familial history in patients with *MKRN3* mutations in our cohort must be interpreted cautiously, because familial history was an inclusion criterion to perform genetic analysis in some cases.

Whether or not the severity of *MKRN3* mutation has any influence on the clinical and hormonal phenotype of CPP remains unknown. Valadares et al. [32] showed that the age during diagnosis of CPP was significantly low in patients with severe *MKRN3* mutations. The impact of the subtype of *MKRN3* mutations on long-term follow-up of these patients was not evaluated in the present study because most of the patients (10/11) had severe mutations.

Patients with CPP caused by *MKRN3* mutations or idiopathic form were treated with a monthly or 3-month low dosage of leuporelin acetate, 3.75 or 11.25 mg, respectively, and the efficacy of GnRHa did not differ be-

tween these groups, resulting in satisfactory anthropometric outcomes. The FH within TH range was reached in all except 1 patient with CPP due to *MKRN3* mutations and in all but 1 patient with idiopathic CPP. In patients with CPP due to *MKRN3* mutations, we were not able to rule out that the father harboring *MKRN3* mutation had not been affected by CPP, which could negatively influence the TH. No statistically significant difference was found between mean FH and mean TH in treated patients having CPP due to *MKRN3* mutations and treated patients with idiopathic CPP. The present study is the first report on the FH of patients with CPP due to *MKRN3* mutations treated with GnRHa, indicating that *MKRN3* defect did not influence FH.

With regard to the BMI and body composition, the prevalence of overweightness and obesity during diagnosis of CPP was equally elevated in patients with and without *MKRN3* mutations, which is in accordance with several studies that reported high BMI during diagnosis of CPP [11, 12]. At the end of GnRHa treatment, the prevalence of overweight and obesity decreased in both groups. The effect of GnRHa treatment on BMI and body composition was explored in several studies, indicating that during GnRHa treatment, no significant mean or individual BMI-SDS change occurs [11, 12, 33, 34]. However, an increase in the percentage of total fat mass has been demonstrated during GnRHa treatment [16]. At the FH, it is noteworthy that a significant reduction in the prevalence of overweight or obesity was observed in both CPP groups. Other studies have also reported a reduction in BMI in patients with idiopathic CPP [17, 35]. These data suggest that changes in BMI and body composition were not influenced by *MKRN3* mutations. The longitudinal analysis of categorical BMI in both groups revealed similar evolution. This information was lacking in previous studies on *MKRN3* mutations [32].

In a large cohort of treated and untreated women with idiopathic CPP, Lazar et al. [14] demonstrated that the prevalence of obesity-related complications, such as metabolic dysfunctions and cancer comorbidities, was not increased, regardless of GnRHa treatment. In this cohort, the prevalence of metabolic dysfunctions at adulthood was not significantly different between patients with CPP due to *MKRN3* mutations and those with idiopathic CPP. These data can indicate that *MKRN3* has no direct role in metabolic regulation.

As already described, for women with idiopathic CPP and were treated, the gonadal function is restored at the predicted time after cessation of GnRHa treatment, with subsequent menarche and regular ovulatory menstrual

cycles [11, 15]. In our study, female patients with CPP due to *MKRN3* mutations and treated with GnRHa also had normal recovery of the gonadotropic axis after discontinuation of GnRHa treatment. Furthermore, spontaneous menarche occurred at the same CA in patients with CPP due to *MKRN3* mutations and idiopathic form. The prevalence of PCOS was equally low in both groups.

In conclusion, we reported the initial presentation and the longest follow-up of patients with CPP due to *MKRN3* mutations, treated with GnRHa, including anthropometric, hormonal, metabolic, and reproductive profiles. All patients reached normal FH, demonstrating the efficacy of GnRHa in preserving genetic adult height potential, regardless of the etiology of CPP. The prevalence of metabolic and reproductive disorders was similar in patients with CPP due to *MKRN3* mutations and idiopathic CPP, indicating that *MKRN3* mutations are not associated with a distinct short- and long-term follow-up compared with idiopathic CPP.

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Statement of Ethics

The protocol was approved by all the local Ethics Committee. Written informed consent was obtained from all participants and/or their guardians.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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