The Italian Register of primary hypoparathyroidism

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Summary

No information is available on prevalence of primary hypoparathyroidism in the general population. This report describes the establishment and development of a national epidemiological survey on primary hypoparathyroidism in Italy through a Register, named RIIP. The services offered to the referring Centers and the data collected through the Italian Register are detailed and discussed. This example offers a possibility that others could follow, allowing the construction of databases necessary for epidemiological studies on rare diseases and the design of clinical trials in such rare diseases.

KEYWORDS: primary hypoparathyroidism, National Register, epidemiology.

Introduction

Calcium is an important bioinorganic ion performing a variety of intra- and extra-cellular functions and playing an important role in maintaining normal physiologic processes. It is also an important component or co-factor required for the proper functioning of coagulation factors and adhesion molecules.

Homeostasis of calcium and phosphorus is maintained by a combined activity of the calciotropic hormones: parathyroid hormone (PTH), Vitamin D [25 OH_2D_3 and 1-25 $(OH)_2D_3$], and calcitonin (CT). PTH, the product of the parathyroid glands, regulates serum calcium concentrations and bone metabolism. In turn serum calcium concentrations regulate PTH secretion by means of a calcium-sensing receptor (CaSR) on the surface of parathyroid cells.

The term of primary hypoparathyroidism refers to a group of inherited disorders in which the relative or absolute deficiency of PTH leads to hypocalcemia and hyperphosphatemia (Table I). These disorders may be caused by developmental defects in the parathyroid glands, by autoimmune endocrinopathies, by defects in PTH synthesis, by impaired regulation of PTH secretion, and by defective PTH action. The latter form termed pseudo-hypoparathyroidism are unique in that PTH secretion is increased rather than deficient.

A cause of hypoparathyroidism is represented by an anomalous development of parathyroid glands. The disease called DiGeorge syndrome can be sporadic or familiarly transmitted as autosomal dominant trait (1, 2).

The polyglandular disorder called autoimmune polyglandular syndrome type 1 (APS 1) is characterized by autoimmune Addison's disease, moniliasis, and hypoparathyroidism. A gene encoding for a putative regulator of transcription featuring two PHD-type zinc-finger motifs (*AIRE:* AutoImmune Regulator) has been discovered as the primary cause of APS 1 (3).

Mutations of the *prepro-PTH* gene are commonly involved in the pathogenesis of familial isolated hypoparathyroidism transmitted in autosomal mode. Familial hypoparathyroidism can also be X-linked (4). In other cases, linkage of hypocalcemia to the locus of the *CaSR* gene (3q21-24) has been demonstrated in some forms of familial hypoparathyroidism (5, 6). Hypoparathyroidism has been also reported to occur in two disorders associated with mitochondrial dysfunctions (7, 8).

Table I - Categories of primary hypoparathyroidism.

Causes	Diagnosis
Developmental defects in the parathyroid glands	• DiGeorge syndrome • Autosomal recessive hypoparathyroidism • Kenney-Caffey syndrome • Mitochondrial neuromyopathy
Autoimmune disorders	• APS 1
Defects of the parathyroid hormone molecule	• Mutations of *PTH* gene
Defective regulation of parathyroid hormone	• Activating mutation of the *CaSR* gene
Defect of the type 1 PTH receptor	• Jansen's chondrodystrophy • Blomstrand's chondrodystrophy
Defect of the stimulatory $G_s\alpha$ subunit	• PHP-Ia and PHP-Ib • PPHP

Mutations of the $G_s\alpha$ subunit gene (*GNAS1*) (20q13-11) have been identified in patients with pseudohypoparathyroidism type Ia (PHP-Ia) and with pseudo-pseudohypoparathyroidism (PPHP). PHP is an autosomal dominant disorder characterized by a typical phenotype (9, 10).

Italian Register of Primary Hypoparathyroidism

In 1996 an Italian Register of Primary Hypoparathyroidism was created in Florence and named RIIP (Registro Italiano Ipoparatiroidismo Primitivo: www.dmi.unifi.it/ipopara/default.htm). It is a passive register and its Central Secretariat has been established in Florence. RIIP collects clinical records both on sporadic and familial cases of primary hypoparathyroidism. Information was obtained from Italian endocrine, neurological and pediatric Centers through the compilation of a simple form, that includes the identification code of the patient, the date of birth, the diagnosis of the type of hypoparathyroidism, the presence of other associated diseases (typical or not), the data on organ and non-organ antibodies, the results of PTH infusion and genetic tests. Each subject is requested to give informed consent and at any time the patient can ask to obtain his/her clinical data deleted from the official file. The form (available on request) has been arranged to be sent by fax and e-mail (Table II).

Goals of the RIIP

The primary goal of the RIIP is the collection of clinical data in order to elaborate epidemiological results on incidence, prevalence and geographical clustering of primary hypoparathyroidism in Italy. The Register supports also the genetic test for Centers not equipped for molecular diagnostic procedures. The collection of these data will make possible a nation-wide survey of the problem and the definition of the prevalence of typical and atypical lesions both in affected patients and their relatives. Through this approach the incidence and prevalence of the various disorders in Italy will be compared to what described in other countries. Finally, the possibility of collecting kindreds affected by rare disorders will make available large patient cohorts necessary in clinical trials designed to recognize therapeutical interventions suitable to a given disorder.

Several genes are involved in the pathogenesis of hypocalcemic disorders due to parathyroid tissue/PTH response dysfunction. The mutational analysis of *GNAS1* gene, the parathyroid hormone (*PTH*) gene, the PTH/PTHrP receptor (*PTH/PTHrPr*) gene, the *CaSR* gene, the human orthologue gene of the *Drosophila glial cells missing gene* dGCM (*GCMB*) gene, the GATA3 transcription factor (*GATA3*) gene and the *AIRE* gene in patients recorded at the RIIP should make possible to identify mutations that have been described in the literature and eventual new mutations responsible for the various (typical or not-typical) forms of primary hypoparathyroidism. In addition, microarrays will make possible to study the expression profile and haplotypes of genes involved in the pathogenesis of hypocalcemic disorders through the construction of a “gene cassette” useful to clarify the metabolic pathways underlying the hypocalcemic dysfunctions. Future advances will be important for the discovery both of novel genetic markers and of the pathogenesis of these disorders, making possible to identify early latent states of hypocalcemia and to create guidelines for diagnostic and clinical management of hypoparathyroid patients in Italy.

Results of the RIIP

So far 109 hypocalcemic patients have been registered in the RIIP of Florence. Subjects had an age ranging from 6 to 71 years. Both sexes were represented (48 female and 61 males). There were 14 cases of PHPIa; 8 cases of PHPIb; 8 cases of PPHP; 57 cases of idiopathic hypoparathyroidism; 2 case of DiGeorge syndrome; 8 cases of isolated parathyroid agenesis; 9 cases of APS 1; and 3 cases of universal calcinosis (Table III). The geographical distribution of the patients was the following: 51% from the South, 39% from the Center, and 10% form

Table III - Subjects registered at the RIIP.

Disease	Number
Isolated parathyroid agenesis	8
DiGeorge syndrome	2
Idiopathic hypoparathyroidism	57
APS 1	9
PHP-Ia	14
PHP-Ib	8
PPHP	8
Universal calcinosis	3

Table II - Form for collecting information on patients affected by primary hypoparathyroidism.

Patient’s code #	Surname and name	Date of birth	Type of hypoparathyroidism*	Associated diseases**	Organ-specific antibodies	Non Organ-specific antibodies	Test for pseudohypoparathyroidism diagnosis***	Genetic diagnosis

* A) Hypoparathyroidism 1) Parathyroid agenesis or 2) DiGeorge syndrome; B) Autoimmune hypoparathyroidism 1) Isolated or 2) Polyglandular autoimmune syndrome type I; C) Hypoparathyroidism 1) Deficient parathyroid hormone secretion; D) Pseudohypoparathyroidism; E) Pseudo-pseudohypoparathyroidism.
** A) Insulin dependent diabetes; B) Hypogonadism; C) Adrenal insufficiency; D) Pernicious anemia; E) Alopecia; F) Candidiasis; G) Hypothyroidism.
*** PTH test.

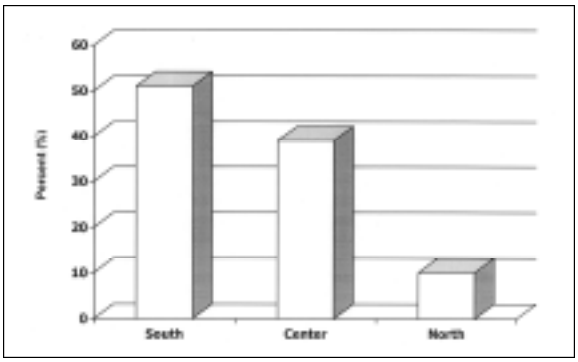

Figure 1 - Geographical distribution of the patients recorded at the RIIP. Of the total subjects registered at the RIIP 51% were from the South, 39% from the Center, and 10% form the North of Italy.

the North of Italy (Fig. 1).

Thirty-nine patients (10 women and 29 men) with a mean age 40±22.4 SEM years (range 7-66) underwent genetic test to evaluate the presence of *GNAS1* gene mutations. For 4 of them, blood samples for genetic analysis from relatives were made available.

Developmental status of the patients was appropriate. The entire cohort of patients was biochemically characterized by serum evaluation of calcium, phosphorus, magnesium, alkaline phosphatase, PTH, vitamin D (25 OH_2D_3 and 1-25 OH_2D_3), and organ- and non-organ specific antibodies. Thyroid function was evaluated by measurement of serum TSH, fT3 and fT4. A sample of urine was collected in order to evaluate the excretion of calcium, phosphorus, magnesium deoxypiridinoline and cyclic AMP. In addition, routine exams were performed in all patients. Lumbar spine BMD (LS-BMD) was also measured by DEXA (Hologic QDR 4500). Electrorcardiogram, electromyography, ocular inspection and skull X-ray and/or CT were performed in all patients.

A new T>C polymorphic site of the *GNAS1* gene was found in 7 patients and 4 relatives from different families indicated by A, B, C, D1, D2, D3, E1, F1, F2, G1, and G2 and it was not associated with modifications of restriction endonuclease recognition sequences (Table IV). The clinical characteristics of patients and of their first-degree relatives are summarized in Table IV. A patient affected (D1) and his sons (D2 and D3)

Table IV - Clinical and biochemical data of the hypocalcemic patients with *GNAS1* gene mutations.

Code #	Sex	Age (Yr)	S-Ca (8.5-10.5 mg/dL)*	UrCa (100-300 mg/24h)	s-P (2.8-4.5 mg/dL)	PTH (10-60 ng/mL	TSH (0.35-3.5 mU/mL)	Diagnosis and clinical signs	Imaging	GNAS1 gene mutation
A1	F	65	6,8	362	4.[illegible]	26	<0.05	Late idiopathic hypocalcemic crisis hyperthyroidism	IC	Heterozygous intron 5 T>C variant nuc.433^{-18}
B1	F	59			3.9	99		Late idiopathic hypocalcemic crisis	IC	Heterozygous intron 5 T>C variant nuc.433^{-18}
C1	M	66	7.7	244	3.8	45	NA	Late idiopathic hypocalcemic crisis	IC	Heterozygous intron 5 T>C variant nuc.433^{-18}
D1	M	17	5.9	235	4.68	137	3.6	PHP Ib, Subclinical hypothyroidism cataract, Br	IC	Heterozygous intron 5 T>C variant nuc.433^{-18}
D2 (S)	M	15	9.8	200	4.7	42.3	NA	N	NA	Heterozygous intron 5 T>C variant nuc.433^{-18} + Heterozygous exon 13 C>T variant c.1113
D3 (S)	M	17	9.7	230	4.5	58	NA	N	NA	Heterozygous intron 5 T>C variant nuc.433^{-18}
D4 (W)	F	45	10.1	245	3.5	62	NA	N	NA	NP
E1	M	48					NA	Late idiopathic hypocalcemic crisis		Heterozygous intron 5 T>C variant nuc.433^{-18}
F1	F	7	7.9	288	4.5	143	6.64	PHP Ia, Br, Ob, RF, SC	SM	Heterozygous intron 5 T>C variant nuc.433^{-18}

continued

Table IV - *continued*

Code #	Sex	Age (Yr)	S-Ca (8.5-10.5 mg/dL)*	UrCa (100-300 mg/24h)	s-P (2.8-4.5 mg/dL)	PTH (10-60 ng/mL)	TSH (0.25-3.5 mU/mL)	Diagnosis and clinical signs	Imaging	*GNAS1* gene mutation
F2 (M)	F	36	10	160	3.9	40	NA	Br	NA	Heterozygous intron 5 T>C variant nuc.433^{-18}
F3 (F)	M	50	9.8	185	3.5	65	NA	N	NA	NP
G1	M	17	8.2	180	4.2	56	2.9	Br, osteopenia, hyperprolactinemia, SM	NA	Heterozygous intron 5 T>C variant nuc.433^{-18}
G2 (B)	M	21	9.6	213	3.9	57	NA	N	NA	Heterozygous intron 5 T>C variant nuc.433^{-18}
G3 (M)	F	62	9.5	228	2.9	60	NA	N	NA	NP
H1	F	15	8.1	282	4.6	157	7.9	PHP Ia, Br, Ob, RF	NA	Heterozygous exon 5 Pro→Leu c. 115
L1	F	56	8.4	300	3.5	48	NA	Late idiopathic hypocalcemic crisis	NA	Homozygous exon 13 C>T variant c. 11[illegible]
M1	F	48	7.9	340	4.7	6	2.8	Hypoparathyroidism	NA	Heterozygous exon 13 C>T variant c.1113
M2 (F)	M	78	8.2	380	4	12	NA	Hypoparathyroidism	NA	Homozygous exon 13 C>T variant c.1113
M3 (M)	F	76	9	267	3.4	15	NA	N	NA	NP
N1	F	56	8.5	280	[illegible]	1[illegible]	NA	Late idiopathic hypocalcemic crisis		Heterozygous exon 13 C>T variant c.1113

Proband: bold characters
* Serum calcium levels were corrected for albumin concentration.
Abbreviations: F: Female; M: Male; (F): Father; (M): Mother; (S): Son; (W): Wife.
In alphabetic order: Br: Brachidactyly; IC: Intracranic Calcification; N: Normal; NA: Not Available; Ob: Obesity; NP: Not Polymorphic; PHP: Pseudohypoparathyroidism; RF: Round Face; SC: Subcutaneous Calcification; SM: Shortening of Metacarpals.

showed the heterozygous T>C mutation and no mutations were found in the mother (D4). Patient D2 had a polymorphism at the exon 13 together with the T>C polymorphism at the intron 5 (11). In addition, 13 patients had a polymorphism at the exon 5 of the *GNAS1* gene previously described by Miric et al. (12). Two subjects were homozygous (Table IV). The clinical characteristic of 4 of the patients was available and reported in Table IV (subjects D2, L1, M1, M2, and N1). One patient (H1) affected by PHP-Ia had a missense mutation of the *GNAS1* gene, characterized by Pro→Leu at the codon 115 in the exon 5, previously described by de Sanctis et al. (13).

Three patients affected by APS 1 and their first-degree relatives underwent genetic test to evaluate the presence of *AIRE* gene mutations. Table V shows the characteristics of these patients and relatives. The probands A1 and A2 had a homozygous mutation Thr→Met at the codon 16 in the exon 1 and a heterozygous mutation Prol→Leu at the codon 252 in the exon 6 already described in the literature (14, 15). The mother (A3) had the heterozygous mutation Thr→Met at the codon 16 in the exon 1 and the father (A4) had both heterozygous mutation Thr→Met at the codon 16 in the exon 1 and the heterozygous mutation Prol→Leu at the codon 252 in the exon 6.

The B1 proband had a homozygous Arg→Stop Codon mutation in the exon 5 previously described by Scott et al. (16). The mother (B2), the father (B3), and the brother (B4) had the same heterozygous mutation.

Conclusions

The recognition of the pathogenetic basis of hypocalcemic disorders is important for patient care, providing important clues for management, as subjects with activating *CaSR* mutations cannot be treated with vitamin D but would benefit most from PTH injections (17). Therapy of hypoparathyroid patients is not a primary outcome of the RIIP, however, the collection of patient populations clinically and genetically characterized, represents the necessary basis for the recognition of selected populations for clinical trials. Finally, a careful genetic study of these patients will be useful in: a) precocious diagnosis of patients affected by a hypocalcemic disorder; b) prevention of complications due to chronic hypocalcemia; and c) early treatment of

Table V - Clinical characteristics of the subjects with *AIRE* gene mutations.

Code #	Sex	Age (Yr)	Diagnosis	*AIRE* gene mutation
A1	F	10	APS 1	Homozygous exon 1 Thr→Met c.16 + Heterozygous exon 6 Prol→Leu c. 252
A2	M	12	APS 1	Homozygous exon 1 Thr→Met c.16 + Heterozygous exon 6 Prol→Leu c. 252
A3 (M)	F	56	N	Heterozygous exon 1 Thr→Met c.16
A4 (F)	M	60	N	Heterozygous exon 1 Thr→Met c.16 + Heterozygous exon 6 Prol→Leu c. 252
B1	F	15	APS 1	Homozygous exon 5 Arg→Stop Codon
B2 (M)	F	59	N	Heterozygous exon 5 Arg→Stop Codon
B3 (F)	M	64	N	Heterozygous exon 5 Arg→Stop Codon
B4 (B)	M	17	N	Heterozygous exon 5 Arg→Stop Codon

Proband: bold characters
Abbreviations: F: Female; M: Male; (F): Father; (M): Mother; (B): Brother.
In alphabetic order: APS 1: Autoimmune Polyglandular Syndrome Type I; N: Normal.

associated disorders.

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