

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

**Inactivating PTH/PTHrP signaling disorders (IPPSDs): evaluation of the new classification in a multicenter large series of 544 molecularly characterized patients**

**This is a pre print version of the following article:**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1767409> since 2021-01-19T10:41:46Z

*Published version:*

DOI:10.1530/EJE-20-0625

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

# 1 Inactivating PTH/PTHrP signaling disorders (iPPSDs): validation of the new 2 classification in a multicenter large series of 544 patients

3 Arrate PEREDA\*<sup>1</sup>, Francesca M ELLI\*<sup>2</sup>, Suzanne THIELE<sup>3</sup>, Luisa de SANCTIS<sup>4</sup>, Anya ROTHENBUHLER<sup>5</sup>, Patrick  
4 HANNA<sup>6</sup>, Bruno FRANCOU<sup>6,7</sup>, Alexandra ERTL<sup>8</sup>, Guiomar PEREZ de NANCLARES<sup>#1</sup>, Agnès LINGLART<sup>§5,6</sup>,  
5 Giovanna MANTOVANI<sup>§2</sup>

## 6 **Authors**

7 \*First co-authors

8 <sup>§</sup>Last co-authors

9 <sup>#</sup>Corresponding author

10 <sup>1</sup>Molecular (Epi)Genetics Laboratory, BioAraba Research Health Institute, Hospital Universitario Araba-  
11 Txagorritxu, Vitoria-Gasteiz, Alava, Spain.

12 <sup>2</sup>Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Endocrinology Unit, Milan, Italy University of  
13 Milan, Department of Clinical Sciences and Community Health, Milan, Italy

14 <sup>3</sup>Lubeck

15 <sup>4</sup>Department of Public Health and Pediatric Sciences, University of Torino, Regina Margherita Children's  
16 Hospital-AOU Città della Salute e della Scienza, Torino, Italy.

17 <sup>5</sup>AP-HP, Endocrinology and diabetes for children, Reference Center for Rare Disorders of Calcium and  
18 Phosphate Metabolism, Platform of Expertise Paris-Saclay for Rare Diseases and Filière OSCAR, Bicêtre Paris  
19 Saclay Hospital, Le Kremlin-Bicêtre, France

20 <sup>6</sup>INSERM U1185, Bicêtre Paris Sud - Paris Saclay University, Le Kremlin-Bicêtre, France.

21 <sup>7</sup>AP-HP, department of molecular genetics, Platform of Expertise Paris-Saclay for Rare Diseases, Bicêtre Paris  
22 Saclay Hospital, Le Kremlin-Bicêtre, France

23 <sup>8</sup>Vienna

## 24 **#Correspondence:**

25 Dr Guiomar Perez de Nanclares, Molecular (Epi)Genetics Laboratory, BioAraba Research Health Institute,  
26 Hospital Universitario Araba-Txagorritxu, Vitoria-Gasteiz, Alava, Spain

27 [gnanclares@osakidetza.eus](mailto:gnanclares@osakidetza.eus)

28 **Keywords:**

29 Pseudohypoparathyroidism, inactivating PTH/PTHrP signaling disorder; classification, PTH resistance,  
30 brachydactyly, ossifications, genomic imprinting

31 **Abstract**

32 Pseudohypoparathyroidism (PHP) and related disorders belong to group of heterogeneous rare diseases that  
33 share an impaired signaling downstream of G $\alpha$ -protein coupled receptors. Affected patients may present  
34 with various combination of symptoms including resistance to PTH and/or to other hormones, ectopic  
35 ossifications, brachydactyly type E, early onset obesity, short stature and cognitive difficulties. Several years  
36 ago, the delay in diagnosis, the variability in disease presentation, as well as the increasing molecular diversity  
37 causing the PHP spectrum have prompted us to propose a novel nomenclature under the term of inactivating  
38 PTH/PTHrP signaling disorders (iPPSD). This novel classification relied on criteria chosen by a group of experts  
39 based on literature evidence. It is now of utmost importance to validate these criteria and/or improve the  
40 basis of this new classification through the thorough analysis of a large international series of 459 probands  
41 and 85 relatives molecularly characterized. In this report, we demonstrate that more than 98% of the  
42 probands met the criteria initially defined, i.e. resistance to PTH (rPTH) and/or ectopic ossifications (EO)  
43 and/or brachydactyly (BR) associated with 2 minor criteria. Noteworthy, most patients (85%) presented a  
44 combination of symptoms rather than a single sign suggestive of iPPSD. Although specific clinical patterns did  
45 show up such as isolated PTH resistance as the main manifestation of iPPSD3 due to *GNAS* methylation  
46 defect, our study confirmed the overlap among the different genetic forms of iPPSD. The clinical and  
47 molecular characterization of iPPSD relatives identified familial history as an additional important criterion  
48 predictive of the disease.

49 Overall, the phenotypic analysis of this large cohort confirmed the validity of the major and minor criteria  
50 and their combination to diagnose iPPSD. This report shows the importance of having simple and easily  
51 recognizable signs to diagnose with confidence these rare disorders and supports a better management of  
52 patients.

53 **Introduction**

54 Pseudohypoparathyroidism (PHP) encompasses a spectrum of related, highly heterogeneous and frequently  
55 overlapping disorders deriving from molecular defects that impair the hormonal signaling *via* receptors  
56 coupled to the adenylyl cyclase by the  $\alpha$ -subunit of the stimulatory G protein (G $\alpha$ ).<sup>1-3</sup>

57 The term PHP includes several subtypes, including PHP type 1A (PHP1A, MIM#103580), PHP type 1B (PHP1B,  
58 MIM#603233) and PHP type 1C (PHP1C, MIM#612462), characterized by biochemical features of  
59 hypoparathyroidism due to peripheral resistance to the action of the parathyroid hormone (PTH) caused by  
60 genetic or epigenetic defects within or upstream the *GNAS* locus, that encodes for the G $\alpha$ .<sup>3</sup> Frequently,  
61 patients also suffer from resistance to other hormones acting through G $\alpha$ -coupled receptors, such as the  
62 thyroid-stimulating hormone (TSH), gonadotropins, growth hormone-releasing hormone (GHRH) and  
63 calcitonin.<sup>3,4</sup> Additionally, individuals with PHP1A and PHP1C variably express early-onset obesity together  
64 with a series of physical features (pre- and/or post-natal growth retardation, dysmorphic facies, varying  
65 degrees of intellectual and/or cognitive impairment and development delay, brachydactyly and ectopic  
66 ossifications) termed Albright hereditary osteodystrophy (AHO).<sup>5</sup> The presence of AHO without PTH  
67 resistance is defined as pseudopseudohypoparathyroidism (PPHP, MIM#612463), while, in case of ectopic  
68 ossifications extending into deep muscles and connective tissues, as progressive osseous heteroplasia (POH;  
69 MIM#166350).<sup>6</sup> Acrodysostosis (ACRDYS, MIM#101800), that is associated with genetic defects at the  
70 *PRKAR1A* and *PDE4D* genes, does also present signs similar to PHP such as brachydactyly, extensive facial  
71 dysmorphism, developmental delay and, frequently, PTH and TSH resistance.<sup>7-9</sup> Molecular alterations at  
72 another PDE gene, the *PDE3A* gene, are finally associated with the autosomal dominant hypertension and  
73 brachydactyly type E syndrome (HTNB, MIM#112410), characterized by brachydactyly type E, severe salt-  
74 independent but age-dependent hypertension, increased fibroblast growth rate, altered baroreflex blood  
75 pressure regulation and juvenile death from stroke when untreated.<sup>10</sup>

76 Several research studies on the clinical and molecular background associated with different PHP subtypes  
77 demonstrated that the delay in obtaining a specific diagnosis often derives from the extremely variable  
78 presentation, the severity of PHP signs and symptoms among patients, even in those carrying the same  
79 genetic alteration, as well as from the significant clinical and molecular overlap both among PHP subtypes  
80 and between PHP and the above mentioned related diseases.<sup>11-15</sup> Moreover, a correct early diagnosis in  
81 infants and in individuals with atypical features is very rarely achieved because clinical symptoms may be  
82 isolated in infancy and considered as poorly specific; biochemical abnormalities typically worsen during  
83 childhood.

84 In 2016, the European Network for the study of PHP (EuroPHPnetwork) conducted an expert initiative to  
85 produce a new nomenclature and classification encompassing all disorders with impairments in PTH and/or

86 PTHrP cAMP-mediated pathway in order to overcome the limits of the historical classification that  
87 disregarded related disorders. More importantly, the former classification did not consider molecular defects  
88 as distinctive criteria, thus failing to stratify many disorders including PHP and AHO. According to the novel  
89 proposal, the term inactivating PTH/PTHrP signaling disorder (iPPSD) was proposed instead of PHP, followed  
90 by a numbering for specific subtypes that allows the description of both clinical and molecular features  
91 (iPPSD1, loss-of-function variant in *PTHR1*; iPPSD2, loss-of-function alteration in *GNAS*; iPPSD3, methylation  
92 defects at one or more *GNAS* DMRs; iPPSD4, *PRKAR1A* pathogenic variant; iPPSD5, *PDE4D* pathogenic variant;  
93 iPPSD6, *PDE3A* pathogenic variant; iPPSDx, no molecular defect identified).<sup>6</sup> Such nomenclature will be used,  
94 together with the classical one when necessary, through the text.

95 The main advantages of the new suggested terminology can be summarized as 1) the definition of a common  
96 mechanism responsible for all diseases, 2) the inclusion of non-genetically characterized patients into the  
97 classification, 3) the avoidance of the ambiguous terms like “pseudo” and 4) the erasure of the clinical and  
98 molecular overlap between diseases.

99 Consequently, it is now of major importance to validate, and improve if necessary, the newly proposed  
100 classification. Hence, we propose a second position paper produced by the EuroPHPnetwork on the  
101 terminology and the classification of disorders characterized by the inactivation of the PTH/PTHrP signalling  
102 pathway. The aim of the present work was to evaluate a large, international case series of highly clinically  
103 and molecularly characterized patients by using the criteria recently proposed. In this large cohort of  
104 genetically confirmed patients, we investigated whether patients met the clinical major and minor criteria.<sup>16</sup>  
105 In addition, we considered still unexplored features to design additional objective criteria to guide an efficient  
106 distinction and stratification of iPPSD subtypes.

107

## 108 **Patients and methods**

109 This work was designed by clinicians and scientists from 3 tertiary centers (Italy, Spain and France) of the  
110 EuroPHPnet. Clinical and molecular data from 459 index patients (Supp.Tab.1) and 85 relatives followed in  
111 their clinical centers and laboratories over the last decades were collected.

112 Inclusion criteria for the study were the availability of complete clinical data at the time of the clinical  
113 diagnosis of each patient and a confirmative molecular diagnosis of a (epi)genetic alteration at *GNAS*,  
114 *PRKAR1A*, *PDE4D* or *PDE3A* loci.

115 Clinical features were divided into major [PTH resistance (rPTH), ectopic ossifications (EO) and brachydactyly  
116 (BR)] and minor criteria (TSH resistance, additional hormone resistances, motor and/or cognitive retardation  
117 or impairment, intrauterine growth retardation and/or post-natal growth retardation, obesity or overweight,  
118 and flat nasal bridge and/or maxillar hypoplasia and/or round face) according to the new proposal for

119 diagnosis and classification.<sup>6</sup> The minimum criteria for a clinical diagnosis of iPPSD were initially defined as at  
120 least one major criterion, either PTH resistance or ectopic ossifications or brachydactyly; in case of  
121 brachydactyly, 2 additional minor criteria were required as well.<sup>6</sup>

122 The molecular workout to identify iPPSD/PHP-related alterations has been described previously.<sup>6,16</sup>

123 Only index cases were included in the analysis to prevent bias, while relatives were evaluated separately. All  
124 patients, legal guardians for minors and relatives involved in the study subscribed the informed consent for  
125 genetic studies and the treatment of personal and clinical data. All procedures were performed in compliance  
126 with relevant legislation and institutional guidelines and were approved by the IRCCS Fondazione Cà Granda  
127 Ospedale Maggiore Policlinico institutional committee (PHP2019, parere 15\_2019bis), the comité consultatif  
128 sur le traitement de l'information en matière de recherche dans le domaine de la santé (CCTIRS, #13-028)  
129 under the promotion of the INSERM (Institut national de la santé et de la recherche médicale) (#DC-2013-  
130 1762), and the Basque Ethics Committee (IRB #PI2013214 and PI2017018).

131

## 132 **Results and Discussion**

### 133 ***Validation of minimum criteria for iPPSD clinical diagnosis***

134 The first and main aim of the present study was to test the detection rate achieved by re-evaluating our  
135 cohort of 459 PHP patients (205 males and 254 females) with a confirmatory molecular diagnosis following  
136 the proposals given by the EuroPHPnetwork in 2016. This meant to determine whether patients showed at  
137 least one major criterion between resistance to PTH (rPTH) and ectopic ossifications (EO) or brachydactyly  
138 (BR) associated with 2 minor criteria.

139 Out of the 459 patients, all but 8 patients (1.7%) met these minimum criteria, demonstrating a 98.3%  
140 detection rate. Thus, the proposed classification showed to work properly and allowed to identify almost all  
141 iPPSD patients at a first screening.

142 Of the 451 patients meeting the minimum criteria, 70 patients (15.5%) presented only 1 major criterion while  
143 381 (84.5%) had either 1 major criterion associated with minor criteria or from 2 up to 3 major criteria with  
144 or without minor criteria (Fig. 1). As expected, although the age at diagnosis was not significantly different  
145 between these two subgroups, the mean age at diagnosis was slightly lower in the group of individuals with  
146 a more complex phenotype (14.9 years vs 21.8 years). Altogether, this suggests that the association of several  
147 symptoms allows an earlier detection of iPPSD patients. No significant difference associated with gender was  
148 found in both groups (Tab.1).

149 Affected subjects without 1 major criterion, therefore not meeting the new criteria for diagnosis, were 7  
150 females and one male; they were diagnosed clinically between the age of 3 and 15 and genetically between

151 the age of 6 and 15 years. Two of them presented with brachydactyly only (IT11 and IT231), two other  
152 patients (SP85, SP130) presented with brachydactyly and pre- and/or post-natal growth retardation and 4  
153 patients (IT137, FR83, IT16 and FR76) did not match any major criteria for iPPSD; the latter patients presented  
154 with TSH resistance (IT16 and FR83) isolated or in association with additional minor criteria or isolated obesity  
155 (FR76). Patient IT137 was referred for suspected mild PTH resistance associated with overweight and  
156 endocrine hyperfunction (subclinical hyperthyroidism, syndrome of inappropriate natriuresis and mild  
157 hypercortisolism) but PTH resistance reverted after vitamin D supplementation (Supp.Tab.1). For most of  
158 these patients, we cannot exclude that some features, at an early stage of their development, could be  
159 unnoticed at the first evaluation or that, given the young age of the patients, they could develop other signs  
160 overtime. Among these 8 patients, we identified 6 iPPSD2 patients carrying a *GNAS* coding mutation, one  
161 iPPSD3 patient and one iPPSD5 patient. We can rule out the possibility that the phenotypic variability is due  
162 to the genetic background as 2 out of 6 iPPSD2 patients carry molecular defects located in the hot spot  
163 regions of *GNAS*, i.e. an exon 1 pathogenic variant (patient IT11) and the only recurrent 4bp-deletion in exon  
164 7 (c.568\_571del, patient IT231), while the remaining 4 iPPSD2 subjects showed genetic variants located at  
165 aminoacidic positions whose replacement is predicted to have a damaging effect both by bioinformatics,  
166 previous reports from the literature<sup>17,18</sup> and Leiden Open Variation Database database (LOVD at  
167 <https://databases.lovd.nl/shared/genes/GNAS>). In addition, among the 6 iPPSD2 patients, 2 were  
168 paternal mutations (former pseudopseudohypoparathyroidism), one was a maternal *GNAS* coding mutation  
169 while the other 3 were of unknown allele origin.

#### 170 ***Age at clinical diagnosis of iPPSD and signs at diagnosis***

171 We divided our cohort in age groups including infancy (from birth up to 2 years, n=53, 11.5%), early childhood  
172 (from 3 to 8 years, n=104, 22.7%), middle childhood (from 9 to 11 years, n=64, 13.9%), adolescence (from 12  
173 up to 18 years, n= 94, 20.5%) and adulthood (over 18 years, n=138, 30.1%). The age at clinical diagnosis was  
174 not available in 6 patients. We found that, at the time of iPPSD diagnosis, most probands (68.6%) were  
175 children or adolescents, with a peak in early childhood (from 3 to 8 years) and in adolescence (from 12 up to  
176 18 years), and the remaining 30.1% were adults (>18 years). In particular, within the whole cohort of  
177 probands, the age range at clinical diagnosis was 0-68 years and the mean age was 15.8±13.6 years. Thus, a  
178 prompt identification of possible iPPSD cases by pediatricians is very important. We did not observe any  
179 gender difference both considering the case series as a whole and the different age groups (Tab. 2).

180 The next step was to define the clinical presentation at diagnosis, in particular the number and combination  
181 of major criteria with or without minor criteria, both considering the whole cohort and the division into age  
182 groups (Fig. 2). The most frequent presentation was isolated rPTH, that was found in 57 (12.4%) of patients.  
183 The second most frequent isolated sign was BR (associated with two minor criteria) in 12 (2.6%) of probands.



184 Finally, the less frequent isolated presentation was EO in one (0.2%) patient. On the other hand, 84.8% of the  
185 patients presented one of the major criteria combined with either another major or minor criteria.

186 When the number of criteria used for the diagnosis was analyzed in the different age ranges, we observed  
187 that younger people presented more clinical features than elder people (Fig. 2). During infancy, childhood,  
188 and adolescence and adulthood, the presence of complex phenotypes associating at least one major criterion  
189 and other criteria, minor or majors, was found in 94%, 81-90%, and 76-78% of the patients, respectively (Fig.  
190 2). In the minority of patients presenting with only one major criterion, isolated PTH resistance was the most  
191 common.

### 192 ***Clinical presentation and age at diagnosis in patients affected with the different iPPSD subtypes***

193 The identification of a causing molecular defect testing is fundamental to confirm the clinical diagnosis and  
194 categorize each patient into a specific iPPSD subtype. No clear genotype-phenotype correlation has been  
195 identified so far and clinical and molecular overlap exists among different PHP and PHP-related disorders.  
196 We thus investigated the correlation between the genetics and the patients' clinical presentation in our  
197 cohort (Tab. 3).

198 As previously observed in our study on the prevalence of PHP-associated molecular defects<sup>17</sup> we found that  
199 the 57 probands presenting with isolated PTH resistance were iPPSD3 due to *GNAS* methylation alterations.  
200 In particular, 70.2% had broad methylation defects and no known underlying primary genetic alteration,  
201 26.3% had a deletion at the *STX16* gene and 3.5% presented broad methylation alterations secondary to UPD.  
202 As expected, the only patient showing ectopic ossifications with no additional signs carried a paternal *GNAS*  
203 point variant. Brachydactyly and 2 minor criteria was found almost in all types of iPPSDs, nearly half of them  
204 being iPPSD5, former acrodysostosis. We also confirmed that iPPSD4 and iPPSD5 diagnoses associate with a  
205 more complex and dysmorphic phenotype (31/37 patients with several major and minor criteria).

206 Several cases confirmed that there is a considerable overlap between what we have historically considered  
207 as different diseases, the most striking overlap being observed between the two most represented subtypes,  
208 i.e. iPPSD2 and 3 (historically PHP1A and 1B) (Fig. 3). As an example, patient IT208 who displayed  
209 brachydactyly plus 2 minor criteria, was diagnosed as iPPSD3 due to loss-of-imprinting at all 4 *GNAS* DMRs.  
210 Several iPPSD3 patients with resistance to PTH presented additional major criteria and/or signs of AHO  
211 (mainly brachydactyly). In addition, patient IT108 diagnosed with iPPSD4 and a *PRKAR1A* pathogenic variant  
212 did not develop PTH resistance while 1 iPPSD2 case with *GNAS* alterations on the paternal allele (patient  
213 FR87) and 4 iPPSD5 patients (patients FR85, FR74, IT8p and FR62) showed resistance to the action of PTH.  
214 These findings further support the usefulness of the new classification and the impossibility for the former  
215 one to predict specific phenotypes, therefore preventing a proper follow-up.

216 Except for an increased prevalence of females in iPPSD5 (4 males vs 14 females), and increased age at  
217 diagnosis in iPPSD3, we found similar sex ratio and similar age at diagnosis in the different iPPSDs. Only 1  
218 patient with iPPSD3 caused by methylation defects at the *GNAS* locus was identified during infancy; this  
219 number increased proportionally with patients' age (48 patients diagnosed in childhood, 48 in adolescence  
220 and 94 in adulthood). This might be related to the lack of symptoms associated to the PTH resistance and  
221 hypocalcemia (Supp. Tab. 1); we know that, in patients with *GNAS* molecular defects, PTH resistance is absent  
222 at birth and develops overtime.<sup>17,19,20</sup> In addition, hypocalcemia may be underdiagnosed for years, when  
223 developing slowly.<sup>16,17,19</sup>

224 In 76 of the 224 iPPSD2 probands, we were able to define the parental inheritance of the genetic defect (63  
225 on the maternal allele and 13 on the paternal one). The iPPSD3 group was mainly represented by patients  
226 with sporadic imprinting defects affecting all 4 *GNAS* DMRs. In iPPSD3 patients affected by the autosomal  
227 inherited *STX16* deletion, we were able to demonstrate the maternal origin in 8 out of 10 for whom  
228 information on parents, mother or father, were available. In addition, 4 women affected with iPPSD3 caused  
229 by *STX16* deletion and isolated loss of methylation at the at the *GNAS A/B:TSS-DMR* transmitted the deletion,  
230 the methylation defect and the iPPSD3 phenotype to their children. Out of 39 probands affected with non-  
231 imprinted genes like (*PRKAR1A* in 19 iPPSD4, *PDE4D* in 18 iPPSD5 and *PDE3A* in 2 iPPSD6), we identified only  
232 2 autosomal transmissions within the same family from mother to son and daughter (iPPSD4).

233 All the above-mentioned data further support the absence of clear genotype-phenotype correlations. It  
234 reinforces the claim that the diagnosis of iPPSDs should be primarily clinical.<sup>16</sup> Nevertheless, the same data  
235 strongly support the need to confirm the genetic diagnosis as the the only way to identify a specific subtype,  
236 because of the dramatic clinical and molecular overlap among these heterogenous disorders.<sup>16</sup>

### 237 ***Minor criteria: frequency and association with major criteria***

238 Many symptoms of iPPSD are non-specific that exist in many endocrine and syndromic diseases different  
239 from this group of disorders. Moreover, the number, the age of appearance and the severity of such features  
240 are extremely variable among patients, even when bearing the same molecular alteration. Indeed, patients  
241 may develop a sequence of AHO features over time or clinical features may be faint and unnoticed at first  
242 examination. Therefore, we decided to determine, among the signs of AHO and other minor criteria, which  
243 ones could be considered as pathognomonic and more predictive of the diagnosis. We evaluated, in our  
244 cohort, the frequency, age of presentation and possible association of a series of symptoms with specific  
245 major criteria.

246 We counted how many times each single minor criterion was seen in patients, both alone and in combination  
247 with additional minor features. The most common symptoms identified were resistance to TSH, dysmorphic  
248 facies marked by a flat nasal bridge and/or a maxillar hypoplasia and/or a round face, obesity or overweight,

249 intrauterine growth retardation and/or post-natal growth retardation, motor and/cognitive retardation or  
250 impairment and additional hormone resistances, e.g. to calcitonin, gonadotropins and/or GHRH in 282  
251 (61.4%), 230 (50.1%), 199 (43.4%), 192 (41.8%), 148 (32.2%) and 90 (19.6%) iPPSD probands, respectively  
252 (Tab. 4 and Supp. Tab. 1)

253 When we considered these clinical features as the unique minor criterion present in a given patient (n=91),  
254 we found that resistance to TSH, intrauterine growth retardation and/or the post-natal growth retardation,  
255 obesity and overweight, and facial dysmorphism were the most frequent features in 41 (45.1%), 19 (20.9%),  
256 13 (14.3%) and 9 (9.9%) patients, respectively (Tab. 4 and Sup. Tab. 1). Noteworthy, intrauterine growth  
257 retardation and/or post-natal growth retardation were the unique minor criteria found in infants; mental  
258 and cognitive impairment was the most frequently reported in early childhood; resistance to TSH was the  
259 most frequent sign in the older groups; finally, the number of obese patients increased significantly from  
260 early childhood to adulthood (Fig. 4).

261 Overall and unfortunately, we were not able to detect a specific minor sign nor a combination of signs  
262 allowing to establish a precise clinical diagnosis, i.e. the iPPSD subtype, or to predict the underlying genetic  
263 alteration. We conclude from our findings that these minor and major signs should be carefully searched  
264 during the first examination in order to promote an earlier detection of iPPSD in patients.

### 265 ***Relatives of index iPPSD patients***

266 The great intrafamilial variability in clinical presentation has been largely reported.<sup>16,21</sup> We took advantage  
267 of this rare, large and unique collection of 459 probands and 85 relatives to investigate this phenotypic  
268 diversity and the iPPSD detection in the patient's family circle.

269 The cohort of 85 relatives includes 36 mothers (M), 3 fathers (F), 12 descendants (D), i.e. 5 sons and 7  
270 daughters, 30 siblings, i.e. 14 brothers and 16 sisters, 3 cousins (C) and one aunt (A) (Table 5).

271 It is remarkable that one third of the relatives (n=28, 32.9%) were diagnosed through the family history and  
272 did not meet the minimum criteria to be classified as iPPSD. Among them, we identified 6 iPPSD3 and 22  
273 iPPSD2 patients, including 6 with a paternal mutation at the *GNAS* gene. In particular, 18 subjects were  
274 apparently healthy with no major nor minor criteria, 5 patients had brachydactyly plus one minor criterion  
275 (4 growth retardation and one facial dysmorphism) and 5 patients showed minor criteria only (growth  
276 retardation, obesity, dysmorphic facies or resistance to TSH). The familial history therefore allowed a  
277 diagnosis of iPPSD before the occurrence of symptoms.

278 We then investigated the already known intrafamilial phenotypic variability of the disease and we observed  
279 that all index patients developed a more severe and complex clinical presentation compared to the parent  
280 from whom they inherited the molecular defect, either genetic or epigenetic, displaying a greater number of  
281 major and/or minor criteria (the overall presence of major and minor criteria in probands and relatives is

282 summarized in Fig. 6 Agnes). The same was true for the only aunt of the series. In a specular way, when we  
283 considered offsprings of affected patients, we found that, in half cases, the clinical phenotype was aggravated  
284 in the next generation. There were few exceptions, most of which being very young patients (1 year or less)  
285 in whom probably the phenotype had not become apparent yet (IT191d, FR32d2, FR64d, FR41d); in addition,  
286 IT84d is a healthy adult daughter of an iPPSD3 mother with rPTH and FR16d is a patient with 2 major and 1  
287 minor criteria whose mother displayed 3 major and 6 minor criteria.

288 All mutated siblings were affected and the clinical presentation was comparable to that of the index sibling.  
289 Patient IT217s, brother of a proband, was not considered in the analysis since no clinical data were available.  
290 Finally, the clinical features of the available couple of cousins were identical to the index case, similarly to  
291 what we found in siblings (Tab. 5).

292 Altogether, and in accordance to observations made in many other complex genetic disorders, our data  
293 suggest that, when present, the familial history of iPPSD should be also considered as a major criterion.

294

#### 295 **Concluding remarks**

296 The investigation of a large, and unique cohort of 544 patients characterized by the inactivation of the  
297 PTH/PTHrP signalling pathway allowed us to propose this second position paper on the terminology and the  
298 classification of iPPSDs. The term “pseudohypoparathyroidism” has been widely used to describe several  
299 highly related, metabolic disorders based on disputable clinical and biochemical grounds. Performing an early  
300 and correct diagnosis and a stratification into subtypes is challenging, due to the overlap between PHP and  
301 related disorders, and even among PHP subtypes. Additionally, the presentation and the severity are  
302 extremely variable among affected individuals, even among those carrying the same molecular alteration.  
303 For this reason, in the recent past a new nomenclature and classification has been proposed,<sup>6</sup> and  
304 recommendations for the diagnosis and management of these patients have been published as a first  
305 international Consensus Statement.<sup>16</sup>

306 The present study investigated the largest cohort of deeply clinically and molecularly characterized patients  
307 affected by iPPSDs and allowed to validate the recently proposed criteria to define and classify these patients,  
308 although further prospective studies are needed to prospectively confirm these observations. Overall, the  
309 phenotypic analysis of this large cohort confirmed the validity of the major and minor criteria and their  
310 combination to diagnose iPPSD, the new classification being able to correctly identify more than 98% of index  
311 patients at the time of their first clinical presentation. The further clinical characterization of iPPSD relatives  
312 importantly identified familial history as a new major criterion predictive of the disease.

313 In conclusion, our report shows the importance of having simple and easily recognizable signs to diagnose  
314 with confidence these rare disorders and support a better management of patients.

## 315 References

- 316 1 Levine MA. An update on the clinical and molecular characteristics of pseudohypoparathyroidism. *Curr*  
317 *Opin Endocrinol Diabetes Obes* 2012; **19**: 443–51.
- 318 2 Bastepe M. Genetics and epigenetics of parathyroid hormone resistance. *Endocr Dev* 2013; **24**: 11–24.
- 319 3 Mantovani G, Spada A, Elli FM. Pseudohypoparathyroidism and Gs $\alpha$ -cAMP-linked disorders: current view  
320 and open issues. *Nat Rev Endocrinol* 2016; **12**: 347–56.
- 321 4 Albright F, Forbes AP, Henneman PH. Pseudo-pseudohypoparathyroidism. *Trans Assoc Am Physicians*  
322 1952; **65**: 337–50.
- 323 5 Shore EM, Ahn J, Jan de Beur S, *et al.* Paternally inherited inactivating mutations of the GNAS1 gene in  
324 progressive osseous heteroplasia. *N Engl J Med* 2002; **346**: 99–106.
- 325 6 Thiele S, Mantovani G, Barlier A, *et al.* From pseudohypoparathyroidism to inactivating PTH/PTHrP  
326 signalling disorder (iPPSD), a novel classification proposed by the EuroPHP network. *Eur J Endocrinol* 2016;  
327 **175**: P1–17.
- 328 7 Linglart A, Menguy C, Couvineau A, *et al.* Recurrent PRKAR1A mutation in acrodysostosis with hormone  
329 resistance. *N Engl J Med* 2011; **364**: 2218–26.
- 330 8 Linglart A, Fryssira H, Hiort O, *et al.* PRKAR1A and PDE4D Mutations Cause Acrodysostosis but Two Distinct  
331 Syndromes with or without GPCR-Signaling Hormone Resistance. *J Clin Endocrinol Metab* 2012; **97**: E2328–  
332 38.
- 333 9 Lee H, Graham Jr. JM, Rimoin DL, *et al.* Exome Sequencing Identifies PDE4D Mutations in Acrodysostosis.  
334 *Am J Hum Genet* 2012; **90**: 746–51.
- 335 10 Maass PG, Aydin A, Luft FC, *et al.* PDE3A mutations cause autosomal dominant hypertension with  
336 brachydactyly. *Nat Genet* 2015; **47**: 647–53.
- 337 11 de Nanclares GP, Fernández-Rebollo E, Santin I, *et al.* Epigenetic defects of GNAS in patients with  
338 pseudohypoparathyroidism and mild features of Albright’s hereditary osteodystrophy. *J Clin Endocrinol*  
339 *Metab* 2007; **92**: 2370–3.
- 340 12 Mariot V, Maupetit-Méhouas S, Sinding C, Kottler M-L, Linglart A. A maternal epimutation of GNAS leads  
341 to Albright osteodystrophy and parathyroid hormone resistance. *J Clin Endocrinol Metab* 2008; **93**: 661–  
342 5.
- 343 13 Unluturk U, Harmanci A, Babaoglu M, *et al.* Molecular diagnosis and clinical characterization of  
344 pseudohypoparathyroidism type-Ib in a patient with mild Albright’s hereditary osteodystrophy-like  
345 features, epileptic seizures, and defective renal handling of uric acid. *Am J Med Sci* 2008; **336**: 84–90.
- 346 14 Mantovani G, de Sanctis L, Barbieri AM, *et al.* Pseudohypoparathyroidism and GNAS epigenetic defects:  
347 clinical evaluation of albright hereditary osteodystrophy and molecular analysis in 40 patients. *J Clin*  
348 *Endocrinol Metab* 2010; **95**: 651–8.

- 349 15 Brix B, Werner R, Staedt P, Struve D, Hiort O, Thiele S. Different pattern of epigenetic changes of the GNAS  
350 gene locus in patients with pseudohypoparathyroidism type Ic confirm the heterogeneity of underlying  
351 pathomechanisms in this subgroup of pseudohypoparathyroidism and the demand for a new classification  
352 of GNAS-related disorders. *J Clin Endocrinol Metab* 2014; **99**: E1564-1570.
- 353 16 Mantovani G, Bastepe M, Monk D, *et al.* Diagnosis and management of pseudohypoparathyroidism and  
354 related disorders: first international Consensus Statement. *Nat Rev Endocrinol* 2018; **14**: 476–500.
- 355 17 Elli FM, Linglart A, Garin I, *et al.* The Prevalence of GNAS Deficiency-Related Diseases in a Large Cohort of  
356 Patients Characterized by the EuroPHP Network. *J Clin Endocrinol Metab* 2016; **101**: 3657–68.
- 357 18 Ahmed SF, Dixon PH, Bonthron DT, *et al.* GNAS1 mutational analysis in pseudohypoparathyroidism. *Clin*  
358 *Endocrinol (Oxf)* 1998; **49**: 525–31.
- 359 19 Usardi A, Mamoune A, Nattes E, Carel J-C, Rothenbuhler A, Linglart A. Progressive Development of PTH  
360 Resistance in Patients With Inactivating Mutations on the Maternal Allele of GNAS. *J Clin Endocrinol Metab*  
361 2017; **102**: 1844–50.
- 362 20 Fernández-Rebollo E, Lecumberri B, Gaztambide S, *et al.* Endocrine profile and phenotype-(epi)genotype  
363 correlation in Spanish patients with pseudohypoparathyroidism. *J Clin Endocrinol Metab* 2013; **98**: E996-  
364 1006.
- 365 21 Mann JB, Alterman S, Hills AG. Albright's hereditary osteodystrophy comprising  
366 pseudohypoparathyroidism and pseudo-pseudohypoparathyroidism. With a report of two cases  
367 representing the complete syndrome occurring in successive generations. *Ann Intern Med* 1962; **56**: 315–  
368 42.
- 369

370

371 **Fundings**

372 This work was supported by the grant “Platforms 2018” to GM from Fondazione IRCCS Ca’ Granda Policlinico  
373 Ospedale Maggiore , by grant PRIN 2017HBHA98 from MIUR-Italian Ministry of University and Research to  
374 GM, AP-HP recurrent funds from the rare disease plan, a grant from the French Society of Pediatric  
375 Endocrinology and Diabetology (SFEDP) (to PH), a grant from the Instituto de Salud Carlos III [Institute of  
376 Health Carlos III] of the Ministry of Economy and Competitiveness [Spain] (to GPdN and AP), co-financed by  
377 the European Regional Development Fund (PI16(00073)), a grant from the Department of Health of the  
378 Basque Government (GV2017/111040 to GPdN), a 2019 fellowship grant from ESPE (to AE), and the 2019  
379 research unit grant from ESPE (to GdPN, GM, ST, AE, BF and AL).

380 **Acknowledgements**

381 The authors are members and acknowledge the Euro-iPPSDnet (formerly Euro-Pseudohypoparathyroidism  
382 network-EuroPHP), the EUCID.net (past COST action BM1208 on imprinting disorders; [www.imprinting-](http://www.imprinting-disorders.eu)  
383 [disorders.eu](http://www.imprinting-disorders.eu)), the European Society of Pediatric Endocrinology (ESPE), the European network for rare  
384 endocrine diseases (ENDO-ERN) and the European Network in Rare Bone Disorders (BOND-ERN).

385 **Conflict of Interest Statement**

386 The authors declare no competing interests.

387 **FIGURE LEGENDS**

388 **Supp.Tab.1**

389 Table resuming clinical and molecular data of the whole investigated cohort, including the 459 index cases.  
390 Abbreviations: BD, date of birth; iPPSD, inactivating PTH/PTHrp signaling disorder; rPTH, resistance to PTH;  
391 EO, ectopic ossifications; BR, brachydactyly; rTSH, resistance to TSH; add HR, additional hormone resistances;  
392 M/C imp, motor and/or cognitive impairment or retardation; IUGR/PNGR, intrauterine and/or postnatal  
393 growth retardation; OB/OW, obesity or overweight; DF, facial dysmorphism; ~~P, proband; M, mother; F,~~  
394 ~~father; D, descendant (son or daughter); S, sibling (brother or sister); C, cousin; A, aunt;~~ M, male; F, female;  
395 mat, maternal inheritance; pat, paternal inheritance; 0, absence of the criterion; 9, criterion not investigated;  
396 1, presence of the criterion; IVS, intron. Legend of *GNAS* methylation defects: 2, uniparental isodisomy  
397 (iUPD); 6, overall methylation defects without known causes; 10, overall partial methylation defects without  
398 known causes; 11, partial (p) loss-of-methylation (LoM) at *XL*, gain-of-methylation (GoM) at *NESP*, LoM at *AB*  
399 and LoM at *GNAS-AS*; 12: LoM at *XL*, GoM at *NESP*, LoM at *AB* and pLoM at *GNAS-AS*; 13, pLoM at *XL*, GoM  
400 at *NESP*, LoM at *AB* and pLoM at *GNAS-AS*; 15, isolated LoM at *AB* and *STX16* deletion; 16, isolated LoM at  
401 *AB* without *STX16* deletion; 17, isolated pLoM at *AB* and *STX16* deletion; 18, isolated pLoM at *AB* without  
402 *STX16* deletions.