Brief report

Hypoparathyroidism due to autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy: long-term clinical follow-up

Silvano Bertelloni
Roberta Parrino
Giampiero I. Baroncelli
Filomena Cetani*

Department of Pediatrics, Santa Chiara Hospital, Pisa, Italy and *Department of Endocrinology and Metabolism, University of Pisa, Pisa, Italy

Address for correspondence:
Silvano Bertelloni, M.D.
Department of Pediatrics, Santa Chiara Hospital
Via Roma 67, 56125 Pisa, Italy
Ph. +39 050 992743
Fax +39 050 993181
E-mail: s.bertelloni@med.unipi.it

KEY WORDS: hypoparathyroidism, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, AIRE gene, long-term follow-up.

Introduction

Hypoparathyroidism is a heterogeneous clinical condition (Table I) (1). It manifests when parathyroid hormone (PTH) produced by parathyroid glands is unable to maintain normal extracellular fluid concentrations of calcium ions, for insufficient secretion or inadequate action on target tissues despite normal or high circulating levels of the hormone (*pseudo-hypoparathyroidism*) (1, 2). 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] production is also decreased, leading to a hormonal deficiency (1).
The predominant clinical manifestations of hypoparathyroidism are related to hypocalcemia. In the acute setting, neuromuscular irritability, including perioral paresthesias, tingling of the fingers and toes and spontaneous or latent tetany with generalized tonic-clonic seizures and laryngeal spasm can be evident. Chronically, hypocalcemia can be asymptomatic and can be recognized after routine blood screening. Alternatively, it can manifest with mild neuromuscular irritability, calcification of the basal ganglia, extrapyramidal disorders, cataracts, alopecia, abnormal dentition, coarse brittle hair, mental retardation or personality disorders (1, 2).
Biochemically, hypoparathyroidism is characterized by low serum calcium concentrations and increased serum phosphate levels in the presence of normal renal function. Serum concentrations of immunoreactive PTH are low or undetectable, except in the setting of pseudo-hypoparathyroidism. 1,25$(OH)_2$D serum levels as well as nephrogenous cyclic AMP excretion are low, whereas renal tubular reabsorption of phosphate is elevated (1).
Autoimmune PolyEndocrinopathy-Candidiasis-Ectodermal Dystrophy (APECED), also known as *autoimmune polyglandular syndrome type I* (OMIM 240300) is an autosomal recessive disorder affecting many tissues, mainly endocrine glands (3, 4). Hypoparathyroidism is one of the earliest and commonest manifestations of the syndrome (3). APECED is caused by mutations in a single gene on chromosome 21q22.3, named *AIRE* (for *autoimmune regulator*), which encode a protein with the characteristics of a transcription factor (3, 4).

In this report, we outline the long-term follow-up of a girl affected by APECED, in whom hypoparathyroidism represented the first recognized manifestation.

Patient report

The patient was the third daughter of unrelated Italian parents from a small town of the Liguria region. She was born in April 1978 after an uncomplicated pregnancy and delivery. At birth, height was 49 cm and weight was 3150 g. Perinatal period was normal.

Table I - Hypoparathyroidism: clinical forms (2).

Agenesis or dysgenesis of the parathyroid glands
X-linked autosomal recessive hypoparathyroidism
DiGeorge syndrome
Hypoparathyroidism, sensorineural deafness and renal dysplasia syndrome
Hypoparathyroidism-retardation-dysmorphism syndrome
Kenny Caffey syndrome
Mitochondrial neuromyopathies
Kearns-Sayre syndrome
Pearson syndrome
Long-chain hydroxyacyl-CoA dehydrogenase deficiency
Destruction of the parathyroid glands
Postsurgical hypoparathyroidism
Hypoparathyroidism after radioactive iodine thyroid ablation
External radiation
Infiltrative disorders
Hemochromatosis
Wilson's disease
Granulomatous diseases
Metastases
Autoimmune polyendocrinopathy candidiasis-ectodermal dystrophy syndrome
Impaired PTH secretion
Primary
PTH gene mutations - autosomal recessive /autosomal dominant
Activating mutations of the Ca^{2+}-sensing receptor
Secondary
Maternal hyperparathyroidism
Hypomagnesemia
Target organ resistance
Primary
Pseudohypoparathyroidism (1A, pseudo, 1B, 1C, 2)
Secondary
Hypomagnesemia

At the age 3 years, after some episodes interpreted as syncope, the girl was referred to our Department for generalized seizures. At admission, she presented with hypocalcemia, increased levels of phosphate and PTH deficiency (Table II). Family and personal history were uninformative, with the exception of mild oral candidiasis in the first year of life, completely recovered with regular antimycotic treatment. Height and weight were normal for age and sex (Table II) and for her mid-parental height [160 cm (-0.4 SDS)] (5). No other specific clinical signs were found.

After correction of hypocalcemia by intravenous calcium infusion, treatment with $1,25(OH)_2D_3$ (0.25 µg thrice/day) and oral calcium (500 mg twice/day) was successfully started. Therapy was monitored and adjusted to maintain adequate serum calcium levels. The girl was well until the age 10 years, 8/12 when she was admitted for hypovolemic shock. She presented with dark skin and biochemical evaluation demonstrated sudden adrenal crisis (Table II). Addison's disease was diagnosed and the patient started treatment with hydrocortisone (10-15 mg/m^2/daily) and 9α-fludrocortisone (0.1 mg/daily). APECED syndrome was suspected and auto-antibodies against adrenal gland, ovary, skin, gastric parietal cells were found to be positive (C. Betterle, Padua).

Pubertal development started around the age 12 years, but it arrested at the stage B2 (5); at this age pituitary gonadal-axis evaluation showed hypergonadotropic hypogonadism and sonography demonstrated small ovarian glands (Table II). Female secondary sexual characteristics was then induced by administration of estro-progestin treatment.

In the following years, the patient developed severe nail candidiasis and recurrent periods of malabsorption, requiring to increase $1,25(OH)_2D_3$ (3-4 µg daily) and calcium (3-4 g daily) doses to maintain low-normal calcium values; she also developed vitiligo and tooth enamel dystrophy, requiring teeth reconstruction. When the patient was 22 year old, she had a short period (3 months) of isolated hypertransaminasemia (2.3 times the upper normal value) spontaneously recovered. Positive antiglutamic acid decarboxylase (GAD) and anti-insulin (AIA) antibodies was detected at the age 24 years [94.5 U/ml (n.v. < 0.9) and 16.1% (n.v. < 0.2)], but parameters of glucose homeostasis remained in the normal range (Table II). *AIRE* gene analysis demonstrated, in a homozygous state, a missense mutation in exon 2 (W78R).

Clinical and biochemical data as well as therapies at last follow-up are summarized in Table II.

Table II - Clinical data at diagnosis and during follow-up.

Parameter	Patient	Note
At first admission		
Age, years	3, 4/12	–
Height, cm	93.5	-0.81 SDS*
Weight	13.2	-12%**
Calcium, mg/dL	4.5	n.v. 8.5-10.5
Phosphate, mg/dL	6.7	n.v. 2.5-5.0
Alkaline phospatase, UI/L	457	n.v. 380-640
PTH, pmol/L°	undetectable	n.v. 40-70
At adrenal crisis		
Age, years	10, 8/12	–
Height, cm	137.0	-0.60 SDS*
Weight	24.7	-21.6 %**
Calcium, mg/dL	8.0	n.v. 8.5-10.5
Phosphate, mg/dL	5.6	n.v. 2.5-5.0
Sodium, mEq/L	125.0	n.v. 136-142
Potassium, mEq/L	6.1	n.v. 3.5-5.5
Cortisol, ng/mL	0.8	n.v. 60-300
ACTH, pg/mL	>1250	n.v. 9-52
At diagnosis of hypogonadism		
Age, years	11, 11/12	–
Height, cm	143.5	-0.76 SDS*
Weight	30.7	13.4%**
Calcium, mg/dL	8.0	n.v. 8.5-10.5
Phosphate, mg/dL	5.6	n.v. 2.5-5.0
Sodium, mEq/L	139.0	n.v. 136-142
Potassium, mEq/L	4.14	n.v. 3.5-5.5
17β-estradiol, pg/mL	12.7	29-270^
LH, UI/L	46.0	1.5-20^
FSH, UI/L	79.5	1.5-10^
Mean ovarian volume, mL	1.2	-2.0 SDS
At last follow-up		
Age, years	26	–
Height, cm	161.5	-0.11 SDS*
Weight	66.5	18.7%**
Calcium, mg/dL	8.8	n.v. 8.5-10.5
Phosphate, mg/dL	4.2	n.v. 2.5-5.0
Sodium, mEq/L	140.0	n.v. 136-142
Potassium, mEq/L	4.28	n.v. 3.5-5.5
Fasting glucose, mg/dL	79.0	n.v. 65-110
Glucose peak°°, mg/dL	133.0	n.v. < 140
HbA1c, %	4.6	n.v. 3.4-5.8
Fasting insulin, mU/mL	8.5	n.v. 4.0-25
Lumbar BMD, g/cm^2	1.142	-0.69***
$1,25(OH)_2D_3$, µg/day	0.75	–
Calcium, g/day	1.5	–
Hydrocortisone, mg/day	15	–
9α-fludrocortisone mg/day	0.5	–
17β-estradiol (20 µg/day) + norelgetromin (150 mg/day)	21 days/28 days^^	–

* According to Tanner et al. (%); ** of ideal body weight for height; ° RIA for M-M PTH (44-68 region of human PTH); *** according to Boot et al. (J Clin Endocrinol Metab 1997;82:57-62); ^ n.v. for follicular phase; ^^ transdermal.

Discussion

In this paper, we report on long-term follow-up of a female patient affected by APECED. Despite severe expression of the disease, the patient attained a final height adequate for her mid-parental and normal bone mineral density in young adulthood, suggesting that an accurate multi-hormonal substitutive treatment may permit good somatic and sexual development. Hypoparathyroidism represented an isolated endocrine finding for several years. Oral candidiasis was recorded in the first months of life but, because it had been easily treated, this feature was not adequately taken into consideration. Severe candidiasis developed only at adolescence. So, the girl was considered affected by primary "isolated" hypoparathyroidism until she was acutely admitted for an adrenal crisis.

In fact, hypoparathyroidism in APECED appears early in childhood, usually between the age 5-10 years (6), while the other main clinical components of the syndrome may did not develop up to adolescence and some manifestations may not evolve until the fifth decade of life or also later (4, 6). Thus, an extensive history and accurate clinical and endocrinological surveil-

lance should be guaranteed to each child presenting with "idiopathic" hypoparathyroidism. Today, molecular evaluation of *AIRE* gene may permit an early diagnosis (3), when other known causes of hypoparathyroidism (Table I) have been ruled out (2). Indeed, molecular analysis of *AIRE* and other genes associated with hypoparathyroidism requires expert professionals and adequate technology. Reference laboratories to perform the molecular analysis in patients selected by restrictive clinical and biochemical criteria should be established for large macroareas. In fact, APECED is a rare disease (incidence ~1:100.000/year) (3, 4), and the health organization for these patients should forecast laboratory and clinical reference centers connected with clinicians working at peripheral level by an effective network. As other recessive genetic disorders, an higher incidence has been found in some populations, who are characterized by a high degree of consanguinity. In Italy, the syndrome is more prevalent in Sardinian population (1:14.400) (8). High incidence of APECED is also reported in Iranian Jews (1:600 to 1:9.000) (9) and Finns (1:25.000) (10). The female to male ratio in APECED varies from 0.8:1 to 2.4:1 (6, 11).

APECED is due to mutation in *AIRE* gene (3). This gene consists of 14 exons spanning approximately 13 kb of genomic DNA and encodes for a 545 aminoacid protein. It is especially prominent in the nucleus of thymic medullary epithelial and dendritic antigen presenting cells (3), and not in the target organs of APECED disease process (3, 12). *AIRE* gene likely plays a role in the induction of self-tolerance, enhancing the expression of peripheral antigens in the thymus and by acting as co-activator of nuclear receptors involved in the process of clonal deletion (3, 4, 14).

AIRE is expressed in the medullary epithelium of the thymus (3); there is a close relationship between the thymic and parathyroid epithelia because they originate from the third pharyngeal pouches (14). Lack of AIRE may affect the negative selection of T-cell normally executed by the thymic medullary epithelial cells and thymic dendritic cells. The defective apoptosis of self reactive T-cells would represent the pathogenesis of the disease (3, 4, 15).

At least 49 mutations have been described in the *AIRE* gene (3), the most frequent of them include a typical Finnish mutation (R257X) (3), also described in some patients from Northern Italy (15), the Sardinian mutation (R139X) (8), and the Jewish-Iranian mutation (Y85C) (3). Previously unreported mutations have been described in 11 patients (from 8 families) originating from a restricted area of Southern Italy (15). The W78R missense mutation was relatively common in this group of patients, suggesting a founder effect (16). The same W78R mutation has been detected in our girl, even if the evaluation of her pedigree did not show any evidence of relatives from Salento peninsula, as previously reported in the other Italian patients sharing the same mutation (16). Indeed, the W78R mutation has been also described in one patient of Czech origin (17), suggesting that sporadic mutations can occur outside from Salento area.

The W78R mutation replacing a non polar aminoacid with a polar basic residue (16) likely determines a large derangement of *AIRE* gene as indicated by the early onset of APECED and the severe clinical expression of the disease in our girl as well as in the other reported subjects (16).

The APECED phenotype is inherited as an autosomal recessive fashions homozygous or compound heterozygous state (3). Indeed, recent data suggest that some mutations may act as dominant, leading to overt APECED syndrome also in heterozygous subjects (18, 19).

Clinically, candidal infection of the skin and mucous membranes is one of the more frequent feature of APECED, being present in the large majority of reported patients (6, 11, 15, 16, 18, 19), with slight differences between Northern Europe and Italy (Table III). It is likely due to T-cell defect, but these subjects do not show other clinical evidence of T-cell immunodeficiency (4). As in the present girl, muco-cutaneous candidiasis may represent the first clinical manifestation (3, 6, 18), but its importance for diagnosis may be underestimated until the appearance of endocrine manifestations. In fact, candidiasis is often mild, in contrast with the extensive severe lesions of other forms of chronic muco-cutaneous candidiasis (4), and affects the skin in only 10% of the patients (6). As underlined in present report, candidiasis is usually followed by hypoparathyroidism just in early childhood (4, 6, 11), mainly in females (13) in whom it becomes manifest between the age of 5 to 10 years by generalized seizures associated with hypocalcemia and low

Table III - Main characteristics of the APECED syndrome reported in literature and in the present girl.

	North Europe patients*	Italian patients**	Present girl
n	90	53	
Male/female	45/45	18/35	
Endocrine manifestations (%)			
Hypoparathyroidism	81	94	+
Addison's disease	79	73	+
Hypogonadism	31	40#	+
Type I diabetes	12	2	–
Thyroid disease	4°	17	–
Nonendocrine autoimmune manifestations (%)			
Mucocutaneous candidiasis	94	85	+
Enamel hypoplasia	77°	100°°	+
Alopecia	31	41	–
Intestinal dysfunction	22	34	+
Vitiligo	21	13	+
Pernicious anemia	16	13	–
Autoimmune hepatitis	16	21	–^

* Data from ref. 14; ** data from ref. 11, 16, 19; ° data from ref. 6; °° data only from ref. 16, 19; # of post-pubertal patients; ^ transient hypertrasaminasemia.

Table IV - Main autoantigens in APECED.

Disease	Tissue	Antigens	Prevalence autoantibodies, %*
Addison's disease	Adrenal	P450c21	66
		P450c17	44
		P450scc	52
Hypoparathyroidism	Parathyroids	Epithelia	19
		Ca^{++}-sensing receptor	–
Hypothyroidism	Thyroid gland	Thyroid peroxidase	–
		Thyroglobulin	–
Type I diabetes	Endocrine pancreas	GAD65	37
		IA-2	7
		GAD67, ICA,	–
Autoimmune hepatitis	Liver	P450CYP1A2	8
		P450CYP2A6	–
		P450CYP1A1	–
		P450CYP2B6	–
		AADC	51
Autoimmune gastritis	Stomach	H^+K^+ATPase	–
Malabsorption	Gut	Tryptophan hydroxylase	45
Penicious anemia	Gastric mucosa Red blood cells	Intrinsic factor	–
Vitiligo	Skin	SOX9, SOX10	–
Alopecia	Scalp	Tyrosine hydroxylase	4

* Data from ref. 13, 14.

or undetectable PTH concentrations (1, 4). About ¼ of patients, mainly males (13), do not develop hypoparathyroidism. The males also showed a later age at onset of hypoparathyroidism (13). The reason for this different evolution of the syndrome seem to be elucidated (3, 4), but the evidence that most of the patients without hypoparathyroidism are males suggest that sex may have some influences on APECED phenotype (13). Autoantibodies against several antigens of parathyroid gland have been described (Table IV), but their involvement in the pathogenesis of impaired PTH secretion remain to be better elucidated (3, 14).

Addison's disease is the second most frequent endocrine disorders in APECED with similar prevalence in Northern Europe and Italy (Table III) and usually appear before the age of 15 years (4, 6, 11). Adrenal autoantibodies are directed against some P450 cytochromes antigens [P450c21 (21-hydroxylase), P450c17 (17-α-hydroxylase), P450scc (side chain cleavage enzyme)] (3). These antigens are in adrenal cortex, and the latter two are also in the gonads (3, 14) (Table IV), suggesting their direct involvement in both Addison's disease and gonadal failure. The autoantibodies inhibit the steroidogenic enzyme activities *in vitro* (14), even if a clear pathological role *in vivo* has not yet been demonstrated (20).

Other endocrine deficiencies are less frequent in APECED (Table III) and are associated with specific autoantibodies (Table IV) (3, 6, 11, 14). In this patient, high levels of GAD antibodies were found, but she did not develop up to now any biochemical signs of impaired glucose homeostasis.

Several ectodermal manifestations of APECED are present in the present patient (Table III). The pathogenetic mechanisms remain to be elucidated (3), but they are usually associated with autoantibodies directed against specific tissue antigens (Table IV) (3, 11, 19). About 20% of patients with APECED have fat malabsorption which is often associated with weight loss, growth retardation and malabsorption of medications (21, 22). The latter finding complicated the clinical follow-up of this girl, leading to some episodes of hypocalcemia likely related to an abnormal absorption of $1,25(OH)_2D_3$ and calcium. In addition, when malabsorption recovered, the patient had some episodes of hypercalcemia due to the increased drug dosages. So, a more strict control of electrolytes may be request during these periods.

Malabsorption has been considered to be a non endocrine manifestation of APECED (22). However, some evidences indicate that an autoimmune attack against the cells of the gastrointestinal-associated system may be responsible of this dysfunction by tryptophan hydroxylase antibodies (23).

In conclusion, APECED syndrome is a rare disease, that should be suspected in all patients affected by chronic candidiasis and autoimmune disorders. Diagnosis still rely on the clinical evidence of two of the three major features (hypoparathyoidism, primary adrenocortical failure, and chronic muco-cutaneous candidiasis) in a single patient; it should be confirmed by the detection of specific autoantibodies and, possibly, by *AIRE* gene analysis. One major manifestation is sufficient to diagnose APECED in siblings of patients (3, 6, 11). After diagnosis all patients require close monitoring and long-term follow-up to prevent illness and lifetreating associated with delayed diagnosis of additional autoimmune diseases (4).

Further works are needed to fully explain why parathyroid glands are affected so often in the patients lacking the functional products of the *AIRE* gene (13).

References

1. Perheentupa J. Hypoparathyroidism and mineral homeostasis. In: Lifshitz F, ed. Pediatric Endocrinology. 4th ed. New York, USA: M. Dekker Inc, 2003:421-467.
2. Garfield N, Karaplis AC. Genetics and animal models of hypoparathyroidism. Trends Endocrinol Metab. 2001;12:288-294.

3. Peterson P, Pitkanen J, Sillanpaa N, et al. Autoimmune polyendocrinopathy-candidiasis-ectodermal distrophy syndrome (APECED): a model disease to study molecular aspects of endocrine autoimmunity. Clin Exp Immunol. 2004;135:348-357.
4. Eisenbarth GS, Gottlieb PA. Autoimmune polyendocrine syndromes. N Engl J Med. 2004;350:2068-2079.
5. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity and stages of puberty. Arch Dis Child. 1976;51:170-179.
6. Ahonen P, Myllarniemi S, Sipila I, et al. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. N Engl J Med. 1990;322: 1829-1836.
7. Meyer G, Badenhoop K. Autoimmune regulator (AIRE) gene on chromosome 21: implications for autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) any more common manifestations of endocrine autoimmunity. J Endocrinol Invest. 2002;25:804-811.
8. Rosatelli MC, Meloni A, Meloni et al. A common mutation in Sardinian autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients. Hum Genet. 1998;103:428-434.
9. Zlotogora J, Shapiro MS. Polyglandular autoimmune syndrome type I among Iranian Jews. J Med Genet. 1992;29:824-826.
10. Bjorses P, Aaltonen J, Vikman A, et al. Genetic homogeneity of autoimmune polyglandular disease type I. Am J Hum Genet. 1996; 59:879-886.
11. Betterle C, Greggio NA, Volpato M. Autoimmune polyglandular syndrome type I. J Clin Endocrinol Metab. 1998;83:1049-1055.
12. Heino M Peterson P, Kudoh J, et al. Autoimmune regulator is expressed in the cells regulating immune tolerance in thymus medulla. Biochem Biophys Res Commun. 1999;257:821-825.
13. Gylling M, Kaariainen E, Vaisanen R, et al. The hypoparathyroidism of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy protective effect of male sex. J Clin Endocrinol Metab. 2003;88:4602-4608.
14. Soderbergh A, Myhre AG, Ekwall O, et al. Prevalence and clinical association of 10 defined autoantibodies in autoimmune polyendocrine syndrome type I. J Clin Endocrinol Metab. 2004;89:557-562.
15. Scott HS, Heino M, Peterson P, et al. Common mutations in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients of different origins. Mol Endocrinol. 1998;12:1112-1119.
16. Meloni A, Perniola R, Faa V. Delineation of the molecular defects in the AIRE gene in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients from Southern Italy. J Clin Endocrinol Metab. 2002;87:841-846.
17. Cihakova D, Trebusak K, Heino M, et al. Novel *AIRE* mutations and P450 cytochrome autoantibodies inCentral and Eastern European patients with APECED.Hum Mutat. 2001;18:225-232.
18. Buzi F, Badolato R, Mazza C, et al. Autoimmune polyendocrinopathy-candidiasis-ectodermal distrophy syndrome:tyme to review diagnostic criteria? J Clin Endocrinol Metab. 2003;88: 3146-3148.
19. Cetani F, Barbesino G, Borsari S, et al. A novel mutation of the autoimmune regulator gene in an Italian kindred with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, acting in a dominant fashion and strongly cosegregating with hypothyroid autoimmune thyroiditis. J Clin Endocrinol Metab. 2001; 86:4747-4752.
20. Furmaniak J, Kominami S, Asawa T, Wedlock N, Colls J, Smith BR. Autoimmune Addison's disease: evidence for a role of steroid 21-hydroxylase autoantibodies in adrenal insufficiency. J Clin Endocrinol Metab. 1994;79:1517-1521.
21. Boscaro M, Betterle C, Volpato M, et al. Hormonal responses during various phases of autoimmune adrenal failure: no evidence for 21-hydroxylase enzyme activity inhibition in vivo. J Clin Endocrinol Metab. 1996;81:2801-2804.
22. Ringenauer C, Meyer RL, Netto GJ, et al. Malabsorption due to cholecystokinin deficiency in a patient with autoimmune polyglandular syndrome type I. N Engl J Med. 2001;344:270-274.
23. Ekwall O, Hedberg Y, Abrahamsson R, et al. Tryptophan hydroxylase autoantibodies and intestinal disease in autoimmune polyendocrine syndrome type 1. Lancet. 1999;354:568.