Pseudohypoparathyroidism (Ia and Ib) and hypercalcitoninemia: a clinical long-term follow-up of two patients

Riccardo G. Gheri[1]
Anna Antonielli[1]
Silvia Tommasi[2]
Vania Vezzosi[3]
Roberto Panconesi[4]
Paola Tozzi[5]
Silvia Pedercini[1]
Chiara Francesca Gheri[1]
Ester Romoli[1]
Mario Serio[1]

[1]Endocrine Unit, Department of Clinical Physiopathology, [2]Nuclear Medicine Unit, [3]Pathology Unit, [4]General Surgery, Department of Surgery and [5]Hormonal Section, Central Laboratory, Azienda Ospedaliera Universitaria Careggi, Florence, Italy

Address for correspondence:
Riccardo G. Gheri, M.D.
Endocrinology Unit
Azienda Ospedaliera Universitaria Careggi
Viale Pieraccini 85, 50134 Florence, Italy
E-mail: riccardogionata.gheri@virgilio.it

KEY WORDS: pseudohypoparathyroidism 1a, pseudohypoparathyroidism 1b, hypercalcitoninemia

Introduction

Pseudohypoparathyroidism (PHPT) is an unusual disease, which is characterized by the resistance of bones and kidney to PTH, followed by hypocalcaemia, hyperphosphataemia, glandular hypertrophy and hypersecretion of PTH. Patients with PHPT clinically manifest tetany seizures, soft tissue calcifications and many congenital malformations. Clinical symptoms may be different and depend on genetic defect or its selectivity with reference to the tissues.
At present we can distinguish three types of PHPT and pseudo-PHPT. The disease usually appears in the infancy. Early diagnosis and vitamin D3 or calcium treatment seem to be the most important for patient's condition. Too late treatment threatens with brain calcification followed by neurological defects and mental retardation.
In this paper we discuss two different patients (A.S., 32 years old male patient with PHPT type 1b, and L.M., 66 years old female patient with 1a PHPT) that during the treatment of hypocalcemia developed hypercalcitoninemia.

Subjects and methods

Our institutional ethics committee approved this study and written informed consent was obtained from the subjects. The clinical study was performed in accordance with the principles of the Helsinki Declaration.

Patients

A.S.
A 32-year-old Italian man was referred to "Azienda Ospedaliera Careggi" hospital (Florence) in 1986 because of recent complain of severe asthenia, dating back at least 5-6 years, and paresthesias of the legs. His father was affected by Alzheimer disease since many years; he had five brothers which were in apparently good health. In the past, he had presented signs and symptoms of tetany during severe febrile episodes, characterized by muscle twitches, cramps and carpopedal spasms. He had no ever smoked. Moreover, he had arterial hypotension (105/80) with 70 b/min; his height was 173 cm, and his weight was 68 kg. There were no somatic phenotypic abnormalities but Chvostek and Trousseau signs (after 25-30 sec) at the first visit were clearly positive. Electromyography (EMG) and electroencephalogram (EEG) confirmed hypocalcemic signs. Intracranial calcifications of the basal ganglions were detected when computed tomography (CT) was performed.
Serum total calcium varied from 1,51 to 1,61 mmol/L, albumin 37-44 g/L, phosphate 1,51-1,69 mmol/L and corrected calcium 0,52 - 0,55 mmol/L. Serum intact PTH levels were 153 and 155 ng/L (normal range: 10-65) and 25(OH)D3 was 40 nmol/L (normal range: 2,25-10,75); serum 1-25$(OH)_2$D3 was 51,60 pmol/L (normal range: 36-144). Serum and urine magnesium and creatinine clearance were normal. Twenty-four-hour urine calcium was 28 mg. Bone formation and resorption markers were normal. Bone mineral densities measured by dual-energy X-ray absorptiometry (DEXA) were within normal limits at the lumbar spine. Intravenous infusion of 200 units of synthetic 1-34 PTH (Parathar®) over 10 minutes was associated with a failure to significantly rise urinary cyclic AMP (from 1,50 mmol/mg creatinine to 4,09 mmol/mg creatinine) and phosphate in urine (that increased only from 112.69 to 243.46 mmol/g creatinine) (Tab. I) in comparison with normal subjects and with hormonopenic hypoparathyroidism patients.
A series of standard provocative test were performed in this patient. TRH test (for TSH, GH and prolactin-PRL) (Tab. II), GNRH test (for LH, FSH, PRL) (Tab. III), AVP infusion test (for serum osmolality) (Tab. IV), glucagon test (for plasma glucose, Insulin, serum Insulin C peptide, GH and cortisol) (Tab. V) were normal.
Basal serum calcitonin was in the normal range but after calcium and calcitriol supplementation significantly increased, during years 1986-2003 (Tab. VI). Thyroid ultrasound examination was always considered normal; no nodules were seen. Calcitonin values of relatives were in the normal range. Serum CEA was always in the normal range. Genetic analysis of GNAS1 gene showed an heterozygous T>C variant at the nucleotide position 433^{-18} (intron 5).

L.M.
A 66-year-old Italian lady was referred to "Azienda Ospedaliera Careggi" Hospital (Florence) in 1999 because of severe hypocalcemia (1,25 mmol/L) and marked increase of plasma PTH (772 ng/L) detected during an evaluation for unexplained seizures. Serum magnesium levels were in the normal range. CT scan detected intracranial severe basal ganglion calcifications. She was born in a very small town near Napoli (Southern

Table I - 1-34 PTH intravenous infusion test (200 UI) over 10 minutes.The peak response in normal subjects is 50- to 100-fold times basal; patient shows only a 2-fold increase.

	B1	B2	0-30 min	30-60 min	60-120 min	
P (ur)	104.29	112.69	243.46	238.95	129.16	mmol/g creat
Nephrogenous cAMP	1.84	1.5	2.34	2.61	4.09	nmol/mg creat

Table II - TRH test (iv injection of 200 µg) (1987).

	Basal	15 min	30' min	60 min	120 min
GH (ng/mL)	1.2	0.5	0.4	0.8	0.5
TSH (mU/L)	4.64	26.69	26.31	18.40	9.72
PRL (mU/L)	2.5	8.2	8.0	4.1	12.1

Table III - GNRH test (iv injection of 100 µg) (1987).

	Basal	15 min	30 min	60 min	120 min
FSH (mU/L)	5.0	5.6	5.9	6.8	6.5
LH (mU/L)	14.9	62.5	61.0	45.7	30.3
PRL (mU/L)	3.9	3.1	2.5	1.9	1.5

Table IV - AVP test (iv injection of 4 µg).

	Basal	3 h	5 h	15 h
Plasma osmolality (mOsm/L)	279	276	275	274

Table V - Glucagon test (iv injection of 500 µg) (1991).

	0	30'	60'	90'	120'	150'	180'	210'	240'
Glucose (mg/dL)	77	131	83	71	60	71	79	78	78
Insulin (mU/L)	5.3	57.9	10.6	5.5	4.0	3.6	4.0	4.5	4.1
C-peptide (pg/mL)	0.6	37.0	2.7	1.1	0.7	0.7	0.7	0.7	1.1
GH (ng/mL)	0.73	0.08	0.23	0.77	9.70	10.2	3.57	0.89	0.47
Cortisolo (nmol/L)	369.0	255	329	260	653	863	1012	982	815

Table VI - Calcitonin values during years of patient A.S.

	1986	1992	1997	1998	2001	2003
Calcium (mmol/L)	1.53	2.10	2.21	2.00	1.95	2.18
PTH (nmol/L)	18.3	14.5	21.6	22.1	14.6	18.2
Calcitonin (2-10 pg/mL)	11.3	10.5	8.99	16.7	51.9	15.4

Italy). She was the second of 8 relatives (5 sons, 3 daughters, Fig. 1); the relatives II-6 (male) and II-7 showed intracranial ganglion calcifications but they refused any other examination. Her menses were regular until the age of 52 years, but she had no pregnancy. She was operated on for bilateral eye cataract. Her heigth was 145 cm and her weight was 55 kg; she exhibited also a round face, with minimal mental retardation. No signs of brachydactyly neither of heterotopic calcifications were present at the clinical and radiological examinations. From many years she suffered by diffuse unexplained paresthesias.

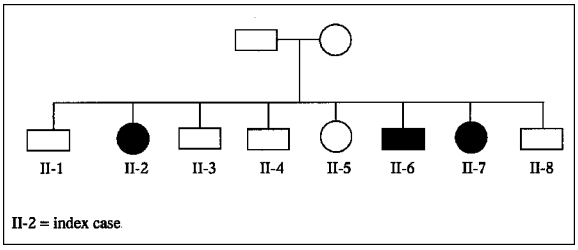

Figure 1 - Genealogic tree of L.M. kindred; filled circles indicate patients with Fahr syndrome (basal ganglion calcifications).

Creatinine clearance, as well as 25(OH)D3 was 54,66 nmol/L (2.25-107.5); serum 1-25$(OH)_2$D3 was 58,32 pmol/L (36-144) were normal. Bone mineral densities measured by dual-energy X-ray absorptiometry (DEXA) were lower than the normal limits at the lumbar spine (BMD: 0,758; T: –2,92). She manifested also a severe olfactory impairment, confirmed by olfactory evoked potentials test; no gustatory neither auditory abnormalities were found. Organ and non-organ specific antibodies were studied, but only anti-parietal cells in gastric mucosa antibodies were found positive; basal gastrin serum levels was very high (356 pg/mL). Calcitonin serum basal levels were high (17,7 pg/mL). No pancreatic endocrine abnormality was found.

Patient started a therapy with calcium (500 mg t.i.d. and calcitriol 0,5 mg o.i.d.). Calcitonin significantly raised during the years (Tab. VII). Ultrasound examination of thyroid gland revealed a multinodular euthyroid goitre (prevailing in the right lobe); suspecting the presence of medullary thyroid carcinoma, a pentagastrin test was performed, that showed a marked rise of stimulated calcitonin (Tab. VIII). For this reason L.M. was operated on (total thyroidectomy); histopathology of excised thyroid gland showed diffuse hyperplasia (not nodular) of C-cells in colloid goitre and immunohistochemistry examination detected positive reaction for calcitonin, cromogranin and CEA. During surgery no hypertrophic parathyroid glands were found.

After the surgical intervention, patient was substituted with l-thyroxine (100 mg/die); calcitonin rapidly decreased to very low serum levels (Tab. VII). The diffuse paresthesias decreased in few days and at the end of the first month after surgery completely stopped; olfactory impairment remained. No mutation of GNAS1 gene were observed in this patient.

Hormone assays

All the hormonal determinations (fT3, fT4, TSH, Insulin, c-Peptide, LH, FSH, GH, prolactin, c-AMP, cortisol, PTH) were performed by standard laboratory methods (radioimmunometric or immunochemiluminometric or immunoenzymometric assays, as appropriate) in the Hormonal Section of the General Laboratory of Azienda Ospedaliera di Careggi and in the Nuclear Medicine Laboratory Unit of the University of Firenze. Serum calcitonin was evaluated by a two-site immunoradiometric assay using ELISA-hCT kit (CIS, Gif-sur-Yvette, France); normal basal levels were 2-10 pg/mL.

Genetic analysis of the GNAS1 gene

Genomic DNA was isolated from peripheral EDTA blood samples of the hypocalcemic and healthy population with the phenol/chloroform procedure. Exons 1-13 of the $G_s\alpha$ encoding gene (GNAS1) were amplified in 50 mL by PCR reaction containing 67 mM Tris-HCl 16.6 mM $(NH_4)_2SO_4$, 5 mM $MgCl_2$, 0.01% Tween-20, 200 μM each of the four deoxyribonucleotides, 0.4 mM of oligonucleotide primers and 1U of Polytaq (Polymed, Florence, Italy) (1). The length of the PCR products was analyzed on 3% agarose gels, stained with ethidium bromide and visualized with UV light. In each subject both strands of each exon were sequenced. Sequencing of the PCR products using both sense and antisense primers was performed using AmpliTaq BigDye Terminator kit and 3,100 Genetic Analyzer (Applied Biosystems).

Discussion

Pseudohypoparathyroidism is a rare disorder characterized by increased levels of PTH in spite of severe hypocalcemia and

Table VII - Calcitonin values during years of patient L.M.

	1999	Jan 2000	May 2000	Nov 2000	Jun 2001*
Calcium (mmol/L)	1.25	2.08	2.28	2.37	2.26
PTH (ng/L)	772	526	430	240	291
Calcitonin (2-10 pg/mL)	17.7	29	54	82.3	2.2

* After total thyroidectomy.

Table VIII - Pentagastrin test (iv bolus injection of 0,1 μg/kg). CEA was 3,1 ng/mL, gastrin was 308 pg/mL (<108), calcium was 2,13 mmol/L.

	-15 min	0	1 min	2 min	3,5 min	5 min	7 min
Calcitonin (pg/mL)	64	69	160	192	151	148	128

hyperphosphatemia. Several forms of PHPT have been described: PHPT type 1a (typical Albright hereditary osteodystrophy [AHO], absent response to PTH infusion, generalized hormone resistance, reduced $G_s\alpha$ activity); pseudo-PHPT (typical AHO, normal serum calcium levels); PHPT type 1b (normal phenotype, defective response to PTH infusion, low serum calcium, normal $G_s\alpha$ activity); PHPT type 1c (typical AHO, defective response to PTH infusion, generalized hormone resistance, normal $G_s\alpha$ activity); PHPT type 2 (low serum calcium, normal cAMP response to PTH infusion, defective urine phosphorus response to PTH infusion).

Very few data exist about C-cells dysfunction in patients with PHPT; moreover, both normal calcitonin (CT) levels (2-4) and increased CT levels (5-7) have been reported. Calcitonin is a 32 amino acid hormone secreted by thyroid C-cells; its receptor is a member of the family of G protein-coupled receptors (8). Usually hypercalcitoninemia is due to medullary thyroid carcinoma, and to several other conditions, such as hypergastrinemia, chronic lymphocytic thyroiditis and renal failure. The recent prospective study of C-cell function in patients with PHPT (9) seems to have demonstrated that high levels of serum calcitonin may be found only in patients with PHPT 1a; it may be associated to resistance to calcitonin, as suggested by the usual impairment of G-protein transduction in patients with PHPT 1a (10) and confirmed by the normal plasma calcitonin levels in patient with PHPT 1b, in which resistance is typically restricted to PTH only (11).

We studied two patients with PHPT, A.S. with type 1b and L.M. with type 1a. In contrast with Vlaeminck-Guillem data (9) we observed high calcitonin serum levels also in the patient with PHPT 1b. No good explanation for the high calcitonin basal level and secretion was found for the patient with PHPT 1b. We were unable to demonstrate, during many years of follow-up, any cause of hypercalcitoninemia: no renal impairment, lymphocytic chronic thyroiditis, hypergastrinemia or medullary thyroid carcinoma were found. However we have to consider that patient's calcitonin serum levels increased after calcium supplementation. Calcium is one of the usual provocative stimulus agent used worldwide to induce calcitonin response. The mechanism underlying calcium-evoked calcitonin release is not fully understood, depending on also by the rate of increase in plasma Ca^{2+} (12). Calcium can operate directly on parafollicular cells or indirectly on gastrin-cells, inducing an increased release of gastrin and secondarily calcitonin increase. Calcium-sensing receptor is expressed in human gastrinoma cells and could be involved in the mechanism of calcium-evoked gastrin release (13). So we may hypothesize that chronic calcium administration is the mechanism by which our patient with PHPT Ib developed hypercalcitoninemia.

In the patient with PHPT 1a we observed high plasma levels of calcitonin since the first examination. C-cells function in this subject may be deranged by several different causes and mechanisms. She had basal hypergastrinemia due to atrophic autoimmune gastritis; hypergastrinemia is one of the known conditions able to induce hypercalcitoninemia (14). Moreover chronic calcium administration to normalize calcium serum levels could directly stimulate calcitonin serum levels. So, in this particular patient, three different mechanism seems to take part in hypercalcitoninemia: resistance to calcitonin, hypergastrinemia for atrophic gastritis, calcium administration. However C-cells appear to respond adequately to the stimulating pentagastrin infusion inducing a marked rising in calcitonin, confirming the normality of C-cell function. An abnormal pentagastrin response is known to be a specific marker for medullary thyroid carcinoma, but in our PHPT 1a patient pathological examination failed to show any evidence of tumor in the excised gland, in agreement with the literature data (5, 15, 16), finding only C-cell hyperplasia.

C-cell hyperplasia precedes the development of medullary thyroid carcinoma in multiple endocrine neoplasia type 2A (MEN2A). Identification of abnormal calcitonin levels after a provocative stimulus is a technique that has been widely used to diagnose this preneoplastic condition in an early stage during the development of medullary thyroid carcinoma, when total thyroidectomy is likely to be curative. C-cell hyperplasia due to some mechanism other than the presence of the PHPT gene mutation may also happen in PHPT 1a kindreds. We cannot exclude the likelihood of developing a medullary thyroid carcinoma in our patient, for the several different mechanism contributing to hypercalcitoninemia. At last the molecular basis of the severe olfactory abnormality, without any other abnormalities regarding gustatory and auditory pathways, is obscure considering the unique G proteins that regulate signal transduction pathways related to vision, olfaction and taste (17-20).

In conclusion, hypercalcitoninemia may be mostly correlated to calcium therapy in PHPT 1b subjects rather than the presence of the genetic abnormality. On the contrary, hypercalcitoninemia seems to be a new described feature of patient with PHPT 1a, of which you to take in account when other different factors for rising calcitonin levels are present, for the risk to develop medullary tumors of the thyroid.

Acknowledgements

We acknowledge that the genetic evaluation was performed in the Laboratory of the Regional Center for HereditaryEndocrine Tumors, directed by Prof. Maria Luisa Brandi.

References

1. Mantovani G, Romoli R, Weber G, Brunelli V, De Menis E, Beccio S, Beck-Peccoz P, Spada A. Mutational analysis of GNAS1 in patients with pseudohypoparathyroidism: identification of two novel mutations. J Clin Endocrinol Metab. 2000;85(11):4243-8.
2. Birkenhager JC, Seldenrath HJ, Hackeng WH, Schellekens AP, Veer Alvd, Roelfsema F. Calcium and phosphorus metabolism parathyroid hormone, calcitonin and bone histology in pseudohypoparathyroidism. Eur J Clin Invest. 1973;3:27-34.
3. Deftos LJ, Powell D, Parthemore JG, Potts Jr JT: Secretion of calcitonin in hypocalcemic states in man. J Clin Invest. 1973;52:3109-3114.
4. Monn E, Osnes JB, Oye I, Wefring KW. Pseudohypoparathyroidism: a difficult diagnosis in early childhood. Acta Paediatr Scand. 1976;65:487-493.
5. Wagar G, Lehtivuori J, Salven I, Backman R, Sivula A. Pseudohypoparathyroidism associated with hypercalcitoninaemia. Acta Endocrinol (Copenh). 1980;93:43-48.
6. Fujii H, Higashi K, Morita M, Sato T. A case of pseudohypoparathyroidism (PHPT) associated with multiple hormonal abnormalities. Jpn J Med. 1984;23:237-241.
7. Kageyama Y, Kawamura J, Ajisawa A, Yamada T, Iikuni K. A case of pseudohypoparathyroidism type 1 associated with gonadotropin resistance and hypercalcitoninaemia. Jpn J Med. 1988;27:207-210.
8. Segre GV. Receptors for parathyroid hormone and parathyroid hormonerelated protein. In: Bilezikian JP, Marcus RM, Levine MA eds. The parathyroids. Basic and clinical concepts. New York: Raven Press; 213-229, 1994.
9. Vlaeminck-Guillem V, D'Herbomez M, Pigny P, Fayard A, Bauters C, Decoulx M, Wemeau JL. Pseudohypoparathyroidism 1a and hypercalcitoninemia. J Clin Endocrinol Metab. 2001;86:3091-3096.
10. Patten JL, Johns DR, Valle D. Mutation in the gene encoding the stimulatory G protein of adenylate cyclase in Albright's hereditary

osteodystrophy. N Engl J Med. 1990;322:1412-1419.
11. Levine MA, Schwindinger WF, Downs RW, Moses AM. Pseudohypoparathyroidism. Clinical, biochemical, and molecular features. In: Bilezikian JP, Marcus RM, Levine MA eds. The parathyroids. Basic and clinical concepts. New York: Raven Press; 781-800, 1994.
12. Wang W, Lewin E, Olgaard K. Rate-dependency of calcitonin secretion in response to increased plasma Ca^{2+}. Eur J Clin Invest. 2002;32(9):669-673.
13. Itami A, Kato M, Komoto I, Doi R, Hosotani R, Shimada Y, Imamura M. Human gastrinoma cells express calcium-sensing receptor. Life Sci. 2001;70(2):119-29.
14. Wion-Barbot N, Schuffenecker I, Niccoli P. Results of the calcitonin stimulation test in normal volunteers compared with genetically unaffected members of MEN 2A and familial medullary thyroid carcinoma families. Ann Endocrinol (Paris). 1997;58:302-308.
15. Mazzuoli GF, Coen G, Antonozzi I. Study on calcium metabolism, thyroid calcitonin assay and effect of thyroidectomy in pseudohypoparathyroidism. Isr J Med Sci. 1967;3:627-636.
16. Lee JB, Tashjian AH Jr., Streeto JM, Frantz AG. Familial pseudohypoparathyroidism. Role of parathyroid hormone and thyrocalcitonin. N Engl J Med. 1968;279:1179-1184.
17. Weinstock RS, Wright HN, Spiegel AM, Levine MA, Moses AM. Olfactory dysfunction in human with deficient guanine nucleotide-binding. Nature. 1986, 14-20.
18. Lerea CL, Somers DE, Hurley JB, Klock IB, Bunt Milam AH. Identification of specific transducin alpha subunits in retinal rod and cone photoreceptors. Science. 1986;234:77-80.
19. Lochrie MA, Hurley JB, Simon MI. Sequence of the alpha subunit of photoreceptor G protein: homologies between transducin, ras, and elongation factors. Science. 1985;228:96-99.
20. McLaughlin SK, McKinnon PJ, Margolskee RF. Gustductin is a taste-cell specific G protein closely related to the transducins. Nature. 1992;357:563-568.