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MKRN3 circulating levels in girls with central precocious puberty caused by MKRN3 gene mutations

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Abstract

Purpose *MKNR3* is a paternally expressed gene whose mutations are the main cause of central precocious puberty (CPP). Protein circulating levels can be easily measured, as demonstrated in idiopathic CPP and healthy controls. No data are available for patients harboring an *MKRN3* mutation. Our aim was to perform *MKRN3* mutation screening and to investigate if circulating protein levels could be a screening tool to identify *MKRN3* mutation in CPP patients.

Methods We enrolled 140 CPP girls and performed *MKRN3* mutation analysis. Patients were stratified into two groups: idiopathic CPP (iCPP) and MKRN3 mutation-related CPP (MKRN3-CPP). Clinical characteristics were collected. Serum MKRN3 values were measured by a commercially available ELISA assay kit in MKRN3-CPP and a subgroup of 15 iCPP patients.

Results We identified 5 patients with *MKRN3* mutations: one was a novel mutation (p.Gln352Arg) while the others were previously reported (p.Arg328Cys, p.Arg345Cys, p.Pro160Cysfs*14, p.Cys410Ter). There was a significant difference in circulating MKRN3 values in MKRN3-CPP compared to iCPP ($p < 0.001$). In MKRN3-CPP, the subject harboring Pro-160Cysfs*14 presented undetectable levels. Subjects carrying the missense mutations p.Arg328Cys and p.Gln352Arg showed divergent circulating protein levels, respectively 40.56 pg/mL and undetectable. The patient with the non-sense mutation reported low but measurable MKRN3 levels (12.72 pg/mL).

Conclusions *MKRN3* defect in patients with CPP cannot be predicted by MKRN3 circulating levels, although those patients presented lower protein levels than iCPP. Due to the great inter-individual variability of the assay and the lack of reference values, no precise cut-off can be identified to suspect MKRN3 defect.

Keywords MKRN3 · Molecular screening · Central precocious puberty · Children · Serum MKRN3 · Mutation screening

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Introduction

Puberty is a complex phenotypic trait whose expression is regulated through an interactive crosstalk of several environmental, genetic, and epigenetic factors [1].

The disruption of this fragile network can lead to disrupted timing of pubertal initiation. Central precocious puberty is the most prevalent disorder of puberty affecting children worldwide with a strong female prevalence [2].

The clinical hallmark of this endocrine disorder includes development of secondary sexual characteristics before the age of 9 in boys and 8 in girls associated with enhanced growth velocity and bone maturation due to premature activation of hypothalamic GnRH secretion.

Despite the increasing incidence of the disease, the etio-pathogenetic mechanism of CPP remains unclear in most cases. Monogenic causes represent less than 15% of all cases of central precocious puberty: among them, the maternally imprinted gene *MKRN3* is the most common one [3]. To date, 65 inactivating mutations of *MKRN3* have been described in children with central precocious puberty (CPP) with a particularly high prevalence in familial CPP cases and in males [4–8].

The physiological role of MKRN3 in pubertal regulation is still under investigation albeit inactivating mutation in this gene is closely related to pubertal onset disruption [9]. The gene encodes a protein, makorin 3, an E3-ubiquitin ligase that plays an inhibitory role in the secretion of GnRH during the prepubertal period both by counteracting stimulatory inputs [10, 11] and by repressing the expression of GnRH1 through the various mechanisms of transcriptional repression and protein degradation [12, 13]. Furthermore, previous studies demonstrated that MKRN3 circulating levels decline throughout puberty in normal subjects and idiopathic CPP confirming its inhibitory role on puberty onset [14–16].

Identifying one or more clinical parameters that could help to easily identify CPP patients carrying *MKRN3* mutation is the next clinical challenge, as not all clinical centers can offer universal genetic screening for *MKRN3* to their patients. To date, no study could find a strong clinical predictor able to distinguish patients harboring *MKRN3* mutation from idiopathic ones. In some studies, FSH levels appeared slightly higher in *MKRN3* mutated patients compared to idiopathic ones [16–18]; however, this finding has not been confirmed by other studies and is not useful in clinical practice [19, 20].

To the best of our knowledge, no information is available on MKRN3 circulating levels in patients with CPP and a loss-of-function mutation in *MKRN3* gene.

In order to investigate if plasma MKRN3 dosage could be a potential screening assay for the diagnosis of CPP due to *MKRN3* inactivation, we screened a large homogeneous Caucasian cohort of CPP girls for *MKRN3* mutations and investigated circulating levels in those patients harboring different pathological *MKRN3* mutations.

Materials and methods

Patients

We performed mutational screening on a large Italian multicenter cohort of 140 unrelated girls with CPP. Patients were recruited from the outpatient Endocrinology service of 14 Italian centers. The patients were mainly recruited by 6 main centers: Pediatric Units of the University of Campania "Luigi Vanvitelli", Naples, the Department of Pediatrics, IRCCS G. Gaslini, University of Genoa, Institute for Maternal and Child Health—IRCCS "Burlo Garofolo"—Trieste, the Pediatric Hospital Giovanni XXIII of Bari and Department of Human Pathology in Adulthood and Childhood, University of Messina, Messina, Italy and the Pediatric and Adolescent Unit, Department of Internal Medicine and Therapeutics, University of Pavia.

According to the Declaration of Helsinki, a written informed consent for blood sample collection and genetic investigation was obtained from all the pro-bands and their relatives to participate in the study.

All the patients recruited were Caucasian. Central activated puberty was defined by pubertal basal luteinizing hormone (LH) levels > 0.7 IU/L and/or GnRH-stimulated (0.1 mg Relefact LHRH; Sanofi-Aventis, Frankfurt am Main, Germany) LH levels > 5 IU/L. A normal brain magnetic resonance imaging defined CPP as idiopathic. Familial CPP was defined as having at least two family members affected.

All clinical and biochemical assessments were performed by the referring physicians. Molecular analysis of the whole cohort has been centralized at the Laboratory of the Pediatric Units of the University of Campania "Luigi Vanvitelli", Naples.

Clinical parameters

All patients underwent a complete clinical examination, including weight, height measurement, BMI SDS calculation using LMS method [21] and staging of breast development according to Tanner's classification [22].

Biochemical measurements

All blood samples were drawn at 8:00 a.m. from an antecubital vein, clotted, and centrifuged, and serum was readily analyzed. Serum protein levels were measured in the patients carrying MKRN3 mutations using a soluble MKRN3

enzyme-linked immunosorbent assay (ELISA) using the commercially available Human MKRN3 ELISA (MyBioSource, San Diego, CA, USA) with a sensitivity of 4.68 pg/mL and a detection limit of 7.8 pg/mL. The intra- and inter-assay variations were $< 8\%$ and $< 10\%$, respectively.

Time-resolved immuno-fluorometric assay (DELFIA; Wallac, Inc.) was used to measure FSH and LH concentrations. Detection limits were 0.06 U/L for FSH and 0.05 U/L for LH with an intra-assay and inter-assay CV less than 5%.

Radioimmunoassay was used to measure serum 17-B-estradiol (CisBio International). The detection limit for plasma estradiol was 4 pg/ml.

Serum leptin levels were measured by commercially available ELISA kit (Mybiosource) in patient subgroups whose MKRN3 levels were measured. The coefficients of variations within and between assays were $< 8\%$ and $< 10\%$ respectively. Detection range was between 0.156 ng/mL and 10 ng/mL.

Molecular analysis

DNA was collected from the index case and their parents when available. Genomic DNA was extracted from peripheral blood lymphocytes according to standard protocols.

The entire MKRN3 gene was amplified by polymerase chain reaction (PCR) followed by purification and automatic sequencing was performed. In addition, in the six patients carrying MKRN3 mutations, automatic sequencing of the coding region of other candidate genes associated with central precocious puberty, such as KISS1, PROKR2, GPR54R (KISS1R), and DLK1, was also performed. PCR primers and conditions are available upon request. The effects of missense variants were predicted by performing a prediction analysis through the basic version of the VarSome search engine and selecting four of the most widely used prediction software: CADD, PROVEAN, SIFT, Polyphen.

Allelic expression analysis by RT-PCR

Total RNA was extracted from the peripheral blood lymphocytes in one out of five index cases (carrying the mutation p.Gln352Arg). RNA extraction kit (Qiagen, Germany) and DNase-I treatment were performed to remove possible contamination of genomic DNA. The OD value was measured using an ND-1000 spectrophotometer (NanoDrop, Thermo scientific), and a final concentration of 1000 ng/μL was prepared for reverse transcription (RT) experiments. The RT reaction was performed using oligo (dT) primers with a reverse transcription (RT) kit (Applied biosystems, Lithuania) according to the manufacturer's guidelines. The amplified products of RT-PCR were sequenced directly using the same MKRN3 gene-specific primers previously used for the amplification of genomic DNA. To confirm that the amplification product of MKRN3 is strictly derived from mRNA (namely only transcriptome) amplification, two control genes were also amplified after the RT-PCR reaction: a positive control as β-actin, whose primers are specific for cDNA template and a negative control as DLK1, whose primers are designed to straddle intronic sequences. The lack of an amplification product for *DLK1* gene confirms the absence of genomic DNA.

Statistical analysis

Data were analyzed by SPSS 28.0 software (International Business Machines Corporation). Descriptive statistics were applied to characterize the whole cohort and data were presented as frequencies or median values (25th and 75th percentile) as appropriate. Lilliefors' test was run to investigate normal distribution: nonparametric data were analyzed using the Mann–Whitney U test. Spearman rank test was used to performed correlation analysis. Statistical significance was set at $p < 0.05$.

Results

Of 140 girls screened, 5 unrelated girls were identified as carrying five heterozygous *MKRN3* mutations; in these patients, no rare variants were found in the other genes analyzed (*DLK1*,*PROKR2, KISS1, a*nd *KISS1R*). Among the 29 cases with a paternal family history of CPP, 5 had mutations in *MKRN3* (17.2%). We identified 5 different heterozygous *MKRN3* mutations in 5 unrelated girls: one was a novel missense mutation (c.1055 > G; p.Gln352Arg) while 2 missense mutations (p.Arg345Cys, p.Arg328Cys), 1 frameshift (p.Pro160Cysfs*14) and one non-sense mutation (p.Cys410Ter) had been previously described [23, 24]. Table 1 synthetizes the mutations retrieved. Additionally, a paternally inherited variant, namely p.Pro161Leu was identified in one girl with familial progressive CPP whose molecular screening was negative for other gene associated with precocious puberty. Since this variant results to be likely benign according ACMG classification, we decided not to include this patient in the cohort analysis. Figure 1 shows localization of the 6 variants. Clinical and biochemical data of this patient are available as supplemental material.

We compared clinical and biochemical data of the 5 girls with *MKRN3* mutations versus those without mutation. Table 2 synthesized all the clinical and biochemical characteristics of our cohort. No clinical difference was identified between the groups.

Table 1 Pathogenic variants identified and prediction software score

Patient	Pathogenic variant	RSID	Allele count	Allele frequency (General and European)	N of homozygotes	Prediction software (score)	ACGM classification
1	c.587_594delCCCCCGGC p.Pro160CysfsTer14	NA	NA	NA	NA	NA	NA
2	c.982C > T p.Arg328Cys	rs1264639964	1	3.98e-6 8.79e-6	0	CADD: 28 PROVEAN: − 7.42 SIFT: 0.01 PolyPhen: 0.998	Likely pathogenic
3	c.1033C > T p.Arg345Cys	rs373441581	2	7.95e-6 8.79e-6	0	CADD: 31 PROVEAN: -7.91 SIFT: 0 PolyPhen: 0.998	Likely pathogenic
4	c.1055A > G p.Gln352Arg	NA	NA	NA	NA	CADD: 22.90 PROVEAN: -3.23 SIFT: 0.002 PolyPhen: 0.80	Uncertain Significance
5	c.1229G > A, c.1230 C > A p.Cys410Ter	NA	NA	NA	NA	NA	NA

Table 2 Patients clinical characteristics

	Whole cohort	CPP girls without MKRN3 mutations	CPP girls with MKRN3 mutations	*P* value
	140	134	5	
Age at first visit	7.5 (6–9)	7.5 (6.8–8)	7.24 (7.3–7.5)	.50926
Age at presentation	7.1 (6.4–7.8)	7.1 (6.7–7.9)	6.5 (6.4–7)	.50926
Height SDS	0.63 (− 0.22; 1.24)	0.62 (− 0.23;1.22)	0.46 (0.09–1.98)	.89656
BMI SDS	0.48 (− 0.35–2.23)	0.44 (− 0.35; 1.23)	0.82 (0.66–0.89)	.8493
Δ Bone Age-Chronological Age	1.9 (1.3–2.7)	1.78 (1.1–2.7)	2.9 (3.63.8)	.5552
Familial case, N(%) of which paternal, N (%)	56 (40%) 29 (51.8%)	51 (6.9%) 26 (51%)	4 (80%) 4 (100%)	.0703
Breast (Tanner)	2 (2–3)	2 (2–3)	3 (2–3)	.32218
Pubarche (Tanner)	2 (1–2)	2 (1–2)	1 (1–2)	.22628
Basal LH (mUI/mL)	0.8 (0.4–2.2)	0.8 (0.35–2)	2.6 (2.2–3.57)	.72634
Peak LH, mUI/mL	9.3 (7.6–15.5)◆	9.25 (7.47–13.5)#	Performed in one patient: 64.6 mUI/mL	N.A
Basal FSH, mUI/mL	4.3 (3.2–6.4)	4.3 (3.2–6.1)	7.2 (6.3–7.9)	.99202
Estradiol, pg/mL	18.9 (11.5–33)	17.8 (11.4–31.9)	39.5 (15–47)	.95216
MKRN3 levels, pg/mL	N.A	879.9* (78.7–1599.75)**	12.72 (40.56–0)**	< .001
Leptin levels, ng/mL	2.86 (2.48–3.58)	2.78* (2.52–3.30)	3.6 (2.9–4.1)	.862

N.A not applicable

◆Calculated for 70 patients

[#]Calculated in 69 patients

*Data calculated on a subgroup of 15 patients

**Values in brackets refer to minimum and maximum value

Clinical features of the CPP girls carrying an MRN3 mutation

Patient 1: Patient 1 was a girl with a heterozygous deletion (c.587_594 delCCCCCGGC; p.Pro160Cysfs*14) also harbored by her father as described in our previous paper [7]. She presented a positive family history for CPP (a paternal cousin received the same diagnosis). Pubertal signs appeared at 6.0 years of age and she underwent the first clinical examination at 7 years of age. Personal antecedents

were uneventful. She presented with a Tanner stage B2PH1. Her height was 136.6 cm (+2.54 SDS), BMI 15.5 kg/m^2 (1.5 SDS), bone age 10 years 3/10 (TW2 methods).

Patient 2: A missense variant, c.982C > T; p.Arg328Cys, in C3HC4 RING finger region, was identified in a girl with apparently sporadic CPP. She presented at 6.5 years of age with advanced pubertal signs (Tanner stage B3PH2). Clinical examination showed height 132.3 cm (+2.4 SDS), weight 32 kg (+1.52 SDS), BMI 18.3 kg/m^2 (+0.85 SDS), advanced bone age of 8 years 7/10.

Patient 3: The heterozygous missense variant, c.1033C > T; p.Arg345Cys, was identified in a girl with familial CPP. Personal antecedents were uneventful. Premature thelarche and pubarche appeared at 6.5 years of age. At the first clinical visit, her height was 126.4 cm (+0.63 SDS), a BMI of 20.72 kg/m^2 (+1.4 SDS), Tanner stage B3 PH3. She presented a bone age advancement of 3.7 years.

Patient 4: A novel missense variant, c.1055 > G; p.Gln352Arg was identified in a girl with familial CPP. The parental segregation analysis confirmed the paternal origin of the mutation. Family history pointed out early menarche at 9 years of age in a paternal aunt. She had a personal history of acute lymphoblastic leukemia at the age of 5, treated with chemotherapy. At 24 months of oncological follow-up, she presented an increased growth velocity of 12 cm per year (+6.9 SDS) and premature thelarche. At the first endocrinological visit, at 7.3 years of age, her height was 125.2 cm (+0.46 SDS), weight was 29.5 (+0.79 SDS), with a BMI of 18.8 kg/m^2 (+0.91 SDS), Tanner stage B2PH2. She presented a bone age of 8 years 8/10.

Patient 5: A pathological variant, c.1229G > A; p.Cys410Ter, and c.1230 C > A; stop gain TGC > TAA, in C3H1 motif at N-terminal region was identified in a girl with familial CPP. Pubertal signs appeared at 6.8 years of age, anticipated by a sustained increased linear growth velocity one year previously. Clinical examination at 7.5 years showed height 140.2 cm (+2.57SDS), weight 36.4 kg (+1.45 SDS), BMI 18.5 kg/m^2 (+0.74 SDS), Tanner stage B2PH2, bone age was 11 years and 1/10 (Fig. 1).

All patients clinical and biochemical characteristics are shown in Table 3.

Circulating MKRN3 levels

The patient carrying p.Arg345Cys mutation was not available for further biochemical investigation, thus was excluded in this pivotal sub-analysis. Table 4 synthetizes detected plasma levels for each variant.

Patient 1 (p.Pro160Cysfs*14) and patient 5 (p.Gln352Arg) presented undetectable plasma MKRN3 level. Subjects 2 carrying the missense mutation p.Arg328Cys showed circulating MKRN3 levels of 40.56 pg/mL, with values still detectable. Interestingly, patient 5 with a non-sense mutation

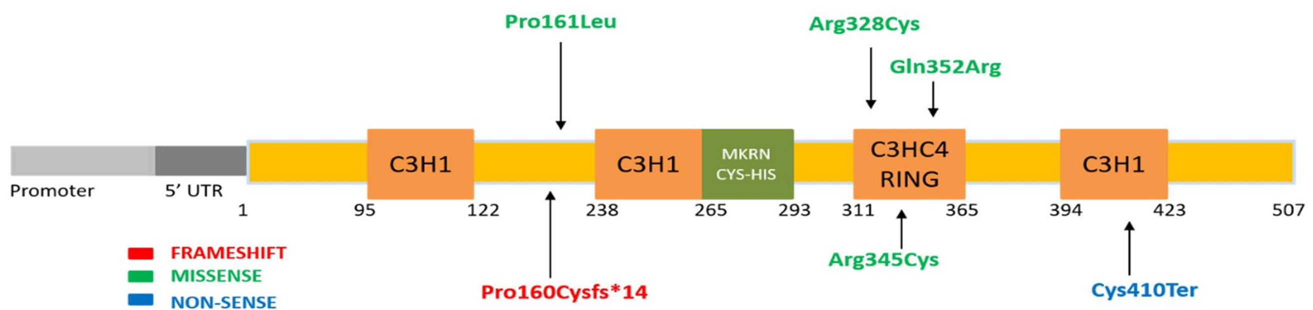

Fig. 1 Synthetizes localization of all 6 variants in MKRN3 gene

Table 3 Patients' clinical characteristics

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
MKRN3 variant	p.Pro160Cysfs*14	p.Arg328Cys	p.Arg345Cys	p.Gln352Arg	p.Cys410Ter
Age at first visit	6.7	6.8	7.3	7.9	7.5
Familial case	Yes	No	Yes	Yes	Yes
Breast (Tanner)	II	III	III	II	III
Pubarche (Tanner)	I	II	III	I	I
Basal LH (mUI/mL)	4.2	2.6	3.57	1.3	2.2
Peak LH (mUI/mL)	N.A	N.A	64.6	N.A	N.A
Basal FSH (mUI/mL)	9	5.1	6.3	7.9	7.2
Estradiol (pg/mL)	15	52.4	47	10	39.5

p.Cys410Ter reported low but measurable MKRN3 circulating levels (12.72 pg/mL).

Patient carrying the likely benign variant p.Pro161Leu presented MKRN3 levels of 27.92 pg/mL but she was excluded from analysis.

Additionally, serum levels of this protein were measured in a subgroup of 15 idiopathic CPP who gave consent for further investigation. Clinical characteristics were comparable between the two groups (see Table 1, supplemental material). Mean circulating levels in idiopathic CPP patients was 676.7 ± 317 pg/mL, median value was 673.5 pg/mL (IQR25–75: 561.665–971.3 pg/mL). Minimum and maximum levels detected were 78.7 pg/mL and 1044.8 pg/mL, respectively.

A significant difference was found between MKRN3 levels in patients carrying *MKRN3* mutations versus idiopathic CPP ($p < 0.001$) [see supplemental material].

No correlation was found between circulating MKRN3 and BMI ($\varrho = -0.253$; p-value = 0.72) and bone age advancement ($\varrho = -0.16$; p-value:0.94). In our cohort, MKRN3 neither correlated with leptin levels ($\varrho = -0.46$; p-value: 0.072).

Allelic expression analysis

Screening performed on our cohort identified 6 patients with mutations in the MKRN3 gene. For these patients, analysis of the parental genotypes revealed that the mutation was derived from the paternal allele. Subsequently, since the circulating levels of MKRN3 showed conflicting results, a possible reactivation of the maternal allele was hypothesized. To study the allelic expression of MKRN3, RT-PCR was performed on the total RNA from peripheral blood of patient 4, the only one whose biological material was available. Direct sequencing of the amplified product confirmed the presence of expression of only the mutated paternal allele, with the presence of mutation p.Gln352Arg, as expected.

Discussion

Inactivating mutations of *MKRN3* are the most frequent monogenic cause of CPP, with an overall prevalence of 9% [25] and are especially in familial forms and in males. In our cohort, *MKRN3* mutations account for 3.6% of CPP in the whole cohort and 13.7% of those with a positive family history of CPP in paternal pedigree. These frequencies are remarkably lower than those reported in the literature which was around 9% in isolated CPP and ranging from 33 to 46% in familial forms [4]. However, our data are in line with our previous report [24], other investigations in the Mediterranean area [26, 27], and similar to a recent study by Kırkgöz et al. [28]. Considering the variation in the prevalence of *MKRN3* mutations according to ethnic distribution [28, 30], the homogeneity of our cohort, exclusively composed of Caucasian females, probably reflects the prevalence of the mutation in Caucasian female population.

Clinical features do not help to identify patients with MKRN3 gene defects. Although a trend to higher FSH levels was found in the MKRN3-CPP group, it does not reach statistical significance, probably due to a limited cohort of mutated patients.

In patients with another form of monogenic CPP, related to *DLK1* gene mutations, circulating DLK1 protein levels are undetectable, suggesting a simple serum assay could be a reliable screening tool to decide which CPP patient should be a candidate for genetic analysis for *DLK1* mutation [31–33].

Thus, our study had the scope to investigate if a simple serum MKRN3 assay could be a useful screening tool for identifying patients with MKRN3 defects.

To the best of our knowledge, this is the first study investigating serum MKRN3 levels in girls harboring an *MKRN3* mutation who presented with non-syndromic CPP. Our results suggest unpredictable circulating MKRN3 levels in mutated patients.

Surprisingly, the novel missense mutation, p.Gln352Arg, resulted in undetectable protein levels. Even if the mutation is classified to have uncertain significance, it is located inside the C3HC4 ring zinc finger, a highly conserved region of the protein, fundamental for ubiquitination activity [20]. As demonstrated by Magnotto et al., western blot analysis of missense mutations in the RING finger markedly reduced ubiquitination in MKRN3 compared to wild-type protein suggesting a decreased ubiquitination function and an increase of other forms following post-translational modifications [20]. Such modifications could change the prevalent form of circulating MKRN3 protein and alter, in different ways, the affinity between the ELISA antibody and the protein epitope, thus explaining the variable and unexpected results obtained by the assay [12].

The frameshift mutation p.Pro160Cysfs*14, carried by patient 1 was previously described by our group in 2015 and resulted in a premature stop codon encoding a truncated protein undetectable in serum [26]. The mutant makorin 3 was predicted to lack both the C3HC4 RING motif and MKRN-specific Cys–His domain, causing probably rapid protein degradation.

Patient 5 harbored a non-sense mutation, p.Cys410Ter, which showed low but detectable levels of circulating protein. The terminal location of the non-sense mutation (c.1229G > A; p.Cys410Ter) could allow the generation of a truncated protein that was still recognizable by ELISA kit antibodies.

An alternative explanation of our results could be found in the technical limits of the ELISA assay used. No

information is available for the type of epitope and precise location within the protein targeted by the human MKRN3 antibody in the available ELISA assay. As MKRN3 shares some grade of similarity with other Makorin proteins of the same family, a possible cross-reaction between different proteins cannot be excluded.

Further analysis was performed to investigate the correlation between MKRN3 levels measured in the entire cohort (iCPP and MKNR3-CCP) and some biochemical parameters, such as BMI, bone age, and leptin. We did not find a statistically significant association between MKRN3 levels and these parameters. Because the analysis was conducted on a small cohort, no firm conclusions could be drawn; however, our results seem to be in line with data obtained by Roberts et al. in mice models [34]. Interestingly, the same unpredictable biochemical behavior was found in patients with Prader–Willi syndrome. As MKRN3 is a gene located within the locus associated with Prader–Willi syndrome (PWS) (15q11.2-q13), an imprinting disorder mainly characterized by the inactivation of the paternal allele, it has been speculated no circulating MKRN3 protein would be found in PWS patients. On the contrary, in 2022, Mariani et al. demonstrated 61% out of 80 patients with PWS studied presented low but detectable MKRN3 levels, suggesting a possible maternal allele reactivation in this population [35].

In one of our patients with CPP and MKRN3 mutation, paternal allele expression was confirmed by expression analysis, thus excluding maternal allele reactivation.

Interestingly, in our study, CPP patients with MKRN3 mutations showed remarkably lower levels of circulating protein (median 12.72 pg/mL) than those measured in the subgroup of 15 idiopathic CPP patients in our study ($p < 0.001$). In the study of Hagen et al. for pubertal patients, early maturing girls reported circulating levels above 200 pg/mL [14] while Grandone et al. reported higher levels in idiopathic CPP girls at diagnosis (612.67 ± 476.54 pg/mL) [16]. Recently, Wu GE presented even higher levels for healthy pubertal controls (834 pg/mL; 663.5–967.8) [36].

However, given the limited number of observations, the lack of MKRN3 reference values for peripubertal stage in the healthy population and the large variability of serum concentrations in patients with iCPP, larger cohorts are needed to assess whether a reliable cut-off value can be identified.

Taken together, our results suggest that the type of *MKRN3* defect in patients with CPP cannot be predicted by MKRN3 circulating levels although patients carrying *MKRN3* mutations presented lower circulating protein levels than idiopathic CPP. Thus, at the best of the present evidence, we suggest that serum MKRN3 dosage cannot be used at the moment as a screening tool for monogenic CPP due to MKRN3 defects, but further studies are needed on larger cohorts on this intriguing topic.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40618-023-02255-5.

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Author contributions AG conceived the study idea and supervised the work process. AG, SP and GC elaborated the design. Recruitment and clinical management were performed by GT, DF, MW, MFF, CL, AF, EMG. Genetic analysis was performed by SP and GC. FA reviewed the literature, collected the data, wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript. FA prepared the drafts for publication. All authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval This study involved human participants, their data and biological material. Approval was obtained from the ethics committee of University "Luigi Vanvitelli", Naples, Italy. The procedures used in this study adhere to the tenets of the 1964 Declaration of Helsinki and its later amendments.

Research involving human participants and/or animals The modified the ethical approval disclosure to be more specific about this research including human participants.

Consent to participate Written informed consent was obtained from all parents of individual participants included in the study. An oral assent was obtained by the patients themselves.

Consent to publish The authors affirm that human research participants provided informed consent for publication of all the clinical and molecular data inhere presented in the document.

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