Supplementary information
Coexistence of two different pseudohypoparathyroidism subtypes (Ia and Ib) in the same kindred with independent Gsα coding mutations and *GNAS* imprinting defects.
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Supplementary patients and methods

Case 1.- At the age of 25 the patient had PTH and TSH resistance. His skeletal age was lower than his chronologic age (using Greulich and Pyle left hand standards [1]) since he was two years old (average 1 - 2 years of delay) with a "catch-up" at the age of 12. His karyotype was 46, XY. His puberty showed normal timing and onset. At the age of 7 he was diagnosed with Wilkins epiphyseal dysgenesis, and started on thyroxine for his hypothyroidism. Two years later a biochemical analysis revealed elevated serum PTH with normocalcemia and high-normal serum phosphorus (Table 1). At his physical exam, he had a rounded face with an ogival palate, clear macrocephaly, soft shortening of fourth and fifth metacarpals of both hands; no subcutaneous ossifications were detectable. He showed a Puberty Tanner Stage of 5 and his neurologic examination was unremarkable with a negative Chvostek´s sign, but mild to moderate cognitive impairment. Cranial CT scan at 23 years old revealed many bilateral and symmetrical supratentorial calcifications in both globus pallidus and subcortical area of frontal lobes, retraction of frontal cortical wrinkles and a mega magna cistern. The patient had low-normal basal GH levels (0.13 ng/ml, normal values: 0-6) and normal IGF-1 levels, which could suggest some degree of resistance to GHRH (patient refused a GH test). His mother and one of her maternal cousins have thrombocytopenia.

Case 2.- A 71-year-old female was referred to us 17 years ago for consultation regarding upper limb paresthesia. The patient was diagnosed with PHP-I after showing low serum cAMP levels 9.4mmol/dl (N: 11-27) without any cAMP response in the urine after PTH administration and lack of urinary phosphate response. Nonetheless, the patient has always had normal PTH levels (Table 1) and remains asymptomatic without calcium or vitamin D treatment. Thus, definitive clinical diagnosis could not be established. Renal tubular phosphate reabsorption was 74.16%, creatinine clearance 73.3ml/min, and 24-hour urinary calcium 159.6mg/24 h. Serum free T4L and TSH were within the normal range (Table 1). TRH stimulation triggered a normal TSH response; serum TSH was 1.11 µIU/ml, and after 20 and 60 minutes of TRH stimulation, it reached 10.8 and 7.7µIU/ml (normal values: basal: 0.48-4.36; 30min: 6.8-20; 60 min: 6-15.5). Serum FSH/LH levels were within the normal postmenopausal range (Table 1). She was operated for an exocrine pancreatic tumor in 2005 with no sequelae and remains free of disease.
She had a round face and short fourth metacarpals. Cranial X-ray showed subcutaneous calcifications in the posterior part of her neck and intracranial calcifications. Hand X-ray confirmed the shortening of the fourth metacarpals, as well as revealing a short distal phalanx of both thumbs.
Two of her eight children were diagnosed with PHP-Ia (cases 3 and 4); one son was born dead and the rest had no metabolic or phenotypic abnormalities. The son of a healthy daughter has been recently diagnosed with autism.

Case 3.- The daughter of case 2 and sister of case 4, is a 46-year-old woman with moderate to severe mental retardation. When she was 8-9 years old she started treatment

with antiepileptic drugs for sudden onset episodes of tonic convulsion followed by loss of consciousness and generalized muscular hypertony. She was evaluated for short stature and finally diagnosed with PHP, subclinical hypothyroidism, and ovarian resistance at the age of 17 based on the clinical findings and biochemical data (Table 1). Cranial X-ray and CT scan revealed multiple intracranial calcifications. She was operated for cataracts in both eyes at the age of 21. On her exam, the patient exhibited a round face, short limbs, and brachydactyly of second and third metacarpals of the right hand, and fourth metacarpal of the left hand. The rest of her physical exam was unremarkable. She reported to have irregular menses with some missed menstrual periods.

Case 4.- the son of case 2 and brother of case 3, is a 38-year-old male with a moderate cognitive retardation. He was first studied because of short stature and tonic clonic seizure episodes and started on antiepileptic drugs that worsened his symptoms. Biochemical data were consistent with PTH and TSH resistance (Table 1). Radiologic studies revealed intracranial calcifications and shortening of third and fourth metacarpals of both hands, leading to the diagnosis of PHP-Ia.

Family 2

Case 5.- is a 20-year-old male that had been followed in the paediatric outpatient clinic since he was twenty months old, when he was first evaluated for several laryngitis and bronchitis episodes with typical febrile seizures. At that time, he was found hypocalcemic and placed on oral calcium treatment. At the age of five he suffered a tetanic crisis preceded by dysphonia and laryngeal stridor. He showed hypocalcemia, hyperphosphatemia, and elevated PTH (Table 1) with normal serum 1,25 dihydroxy vitamin D3 (28pg/ml; normal = 18-78). Exogenous PTH administration resulted in no changes in serum calcium, serum cAMP, urinary phosphate, and urinary cAMP. Biochemical data were also consistent with TSH resistance (Table 1). Feet X-rays revealed shortness of the left fourth metacarpal bone and a 3 x 2 cm calcification in his left heel, which reappeared two years after initial excision. He had moderate school retardation that required additional academic help since his early childhood school. Based on these findings, PHP-Ia diagnosis was established.

On physical exam, he had a rounded face with short neck, short fourth left metacarpal, and bilateral cubitus valgus that prevents full extension of the elbows. A solid mass was found in his left heel but no other masses were palpable in other locations. He showed a Puberty Tanner Stage of 5. Both of his parents and a 24 year-old sister were healthy.

The family history revealed his mother presented similar phenotype (height: 150cm, weight: 48.5kg; rounded face) without biochemical anomalies, which could be suggestive of PPHP.

Case 6.- is a 53-year-old female who is the second cousin of case 5. At the age of 17 she started with paresthesia and cramps in her legs with an ascendent direction, accompanied by carpopedal spasms. Diphenylhydantoin was initiated, but the crisis was not responsive to this treatment. She was admitted to the Hospital at age 23 years because her symptoms worsened and she showed occasional deviation of the oral commissure and a positive Trousseau's sign after 2 minutes. Based on biochemical analyses (Table 1), diagnosis of pseudohypoparathyroidism was established. Serum 1,25 dihydroxy vitamin D3 levels were normal (24pg/ml; normal = 15-100). TSH and antithyroglobulin antibodies levels were above the normal range, so an autoimmune hypothyroidism was diagnosed. Cranial CT scan showed intracerebral calcifications,

and an ophthalmologic exam revealed cataracts in both eyes. The patient demonstrated no evidence of hypogonadism; her age at menarche was 14 years and continued to have regular menses. At the age of 44 she was investigated for limb bruises without any traumatic cause and she was diagnosed with thrombopenic purpura.
On her exam she was asymptomatic. Although the patient had short stature, none of the other features of AHO was detected. Thus, a final clinical diagnosis of PHP-Ib was established. The patient is the fourth of five siblings and has three brothers and one sister. Her oldest brother exhibits idiopatic thrombopenia. Her sister, two brothers and three female nephews are treated for hypothyroidism.

Methylation specific-Multiplex ligation-dependent probe amplification (MS-MLPA):

Methylation-specific MLPA (MS-MLPA) is a semi-quantitative method for methylation profiling. MS-MLPA is a variant of the MLPA technique in which copy number detection is combined with the use of a methylation-sensitive restriction enzyme.
For the copy number analysis, the data generated were intra-normalized by dividing the peak area of each amplification product by the total area of only the reference probes. The ratios were obtained by dividing the intra-normalized probe ratio in a sample by the average intra-normalized probe ratio of all reference runs. For the methylation analysis, the intra-normalized peak area of each MS-MLPA probe from the digested sample was divided by that obtained in the undigested sample.

References for supplementary patients and methods

[1] Greulich WW, Pyle SI. Radiographic Atlas of skeletal development of the hand and wrist (Second Edition). Stanford University Press ed. 1959.

Supplementary results

Results of the MS-MLPA analysis.

The height of the columns represents the dosage of the respective segments in the genomic DNA for the patient (black columns) and the mean of the controls (white columns). Normal dosage is observed for the patient. Data are representative of two independent experiments with similar results.