# Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome (APECED): report on three cases from Southern Italy

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## Introduction

Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy Syndrome (APECED, OMIM 240300), or Autoimmune Polyglandular Syndrome Type 1 (APS-1), is a rare autosomal recessive disorder with high penetrance, characterized by a variable combination of destructive autoimmune phenomena (1, 2) and caused by a number of different mutations in the autoimmune regulator (AIRE) gene, which maps to 21q22.3 (2).
Endocrine glands are mainly affected, in association with chronic mucocutaneous candidiasis; therefore the progression of the disease will lead to variable degrees of failure of parathyroid glands, adrenal cortex, gonads, pancreatic beta-cells, thyroid, gastric parietal cells. Non-endocrine less frequently associated conditions are represented by malabsorption, chronic active hepatitis, gastric parietal cell atrophy, and pernicious anemia. The most characteristic ectodermal manifestations are dental enamel hypoplasia, pitted nail dystrophy, alopecia, keratopathy, and vitiligo. Two of the classical triad of symptoms (hypoparathyroidism, mucocutaneous candidiasis, adrenocortical insufficiency) are required for the diagnosis (4).
Segregation has been reported in some ethnic groups: the Finnish population has the highest report of patients, (about 1:25.000 inhabitants) (5, 6), followed by Iranian Jews (1:9000) (7) and Norvegian population (8). A small group of patients has been reported in Italy to date, mainly in Sardinia (1:14000 inhabitants) (9), the Salento area in the Puglia region in Southern Italy (10) and the Veneto region in Northern Italy (1). Only one affected family has been reported in Central Italy (11).
We report here three newly diagnosed patients affected by the classic clinical features of the disease (from two families originating from Southern Italy), in which mutations in the AIRE gene were found.

## Clinical case 1

A 16-year-old girl (AC) from healthy consanguineous parents originating from Calabria (Southern Italy, close to the Salento area) was referred to our outpatient clinic by the Dermatologic Unit, where she was hospitalized because of alopecia universalis, for an evaluation of primary amenorrhea and hypocalcaemia.
She had a cerebrospinal meningitis at 18 months of age, and a lymphadenitis from *Mycobacterium tuberculosis* at three years of age; three pneumonia infection followed in the following four years. At the age of seven, alopecia universalis, and nail dystrophy occurred, with an initial good response to oral corticosteroids. After two years a diagnosis of chronic autoimmune thyroiditis with subclinical hypothyroidism was made and for the first time hypocalcaemia with hyperphosphatemia and inadequately low-normal parathyroid hormone (PTH) were reported (serum calcium 7.6 mg/dL, n.v. 8.8-10.3, phosphorus 7.4 mg/dL, n.v. 3.5-5.6, PTH 11.2 pg/ml, n.v. 10-70), together with a modest increase in serum transaminases and keratoconjunctivitis. There was a worsening of the alopecia and nail dystrophy. Only l-thyroxine therapy was prescribed.
Normal growth and school performance are reported until she was 15 years old, with the only exception of abnormal susceptibility to respiratory infections; at that time a marked asthenia developed, and a seizure occurred, after which hypocalcaemic tetany was diagnosed, in association with inadequate PTH levels (11.4 pg/ml). She was hospitalized in an Internal Medicine Unit, and therapy with oral calcium, and 25-hydroxy vitamin D was started: clinical symptoms improved, and serum calcium level increased, but did not returned to normal values. Basal levels of circulating ACTH were normal (27 ng/mL, n.v. 10-49) and cortisol, 17-OH progesterone, DEAS, were in the low-normal range, as well as urine cortisol levels; on the contrary, FSH and LH were markedly increased (FSH 45 mU/L, LH 11.2 mU/L) with very low estradiol levels, suggesting a primary ovarian failure. She did not have menarche; pubertal changes were only limited (Tanner B2, G2) and her bone age showed a 2-year delay with respect to chronologic age. A pelvic ultrasound revealed ovaries with normal dimensions for age, and a transitional uterus, and normal liver characteristics. However a mild transaminase increase was still present, with no signs of cholestasis and a positivity for anti-mithocondrial antibodies was found, suggesting an autoimmune liver involvement; other organ and non organ-specific antibodies were non detectable.
The following findings were obtained when the patients was referred to our Endocrine Unit after one year:
– marked positivity of anti-ovary, anti-adrenal, anti-thyroperoxidase and anti-glutamic acid decarboxylase antibodies; negativity of all the non organ-specific antibodies except for a mild positivity of the anti-mithocondrial antibodies;
– normoglycemia with impaired glucose tolerance, normal levels of C-peptide and mild hyperinsulinemia;
– high-normal basal ACTH levels (74 ng/ml, n.v. <52) with low-

normal basal cortisol levels (162 nM, n.v. 160-690) and serum sodium (135 mEq/L, serum potassium 4.1 mg/dL) and no response to adrenocorticotropic hormone (ACTH test), indicating a subclinical hypoadrenalism;
– normal levels of PRL, IGF-1 and osmolarity; high levels of FSH and LH (117 and 53 U/L respectively);
– normal hepatic indexes (with transaminases in the high-normal limit) and normal liver appearance at ultrasound;
– in therapy with 25-OH vitamin D and calcium: high levels of circulating 25-OH vitamin D (338 ng/mL, n.v. 11-70) with low 1,25-OH vitamin D (18.4 pg/mL, n.v. 20-67), with serum calcium of 8.2 mg/dL (n.v. 8.8-10.7) and calciuria of 110 mg/day (n.v. 100-300);
– worsening of the keratoconjunctivitis; nail dystrophy;
– hypotension with normal basal and stimulated epinephrine and norepinephrine values;
– no actual evidence for mucocutaneous candidiasis.

*Analysis of mutations of the AIRE gene:* homozygous mutation CGA→TGA at codon 203 in exon 5, causing an aminoacidic substitution Arg→STOP in the protein. The same heterozygous mutations. are present in both parents and the brother.

Cortone acetate and low-dose estrogens were added to l-thyroxine, and 25-hydroxy vitamin D was substituted by calcitriol, 0.03 µg/kg/day and low dose oral calcium. After one year there were no signs of worsening of the disease; normocalcaemia and normocalciuria were obtained, there was a progression of pubertal status to Tanner stage III, and a growth spurt, with a predicted final age in the same range of that of her parents. There was a significant decrease in the number and severity of recurrent infections; she recently reported the occurrence of diarrhoea with increasing frequency, and the possibility of malabsorption is under investigation.

**Clinical cases 2 and 3**

*Case 2*

A six years old female (GR), was referred to our outpatient Unit for the suspect of autoimmune polyendocrinopathy. The healthy consanguineous parents are from the Puglia region, in southern Italy, in a area close to the Salento area.

GR had a first seizure when she was one year old; hypocalcaemia and hypophosphoremia were found. Calcium and vitamin D were prescribed for a 2-week period, with no other measurement of serum calcium and phosphorus levels. Infections of the urinary tract presented for the first time, and were recurrent from that time till the present age.

The infant was well till one year later, when a second seizure occurred. Hypoparathyroidism was diagnosed and therapy with 25-hydroxy vitamin D and calcium was started; thyroid hormones, serum sodium and potassium were normal, as well as serum glycemia and routine analysis. Alopecia presented, with rapid progression to a generalized form.

When she was 4 years old GR was again hospitalized for hypocalcaemia (7 mg/dL) with hyperphosphatemia (6.5 mg/dL - she was on 25-hydroxy vitamin D and calcium therapy per os), cervical polyadenopathy, respiratory and urinary tract infection, onicodystrophy. Serum sodium level at the low-normal limit (133 mEq/L) high-normal potassium (5.0 mEq/L) with normal serum cortisol were detected; upper respiratory tract candidosis was diagnosed and nephrocalcinosis was found at ultrasound evaluation. A reduction in NK T lymphocytes (CD16, CD56+), helper lymphocytes (CD3+, CD4+) and B-lymphocytes, with increase of CD3+ and CD8+ were found. Mutational analysis for DiGeorge Syndrome showed no alteration. Oral sodium supplements were added to 25-hydroxy vitamin D and calcium.

The evaluation performed at our Clinic showed:
– generalized alopecia, nail dystrophy and mucocutaneous candidosis; decreased growth rate, more marked in the last year, normal thyroid, no mental or intellectual deficits, absence of ocular involvement;
– marked positivity of anti-adrenal antibodies; negativity of the other organ-specific and non organ-specific antibodies;
– hypoadrenalism with increase in basal ACTH levels (170 ng/dL, n.v. < 50), hyponatriemia (128 mE/L);
– hypocalcaemia (7.2 mg/dl), hyperphosphatemia (6.5 mg/dl) and hypercalciuria (350 mg/day) with undetectable PTH levels;
– normal thyroid function, PRL, IGF-1, LH, FSH, glycemia;
– nephrocalcinosis; no alteration in renal function; *Escherichia Coli* urinary infection.

Therapy with hydrocortisone and calcitriol in substitution for 25-hydroxy vitamin D were started, in addition to oral calcium, and to appropriate therapy for candidosis and urinary tract infection. After two years she has calcemia in the low-normal range and normophosphatemia, with calciuria in the normal range, normal serum sodium and potassium levels; she has normal growth rate and school performances. There are no differences in circulating autoantibodies except for a mild positivity of glutamic acid decarboxilase antibodies. Oral candidosis is not detectable; urinary tract infections are recurrent.

*Case 3*

The previous healthy seven years-old brother of case 2 (VM) had a hypocalcemic tetanic crisis one week before the sister's visit at our Clinic, and therefore he was evaluated at the same time:
– normal growth velocity, mental and school performances; absence of ocular problems, euthyroidism, normoglycemia;
– hypoparathyroidism;
– mild oral candidosis;
– slight positivity for antiadrenal antibodies; negativity of antithyroid, anti glutamic acid decarboxylase, antiparietal cells, and non organ specific antibodies;
– normal circulating ACTH and cortisol levels, and normal cortisol response to ACTH administration.

After two years of treatment with calcitriol and calcium there are no variation in the pattern of circulating autoantibodies, nor a worsening in adrenal basal and stimulated funtion. Oral candidosis is not evident.

*Analysis of mutations of the AIRE gene:* in both children is present a homozygous mutation Thr→Met at codon 16 in exon 1 and a heterozygous mutation Prol→Leu at codon 252 in exon 6. The mother has the heterozygous mutation Thr→Met at codon 16 in exon 1; the father showed both heterozygous mutations. They have normal parathyroid and adrenal function as well as euthyroidism and normoglicemia and absence of ectodermal dystrophies.

**Discussion**

APECED is the first multiple autoimmune disease caused by a defect in a single gene, the autoimmune regulator AIRE; the protein acts as a transcription activator and its genomic target and functions have not been fully elucidated yet. The three cases described here, from two unrelated families originating from areas close to that of the previously described APECED patients in Southern Italy (10), point out interesting aspects of the disease in relation to both diagnostic and therapeutic problems.

The majority of described cases of APECED are familial, with an autosomal recessive mode of inheritance; in the two families studied there is a negative anamnesis for APECED-related

diseases. The clinical spectrum in patients with APECED is broad; within a single family, it has been reported a variation of symptoms between affected sibling. The disease usually occurs in childhood, and the prevalence of most disease components increases with age; new tissue-specific symptoms may appear at any age (4); in some cases the disease may become clinically recognizable only in adulthood. The frequent presentation with dermatological problems associated to the mild signs that are generally associated to chronic hypocalcaemia or hyponatremia or to recurrent respiratory or urinary tract infections before an acute, life-threatening hypocalcemic or hypoadrenal crisis can determine a wrong initial approach to the disease, leading to errors in diagnosis, or a delay in the recognition of the disease, and to underestimation of the importance of life-long follow-up. The delay in the diagnosis can also influence therapeutic errors, such as the choice of 25-hydroxy vitamin D for the management of hypocalcaemia; in the absence of PTH, it is difficult to obtain normocalcemia, with the risk of prolonging the clinical problems related of hypocalcaemia (2). The recognition of subclinical hypoadrenalism is also very difficult for the lack of specific signs for a long period. The suspect of the disease must prompt the assay of plasma ACTH and cortisol levels and an ACTH test, even in the presence of low-normal serum electrolytes, allowing an early beginning of cortisone acetate therapy. The little correlation between the presence of antibodies and the clinical manifestations of APECED, even among members of a same kindred contributes to the diagnostic and therapeutic difficulties; the detection of antibodies in affected individuals may precede eventual endocrine deficiencies; the significance of autoantibodies detection in apparently healthy family members is unclear.

Another important clinical aspect of the disease is represented in our patients by the observation that the earlier the first component appears, the more likely is the development of multiple manifestations; late manifestations of the disease are likely to have fewer components (4, 12). This explains the necessity of an aggressive diagnostic effort in pediatric patients in order to an early detection of adrenal failure, hypoparathyroidism or diabetes.

The understanding of the relevance of major mutations of the AIRE gene to the different pattern of clinical disorders associated to APECED and the analysis of a possible clustering of mutations in definite subpopulations will represent an important contribution to the management of the disease and may become an important tool in its early detection. The stop codon mutation in our clinical case 1 (C203T in exon 5) was previously detected only in two affected siblings of Northern Italy in compound heterozigosity with R257X (a common cause of APECED in Northern Italian and Finnish patients) (13); it was of maternal origin. R257X and R203X are both situated before the first zinc finger motif in the AIRE gene. In this family, the two siblings had a similar disease progression except for the lack of hypoparathyroidism in one of them (up to the time of the report). AC, now seventeen years old, shows a serious multisystemic involvement from the disease: hypoparathyroidism, adrenal, thyroid and ovarian failure, incoming diabetes mellitus, worsening keratoconjunctivitis, generalized alopecia, nail dystrophy, and the suspect of developing hepatic and gastrointestinal involvement. There are no actual signs of candidosis.

The T16M homozygous missense mutation in exon 1 in cases 2 and 3 and their mother was previously described (14) in a Russian patient in heterozigosity with the mutation R257X; he was affected, at time of the report, by hypoparathyroidism, Addison disease, hypothyroidism and mucocutaneous candidosis. Like other missense mutations it seems to accumulate to the N-terminal HSR domain-coding region.

The missense mutation P252L in exon 6 (cases 2 and 3) has been described in one patient from the Salento region in Italy in a compound heterozygous state with the W78R mutation (10). Interestingly, the paternal grandparents of this patient come from the Naples area, as well as those of the father of the probands, also presenting the same mutation. This mutation lies in the amino-terminal SAND domain, a DNA-binding domain, and it probably results in the alteration of the secondary structure of the gene related to its proline-rich composition. GR and AR are now 8 and 9 years old, so the pattern of the disease can be subjected to variations. However, GR presented serious and early manifestations of the disease, similar to our case 1, while VR still has only parathyroid involvement and mild positivity of anti-adrenal antibodies with normal basal and stimulated function.

In conclusion, the clinical expression is highly variable in patients with APECED and in addition some of the manifestation can appear up to the fifth decade; these characteristics lead to life-long diagnostic and therapeutic problems. Improving knowledge of genotype-phenotype correlations will lead to a better understanding and a follow-up of the different patterns of the disease (15).

**References**


1. Betterle C, Greggio NA, Volpato N. Autoimmune polypglandular Syndrome type 1. J Clin Endocrinol Metab. 1998;83:1049-1055.
2. Perheentupa J. APS-I/APECED: the clinical disease and therapy..Endocrin Metab Clin N Am. 2002;31:321-333.
3. Kumar PG, Laloraya M, She JX. Population genetics and functions of the autoimmune regulator (AIRE). Endocrin Metab Clin N Am. 2002;31:295-320.
4. Ahonen P, Myllarniemi S, Sipilä I et al. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. New England J Med. 1990; 322:1829-1836.
5. Ahonen P. Autoimmune polyendocrinopathy-candidosis-ectodermal dystrophy (APECED); autosomal recessive inheritance. Clin Genet. 1985;27:535-542.
6. Aaltonen J, Bjiorses P, Sandkuij J et al. An autosomal locus causing autoimmune disease: autoimmune polyglandular disease type I assigned to chromosome 21. Nat Genet. 1994;8:83-87.
7. Zlotogora J, Shapiro MS. Polyglandular autoimmune syndrome type I among Iranian Jews. J Med Genet. 1992;29:824-826.
8. Myhre AG, Halonen M, Eskein P et al. Autoimmune polyendocrine syndrome in Norway. Clin Endocrinol (Oxf). 2001;54:211-217.
9. Rosatelli MC, Meloni A., Meloni A et al. A common mutation in Sardinian autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients. Hum Genet. 1998;103:428-434.
10. Meloni A, Perniola R, Faa V et al. Delineation of molecular defects in the AIRE gene in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients from Southern Italy. J Clin Endocrinol Metab. 2002;87:841-846.
11. 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.
12. Neufeld M, MacLaren MK, Blizzard RM. Two types of autoimmune e Addison's disease associated with different polyglandular autoimmune (PGA) syndromes. Medicine. 1981;60:355-362.
13. Scott HS, Heino M, Peterson P et al. Common mutations in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients of different origins. Mol Endo. 1998;12:1112-1119.
14. Cihakova D, Trebusak K, Heino M et al. Hum Mutat. 2001;18: 225-232.
15. Buzi F, Badolato C, Mazza C et al. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome: time to review diagnostic criteria? J Clin Endocrinol Metab. 2003;88:3146-3148.