Clinical picture of early infancy PTH resistance syndromes: is it time to improve diagnostic criteria?

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Abbreviations

AHO: Albright hereditary osteodystrophy; iPPSD: inactivating PTH/PTHrP signaling disorder; PHP: pseudohypoparathyroidism; PTH: parathyroid hormone; TSH: thyroid-stimulating hormone

ABSTRACT

Background: Clinical presentation of inactivating PTH/PTHrP signaling disorders (iPPSDs, historically pseudohypoparathyroidism (PHP)) exhibits pronounced age-dependence. Indeed, main features, including PTH resistance and brachydactyly, develop during late childhood, whilst other features (ectopic ossifications, obesity and hypothyroidism) are the most prevalent in toddlers. The latter are included among minor diagnostic criteria; therefore, a significant diagnostic delay has been reported. Aim of this work is to describe the early natural history of a large cohort of iPPSD/PHP patients, in order to improve the diagnosis, with the final goal of proposing new diagnostic criteria for early infancy and reducing the diagnostic delay.

Methods: We included 117 patients regularly followed up in two European Endocrinology tertiary centres and we retrospectively collected data on the age of onset of main clinical and hormonal features.

Results: In our cohort the median age at diagnosis was 5.9 years. Age of onset of PTH resistance and brachydactyly, major criteria for diagnosis, was significantly different from that of both TSH resistance and obesity (median age 6.1, 5.8, 1.85 and 2 years, respectively). Minor diagnostic criteria were more represented than major criteria in children before 2 years (p=0.002). Indeed, in 64% of patients before 2 years none of the major criteria were observed, conversely 71% had already developed at least one minor criterion; in particular, 20% had developed TSH resistance and obesity.

Conclusion: Clinical picture of iPPSD/PHP in early infancy differs from that of mid-late infancy and adults, thus current diagnostic criteria may not be appropriate for children. We suggest that the combination of early onset obesity and elevated TSH should raise the suspicion and trigger genetic screening before 2 years of age.

INTRODUCTION

PTH resistance syndromes include a large spectrum of disorders historically named pseudohypoparathyroidism (PHP) and related disorders and newly referred to as inactivating PTH/PTHrP Signaling Disorders (iPPSD) [1]. This group of syndromes is characterized by physical findings, variably associated in each subtype, as well as hormonal and neurocognitive abnormalities [2].

Fuller Albright and colleagues first described PHP in 1942 as the first hormonal resistance to be characterized [3], since then a remarkable clinical variability and/or atypical phenotypes were observed, leading to the new classification and nomenclature, which is basically conceived around the pathophysiology and the underlying genetic defect [1]. Indeed, all these disorders result from impairments in the cAMP-mediated signal transduction pathway, particularly in the PTH/PTHrP signal transduction cascade. A better understanding of this pathway elucidated the pathophysiology of PTH resistance syndromes and therefore the genetic background. The most frequent subtypes of iPPSD/PHP are caused by *de novo* or autosomal dominantly inherited mutations or methylation defects within the *GNAS* complex locus, an imprinted locus encoding the α-subunit of the stimulatory G protein (Gsα) and several other transcripts [4,5]. Moreover, mutations in *PRKAR1A* and *PDE4D*, which are crucial for Gsα-cAMP-mediated signaling, have been found in patients affected by acrodysostosis type 1 and 2, iPPSD4 and iPPSD5 respectively [6,7]. In addition to *PRKAR1A* and *PDE4D* variants, *PDE3A* mutations have also been identified within the PTH1R/Gsα/Camp/PKA pathway, although they are associated with a distinct phenotype characterized by hypertension and brachydactyly type E [8].

A molecular cause can be identified in about 80-90% of patients with iPPSD/PHP [9,10], but clinical and biochemical overlap is frequently described in these patients. Thus, in the absence of a molecular analysis

the diagnostic classification and the understanding of the natural history can be challenging [5] adding more difficulties in the management of this group of disorders.

As for clinical presentation, iPPSD/PHP encompasses a heterogeneous group of diseases, highly variable in clinical features and disease severity, even among patients carrying the same genetic background [5]. Clinical and biochemical features that show minimal or no overlap with other diseases were defined as major criteria for the diagnosis of iPPSD/PHP [11], as suggested by international experts [1]; these include PTH resistance, ectopic ossifications and brachydactyly, the latter associated with at least one major or two minor features. Conversely, minor criteria are less specific to iPPSD/PHP and common to other clinical conditions, i.e. TSH resistance, other hormone resistances such as GHRH, calcitonin, gonadotropins, glucagon and adrenaline, motor and cognitive retardation or impairment, intrauterine and postnatal growth retardation, early onset obesity and overweight, or facial dysmorphisms [1]. Clinical presentation at diagnosis is highly variable in an age-dependent manner, as many of the features, including the three major criteria, develop during mid and late childhood [5]. PTH resistance, the hallmark of these disorders, is usually absent at birth, and overt subsequent hypocalcemia is detected in most patients by the age of 7 to 8 years [11–13]. Similarly, brachydactyly is absent in most patients during early infancy and it becomes evident during puberty [1,11,14]. On the contrary, other features such as TSH resistance or obesity/overweight, currently included among minor diagnostic criteria, are often already present during early infancy [15–17]. Therefore, the clinical picture of early infancy iPPSD/PHP is quite different and less specific than that of late childhood and adulthood, and in this scenario a significant delay in diagnosis may be common [16,18]. Diagnostic delay in iPPSD/PHP may have several relevant implications. It can lead to severe complications, such as hypocalcemia-related seizures, and limit the opportunity for timely monitoring of growth and initiation of growth hormone replacement therapy. Moreover, delayed diagnosis may hamper appropriate genetic counselling for families and compromise the coordination of multidisciplinary care for affected patients.

The aim of the present work is to describe the early natural history of iPPSD/PHP by analyzing a large cohort of patients, in order to improve the diagnostic work-up and reduce the diagnostic delay in these patients, with the final goal to suggest new diagnostic criteria specific for early infancy.

PATIENTS AND METHODS

Patients

We collected data from 117 patients affected with iPPSD/PHP, regularly followed up at the Endocrinology Unit of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy) and at the Department of Endocrinology and Diabetes for Children of Bicêtre Paris Saclay Hospital (Le Kremlin Bicêtre, France). These patients were extracted from the same cohort of patients that we have recently studied focusing on the occurrence of neonatal complications [19], ruling out patients whose age of onset of main clinical and biochemical features could not be dated. Sixty-seven children and 50 adults were included in this study (67 females and 50 males, median age 16.5 years). All patients had a genetically confirmed diagnosis. Detailed information on genetic variants and inheritance patterns is provided in the suppl. Table 1 [20]. Sixty-two patients (53%) were diagnosed with iPPSD2 (PHP1A), 21 (18 %) with iPPSD3 (PHP1B), 11 (9.4 %) with iPPSD4 (acrodysostosis type 1, mutation in *PRKAR1A*), 5 (4.3 %) with iPPSD5 (acrodysostosis type 2, mutation in *PDE4D*), 3 (2.5%) with iPPSD1 (mutation in *PTH1R*) and 15 (12.8%) with iPPSDx (unknown mutation). The study was approved by the local ethical committee of Milan area 2 (ID: 841, approval No. 15_2019bis) and was conducted in accordance with the Declaration of Helsinki. All patients or their legal guardians provided written informed consent prior to study participation.

Methods

We retrospectively extracted from each medical record data about the age of onset or the age of diagnosis of the main clinical and biochemical features (major and minor diagnostic criteria).

We defined early infancy as the period before 2 years, thus including infants (1 to < 12 months) and toddlers (1 to < or = 2 years).

Resistance to PTH was defined as the association of an increased serum level of PTH and elevated serum phosphate (greater than the normal range for age: birth to 4 years, 1.3 to 2.2 mmol/L; 4 to 10 years, 1.2 to 1.7 mmol/L; 10 to 18 years, 1.1 to 1.8 mmol/L; and >18 years, 0.8 to 1.5 mmol/L) and/or low serum calcium (≤2.20 mmol/L) in the absence of vitamin D deficiency and chronic kidney disease. Serum 25-

hydroxyvitamin D levels were assessed on an individual patient basis as part of the diagnostic biochemical evaluation, consequently vitamin D deficiency was excluded or, when present, adequately supplemented in all patients before establishing the biochemical diagnosis of PTH resistance. The same applies to chronic kidney disease, which was ruled out in all cases based on standard clinical and biochemical evaluations.

Brachydactyly was described at physical examination or as radiological evidence of variable shortening of the metacarpals, occasionally accompanied by relatively shortened metatarsals. Ectopic ossifications were evaluated through a careful clinical examination.

Resistance to TSH was defined as elevated serum levels of TSH with normal or slightly reduced levels of thyroid hormones. In some cases, we considered as the age of onset of hormonal resistances the time at which replacement therapies for PTH and TSH resistance were initiated, with active vitamin D analogs or levothyroxine, respectively. Regarding PTH resistance, treatment was generally initiated when PTH levels exceeded twice the upper limit of normal (ULN), regardless of the presence of hypocalcemia, in line with current recommendations[5].

In both cohorts (French and Italy), neonatal screening for congenital hypothyroidism, based on TSH measurement, was systematically performed according to national standards.

All biochemical measurements were performed in local certified laboratories using standardized and validated assays routinely adopted in clinical practice. For PTH measurements, values were also reported as upper limit of normal (ULN), in order to account for differences among assays used across centers.

The presence of obesity was described according to the specific growth charts as weight > 3 SD before 2 years and BMI > IOTF 30 after 2 years, whereas overweight was defined as weight > 2 SD before 2 years and BMI > IOTF 25 after 2 years. In children older than 2 years, the extended international (International Obesity Task Force; IOTF) body mass index cut-offs were used [21]. We respectively used Italian or French growth charts or World Health Organization growth charts for children of other nationalities.

Neurocognitive impairment was not systematically diagnosed through standardized neuropsychological tests but was defined by the caring physician as global retardation of developmental milestones, psychomotor retardation, delayed speech or the need for an assistant teacher and extra school help.

STATISTICS

Quantitative variables describing patient characteristics, including age (years), age at diagnosis (years) and age of onset of main clinical features (years), were expressed as medians with interquartile range (IQR) or means ± SD according to their distribution. Qualitative data were described in numbers and percentages (%).

Normal distribution was verified using Kolomogorov-Smirnov test.

Wilcoxon Signed Rank test was used to compare age of onset of main clinical and biochemical features of iPPSD/PHP, as the variables were not normally distributed. Categorical data were analyzed using the Chi-squared test or Fisher's exact test if the expected value was < 5.

Kaplan-Meier curves were plotted for each major criterion, showing the proportion of patients remaining event-free over time.

P-values < 0.05 were considered statistically significant. All statistical analysis, tables, and figures were done using SPSS version 27 (IBM), Prism version 7.0 and R software version 4.4.1.

RESULTS

In our cohort of patients, the mean age at diagnosis was 7.2 ± 6.7 years (median 5.9 years, IQR 1.8-11.0), being significantly lower in patients affected by iPPSD2/PHP1A ($p < 0.001$).

As for the age of onset of the main clinical and biochemical features (Figure 1, Table 1), PTH resistance was first detected at a median age of 6.1 years (IQR 2.5-11.8, mean 7.8 ± 6.1) and median PTH ULN values were 2 (IQR 1.3-4). Conversely, the age of onset of obesity (median 2 years, IQR 0.5-3.0, mean 2.4 ± 2.6) and the age at which TSH resistance was first detected (median 1.85 years, IQR 0.3-7.7, mean 5.2 ± 7.7; median TSH at diagnosis 8.5 (IQR 6.8-12.2)) were significantly lower ($p = 0.005$ and $p = 0.01$, respectively).

Similarly, brachydactyly was first described at a median age of 5.8 years (IQR 3.3-8.8, mean 5.9 ± 3.6), which is significantly later than the age of onset of TSH resistance ($p = 0.00$) and obesity ($p = 0.002$).

In contrast, no statistically significant difference was detected between the age of onset of PTH resistance and brachydactyly (p = 0.9) and between the age of onset of TSH resistance and obesity (p = 0.4).

Ectopic ossifications are unique and specific to patients affected by iPPSD2/PHP1A/POH, this has somehow impaired statistical significance of some tests (i.e. the age of onset of PTH resistance and ectopic ossifications are not significantly different, p=ns; while the age of onset of brachydactyly is significantly higher than the age of onset of ectopic ossifications, p=0.04). In fact, almost 65% of patients in our entire cohort have never developed ectopic ossifications.

Therefore, considering major criteria for diagnosis, the median age at which the first sign is detected is 4.2 years (IQR 1.0-9.0); at 2 years 64% of patients did not present any major sign while at 10 years only 20% of patients have not developed any major criteria yet (Figure 2).

In detail, 80% of patients developed ectopic ossifications at 3 years, 50% of patients presented with brachydactyly at 5.6 years and 50% received the diagnosis of PTH resistance at 6 years (Figure 3).

Table 1 shows the differences among iPPSD/PHP subtypes regarding diagnostic age and age of onset and detection of main clinical and hormonal features. Noteworthy that the diagnosis and the detection of main features occur earlier in iPPSD2/PHP1A than in other iPPSD/PHP subtypes.

Moreover, comparing diagnostic age and age of onset of clinical and hormonal features between patients with inherited mutations and patients with no family history of iPPSD/PHP, we found that, as expected, patients who inherited the disease were diagnosed earlier, and hormonal resistances were also detected earlier than in patients with *de novo* mutations. On the other hand, clinical features, including brachydactyly, obesity and ectopic ossifications, did not differ as regards to age of onset between patients with inherited mutations and patients with *de novo* mutations (Table 2).

In details, patients without a family history of iPPSD/PHP accounted for 41 out of 117 cases (35%). Among them, 11 patients (27%) were diagnosed before the age of 2 years (mean age 1.2 ± 0.5 years). In most of these early-diagnosed patients (7/11, 64%), the first clinical feature that prompted clinical evaluation was the presence of ectopic ossifications, while two cases (18%) were brought to medical attention because of TSH abnormalities. In the remaining patients the first signs are ectopic ossifications associated with TSH resistance and brachydactyly, respectively (suppl. Table 2) [20].

As for diagnostic criteria, we studied the distribution of both major and minor criteria in patients before 2 years. In 34.5% of patients TSH resistance was diagnosed before 2 years and 36.1% of patients developed obesity or overweight before that age, while 22.2%, 16.7% and 11.8% of patients were diagnosed with ectopic ossifications, PTH resistance or brachydactyly before 2 years, respectively.

Figure 4 illustrates the number of major (A) and minor (B) criteria present before the age of 2 years.

Minor criteria were more represented than major criteria in this population ($p = 0.002$). Indeed, in 64% of patients before the age of 2 years, no major criteria were described, while 71% had already developed at least one minor criterion.

Minor criteria considered in our study were: intrauterine growth restriction (IUGR), cognitive impairment, TSH resistance, other hormonal resistances, short stature and obesity/overweight.

Therefore, we have evaluated the prevalence of the various associations of major and minor criteria before 2 years (Table 3), with the final goal of proposing new diagnostic criteria more useful and appropriate for early infancy. In 20% of patients TSH resistance and obesity/overweight have been simultaneously detected before 2 years, showing a similar prevalence to main major criteria in that age group (PTH resistance and ectopic ossifications, 16.7% and 22.2% respectively).

Brachydactyly is less specific than the other major criteria and should be combined with at least one major or two minor criteria to trigger the diagnosis of iPPSD/PHP as previously suggested [1,22]. In our cohort, brachydactyly and PTH resistance or ectopic ossifications were simultaneously detected in only 3-4% of

patients before the age of 2 years, which is considerably lower than the 20% observed in patients with TSH resistance and obesity/overweight.

Even comparing the concomitant prevalence of brachydactyly and two minor criteria (i.e. TSH resistance, neurocognitive impairment or obesity/overweight) we found a remarkable difference in respect to the prevalence of TSH resistance associated to early onset obesity/overweight.

DISCUSSION

The term pseudohypoparathyroidism (PHP) newly referred to as inactivating PTH-PTHrP signaling disorder (iPPSD) includes a group of rare diseases whose exact prevalence is unknown and estimated to be 1/295,000 in Japan[23], 1/150,000 in Italy (Orphanet ID#12935), 1.1/100,000 in Denmark [24] and probably closer to 1/20,000 in the United States [25]. The diagnostic work-up leading to the diagnosis of iPPSD/PHP starts from clinical and biochemical features that may vary depending on the age of the patient, on the family history, and on the mode of inheritance. The median age at diagnosis in our study cohort was six years (mean seven years), similarly to the age of onset of the iPPSD/PHP major features described in the literature, i.e., PTH resistance and brachydactyly [22]. In particular, as for patients with iPPSD2/PHP1A, PTH resistance, the central hallmark of these diseases, develops gradually over time and overt symptomatic hypocalcemia is present in most patients by the age of 7 to 8 years [5,12,13,26]. In this scenario, an early diagnosis of iPPSD/PHP can thus be challenging, and a diagnostic delay is commonly described.

As previously reported by *Kayemba-Kay's et al.*, iPPSD2/PHP1A seems to have a bimodal clinical and biological presentation pattern [16]. In fact, in their cohort features such as lunar face, subclinical hypothyroidism, subcutaneous ossifications, and rapid weight gain/obesity were the most prevalent signs in toddlers (age < 2 years), while moderate mental retardation, brachydactyly, hypocalcemic seizures, short stature and overt TSH resistance predominated in children older than 2 years [16].

Thus, iPPSD/PHP can have an atypical pattern of onset and development during early infancy.

We include all these disorders under the common definition of "PTH resistance syndromes" and, as such, we expect the occurrence of PTH resistance to raise the first diagnostic suspicion in almost all cases. Other

clinical features are suggestive of iPPSD/PHP and present minimal overlap with other clinical conditions; for this reason they have been included among major diagnostic criteria: ectopic ossifications and brachydactyly [1]. Nevertheless, the clinical picture is more complex and major criteria are often absent, particularly during early infancy.

As for PTH resistance, patients often do not present with hypocalcemia or elevated PTH levels until after few years of life [12]. In order to explain this latency, knockout mouse models were studied and showed that PTH resistance develops with age, despite the presence of the molecular defect since conception, leading to the hypothesis that there is a gradual silencing of the paternal α-subunit of the stimulatory G protein (Gsα) in the renal proximal tubule [13]. In accordance with these observations, PTH resistance in our cohort was first detected at a median age of 6.1 years (IQR 2.5-11.8) and was described in only 16.7% of patients before 2 years. Only 6 patients (5%) of our entire cohort were diagnosed after a hypocalcemic seizure. Some authors speculate that vitamin D and/or calcium supplementation could also play a role in delaying the occurrence of hypocalcemia in toddlers [16], unfortunately we do not have data about early vitamin D and calcium supplementation in our entire cohort.

Among the major diagnostic criteria, brachydactyly is another specific although not early clinical sign. It becomes clearly recognizable during late childhood or pre-puberty [14], except in patients diagnosed with acrodysostosis in which the shortening of metacarpals and metatarsals is usually observed in early infancy [6]. Similarly to PTH resistance, the median age at first detection of brachydactyly in our cohort was 5.8 years (IQR 3.3-8.8) and brachydactyly was described before 2 years in 11.8% of patients only.

Conversely, ectopic ossifications are specific signs of inactivating mutations of *GNAS* exon 1 to 13 and they usually appear earlier during infancy, even at birth in some cases in the form of osteoma cutis [5,11]. *Riepe* et al. have previously reported the case of a newborn presenting with hypothyroidism and subcutaneous ossifications who was subsequently diagnosed with iPPSD2/PHP1A, and concluded that calcinosis cutis in an infant or child should lead to the suspicion of iPPSD/PHP [27]. In line with this case, ectopic ossifications in our series were described at a median age of 1 year (IQR 0.25-2.75), and we observed that these are the only early manifestations among the major diagnostic criteria.

Indeed, there are some reports in literature on the early clinical and biochemical features in iPPSD/PHP

supporting the hypothesis that major criteria are not so accurate for diagnosis during early infancy [11–13,28]. These are either single case reports or small case series. Our data confirm and better elucidate this aspect, highlighting that the age of onset of PTH resistance and brachydactyly is significantly greater than the age of onset of TSH resistance, obesity and ectopic ossifications. We can therefore speculate that PTH resistance/brachydactyly and TSH resistance/early obesity represent two pairs of clinical features which show a strong age dependence.

TSH resistance has often been reported as one of the first biochemical signs of iPPSD/PHP [29–31] even at birth, being in some case misdiagnosed as congenital hypothyroidism [17,32,33]. Thus, if some authors suggest to screen for iPPSD/PHP all infants presenting with concomitant hypothyroidism and ectopic ossifications [27], others recommend to consider PTH resistance syndromes when there is the simultaneous detection of congenital hypothyroidism and hyperphosphatemia, even if Albright Hereditary Osteodystrophy (AHO) is missing [32].

Early onset obesity is another minor criterion variably associated with some iPPSD/PHP subtypes [15,34–36] and together with TSH resistance it could be considered as one of the earliest manifestations suggesting the diagnosis. Recently, a large cohort of patients with severe early onset obesity was screened for loss-of-function mutations of *GNAS,* revealing an unexpectedly high prevalence (22/2548, 1%) [37]. Interestingly, also in these patients TSH resistance was an early feature, while PTH resistance and overt hypocalcemia emerged over time, confirming the findings of the previous series. The authors seem to suggest that screening for *GNAS* children with early onset of severe obesity may allow to reduce the diagnostic delay, similarly to what previously suggested from *Kayemba-Kay's et al.* in their series of ten patients [16].

In our study cohort, TSH resistance was detected at a median age of 1.85 years (IQR 0.3-7.7) and it was already diagnosed in 34.5% of patients before 2 years. Similarly, the median age of onset of obesity was 2 years (IQR 0.5-3.0) and 36.1% of patients presented with obesity or overweight before the age of 2 years.

Trying to dissect the best association of diagnostic criteria suitable for early infancy, TSH resistance and early onset obesity have shown a higher prevalence in children before 2 years (20%), in comparison to the

association of two major criteria such as PTH resistance and brachydactyly, that were present in only 4% of children before that age, or to the association of brachydactyly and two minor criteria as suggested by current guidelines.

Figure 5 proposes a diagnostic flow-chart for early infancy. In line with our findings, we suggest the importance of suspecting PTH resistance syndromes in children presenting with early onset obesity and increased TSH levels. Specifically, in our proposed diagnostic flow-chart, the coexistence of TSH resistance and rapid weight gain before 2 years of age represents a potential early warning sign that may lead to the genetic testing, after careful clinical evaluation. Conversely, the presence of only one of these two findings should be interpreted in the context of other suggestive features, such as additional hormonal resistances, brachydactyly, elevated PTH levels, neurocognitive impairment etc.

Unfortunately, statistical analysis stratified by iPPSD/PHP subtype could not be performed, due to the small size of some cohorts (i.e., iPPSD4, iPPSD5 or iPPSD1). Notably, iPPSD2/PHP1A represents a more complex clinical entity, characterized by both hormonal resistances and the constellation of physical features defined as Albright Hereditary Osteodystrophy (AHO), which includes a round face, a stocky habitus with short stature, brachydactyly and ectopic ossifications [5]. This could partly explain why diagnostic age, as well as the age of onset of main clinical or biochemical features, is lower in this subgroup of patients compared to the others (Table 1).

However, when comparing patients with inherited mutations to those with *de novo* mutations, no differences in the age of onset of clinical features were observed, whereas biochemical abnormalities were usually detected earlier in patients with inherited disease (Table 2). This finding is rather expected, as patients with a known family history of iPPSD/PHP are tipically screened and followed up earlier, highlighting the need for further studies exploring the natural disease history in newborns of affected adults.

CONCLUSIONS

The major features of iPPSD/PHP usually appear in late childhood with consequent common diagnostic delay. Nevertheless, a few complications and clinical or biochemical features are already present in the neonatal period or early infancy. Our study confirms the previous series reporting a bimodal age-dependent clinical scenario and it points out that current diagnostic criteria may not be accurate to diagnose the disease in toddlers. Reducing the diagnostic delay may have different important consequences. Firstly, it may prevent the most severe clinical complications, such as hypocalcemia-related seizures. Moreover, accounting the important impact of iPPSD2/PHP1A and acrodysostosis on statural growth, an earlier diagnosis could allow to better monitor height and growth rate in order to introduce recombinant human growth hormone (rhGH) replacement therapy during the limited time window for a potentially effective therapy [38]. Finally, reducing the diagnostic delay could also improve both the genetic counselling for families planning to have another child and the multidisciplinary management of the affected children. In our cohort, the most represented clinical and biochemical features in the first years of life were TSH resistance and early onset obesity/overweight, currently included among minor diagnostic criteria, pointing out that diagnostic criteria should be reviewed and differentiated according to age.

LIMITATIONS

We acknowledge that this study is limited by the retrospective nature of the analysis. For this reason, we could not be able to retrieve all data on the age of onset of clinical and biochemical features. Further prospective studies are needed to better clarify the natural early infancy history of iPPSD/PHP, particularly enrolling all newborns who inherited the disease from known adult patients. Thus, our conclusions are to be understood as recommendations and a basis for future research. 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 for each iPPSD/PHP subtype. However, this study set the need of revising diagnostic criteria for early infancy. It is possible that a bimodal diagnostic approach for children and adults is requested.

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Data Availability: Some or all data generated or analyzed during this study are included in this published article or in the supplemental information; additional data are available upon reasonable request.

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Legends for figures and tables

Figure 1. Age of onset and age at first detection of the main clinical and biochemical features of iPPSD/PHP (all patients included) (PTH-R: PTH resistance, TSH-R: TSH resistance, BD: Brachydactyly, OB: obesity, EO: ectopic ossifications; ** = $p < 0.05$, ***= $p < 0.005$).

Figure 2. Percentage of patients presenting major criteria (that is probability of being major criteria free) overtime (Kaplan Meier estimation).

Figure 3. Percentage of patients presenting with PTH resistance, ectopic ossifications and brachydactyly (that is probability of being major criteria free) overtime (Kaplan Meier estimation).

Figure 4. Number of major (A) and minor (B) diagnostic criteria observed before the age of 2 years.

Figure 5. Proposed flow-chart for diagnosis of iPPSD/PHP patients during early infancy.

Table 1. Percentage distribution, age of onset and age at first detection of the main clinical and hormonal features across iPPSD/PHP subtypes.

Table 2. Comparison between patients with inherited diseases and patients with *de novo* mutations regarding age of diagnosis and age of onset and detection of clinical and hormonal features, respectively.

Table 3. Distribution of clinical and biochemical features before 2 years.

Table 1.

	Total	iPPSD1 (*PTHR1* mutation)	iPPSD2 (*GNAS* mutation)	iPPSD3 (*GNAS* methylation defect in the promoter)	iPPSD4 (*PRKAR1A* mutation)	iPPSD5 (*PDE4D* mutation)	iPPSDx (unknown mutation)
N° of patients	117	3	62	21	11	5	15
Sex (F/M)	67/50	2/1	32/30	11/10	8/3	4/1	10/5
Median age at diagnosis (Q1-Q3) years	5.9 (1.8-11.0)	10.3 (10.1-12.3)	2.3 (1.0-5.0)	12.2 (7.0-16.7)	7.0 (2.7-9.5)	8.0 (5.0-11.6)	11.3 (8.7-11.8)
PTH resistance (%)	88 (75)	2 (67)	46 (74)	21 (100)	11 (100)	1 (20)	7 (47)
Median age at PTH resistance diagnosis (Q1-Q3) years	6.1 (2.5-11.8)	13.0 (12.3-13.6)	2.7 (1.4-6.0)	12.0 (5.9-14.0)	8.6 (3.7-11.3)	3.6	13.4 (11.3-14.5)
TSH resistance (%)	77 (66)	1 (33)	51 (82)	13 (62)	11 (100)	0 (0)	1 (7)
Median age at TSH resistance diagnosis (Q1-Q3) years	1.85 (0.3-7.7)	0.7	1.2 (0.1-2.6)	13.1 (9.3-18.0)	7.6 (3.6-9.5)		NA
Brachydactyly (%)	85 (73)	2 (67)	51 (82)	4 (19)	11 (100)	5 (100)	12 (80)
Median age at brachydactyly onset (Q1-Q3) years	5.8 (3.3-8.8)	6.5 (5.8-7.3)	4.9 (2.6-7.3)	6.7 (6.5-9.5)	5.0 (3.1-7.0)	5.0 (4.0-6.0)	10.3 (8.1-11.7)
Ectopic ossifications (%)	40 (34)	0 (0)	39 (63)	0 (0)	0 (0)	0 (0)	1 (7)
Median age at ectopic ossifications onset (Q1-Q3) years	1.0 (0.25-2.75)		1.0 (0.3-2.5)				NA
Obesity (%)	49 (42)	1 (33)	38 (61)	6 (26)	0 (0)	3 (60)	1 (7)
Median age at obesity onset (Q1-Q3) years	2.0 (0.5-3.0)	1.8	2.0 (0.6-3.0)	0.4 (0.3-0.5)		6.7 (4.3-6.8)	NA

NA = Not available = Missing data

Table 2.

Variable	Inherited mutation (n=42)	De Novo mutation (n=41)	p
Median Diagnostic Age (Q1–Q3) years	2.55 (0.5-5.5)	7.8 (2.0-12.2)	**0.002**
Median Age of TSH resistance onset (Q1–Q3) years	0.8 (0.1-2.4)	7.4 (0.8-15.0)	**0.002**
Mean Age of PTH resistance onset (SD) years	4.4 (3.5)	9.2 (8.1)	**0.003**
Median Age of brachydactyly onset (Q1–Q3) years	5.0 (2.2-7.6)	5.5 (3.5-9.0)	**0.28**
Median Age of obesity onset (Q1–Q3) years	2.0 (0.6-2.9)	2.1 (0.4-6.0)	**0.40**

Median Age of ectopic ossifications onset (Q1–Q3) years	1.7 (0.3-5.5)	0.4 (0.2-1.0)	**0.13**

Table 3.

	N	%
Obesity/Overweight (OB/OW)	39/108	36.1
TSH resistance	39/113	34.5
Ectopic ossifications (EO)	26/117	22.2
Neurocognitive impariment (NC)	22/100	22
PTH resistance	19/114	16.7
Brachydactyly (BD)	13/110	11.8
OB/OW + TSH resistance	23/114	20
OB/OW + TSH resistance + EO	10/115	9
OB/OW+ TSH resistance + NC	10/112	9
BD + OB/OW + NC	4/92	4.3
BD + TSH resistance + NC	4/95	4.2
BD + PTH resistance	5/116	4
BD + EO	4/110	3.6
BD + TSH resistance + OB/OW	3/101	3

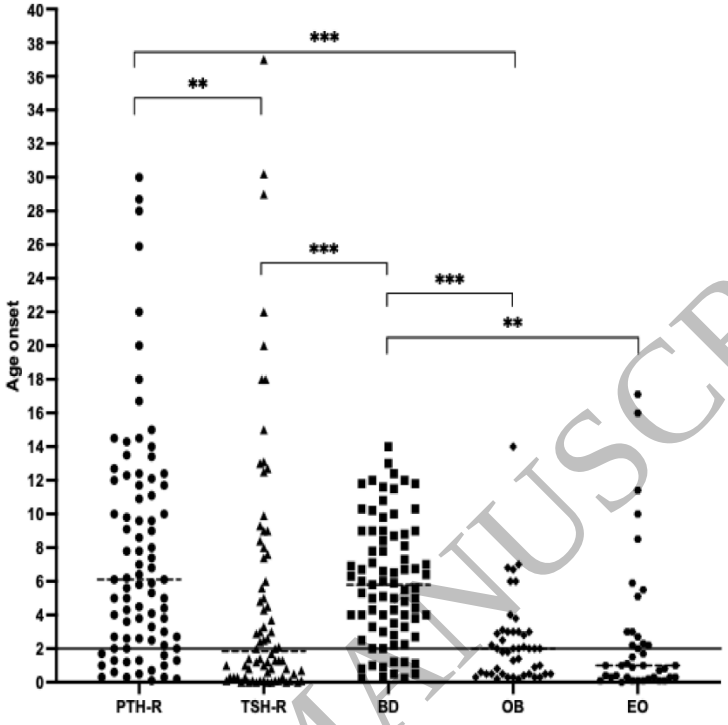

Figure 1
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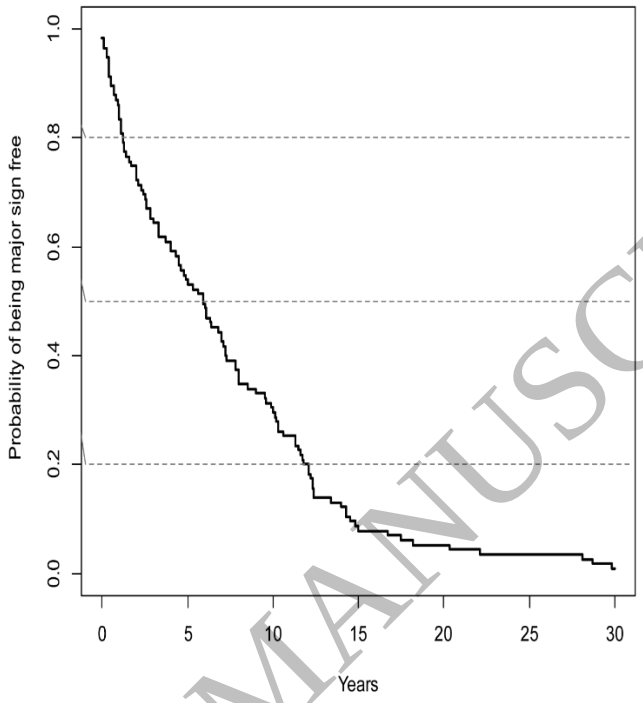

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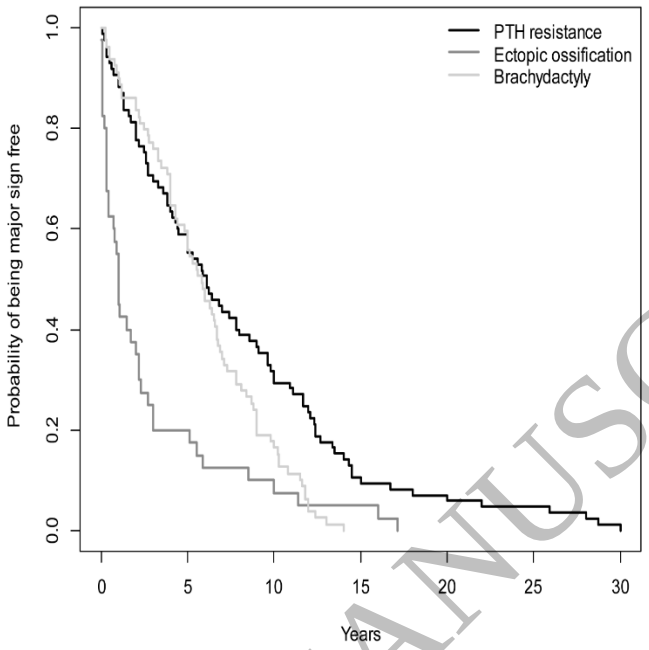

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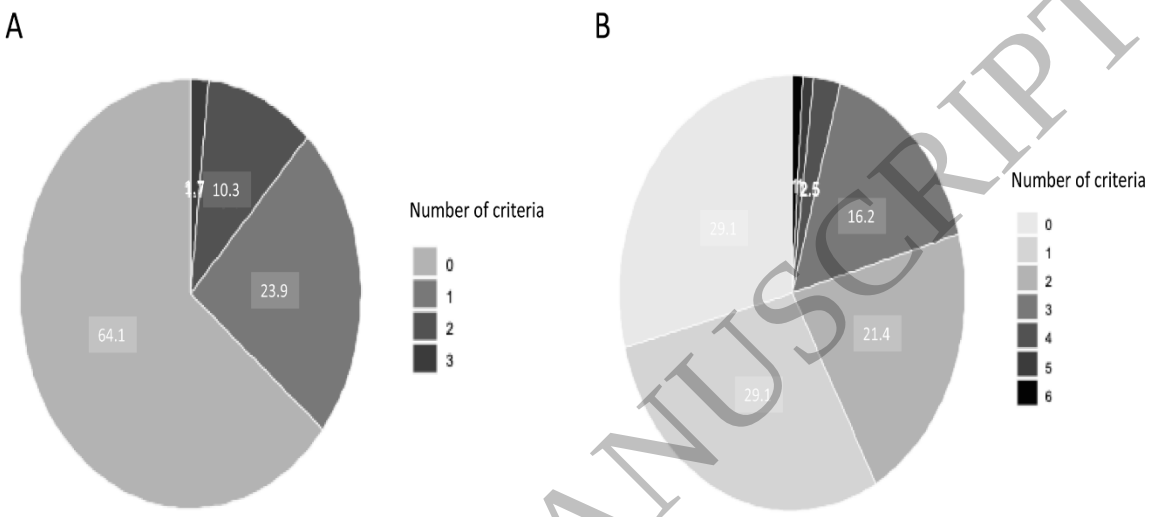

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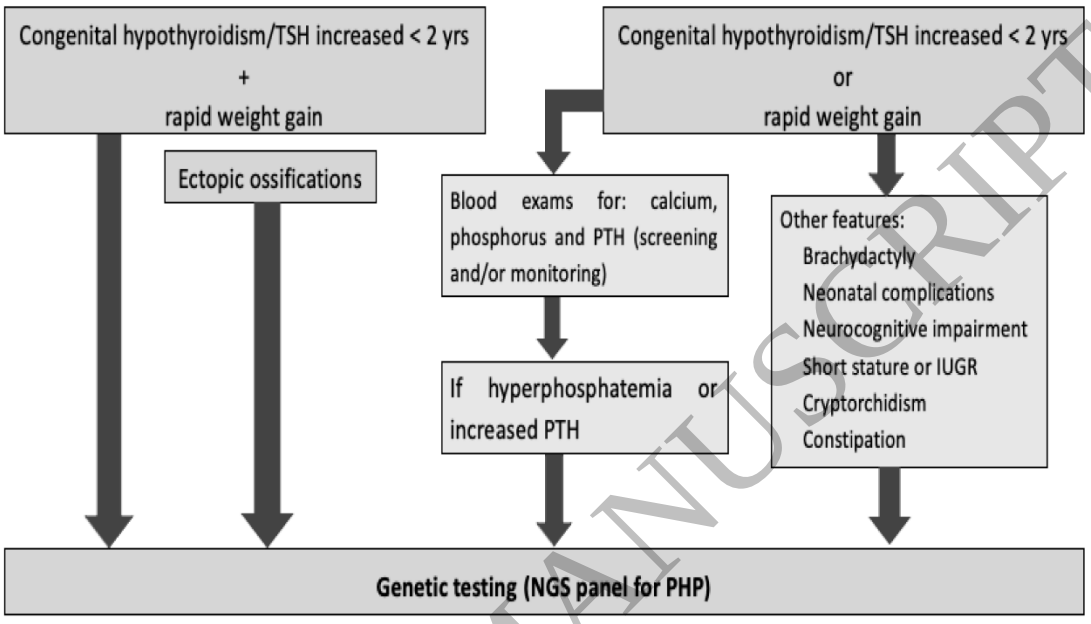

Figure 5
339x190 mm (x DPI)