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Clinical Research Article

High Prevalence of Hypercalcitoninemia in a Large Cohort of Adult and Pediatric Patients With PTH Resistance Syndromes

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Abstract

Context: Pseudohypoparathyroidism (PHP) refers to a group of rare hereditary disorders associated with resistance to parathyroid hormone (PTH) and other hormones now termed *inactivating PTH/PTHrP disorders* (iPPSD). Hypercalcitoninemia has been seldom reported in small series.

Objective: Our aim was to investigate the characteristics of hypercalcitoninemia in pediatric and adult patients with PHP/iPPSD.

Methods: We retrospectively collected data from 2 cohorts from 2 European endocrinology tertiary centers: the pediatric cohort comprised 88 children with available calcitonin (CT) measurements; the adult cohort included 43 individuals with simultaneous CT and PTH measurements.

Results: In the pediatric cohort, 65.9% had hypercalcitoninemia (median CT 15 ng/L); in the adult cohort 53.5% (mean CT 21.6 ng/L). There was no difference between CT in pediatric and adult population; we observed stable CT levels over a median follow-up of 134.5 months in adults. Notably, no correlations were detected between CT and PTH levels. Other etiologies of hypercalcitoninemia were excluded; adult patients underwent regular thyroid ultrasound to screen for medullary thyroid cancer (MTC). We performed 20 calcium stimulation tests in adult patients. While there was a significant difference in basal and peak CT between our patients, healthy subjects, and subjects with MTC, there was no difference with patients with C-cell hyperplasia.

Conclusion: This study underscores the common occurrence of hypercalcitoninemia in both pediatric and adult patients with PHP/iPPSD, in particular with subtypes iPPSD2 and iPPSD3. Furthermore, these patients show hyperresponsiveness to calcium stimulation tests falling between healthy subjects and patients with MTC. These findings contribute to the understanding of CT dynamics in the context of PHP/iPPSD.

Key Words: pseudohypoparathyroidism, calcitonin, hormonal resistance, C-cell hyperplasia

Abbreviations: AHO, Albright hereditary osteodystrophy; cAMP, cyclic adenosine monophosphate; CCH, C-cell hyperplasia; CT, calcitonin; GHRH, growth hormone-releasing hormone; Gsα, stimulatory G protein α; iPPSD, inactivating PTH/PTHrP disorders; IQR, interquartile range; MTC, medullary thyroid cancer; PHP, pseudohypoparathyroidism; PHP1A, pseudohypoparathyroidism type 1A; POH, progressive osseous heteroplasia; PPHP, pseudopseudohypoparathyroidism; PTH, parathyroid hormone; PTHrP, parathyroid hormone-related protein; TSH, thyrotropin (thyroid-stimulating hormone); ULN, upper limit of normal.

The term *pseudohypoparathyroidism* (PHP) refers to a group of heterogeneous and rare disorders that are typically associated with end-organ resistance to parathyroid hormone (PTH) and other hormones due to molecular defects that impair hormonal signaling via receptors that are coupled to activation of adenylyl cyclase through the α-subunit of the stimulatory G protein (Gsα) (1). It was historically the first hormonal resistance to be described in 1942 by Fuller Albright and colleagues (2).

A molecular cause can be identified in about 80% to 90% of patients with PTH resistance syndromes and consist of alterations within or upstream of the *GNAS* complex locus (1, 3). The most common underlying mechanisms are de novo or autosomal dominantly inherited genetic mutations and/or methylation defects within the imprinted *GNAS* gene cluster, whose main transcript encodes Gsα (4, 5). The disease phenotype may vary according to the genetic/epigenetic defect and to

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whether the alteration is on the maternal or the paternal allele (6). In addition, mutations in *PRKAR1A* (encoding cAMP-dependent protein kinase type 1α regulatory subunit) and *PDE4D* (encoding cAMP-specific 3′,5′-cyclic phosphodiesterase 4D), which are also crucial for Gsα–cAMP-mediated signaling, have been found in a subset of patients with acrodysostosis, a group of disorders displaying highly similar clinical characteristics, including hormone resistances (5, 7).

Several phenotypes have been described and classified based on clinical features, presence or absence of hormonal resistances, in vivo response to exogenous PTH, and in vitro assay measuring the Gsα protein activity from erythrocyte membranes (1, 5). However, the identification of genetic and epigenetic defects involving the cyclic adenosine monophosphate (cAMP) signaling cascade as common disease mechanisms led to the definition of a new nomenclature and classification. The new term proposed to identify the disorders is *inactivating PTH/PTHrP signaling disorder* (iPPSD), stressing the common mechanism responsible for all these diseases (8). Furthermore, it proposes a classification in subtypes iPPSD 1 to 6, in which each subtype is identified by a progressive number indicating a specific molecular defect in the cAMP cascade and subtype iPPSDx where no known genetic or epigenetic defect is identified (8). This classification is therefore adopted for the herein manuscript.

Clinically, iPPSDs are characterized by a variable collection of physical features such as short stature, stocky build, round face, brachydactyly, and ectopic ossifications (ie, Albright hereditary osteodystrophy [AHO]) as well as early-onset obesity, neurocognitive impairment, and developmental delay (1, 9). As for hormonal features, most iPPSD patients share biochemical characteristics of hypocalcemia and hyperphosphatemia due to PTH resistance (6). Moreover, resistance to other hormones that act through receptors coupled to Gsα is observed, the most frequent being resistance to thyrotropin (thyroid-stimulating hormone [TSH]), followed by growth hormone-releasing hormone (GHRH) and gonadotropins (1, 10).

In particular, the most frequent subtypes, and also predominant in our study cohort, are iPPSD2 and iPPSD3. iPPSD2 encompasses all disorders associated with loss-of-function mutations of *GNAS* gene and corresponds to previously known PHP1A (pseudohypoparathyroidism type 1A), PPHP (pseudopseudohypoparathyroidism), and POH (progressive osseous heteroplasia), when the mutation is on maternal or paternal allele, respectively. PHP1A was initially defined as the association of resistance to multiple hormones, features of AHO and decreased Gsα activity using in vitro assays, while PPHP shows features of AHO without evidence of hormonal resistances and POH is a disorder characterized by heterotopic ossifications expanding into dermis, muscles, and connective tissue (11). On the other hand, iPPSD3 formerly known as PHP1B, was initially defined as isolated hormonal resistances, absence of AHO, and normal levels of Gsα activity and is associated with abnormal patterns of methylation in the *GNAS* complex locus (1, 8).

Calcitonin is a 32-amino acid hormone produced by thyroid parafollicular C-cells and its receptor belongs to the G protein–coupled receptors (12). Calcitonin decreases bone demineralization by inhibiting osteoclast action (13); it also increases calcium and phosphate renal excretion, and it promotes renal production of 1,25-dihydroxyvitamin D3 and acts as a neuromediator (14, 15). It is mainly used as the most sensitive tumoral marker of medullary thyroid cancer (MTC) (16).

Hypercalcitoninemia has been reported in patients with iPPSD (17, 18); however, there are a limited number of case reports or small series published (19-21) and only one paper focusing on natural history and mechanisms of hypercalcitoninemia in a cohort of 6 patients (12). To our knowledge, there are no data on calcitonin levels in children affected by iPPSD.

This study's aim was to investigate the prevalence and characteristics of hypercalcitoninemia in a large cohort of adults and children with iPPSD.

Patients and Methods

We retrospectively collected data from 2 cohorts of patients. The first cohort was extracted from a cohort of 136 children with iPPSD who were previously studied for neonatal and early infancy characteristics (22). Within this cohort, we included 88 children with at least one available calcitonin measurement; 72 patients and 16 patients are followed-up at regular intervals at the reference center for rare disorders of the calcium and phosphate metabolism at Bicêtre Paris Saclay Hospital (Le Kremlin Bicêtre, France) and at the Endocrinology Unit of Fondazione IRCCS Ca" Granda Ospedale Maggiore Policlinico (Milan, Italy), respectively.

The second cohort included 43 adults with iPPSD with at least one simultaneous measurement of calcitonin and parathyroid hormone (PTH) who were regularly visiting the Endocrinology Unit of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (median follow-up period of 134.5 months, interquartile range [IQR] 55.3-328.5).

For both cohorts, resistance to PTH was defined as the association of an increased serum level of PTH and high serum phosphate according to age-appropriate reference values and/or low serum calcium in the absence of vitamin D deficiency. Resistance to TSH was defined as elevated serum levels of TSH with normal or slightly reduced levels of thyroid hormones. Genetic testing was performed to confirm clinical or biochemical suspicion according to current guidelines to identify molecular mutations. All patients with PTH resistance were treated with oral calcium supplements and vitamin D active metabolites (calcitriol) at different doses throughout the follow-up period depending on PTH, calcium, and phosphorus levels.

In both cohorts, calcitonin levels were measured using different assays, and therefore normal range values varied depending on the assay used. Taking into account that in the first years of life calcitonin levels progressively reduce (16, 23, 24), based on the laboratory reference ranges we used a cutoff of 5 pg/mL for patients > 3 years old and of 15 pg/mL for patients > 6 months and < 3 years old. PTH levels were also measured using different assays and expressed as upper limit of normal (ULN) in order to even out differences in normal range values.

Moreover, C-cell function was assessed by calcium stimulation test in 14 adults with iPPSD; results were compared to a control group of 33 healthy adults, 19 adults with histological evidence of MTC, and 37 adults with C-cell hyperplasia (CCH). All calcium stimulation tests were performed at the same center in Milan as previously described (25). Briefly, calcium gluconate was administered intravenously at the dose of 25 mg/kg at 5 mL/min and blood samples were drawn at 0′, 2′, 5′, and 15′ minutes after the infusion started. To measure calcitonin levels a chemiluminescent assay (CLIA) was used with an analytical sensitivity of 2 pg/mL.

Screening for MTC was carried out in 36/43 adult iPPSD patients by thyroid ultrasonography, of whom 20 had

Table 1. Main demographical features in iPPSD/PHP children with normal basal CT levels compared with children with elevated CT levels

	Normal basal CT (n = 30)	Elevated basal CT (n = 58)	
Sex F (%)	13 (43.3)	35 (60.3)	$P = .12$
Median age at diagnosis, years (IQR)	12.1 (5.6-14.8)	4.3 (1.4-8.0)	$P < .00$
iPPSD2/PHP1A n (%)	9 (30)	40 (68.9)	$P < .00$
iPPSD3/PHP1B n (%)	8 (26.6)	8 (13.8)	$P = .14$
iPPSD/PHP other n (%)	13 (43.4)	10 (17.3)	$P = .01$
No. of resistances			
0 (%)	11 (36.7)	6 (10.3)	$P = .00$
1 (%)	6 (20)	9 (15.6)	$P = .59$
2 (%)	13 (43.3)	43 (74.1)	$P = .00$

Abbreviations: CT, calcitonin; iPPSD2, inactivating PTH/PTHrP signaling disorder subtype 2, also known as PHP1A; iPPSD3, inactivating PTH/PTHrP signaling disorder subtype 3, also known as PHP1B; PHP, pseudohypoparathyroidism.

hypercalcitoninemia. When possible, it was performed in our centers by the same ultrasound operator. Other etiologies of hypercalcitoninemia were excluded, namely, renal failure and drug interferences such as proton pump inhibitor therapy.

Statistical Analysis

Data analysis and graph plotting were performed using SPSS statistical package (software version 27) and Prism (software version 7.0). Quantitative variables that describe the cohort's characteristics were expressed as mean ± SD when normally distributed, while quantitative variables not normally distributed were expressed as median and IQR. Qualitative variables were expressed as numbers and percentages (%). Normal distribution of variables was evaluated using the Kolomogorov-Smirnov test.

The Student *t* test was used to compare mean calcitonin values when samples were normally distributed, while the Wilcoxon-Mann-Whitney test was used to compare mean calcitonin levels when samples were not normally distributed. To evaluate whether hypercalcitoninemia and different categorical variables were related to each other, a Chi-square test was used or Fisher exact test when expected values were < 5. Nonparametric Spearman testing was used to analyze correlation between PTH levels expressed as ULN and basal calcitonin levels. We considered *P* values < .05 statistically significant.

Results

Pediatric Cohort of iPPSD Patients

We analyzed the data of 88 children with iPPSD who had available basal calcitonin levels, of whom 55.7% had iPPSD2 (corresponding to PHP1A), 18.2% had iPPSD3 (PHP1B), and 26.1% had other iPPSD subtypes, that is, 11 iPPSDx (mutation unknown), 2 iPPSD1 (*PTH1R* mutation), 7 iPPSD4 (*PRKAR1A* mutation), and 3 iPPSD5 (*PDE4D* mutation). The main demographical features of this cohort of children are expressed in Table 1, comparing pediatric patients with normal calcitonin values and with hypercalcitoninemia. Thirty-one children (35%) had a known familial history of iPPSD and were therefore screened for hormone resistance starting soon after birth. Among these 88 iPPSD children, 58 (35 girls) had hypercalcitoninemia according to the laboratory's specific age reference range (65.9%), with a median basal calcitonin level of 15 ng/L (IQR 7.2-25.3 ng/L). Basal calcitonin levels were similar between boys and girls (girls median calcitonin levels 15 ng/L, IQR 7.2-25.1 ng/L; boys median calcitonin levels 15.1 ng/L, IQR 6.95-25.5 ng/L, $P = .85$). We analyzed the age at which hypercalcitoninemia was detected for the first time and found that 12.1% (7/58) of patients had elevated basal calcitonin levels before the age of 2 years, 29.3% (17/58) before the age of 5 and 65.5% (38/58) before the age of 10 years. In the youngest patients, with evidence of hypercalcitoninemia before the age of 2 years, 6 of 7 patients (85.7%) had already developed PTH and TSH resistance. Twelve children showed evidence of hypercalcitoninemia before the diagnosis of PTH resistance. Children with hypercalcitoninemia were diagnosed earlier (median age at diagnosis 4.3, IQR 1.4-8 years) than children displaying basal calcitonin levels within the normal range (median age at diagnosis 12.1, IQR 5.6-14.8 years and, $P < .00$); moreover, they showed a higher number of hormonal resistances and had more frequently iPPSD2/PHP1A ($P < .00$) (Table 1). Furthermore, of all children with iPPSD2/PHP1A (49), 40 had hypercalcitoninemia (81.6%); this differed from children with iPPSD3/PHP1B (16), who exhibited hypercalcitoninemia in about 50% of the cases (8 patients).

Adult Cohort of iPPSD Patients

We analyzed data from 43 adults (25 females) with iPPSD who had at least one simultaneous measurement of calcitonin and PTH levels. Among these 43 patients, 55.8% (24/43) were diagnosed with iPPSD3 (PHP1B), 39.5% (17/43) with iPPSD2 (PHP1A), 1 patient had iPPSD4 (*PRKAR1A* mutation), and 1 iPPSDx patient had no mutation identified. Main demographical features of the adults with iPPSD are shown in Table 2, comparing adult patients with normal calcitonin values and patients with hypercalcitoninemia.

We evaluated hormonal resistances in the total population and found that, other than PTH resistance, which was present in all patients, 30 patients had TSH resistance (69.7%), 6 patients had GHRH resistance (13.9%), although of note 9 patients were never tested for growth hormone deficiency, and 23 patients (53.5%) had elevated basal calcitonin levels (12 females and 11 males).

The demographical and clinical features, such as sex, age at diagnosis, iPPSD subtype, or number of hormonal resistances per patient, were similar between the group of iPPSD adults with hypercalcitoninemia and the group of iPPSD adults with normal basal calcitonin levels (Table 2). Of all iPPSD2/PHP1A adults (17), 11 had hypercalcitoninemia (64.7%); in the subgroup with iPPSD3/PHP1B (24), 12 patients had

Table 2. Main demographical features in iPPSD/PHP adults with normal basal CT levels compared with iPPSD/PHP adults with elevated basal CT levels

	Normal basal CT (n = 20)	Elevated basal CT (n = 23)	
Sex, F (%)	13 (65)	12 (52.2)	$P = .5$
Mean age at diagnosis, years	17 ± 10.5	21.6 ± 15	$P = .4$
Mean age at last follow-up, years	32.7 ± 13.4	37.6 ± 9.7	
iPPSD2/PHP1A (%)	6 (30)	11 (47.8)	$P = .3$
iPPSD3/PHP1B (%)	12 (60)	12 (52.2)	$P = .7$
iPPSD4/Acrodys1 (%)	1 (5)	0	
iPPSDx/Unknown mutation (%)	1 (5)	0	
No. of resistances			
0 (%)	1 (5)	0 (0)	
1 (%)	4 (20)	6 (26)	$P = .72$
2 (%)	13 (65)	15 (65.2)	$P = .99$
3 (%)	2 (10)	2 (8.7)	$P = 1.00$

Abbreviations: CT, calcitonin; iPPSD2, inactivating PTH/PTHrP signaling disorder subtype 2, also known as PHP1A; iPPSD3, inactivating PTH/PTHrP signaling disorder subtype 3, also known as PHP1B; iPPSD4, inactivating PTH/PTHrP signaling disorder subtype 4, PRKAR1A mutation; iPPSDx, mutation unknown; PHP, pseudohypoparathyroidism.

hypercalcitoninemia (50%). Mean calcitonin levels in patients with hypercalcitoninemia were 21.6 ± 8.3 ng/L, with no statistical difference between female and male patients (F mean basal calcitonin levels 22.3 ± 7 ng/L, M mean basal calcitonin levels 21 ± 9.4 ng/L, $P = .7$).

We then compared median calcitonin levels in children and adults and found no statistically significant difference ($P = .5$, Fig. 1). Furthermore, calcitonin levels were assessed periodically in 17 adults for a median follow-up time of 134.5 (IQR, 55.3-328.5) months. These measurements are summarized in Fig. 2. Values remained stable throughout time as no statistically significant difference ($P = .4$) was found comparing initial calcitonin levels with the last available ones.

Finally, throughout the follow-up period we simultaneously evaluated PTH levels. In the cohort of patients with hypercalcitoninemia, median PTH ULN levels were 1.5 (IQR, 1.0-2.5). We assessed Spearman correlation between PTH and calcitonin levels and found no correlation between the trend of these hormones over time ($r = 0.09$; $P = .5$) (Fig. 3).

Other causes of secondary hypercalcitoninemia were excluded in all patients. Of note, one patient had a declining renal function with calculated GFR (EPI-CKD) between 60 and 47.7 mL/min, with subsequent increase of calcitonin levels during follow-up.

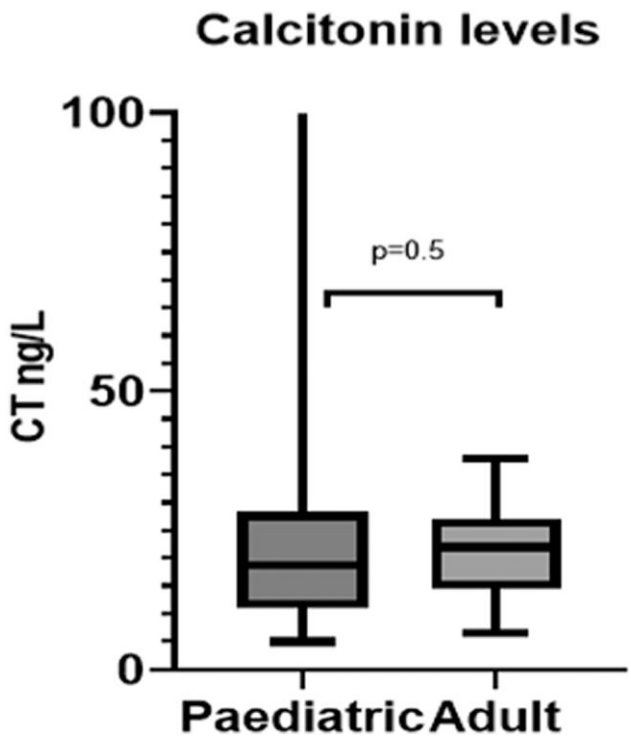

Figure 1. Median calcitonin (CT) levels in children and adults with iPPSD/PHP and hypercalcitoninemia.

Calcitonin Stimulation Test

In a subset of 14 iPPSD adults with elevated basal calcitonin levels we assessed C-cell function through a calcium stimulation test (5 patients with iPPSD2/PHP1A and 9 with iPPSD3/PHP1B); 6 of these patients performed the stimulation test twice (median basal calcitonin levels 19.2, IQR 9.0-24.7 ng/L; median peak calcitonin levels 285, IQR 184.6-573.8 ng/L). We compared both basal and peak calcitonin levels with a control group of 33 healthy patients, 19 patients with pathological evidence of MTC, and 37 patients with CCH, 37 of whom were previously reported (5 MTC and 32 CCH) (25). We found that in both basal and peak calcitonin values there was a statistically significant difference between the healthy control group and the 3 patient groups (all data are shown in Fig. 4 and Table 3). Comparing median values between groups, healthy vs iPPSD: basal calcitonin $P = .00$ and peak calcitonin $P = .00$; healthy vs MTC: basal calcitonin $P = .00$ and peak calcitonin $P = .00$; healthy vs CCH: basal calcitonin $P = .00$ and peak calcitonin $P = .00$. There was also a statistically significant difference between the MTC group vs both iPPSD and CCH (basal calcitonin $P = .00$ and peak calcitonin $P = .01$; basal calcitonin $P = .00$ and peak calcitonin $P = .00$, respectively). However, no statistically significant difference was found between iPPSD patients and the

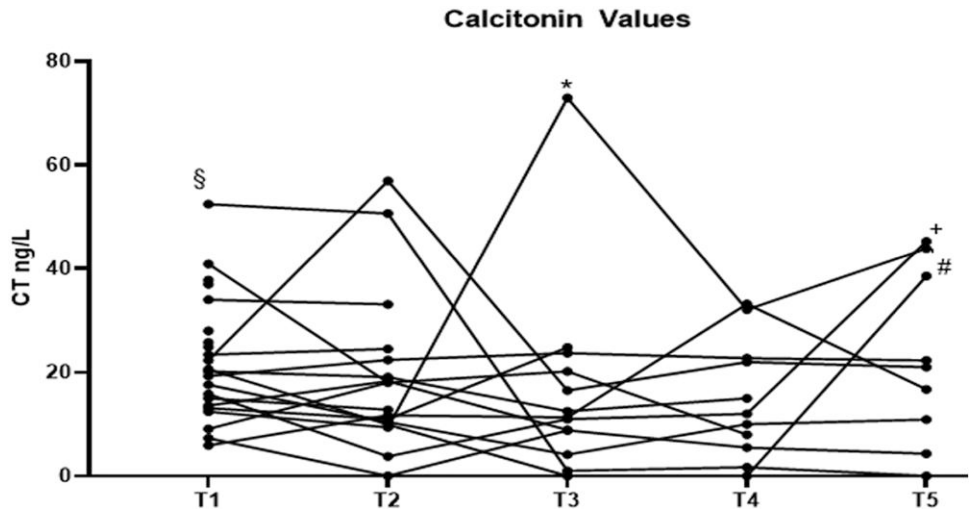

Figure 2. Trend in calcitonin (CT) values throughout follow-up time in the adult cohort (*, # calcitonin was measured in other laboratory; + increase in CT values was due to worsening of renal function; § patient who underwent total thyroidectomy).

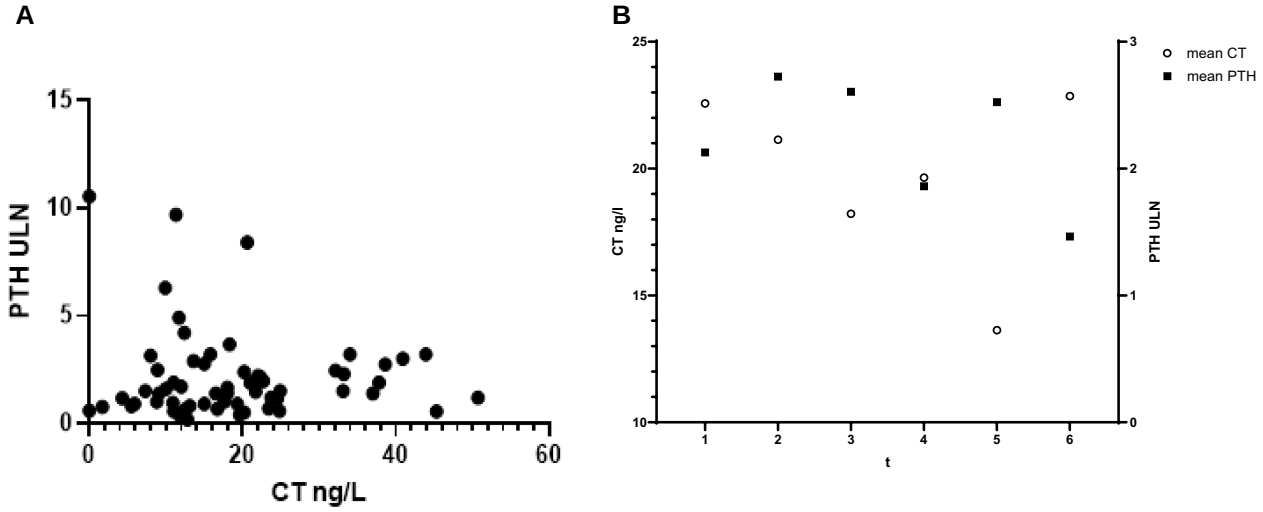

Figure 3. Correlation between parathyroid hormone (PTH) levels (ULN) and calcitonin (CT) levels (ng/L) throughout time. (A) Spearman correlation between individual measurements of PTH and CT levels ($r = 0.09$; $P = .5$). (B) Graphical representation of mean PTH ULN and CT at each time point.

CCH group in both basal ($P = .14$) and peak calcitonin values ($P = .24$).

Because of the small sample size, it was not possible to evaluate gender differences in terms of calcitonin response to calcium stimulation test. However, in the iPPSD group median peak calcitonin levels were 294 (IQR 184-623) ng/L for females and 276 (IQR 152-555.5) ng/L for males; in the CCH group median peak calcitonin levels were 229 (IQR 136.5-469.2) ng/L for females and 238 (IQR 198.2-470.7) ng/L for males; in the MTC group median peak calcitonin levels were 374 (IQR 324-1113) ng/L for females and 862.5 (IQR 431-27 758) ng/L for males.

Screening for Medullary Thyroid Cancer

Thirty-six adult iPPSD patients were screened for MTC and underwent ultrasonography, of whom 20 had hypercalcitoninemia. Four patients out of 16 with normal basal calcitonin values had evidence of thyroid nodules (25%), and 4 patients out of 20 with hypercalcitoninemia were diagnosed with thyroid nodules (20%), showing no significant difference in nodule prevalence between the 2 groups ($P = 1.0$). Among the latter: 1 patient had a cystic nodule of 6 mm, 1 had a solid nodule of 5.7 mm, 1 had a large anechoic nodule of 34 × 26 mm with a solid isoechoic component with bright spots of 16 × 11 mm in diameter, and 1 patient had a solid isoechoic nodule of 12 mm. The latter 2 patients underwent further investigation by calcium stimulation testing and fine needle aspiration. Both patients had a hyper-response to calcium stimulation (respectively 1 female patient, basal calcitonin 52.5 ng/L and peak calcitonin 623 ng/L; 1 male patient, basal calcitonin 34.1 ng/L and peak calcitonin 651 ng/L); while fine needle aspiration resulted negative for features suggestive of malignancy (Tir2) as well as for calcitonin presence on washout fluid. Despite this, the 2 latter patients decided to undergo total thyroidectomy, and, in both cases, pathological examination excluded MTC; however, CCH was described.

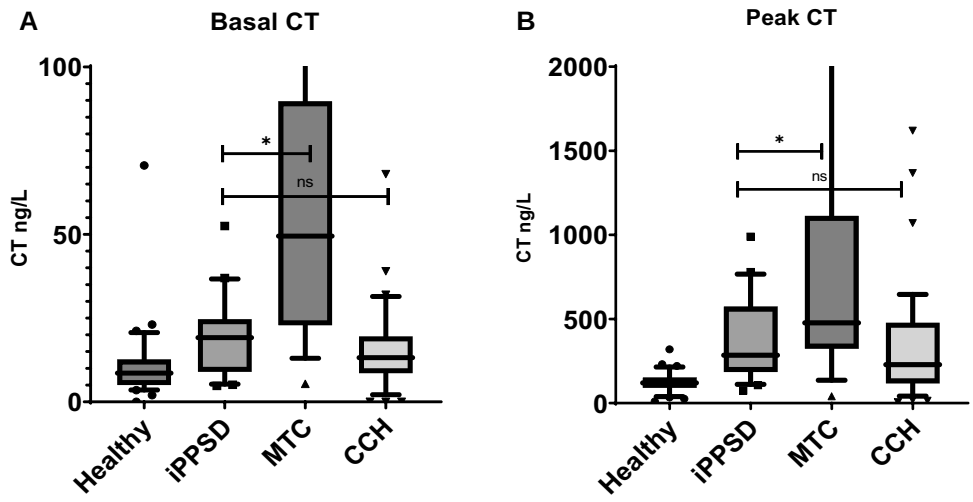

Figure 4. Median basal and peak CT values in adult patients who underwent calcium stimulation test. (A) Comparison of median basal CT levels in control, iPPSD/PHP, MTC, and CCH groups of patients. (B) Comparison of median peak CT levels in control, iPPSD/PHP, MTC, and CCH groups of patients. Abbreviations: CCH, C-cell hyperplasia; CT, calcitonin; iPPSD, inactivating PTH/PTHrP signaling disorder; MTC, medullary thyroid cancer; PHP, pseudohypoparathyroidism.

Discussion

Hypercalcitoninemia has been seldom described in patients diagnosed with iPPSD; however, there are only few case reports published and only one retrospective study has focused on the natural history and mechanisms of hypercalcitoninemia in 6 iPPSD patients (12, 19-21). To our knowledge, no data on calcitonin levels in the pediatric population are available.

In our large cohort we found a significant prevalence of hypercalcitoninemia in iPPSD patients, reaching 65.9% of pediatric patients and 53.5% of adult patients with available calcitonin values. Among our patients, calcitonin levels were only mildly elevated in both cohorts (median basal calcitonin of 15 ng/L, IQR 7.2-25.3 ng/L in the pediatric cohort and of 21.6 ± 8.3 ng/L in the adult cohort). Interestingly, we report that elevated calcitonin levels can be present since an early age; in particular, 65.5% of children developed hypercalcitoninemia before the age of 10 years, and its onset seems to be in line with that of other hormonal resistances. Indeed, 85.7% of patients under the age of 2 years with hypercalcitoninemia had already developed PTH and TSH resistance. Furthermore, we observed a stability of calcitonin levels through time, demonstrated by comparable median calcitonin levels in adult and pediatric populations and unvaried calcitonin levels in the adult population over a long follow-up period.

It is well-known that in the general population serum calcitonin levels are higher in men compared to women, almost certainly due to larger C-cell mass (16). However, we did not find any statistical difference between mean basal calcitonin levels in males and females in both our cohorts, as observed in the CCH group.

Vlaeminck-Guillem et al suggested that elevated calcitonin levels are specific to iPPSD2 (PHP1A subtype) (12); moreover, the majority of case reports describe this condition in iPPSD2/PHP1A patients. In our pediatric population we did indeed find that patients with elevated calcitonin levels more frequently had iPPSD2/PHP1A. However, this subtype was more frequent in children (55.7%), likely associated to an earlier age at diagnosis and more severe clinical manifestations. As a matter of fact, hypercalcitoninemia was linked to an earlier age at diagnosis and higher number of hormonal resistances. On the other hand, in adults with iPPSD3/PHP1B (55.8%) and iPPSD2/PHP1A (39.5%), there was no correlation with iPPSD subtype or with the number of hormonal resistances. Taking into consideration these differences in the 2 cohorts, our final opinion is that hypercalcitoninemia is not correlated to the iPPSD subtype nor associated with disease severity. However, because of the limited number of patients, especially in the adult population and for some subtypes, we can only study differences in iPPSD subtypes due to *GNAS* mutations or epigenetic defects (ie, iPPSD2/PHP1A and iPPSD3/PHP1B) and we cannot give definite conclusions on other subtypes. Furthermore, the retrospective nature of our study limited the description of natural history of hypercalcitoninemia in iPPSD patients and the evaluation of potential predictive factors.

High calcitonin levels are the most sensitive tumoral marker of MTC (16); however, they might be elevated also in patients with chronic renal failure, primary hyperparathyroidism, autoimmune thyroiditis, and other neuroendocrine tumors (16). In our cohort of adult patients MTC was ruled out through periodic clinical and ultrasonographic assessments. Only 4 patients were diagnosed with thyroid nodules and of these patients, 2 underwent total thyroidectomy that excluded MTC. Moreover, in one patient for whom a long follow-up after surgery is available,

Table 3. Median basal and peak CT values in adult patients who underwent calcium stimulation test

	Basal CT ng/L median (IQR)	Peak CT ng/L median (IQR)
Healthy controls	8.6 (5.1-12.6)	120 (91.9-150.5)
PHP/iPPSD	19.2 (9.0-24.7)	285 (184.7-573.8)
MTC	49.5 (22.9-89.7)	477 (324-1113)
CCH	13.2 (8.6-19.5)	238 (117.3-478.5)

Abbreviations: CCH, C-cell hyperplasia; CT, calcitonin; iPPSD, inactivating PTH/PTHrP signaling disorder; MTC, medullary thyroid cancer; PHP, pseudohypoparathyroidism.

both basal and stimulated calcitonin levels became undetectable after thyroidectomy. Other studies have reported lack of any evidence of tumors in surgical specimens from iPPSD patients with elevated calcitonin levels undergoing thyroidectomy (12, 19, 20). Therefore, having ruled out other causes of hypercalcitoninemia, we can conclude that iPPSD may be an independent cause of hypercalcitoninemia.

Serum calcitonin levels in patients with MTC increase in response to calcium or pentagastrin stimulation; on the contrary, there is no increase in patients with various nonthyroidal malignancies and healthy subjects (16, 25). In 1973, Deftos et al were the first to report an abnormal response of calcitonin to calcium stimulation test in iPPSD patients (26), and this was confirmed in later papers (12, 21). Thus, to better assess C-cell function, we performed calcium stimulation test in our cohort of adult patients with elevated basal calcitonin levels.

In this cohort there was a statistically significant difference in both basal and peak calcitonin levels between iPPSD and healthy subjects, thus confirming hyperresponsiveness of calcitonin in iPPSD patients. Furthermore, we found that iPPSD showed significantly lower basal and peak calcitonin values in respect with the MTC group, while this difference was not observed comparing iPPSD and CCH groups. In the authors' opinion, this is an important finding which leads to speculation that iPPSD patients are likely to have CCH, this also being supported by pathological evidence after thyroidectomy in 2 subjects. Further studies are needed to establish a possible cutoff value of calcitonin levels to differentiate the 2 groups; however, the most important clinical implication is the need of a lesser diagnostic burden in iPPSD patients.

The pathogenetic mechanism by which iPPSD is responsible for CCH and hypercalcitoninemia remains unknown (12). Calcitonin receptor is also a transmembrane protein coupled to adenylate cyclase through the G protein; thus, high calcitonin levels and hyperresponsiveness to calcium stimulation test might be due to hormonal resistance (12, 17). Vlaeminck-Guillem's study demonstrated that calcitonin administration, whether intravenous, endonasal, or intramuscular, raises plasma cAMP levels in healthy subjects, but fails to induce a rise in iPPSD2/PHP1A patients, consistent with the unresponsiveness of target tissues to calcitonin (12). Calcitonin resistance could explain the characteristics found and previously discussed such as mildly elevated levels, the nonprogressive trend, and lack of gender difference.

Another hypothesis is that CCH and hypercalcitoninemia are due to an imbalance in the calcium-phosphate system (26, 27). On one hand, the hypocalcemic state might induce an increase in calcitonin thyroidal stores due to a lacking inhibitory effect of serum calcium on calcitonin gene expression (26, 27). On the other hand, hypercalcitoninemia might be favored by low levels of 1,25-dihydroxyvitamin D3 (12, 27) and by elevated PTH levels (16). However, the finding of hypercalcitoninemia in 12 pediatric patients with no concomitant evidence of PTH resistance further supports hormone resistance as the causative mechanism. Finally, we found no correlation between calcitonin and PTH levels, this being also confirmed in other studies (21) and further supporting the proposed underlying mechanism.

Conclusions

Our study reports a high prevalence of hypercalcitoninemia in both adult and children with iPPSD, in particular for subtypes due to *GNAS* mutations or epigenetic defects (ie, iPPSD2/PHP1A and iPPSD3/PHP1B). Adults also show calcitonin hyperresponsiveness to calcium stimulation test, similar to subjects with CCH but different from MTC. iPPSD2/PHP1A and iPPSD3/PHP1B seems to be an independent cause of hypercalcitoninemia and CCH and, although the pathogenetic mechanism is not clearly defined as the calcitonin receptor belongs to the family of transmembrane receptors coupled to the stimulatory G protein, high calcitonin levels are likely be due to insensitivity to the hormone itself, thus adding calcitonin resistance to the list of hormone resistances typical of these rare disorders.

Limitations

We acknowledge that this study is limited by the retrospective nature of the analysis. Although being the largest cohort of patients reported in the literature so far, the relatively small number of subjects limits the conclusions that can be drawn, in particular for some iPPSD subtypes, and precludes the identification of prognostic factors as well as clinical recommendations as to who should be screened for hypercalcitoninemia and when. As previously described, all patients with PTH resistance were treated with oral calcium supplementation and vitamin D active metabolites (calcitriol); unfortunately we are not able to analyze possible correlations between therapies and calcitonin values, as we lack data on the doses used at each time point per patient. However, independently of the therapies, all patients were normocalcemic and normophosphatemic at the time when calcitonin was measured and PTH levels were maintained as low as possible while avoiding overtreatment, following current guidelines for iPPSD.

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Disclosures

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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