What to consider when pseudohypoparathyroidism is ruled out: iPPSD and differential diagnosis

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**Abstract**

**Background:** Pseudohypoparathyroidism (PHP) is a rare disease whose phenotypic features are rather difficult to identify in some cases. Thus, although these patients may present with the Albright's hereditary osteodystrophy (AHO) phenotype, which is characterized by small stature, obesity with a rounded face, subcutaneous ossifications, mental retardation and brachydactyly, its manifestations are somewhat variable. Indeed, some of them present with a complete phenotype, whereas others show only subtle manifestations. In addition, the features of the AHO phenotype are not specific to it and a similar phenotype is also commonly observed in other syndromes. Brachydactyly type E (BDE) is the most specific and objective feature of the AHO phenotype, and several genes have been associated with syndromic BDE in the past few years. Moreover, these syndromes have a skeletal and endocrinological phenotype that overlaps with AHO/PHP. In light of the above, we have developed an algorithm to aid in genetic testing of patients with clinical features of AHO but with no causative molecular defect at the GNAS locus. Starting with the feature of brachydactyly, this algorithm allows the differential diagnosis to be broadened and, with the addition of other clinical features, can guide genetic testing.

**Methods:** We reviewed our series of patients ($n = 23$) with a clinical diagnosis of AHO and with brachydactyly type E or similar pattern, who were negative for GNAS anomalies, and classify them according to the diagnosis algorithm to finally propose and analyse the most probable gene(s) in each case.

**Results:** A review of the clinical data for our series of patients, and subsequent analysis of the candidate gene(s), allowed detection of the underlying molecular defect in 12 out of 23 patients: five patients harboured a mutation in PRKAR1A, one in PDE4D, four in TRPS1 and two in PTHLH.

**Conclusions:** This study confirmed that the screening of other genes implicated in syndromes with BDE and AHO or a similar phenotype is very helpful for establishing a correct genetic diagnosis for those patients who have been misdiagnosed with "AHO-like phenotype" with an unknown genetic cause, and also for better describing the characteristic and differential features of these less common syndromes.

**Keywords:** Brachydactyly, Pseudohypoparathyroidism, Albright's hereditary osteodystrophy, Hormone resistance, Short stature

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Background
Albright's hereditary osteodystrophy (AHO) is a unique phenotype classically associated with pseudohypoparathyroidism (PHP) [1, 2]. This phenotype was initially described by Albright et al. as a constellation of signs, including short stature, obesity with a rounded face, subcutaneous ossifications, mental retardation and brachydactyly. Parathyroid hormone (PTH) resistance was also originally included as a feature of the AHO phenotype as these authors noticed a reduced calcaemic and phosphaturic response to injected bovine parathyroid extract in such patients with normal renal function [1]. However, more patients with this phenotype but lacking hormone resistance were described in 1952, thus this disease was termed pseudopseudohypoparathyroidism (PPHP). Consequently, this type of hormonal resistance was included as a non-obligatory manifestation of AHO [3]. Many years later, genetic and/or epigenetic alterations at the guanine nucleotide-binding protein, alpha-stimulating (Gsα) locus (GNAS) were identified as the cause of this condition in about 70% of patients with a clinical diagnosis of PHP/PPHP [4], or iPPSD2 (inactivating PTH/PTHRP signalling disorder) and iPPSD3 according to the new proposed classification [5].

Despite this high detection rate of GNAS molecular defects, some patients with a clinical suspicion of PHP/PPHP still lack a confirmed molecular diagnosis, possibly due to the variability of the manifestations in terms of both number and severity, especially in cases in which there is no family history ([5–7] and personal data). In addition, the features of the AHO phenotype are not exclusive to PHP/PPHP. For example, AHO-like syndrome, or brachydactyly and mental retardation syndrome (BDMR, OMIM#600430), as its name indicates, includes a group of patients who show several features of AHO (BDE and mental retardation being the most notable) but with normal Gsα levels and with no endocrine abnormality [8]. These patients frequently carry deletions at the 2q37 chromosome or mutations in the gene coding for histone deacetylase 4 (HDAC4), which is found at this locus [8, 9]. Similarly, the biochemical alterations (hypocalcaemia and hyperphosphatemia) observed in PHP are also present in other syndromes associated with calcium homeostasis, such as hypoparathyroidism [10]. PTH resistance and brachydactyly (but in a more severe form) are also present in acrodysostosis with multihormonal resistance ACRDYS1 (or iPPSD4, OMIM#101800) [11–13], which is associated with mutations in the gene coding for the cAMP-dependent protein kinase type 1 regulatory subunit protein (PRKAR1A) [14]. Another type of acrodysostosis, which lacks hormone resistance (ACRDYS2 or iPPSD5, OMIM#6146139), is caused by mutations in the gene coding for phosphodiesterase 4D (PDE4D) [15, 16].

Considering recent publications in which a significant number of patients were clinically misdiagnosed as PHP when they actually had other syndromes [17], our goal was to validate our diagnostic algorithm starting with the brachydactyly feature to guide candidate gene testing in patients with features of AHO who do not carry genetic or epigenetic alterations at the GNAS locus.

Methods
Patients
The current series involved 23 out of a total of 149 patients referred to the Molecular (Epi)Genetics Laboratory at OSI Arava University Hospital for molecular diagnosis with a clinical suspicion of AHO phenotype with or without PTH resistance and the presence of brachydactyly type E or a similar pattern. (Epi)genetic alterations at the GNAS locus had been previously ruled out as described [18].

The clinical details of the whole series studied are summarized in Table 1. Some of these patients had already been reported, as indicated in the Table 1. The clinical history of the patients, including hand(s) (Additional files 1, 2 and 3: Figures S1-S3) and feet radiographs and clinical photos (if available), was requested from the physicians who referred the samples for genetic study.

Candidate gene approach
The patients' clinical features were reviewed to classify them according to the brachydactyly pattern and other clinical features, in accordance with the diagnostic algorithm proposed by us previously [19] and updated to include the most recent findings (Figure 1). The most probable candidate gene(s) were studied in each patient (Additional file 4: Table S1).

The following disorders (all of which present BDE or similar types) were considered: (i) iPPSD4 [14] and iPPSD5 [15, 16]; (ii) hypertension with brachydactyly syndrome or iPPSD6 (HTNB, OMIM#112410), in which the responsible gene, phosphodiesterase 3A (PDE3A), has been identified very recently [20]; (iii) tricho-rhino-phalangeal syndrome type I and III (TRPS-I, OMIM#190350; TRPS-III, OMIM#190351), caused by mutations in the TRPS1 gene [21] or the more severe form, type II (OMIM#150230), which is a contiguous gene syndrome on 8q24.1 involving loss-of-function copies of the TRPS1 and EXT1 genes [22]; (iv) BDMR; (v) brachydactyly type E with short stature, PTHLH type (OMIM#613382), caused by mutations in the gene coding for parathyroid hormone-related protein (PTHRLH) [23–25]; and (vi) isolated BDE in which the HOXD13 gene has been implicated [26, 27].

We should mention at this point that, although Turner syndrome chromosomal disorder (frequently, 45,X) is a relatively well-known entity, patients also show BDE [28] and short stature, which could give rise to some misdiagnoses. However, none of our patients presented clinical features compatible with Turner syndrome.
| PATIENT  | Age at consultation | Age of genetic diagnosis | Sex | Elevated PTH | Ca/P | Vitamin 25(OH)D | BD | MR | Height (cm) | BMI | Facial dimorphisms | Skeletal dysplasia | Advanced bone age | Dental defects | Other features |
|----------|---------------------|--------------------------|-----|--------------|------|-----------------|----|----|-------------|-----|---------------------|-------------------|--------------------|---------------|--------------|
| PHP01    | 7y                  | 12y6m                    | M   | Yes (and TSH) | N    | ND              |    |    | –1.5SD      | 1.3SD | broad face with widely spaced eyes | maxillonasal hypoplasia, severe hypoplasia of the skull, thickened calvarium, increased size of the jaw with severe malocclusion | No              | ND            | –            |
| PHP02    | 6y6m                | 8y6m                     | F   | Yes          | P    | ND              |    |    | –2.5SD      | 0.2SD | broad face with widely spaced eyes | maxillonasal hypoplasia | No              | Yes           | pigmented skin spots |
| PHP03    | 3y                  | 3y10m                    | F   | Yes (and TSH) | N    | N               |    |    | –1.8SD      | 0.9SD | broad face with widely spaced eyes | maxillonasal hypoplasia, severe hypoplasia of the skull, thickened calvarium | yes          | ND            | –            |
| PHP04    | 13y                 | 18y                       | F   | Yes          | N    | ND              |    |    | No          | 135 cm (−3SD) | flat round face | genu valgum, Madelung deformity, eczatosis in the knee (6y) | ND              | ND            | osteoporosis |
| PHP05    | –                   | 3y9m                     | F   | Yes (andTSH) | ND   | Low levels      |    |    | 45 cm (−2.7 SD) | + 1.8 SD | broad nasal root | – | Yes (5-6y) | ND            | short neck, café-au-lait spots |
| PHP06    | 42y                 | 45y                       | F   | No (after Vit. D treatment) | ND | Low levels at first |    |    | 139 cm (−4 SD) | >250 | broad face with flattening of nasal ridge, facial dysostosis, spaced eyes | maxillonasal hypoplasia | – | ND            | short neck, hyperinsulinism |
| PHP07    | 31y                 | 40y                       | F   | Yes (and low GH) | N | N               | MT: III-V outcarving cones of MP & TP | Learning difficulties (no test) | 141.5 cm (−4.5 SD) | 42.7 kg/m2 (>250) | 42.7 kg/m2 (>250) | 42.7 kg/m2 (>250) | round face, thin upper lip and prominent lower lip, pear-shaped nose | – | tooth hypoplasia | sparse hair, hypoplastic arthrogryphosis of both hips and knees |
| PHP08    | –                   | 11y                       | F   | N             | N   | N               | MT: II-V outcarving cones of MP & BP | N | 139.8 cm (−1.5SD) | 26.5 kg/m2 (1.96 SD) | round face, thin upper lip, long philtrum, pear-shaped nose, protruding ears | – | ND            | ND            | sparse hair, lateral sparse eyebrows, type 2 diabetes |
| PHP09    | –                   | 32y                       | F   | No            | N   | ND              | Generalized shortening, severe outcarving of the epiphyses | ND | 152 cm (−2.5SD) | ND | thin upper lip, long philtrum, round nose, sparse eyebrows, prominent forehead | – | – | ND            | Sparse hair |
| PHP09-D  | 5m                  | 9m                        | F   | No            | N   | ND              | NA | ND | –2.8 SD     | −2.4SD | thin upper lip, long philtrum, round nose, sparse eyebrows, prominent forehead, separated eyes | ND | ND | ND | Sparse hair |
Table 1 Clinical description of patients studied (patient data as provided by the clinician at the reference centre) (Continued)

| PATIENT | Age at consultation | Sex | Elevated PTH Ca/P Vitamin 25(OH)D | BD | MRI | Height (cm) | BMI | Facial dimorphisms | Skeletal dysplasia | Advanced bone age | Dental defects | Other features |
|---------|---------------------|-----|---------------------------------|----|-----|-------------|-----|-------------------|------------------|----------------|---------------|---------------|---------------|
| PHP10   | 33y                 | F   | No                              | N  | ND  | Generalized shortening, stubby fingers (no X-ray received) | ND  | ND                | NO               | ND             | NO            | Sparse hair |
| PHP10-S | 1y7m                | M   | No                              | N  | ND  | NA                                      | ND  | ND                | Growth failure  | ND             | ND            | Sparse hair, stabiulemus |
| PHP10-D | 6y                  | F   | No                              | N  | ND  | Yes (no X-ray)                           | ND  | ND                | Growth failure  | ND             | ND            | Sparse hair, stabiulemus |
| PHP11   | 11.5y               | F   | N                               | N  | N   | MT: IV                                  | −1 SD | N                | No              | Yes (13.5 years) | No           | Advanced bone age |
| PHP12   | 10y                 | F   | No                              | N  | ND  | MT: II-V/TP: I & III                   | No  | 148.7 cm (~0.4SD) | 1.7 SD          | round face, long philtrum | –             | short neck, deformed and widely separated nipples |
| PHP13   | 28y                 | F   | No                              | N  | ND  | Severe (especially IV & V MT) (no X-ray received) | Yes | 139 cm (~4SD)     | p3-p10          | small saddle nose, prominent forehead, epicanthal folds, upward slanting palpebral fissures, low and dysplastic ears | Yes (12y) | No   |
| PHP14   | 10y                 | F   | No                              | N  | ND  | MT: V                                   | ND  | 130.6 cm (~1SD)  | 85th            | dindactyly, cone-shaped phalangeal epiphyses | ND            | hypothyroidism |
| PHP15   | 17y                 | F   | No                              | N  | ND  | MT: IV                                  | No  | p45               | p75-p90         | round face, facial asymmetry | ND          | ND   |
| PHP16   | 16y                 | M   | No (after Vit. D treatment)     | N  | ND  | MT: IV & V (no X-ray received)          | ND  | ND                | (Obesity)       | –              | –             | Dental malformations |
| PHP17   | 9y7m                | F   | No                              | N  | ND  | MP: II-V at least (BD A17)              | ND  | −2.25D + 2.75D    | prominent forehead, depressed nasal root | No            | ND   |

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Table 1  Clinical description of patients studied (patient data as provided by the clinician at the reference centre) (Continued)

| PATIENT | Age at consultation | Age of genetic diagnosis | Sex | Elevated PTH | Ca/P | Vitamin 25(OH)D | BD | MR | Height (cm) | BMI | Facial dimorphisms | Skeletal dysplasia | Advanced bone age | Dental defects | Other features |
|---------|---------------------|--------------------------|-----|--------------|------|----------------|-----|----|-------------|-----|-------------------|-------------------|-----------------|---------------|---------------|
| PHP18   | 9y11m               | –                        | M   | No           | N    | N              | Stubby digits MT: III-IV at least | No | 117 cm ($-3.8SD$) | N              | flattening of nasal ridge | stocky build, hip hypoplasia, horizontal acetabulum, vacum deformity, shortened tibia and femur, decreased interpedicular distance, scoliosis, bone dysplasias | ND             | ND            | –             |
| PHP19   | 65y                 | –                        | F   | No           | N    | N              | MT: IV | (SS) | ND             | –              | big nose, thin upper lip | –                | –              | ND            | –             |
| PHP20   | 15y5m               | –                        | M   | No           | N    | N              | MT: IV & V | No | 143.5 cm ($-3.9SD$) | + 4.2SD         | –                | delayed puberty | –             | ND            | –             |
| PHP21   | 10y                 | –                        | F   | (TSH mildly increased) | N    | ND             | MT: IV; TP: I | No | p30 | –              | ND             | prominent forehead, periorbital hyperpigmentation, long palpebral fissure, deep philtrum, thick eyebrows | –                | ND             | ND            | –             |
| PHP22   | 13y                 | –                        | F   | N            | N    | ND             | MP: II & V (BD:A47) | No | p50 | + 1.5SD        | N              | Bilateral cubitus valgus, short forearms, exostosis in both tibia, dorsolumbar hyperkyphosis in D12-L1 | ND             | N             | bicornuate uterus, short neck, wide thorax | –             |
| PHP23   | 8y1m                | –                        | F   | No           | N    | N              | MT: IV & V (mild) and clinodactyly of V | Mild | > 3SD | + 5.5SD | –             | round face, thin upper lip, pear-shaped nose, sparse, arched eyebrows | –                | ND             | ND            | sparse hair, epilepsy |
Genomic DNA was extracted from peripheral blood mononuclear cells using the QIAamp DNA Mini Kit (QIAGEN, Düren, Germany) according to the manufacturer’s instructions.

The DNA obtained was amplified by PCR for PRKAR1A (ref: NM_002734), PDE4D (ref: NM_001104631), TRPS1 (ref: NM_014112), HDAC4 (ref: NM_006037), PTHLH (ref: NM_198965.1) and/or HOXD13 (ref: NM_000523) coding exons and exon–intron junction, using specific primers (primers available on request).

Direct Sanger sequencing was carried out using standard methods and an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA). The reference sequences mentioned in parentheses were employed for mutation description according to the HGVS nomenclature.

Gene dosage analyses by multiplex ligation-dependent probe amplification (MLPA)

Gene dosage analyses were carried out using the SALSA MLPA P228-B1 TRPS1-EXT1, P179-B1 Limb−1 and P264 B1 Human Telomere 9 probemix (MRC-Holland, Amsterdam, The Netherlands), when no alterations were identified by direct sequencing of the TRPS1 gene, HOXD13 gene or HDAC4 gene, respectively.

Microsatellite analysis

2q37 deletions were studied by microsatellite analysis as reported previously [29].

Results

Following the proposed candidate gene approach guided by the aforementioned diagnostic algorithm, 12 out of 23 patients were diagnosed genetically.

Mutations in PRKAR1A in the current series: iPPSD4

Six patients with PTH resistance and severe, generalized brachydactyly, both of which are typical characteristics of iPPSD4 (classically called ACRDYS1), were identified. Four of these patients harbour the recurrent c.1101C>T/p.Arg368* mutation (PHP01 and PHP02 already reported [30], and PHP04 and PHP05) and a fifth one carried the c.854A>G/p.Gln285Arg mutation (PHP03, reported [30]). The functional impact of this substitution was assessed experimentally and it was found to produce a similar impairment to the recurrent mutation, i.e.,
defects in PKA activation characterized by a reduced sensitivity to cAMP [30]. Parental studies suggested that all mutations were de novo. The remaining patient had no mutation in PRKAR1A.

**Mutations in PDE4D in the current series: iPPSD5**
In the patient (PHP06) with no mutation in PRKAR1A, the other gene, PDE4D, associated with iPPSD5 (formerly named ACRDYS2) was sequenced. Initially this patient presented elevated PTH (PTH = 107 pg/ml, normal range: 10–65; 25-OH vitamin D = 13.9 ng/ml, normal range 20–100), which is why iPPSD4 was suspected. However, after vitamin D treatment her PTH level normalized [31] (it was probably secondary to vitamin D deficiency) [32]. A novel heterozygous mutation (c.934C>G/p.Leu312Val) was identified in PDE4D. Functional studies of this mutation confirmed its pathogenicity [31].

**Mutations in TRPS1 in the current series: TRPS-I**
Four patients with features suggestive of TRPS-I (i.e. short stature, severe BDE with outcarving of the phalangeal epiphyses, sparse hair, bulbous tip of the nose or pear shaped nose, long philtrum, thin upper lip, as described in table 1) were positive for TRPS1 gene mutation: two patients (PHP08, previously described [33]; and PHP09) carried the recurrent c.2762G>C/p.Arg921Gln mutation and the remaining two patients each showed a different mutation: c.2830delA/p.Arg944Glyfs*3 in one case (PHP07, previously described [33]) and c.3159_3160delAAinsT/p.Lys1053Asufs* (PHP10) in the other. Although neither of these has been described previously in the literature, the cosegregation in other family members (Additional file 4: Table S1) and frameshift characteristics (i.e., they would lead to truncated proteins if translated) suggested that they were possibly pathogenic.

**Mutations in PTHLH in the current series: BDE with short stature PTHLH type**
The BDE PTHLH type was suspected in two patients with BDE and advanced bone maturation for their chronological age. Indeed, two different novel mutations (c.101+ 3delAAGT, in PHP11 [34] and c.166C>T/p.Arg56*, in PHP12 [35]) were identified in the PTHLH gene in these patients. The characteristics of these mutations (frameshift and nonsense, respectively) suggested that they were causative of the pathology.

**Discussion**
PHP includes a heterogenic group of rare disorders associated with the AHO phenotype [1, 2]. Except for subcutaneous ossifications, the features of AHO are rather nonspecific as they also appear in other disorders, such as AHO-like syndrome [8] or acrodysostosis [11–13]. Less frequently, misdiagnosis with other entities has been observed because of the presence of BDE combined with short stature and obesity, which are also typically associated with other dysmorphic features and sometimes also with hormonal imbalances [33–37]. In this constellation of features, obesity or overweight and short stature could act as confusing factors as both are nonspecific [5, 33]. In addition, although obesity, intellectual disability, and resistance to several hormones are still extensively related to AHO, they may not be directly associated with genetic defects in GNAS [5].

The discovery of new genes implicated in syndromes with a phenotype similar to AHO, as well as other molecular mechanisms causative of iPPSD2 (classically named PHP/PPHP) [38], has been very helpful for establishing a correct genetic diagnosis for patients diagnosed with an “AHO-like phenotype” of unknown genetic cause [11, 14, 17, 33–35, 39], as well as for better describing the characteristic features of these less common syndromes. In ours and previously reported experiences [19, 40], BDE is the most specific and objective feature. For this reason, it was used as the inclusion criterion in this study and as a starting point to classify the aforementioned disorders in the previously proposed diagnostic algorithm [19]. It is well known that BDE was initially described as a variable shortening of the meta-carpals/metatarsals with a more or less normal length of the phalanges [41].

As a result of the clinical re-evaluation of this series of patients, half of them (12/23) could be genetically diagnosed (supporting the importance of a good clinical examination, and the need of multidisciplinary approaches in the follow-up of these patients) and new knowledge acquired regarding these pathologies and the characteristic features detailed below.

We also analysed the features observed in our iPPSD4 (5/12) and iPPSD5 (1/12) patients and other cases described in the literature and noticed that the skeletal dysmorphisms (broad face, widely spaced eyes, maxillonasal hypoplasia, severe and generalized brachydactyly in hands/feet, severe short stature, cone-shaped epiphyses with early epiphyseal fusion, and advanced bone age [17, 30]) are very similar in both groups, although the facial dysmorphisms are often more severe in iPPSD5 [17, 30, 42]. Decreased interpedicular distance and mental retardation also appear to be more specific for iPPSD5 [6, 17, 30] since iPPSD4 patients show only behavioural disorders [30]. Finally, hormone resistance, which was initially used as a main differential characteristic to classify the patients with acrodysostosis, seems not to be as specific as initially appeared because more exceptions are found as more patients are reported (PTH resistance was recorded in 76% of iPPSD4 and 27% of iPPSD5 cases in the last review of Elli et al. [17]).
All the iPPSD4 patients in our series exhibited PTH resistance, and although the iPPSD5 patient (PHP06) initially presented elevated PTH levels, PTH normalized after correcting the vitamin D deficiency, which is consistent with secondary hyperparathyroidism [32]. It is noteworthy that in contrast with the rest of the syndromes reflected in the algorithm, in which brachydactyly is usually not marked until the age of 6 years [39, 40], in acrodyssostosis (both iPPSD4 and iPPSD5) the shortening and cone-shaped epiphyses are manifested during early childhood [16, 30].

Given the presence of brachydactyly and short stature, TRPS could be confused with the AHO phenotype, especially so if obesity (or overweight) and/or PTH resistance [37] and another hormone imbalance (GH deficiency has also been reported in some TRPS autosomal-dominant BDE in 11 families, two of them linked protein (PTHrP), have been identified as a cause of epiphyses, and sparse, slowly growing scalp hair. The involvement of the phalanges in the brachydactyly tip of the nose (or pear-shaped nose), thin upper lip, illustrative features of TRPS syndrome are: bulbous series of patients, and comparing with those reported here. Keeping in mind all the identified cases in our two previously published cases [33] reviewed previously, in our opinion the most characteristic and illustrative features of TRPS syndrome are: bulbous tip of the nose (or pear-shaped nose), thin upper lip, involvement of the phalanges in the brachydactyly pattern and the typical outcarving of the phalangeal epiphyses, and sparse, slowly growing scalp hair.

Alterations which lead to the haploinsufficiency in PTHLH, the gene coding for parathyroid hormone related protein (PTHrP), have been identified as a cause of autosomal-dominant BDE in 11 families, two of them within our series [23, 24, 34, 35, 48, 49]. Although initially named as “BDE with short stature, PTHLH type” (OMIM#613382), because it is almost always associated with short stature [23–25], we have observed in both PTHLH patients in our series (PHP11 [34] and PHP12 [35]) that this short stature may not manifest until middle or late childhood. In both these cases, the patients had normal stature for their age but advanced bone age. Consequently, they experienced early epiphyseal closure, an early halt to growth, and their predicted final height is estimated to be below their target height. Thus, both the progenitors’ final height and bone age should be taken into account when determining whether patients show a height in the lower range of normality.

Overall, our use of a diagnostic algorithm in the current study has helped to determine the genetic cause in 12/23 patients with BDE who were clinically misdiagnosed as PHP/PPHP. Similarly to the 12 cases solved, the remaining cases were also classified and studied using the candidate gene approach guided by the proposed algorithm. However, we did not find any genetic alterations in the candidate genes studied, possibly due to some limitations of the study, such as (i) analysis of putative deletions at PTHLH is lacking; (ii) hand X-rays are missing for four patients, therefore it is difficult to propose any other potential diagnosis, (iii) although a large number of genes have been identified as the cause of BDE in recent years, the genetic cause of some BDE cases remains unknown [50].

Conclusions
We conclude that use of the presented algorithm in patients with idiopathic BDE is helpful for establishing a correct genetic diagnosis for those patients who have been misdiagnosed as PHP/PPHP. [5]

Additional files
Additional file 1:Figure S1. Hand X-rays for patients with acrodyssostosis, caused by mutation at either PRKAR1A (PHP02, panel A) or PDE4D (PHP06, panel B) They presented severe shortening of all hand bones with cone-shaped epiphysis (rows). (TIFF 1641 kb)

Additional file 2: Figure S2. Hand X-rays for a mother (PHP09, panel A) and her daughter (PHP09-D, panel B) with tricho-rhino-phalangeal syndrome caused by the same mutation in TRPS1. The mother’s hands showed severe bilateral shortening of the bone with the characteristic outcarving of the phalangeal epiphysis (rows). However her daughter was too young to manifest this brachydactyly and outcarving. (TIFF 3376 kb)

Additional file 3: Figure S3. Hand X-rays for patients without genetic diagnosis: (A) Patient PHP18 exhibits stubby digits and shortening of at least metacarpals (MT) IV-V; (B) Patient PHP19’s hands show bilateral shortening of MT IV; (C) Patient PHP20 presents shortening of MT IV and V; (D) Patient PHP21’s hands reveal bilateral shortening of MT IV and first telephalanx; (E) Patient PHP22’s hands present bilateral shortening of II and V metacarpals (similar to BDA4); (F) Patient PHP23 presents mild shortening of MT IV and V and clinodactyly of the V digit. (TIFF 3217 kb)

Additional file 4: Table S1. Brief summary of the candidate genes analysed for each patient and the results. (DOCX 21 kb)

Abbreviations
ACRDYS1: Acrodyssostosis type 1 with multihormonal resistance; ACRDYS2: Acrodyssostosis type 2 without hormone resistance; AHO: Albright’s hereditary osteodystrophy; BDE: Brachydactyly type E; BDMR: Brachydactyly and mental retardation syndrome; GH: Growth hormone; GNAS: Gene coding alpha subunit of the stimulatory guanine nucleotide-binding protein; Gs alpha subunit of the stimulatory guanine nucleotide-binding protein; HDAC4: Gene coding for histone deacetylase 4; HOXD13: Gene coding for homeobox D13; HTNB: Hypertension with brachydactyly syndrome; IPPSD: inactivating PTH/PTHP signalling disorder; PDE3A: Gene coding for phosphodiesterase 3A; PDE4D: Gene coding for phosphodiesterase 4D; PHP: Pseudohypoparathyroidism; PRKAR1A: Gene coding for the CAMP-dependent protein kinase type 1 regulatory subunit; PTH: Parathyroid hormone; PTHLH: Gene coding for parathyroid hormone-related protein; PTHrP: Parathyroid hormone related protein; TRPS: Tricho-rhino-phalangeal syndrome; TRPS1: Gene coding for zinc finger transcription factor TRPS1

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Availability of data and materials
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Authors’ contributions
GPN designed the project. AP and IG participated in the molecular analysis and the clinical discussion. AP and GPN designed and wrote the first draft. AP and IG participated in the recruitment, clinical description of the patients and the committee concerned. This project was approved by the Basque Clinical All authors read and approved the final manuscript.

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