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Original Article

Autoimmune Polyendocrinopathy Candidiasis Ectodermal Dystrophy (APECED): Single-Centre Experience

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Abstract

Background: APECED is a rare autosomal recessive disease, caused by mutations in the *AIRE*-gene resulting in a failure of T-cell tolerance, clinically characterized by multiple autoimmunopathies.
The objective of the study was to determine the clinical and genetic features and consequences of APECED in a single-centre Ukrainian cohort of patients.

Material and methods: Out of five families, eight patients with APECED were included in the study. Family history and clinical information of each patient was collected; laboratory studies aimed at identifying endocrine disorders, other autoimmunity, and infections was done. Genetic testing by NGS was performed in four out of five families. Patients were included in the study after the written informed consent had been signed by their parents/legal guardians.

Results: In most patients, the onset of the disease was noted in childhood with manifestations of hypoparathyroidism and/or candidiasis. Adrenal insufficiency was associated later. Diabetes mellitus, hypothyroidism and hypogonadism were observed as other endocrinopathies. Five of eight patients (62.5%) developed autoimmune hepatitis, two patients suffered from autoimmune lung lesions, and two developed enteropathy. Two patients were diagnosed with brain damage: psychosis and autoimmune encephalitis. All the patients that we examined were homozygous carriers of the Finn major mutation R257X (c.769C> T) of the AIRE gene.

Conclusion: One or two endocrinopathies in combination with recurrent candidiasis are the key to a diagnosis of APECED. Most patients also develop organ autoimmunopathy. The mutation R257X (c.769C> T) of the AIRE-gene is predominant in the population of western Ukraine.

Keywords: Polyendocrinopathies; APS-1; Autoimmune Encephalitis; Clinical Presentation

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Introduction

Autoimmune polyendocrine syndrome, comprises a diverse group of clinical conditions characterized by the functional impairment of multiple endocrine glands due to loss of immune tolerance. We have now come to appreciate the fact that these syndromes can be broadly categorized as rare monogenic forms, such as autoimmune polyendocrine syndrome type 1 (APS-1), and a more common polygenic variety, autoimmune polyendocrine syndrome type 2 (1). APS-1, also called autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED; Online mendelian inheritance in man (OMIM) number, 240300), is an autosomal recessive disease caused by mutations in the autoimmune regulator gene (AIRE) (2-4). It is a rare disease that is more common in certain historically isolated populations, such as Persian Jews (1:9,000), Sardinians (1:14,000) and Finns (1:25,000) with higher rates of consanguineous marriages, and a founder effect. Indeed, the missense mutation Y85C, the nonsense mutation R139X, and the nonsense mutation R257X are commonly observed and are somewhat specific for these patient populations, respectively (2, 5, 6). The prevalence of APECED, has also been estimated in other countries such as Norway (1:80,000), Slovenia (1:43,000), and Poland (1:129,000) (6-8). The prevalence of APS-1 in Ukraine is unknown. There are only isolated descriptions of few cases of this disease (9) in Ukrainian patients, and the prevalence of mutations in the AIRE gene in the population of Ukraine remains unknown.

APECED is an inherited autosomal recessive disorder, with more than 100 AIRE mutations reported throughout the gene. The AIRE gene, located on chromosome 21q22.3, is primarily expressed in thymic medullary epithelial cells, with lower amounts in lymph nodes and tonsils. It controls the induction and maintenance of immune tolerance to self-antigens (2, 10). The AIRE gene prevents autoimmunity by regulating the negative selection of autoreactive T-cells. Abnormal AIRE genes result in impaired clonal deletion, thus allowing T-cells expressing autoreactive TCRs to expand to the periphery and induce autoimmunity (11-13). Although AIRE gene mutations are almost exclusively autosomal recessive, an autosomal dominant mutation has been reported in animal models. Depending on the cohort of the patients, homozygous or complex heterozygous, AIRE mutations are more or less common. Most mutations of the AIRE gene that have been associated with APS-1, are located in exons 6, 8, and 10, and cause transcription of a truncated protein. The two most common mutations are a nonsense mutation in exon 6 (R257X, "Finnish major mutation") and a 13 base-pair deletion (967-979del13bp) in exon 8 (2, 4, 6, 14). Homozygosity for the p.Y85C, p.R139X, or p.R257X mutations was observed in the vast majority of the Persian Jews patients, Sardinian, and Finns. Deletions 1094–1106del13 are common in APECED patients in North America, Norway, the United Kingdom, and Ireland. In North American, patients' complex heterozygous AIRE mutations are the most common, consistent with greater genetic and ethnic diversity.

Pathogenic variants in the *AIRE* gene lead to multiorgan autoimmunity. The endocrine system is most commonly involved. Autoimmunity is associated with increased levels of autoantibodies against proteins, produced specifically by the affected organs (15-17). Autoantibodies against interleukin IL-17 and IL-22 were identified in the serum of patients with AIRE deficiency, suggesting that susceptibility to *Candida* infections also has an autoimmune basis (18, 19).

AIRE deficiency/APECED is known for its wide variation in clinical presentations and in the course of disease, even among affected family members exhibiting an identical genetic aberration (20-22).

APECED is typically suspected in patients who have two out of the three basic symptoms: hypoparathyroidism (usually manifested by hypocalcemia), adrenal insufficiency, and mucocutaneous candidiasis. Suspicions are raised further by the presence of other autoimmune manifestations including diabetes, gonadal insufficiency, hepatitis, pernicious anemia, etc. (6, 23, 24). A definitive diagnosis can be made by sequencing the *AIRE* gene.

Materials and methods

Under our observation, there were eight APECED patients from five unrelated families, aged 2 to 25 years (2 boys and 6 girls, a sex ratio of 1:3). All patients were residents of Lviv region,

Table 1. Diagnostic criteria of APECED as reported by Husebye et al. (22)

One of the following three criteria is necessary for a definitive diagnosis:
1) presence of at least two of the three major components: chronic mucocutaneous candidiasis, hypoparathyroidism, or adrenal Insufficiency 2) only one major component if a sibling is affected by APECED 3) disease-causing mutations in both *AIRE* genes
One of the following three criteria suggests a probable diagnosis:
1) presence of one of the three major components (before 30 years of age) and at least one of the minor components 2) any component in the presence of anti-interferon antibodies 3) any component in the presence of antibodies against NALP5, AADC, TPH, or TH

AIRE, autoimmune regulator; NALP5, NACHT leucine-rich repeat protein 5; AADC, aromatic L-amino acid decarboxylase; TPH, tryptophan hydroxylase; TH, tyrosine hydroxylase.

Western Ukraine. The first case was diagnosed as early as 1995. The age of the patients at the time of diagnosis was between two and six years (a median age of 3.5 years). The time of observation ranged from six months to 15 years. Three living patients have reached the age of 18 years. They continue to be followed up at our center. Patients who received a diagnosis of APECED, were included in the study, after the written informed consent had been obtained from the participants and their parents/legal guardians.

The diagnosis of APECED was based on clinical symptoms, genetic and biochemical tests according to the criteria for clinical definitive diagnosis of APECED by Husebye et al. (**Table 1**) (22). A detailed personal and genealogic history was collected, with an emphasis on the detection of possible APECED symptoms, the time of their appearance, the severity of all the detected abnormalities, and the efficacy of all the treatments used for correction of the symptoms. The patients underwent the appropriate routine clinical and laboratory investigations, for the detection and monitoring of endocrinopathies and other disorders. This, in compliance with the clinical presentation of the disease, immunologic studies using flow cytometry, and genetic testing using NGS method. Genetic verification was performed in at least one the patients out of each of the four affected families.

Statistical analysis was carried out using the statistical functions of Microsoft Excel 2013, spreadsheet editor.

Results

In majority of the patients, the first symptoms of the disease were noted at early childhood (at an average age of 3.1 years), manifesting with hypoparathyroidism (HP) and/or chronic mucocutaneous candidiasis (CMC). Later (at an average age of 8.5 years), these early symptoms were accompanied by symptoms and signs of adrenal insufficiency (AI). Thus, a typical triad (CMC, HP, AI) was usually assembled somewhere between the ages of 3 to 11.5 years, which often postponed the timing of an APECED diagnosis. The symptoms at clinical presentation and the age at which they appeared, are summarized in **Table 2.** Other endocrinopathies that were observed, included diabetes mellitus (1 in 8 patients or 12.5%), hypothyroidism (1 in 8 or 12.5%), and hypogonadism (1 in 8, in adolescence). Five out of eight patients (62.5%) developed autoimmune hepatitis, two patients (25%) – autoimmune lung lesions, and two patients (25%) autoimmune enteropathy. Two patients had symptoms of idiopathic chronic urticaria, and two others had fever with facial butterfly-type rash at peak of the fever. Two patients (25%) were diagnosed with brain damage: psychosis, and autoimmune encephalitis. Two others suffered damages to the eyes (keratoconjunctivitis and macular degeneration). Two of the patients (25%) had repeated adrenal crises, demanding urgent therapeutic interventions.

Clinical presentation was characterized by significant variability and severity of the

Table 2. Clinical presentation and age of the symptoms onset

Patient, age (yr) Sign	P1, 25 d	P2, 23	P3, 13 d	P4, 25	P5, 10 d	P6, 5	P7, 7	P8, 2.5	n	%	Age (yr), Median (min-max) (lower-upper quartile)
Classical triad											
CMC	4	2	11	5	3	4	3	2	8/8	100	3.5 (2-11) (3-4)
HP	6	3	2	5	-	-	3	-	5/8	62.5	3.0 (2-6)(3-4.5)
AI	11.5	3.5	11	9	8	-	4	-	6/8	75	8.5 (4-11.5) (6-10)
Other endocrine											
HT	9.5	-	-	-	-	-	-	-	1/8	12.5	
Type 1 diabetes	-	-	6	-	-	-	-	-	1/8	12.5	
Hypogonadism	-	-	-	-	22	-	-	-	1/8	12.5	
Skin/nail/dental											
Urticarial eruption	-	-	3	2	-	-	-	-	2/8	25	2.5 (2-3) (2.25-2.75)
Nail dystrophy	+	+	+	-	+	-	-	-	4/8	50	
Enamel hypoplasia	+	+	-	-	+	-	+	-	4/8	50	
Other											
Autoimmune hepatitis	-	-	6	-	3	4	5	2.5	5/8	62.5	4 (2.5-6) (3.25-4.75)
Intestinal dysfunction	-	-	11	-	-	-	3	-	2/8	25	7 (2-11) (5.25-9.75)
Pneumonitis	-	-	12	-	3	-	-	-	2/8	25	7.5 (3-12) (5.25-9.75)
SLE-like syndrome	-	3.5	-	-	-	-	-	-	1/8	12.5	
Encephalitis	-	-	12	-	-	-	-	-	1/8	12.5	
Psychosis	25	-	-	-	-	-	-	-	1/8	12.5	
Brain calcifications	11								1/8	12.5	
Fever unknown origin			12				6		2/8	25	9 (6-12) (7.5-9.5)
Keratoconjunctivitis							5	2	2/8	25	3.5 (2-5) (2.75-4.25)
Nephritis			12						1/8	12.5	
Nephrocalcinosis	11								1/8	12.5	

+, symptom present but the age onset is unknown; d, dead patients.

symptoms and course of the disease over time. Three patients died in childhood or as young adults, the median age being 16. This was due to progressive multisystem manifestations of the disease, two of them from brain damage, and one from an adrenal crisis.

Genetic diagnostics were performed in four out of five families. All the tested APECED patients appeared to be homozygous carriers of the so-called *Finn major* mutation R257X (c.769C>T) in exon 6 of the *AIRE* gene. This mutation is not, however, restricted to the Finns, being the most common mutation worldwide (4, 6). In one of the families, both parents and two clinically healthy siblings of the proband were genetically tested, and all appeared to be heterozygous carriers of the same mutation.

A familial case of APECED with an extremely severe, dramatically progressing course of disease is presented below.

A 12-year-old girl, the second child from a non-consanguineous marriage, had HP from the age of two. At the age of six she developed autoimmune hepatitis, at the age of 10, type 1 diabetes, and at the age of 11, she developed adrenal insufficiency. She was prone to frequent symptoms of diarrhea, which were mostly resistant to common anti-diarrheal medications. At age 11, genetic testing was done which revealed a homozygote for mutation R257X (c.769C>T) in the AIRE gene.

Her older brother also had APECED with the classic triad: HP, relapsing candidiasis of the nails and oral mucosa, signs of adrenal insufficiency, including one severe adrenal crisis. The girl's disease was well compensated by substitution therapy with vitamin D derivatives (calcitriol), calcium supplementation, insulin therapy, hydrocortisone and fludrocortisone, plus

Table 3. Immunological investigation of the APECED patient with multiorgan autoimmunity

Cells (phenotype)	Patient	Normal ranges
	% (cells/μL)	% (cells/μL)
Lymphocytes	11.75 (693) ↓	25 – 45 (1500 – 4000)
T-cells		
Total T-cells *(CD3+)*	92.5 (641) ↓	58 – 85 (870 – 3400)
Activated T-cells *(CD3+HLA DR+)*	8.2 (57)	3 – 15 (45 – 600)
T-helpers *(CD3+CD4+)*	44.3 **(307)** ↓	30 – 56 (450 – 2240)
CM (CD4+CD45RA-CCR7+)	22.32 (69) ↓	40 – 65 (180 – 1460)
EM (CD4+CD45RA-CCR7-)	18.91 (58)	4 – 16 (18 – 350)
Naïve (CD4+CD45RA+CCR7+)	43.8 (134) ↓	40 – 70 (180 – 1570)
TEMRA (CD4+CD45RA+CCR7-)	14.9 (46)	0 – 2 (0 – 50)
T-cytotoxic *(CD3+CD8+)*	47.8 (323)	18 – 45 (270 – 1800)
CM (CD8+CD45RA-CCR7+)	11.09 (36)	10 – 31 (27 – 560)
EM (CD8+CD45RA-CCR7-)	2.53 (8)	2 – 16 (6 – 290)
Naïve (CD8+CD45RA+CCR7+)	80.76 (261)	35 – 70 (95 – 1260)
TEMRA (CD8+CD45RA+CCR7-)	2.53 (8) ↓	4 – 30 (10 – 540)
RTE (CD4+CD45RA+CD31+)	37.38 (114) ↓	39 – 70 (180 – 1570)
Tregs (CD4+CD25+CD127low)	12.12 (37)	4 – 11 (18 – 250)
B-cells		
Total B-cells *(CD19+)*	2.9 (19) ↓↓↓	7 – 20 (110 – 800)
Sw-B-mem (CD19+CD27+IgD-)	21.63 **(4)**	8 – 20 (9 – 160)
Non-sw-B-mem (CD19+CD27+IgD+)	10.96 **(2)**	8 – 20 (9 – 160)
Naïve B (CD19+CD27-IgD+)	44.1 **(8)** ↓↓↓	60 – 80 (70 – 640)
B1 (CD 20+CD5+)	15.38 **(3)**	15 – 40 (18 – 320)
CD21low B-cells *(CD20+CD5+)*	58.53 (11) ↑↑↑	1 – 15 (1 – 120)
CD21low CD38low (CD19+CD21-CD38-)	9.67 (2)	0.9 – 5 (0 – 120)
Transitional B-cells *(CD19+CD24++CD38++)*	0.4 (0) ↓	3 – 10 (3 – 80)
NK-cells		
NK (CD16+CD56+)	4.4 (31) ↓↓	5 – 25 (70 – 1000)
NKT (CD3+CD16+CD56+)	0 (0)	0.1 – 5 (1 – 200)

CM, central memory; EM, effector memory; NK, natural killer cells; NKT, natural killer T-cells; non-sw-B-mem, non-switched B-memory cells; RTE, recent thymic emigrants; sw-B-mem, isotype-switched B-memory cells; TEMRA, effector memory T cells re-expresses CD45RA; Tregs, regulatory T-cells.

azathioprine and small doses of prednisolone for hepatitis. Up to the age of 12, she had normal physical and psychological development, and reached puberty (Tanner stage II). However, just before turning 12, her condition worsened. She started to have infrequent episodes of unexplained fever, which became permanent after 1 month, with the eruption of a maculopapular rash at the peak of fever, mainly around the joints. At this time, changes in the lungs appeared: scattered, dry, wheezing and small bubble rales, but without breathlessness, cough, or other overt signs of lung involvement (her chest X-Ray and spirometry data were unremarkable). Laboratory tests showed an increase of both ESR (up to 44 mm/Hr) and CRP (12-24 mg/l). This patient was persistently predisposition to low sodium and potassium levels in the blood, despite the administration of gluco- and mineralocorticoids. Her calcium and phosphorus levels remained close to normal, as did ALT and AST, while diabetes was well taken care of with insulin treatment.

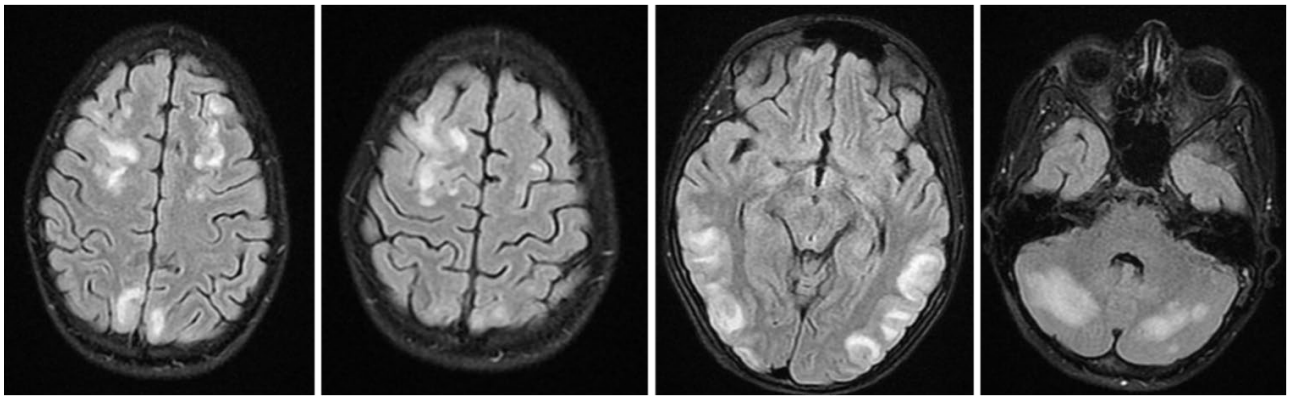
Initial therapy with antibiotics and antifungals appeared to be ineffective: the patient continued to have fever spikes, with the development of oropharyngeal candidiasis. A contrast-enhanced CT screening of the chest and the abdominal cavity was performed, revealing in both lungs, the presence of multiple round foci, 2-6 mm in diameter, and with distinct contours. There was

also a thickening of the wall of the gut in the region of the ileocecal angle, and also atrophic changes in the right adrenal gland.

We suggested that there was a progressive autoimmune multiorgan damage in this patient. As she continued to have fever spikes, and symptoms of general physical and psychological exhaustion, the steroid therapy (prednisolone) was enhanced. After a short period of being in a stable condition, she deteriorated again with the acute development of breathlessness, dry cough, and profound weakness. Her oxygen saturation dropped to 90%. An ultrasound examination of the lungs, revealed the presence of an interstitial tissue reaction. Laboratory data showed moderate leukocytosis (13.0 x 10^9/l) with lymphopenia, increased LDH levels (up to 1125 IU/l), persistent hyponatremia (129-133 mmol/l), and moderate hypokalaemia. Blood and sputum cultures, including sputum microscopy, were negative for bacteria as well as for fungi. The TBC test (quantiferon test), was negative. Blood procalcitonin and faecal calprotectin, were both normal.

Permanent oxygen therapy was started; the therapy was augmented by IV Meropenem and Voriconazole, IV Trimethoprim/ Sulfamethoxazole 120 mg/kg/day to cover any possible pneumocystic disease of the lungs. The patient also received IVIG 0.4 g/kg. Her condition slowly improved: she became more active, regained her appetite, and the fever started to drop, breathlessness gradually decreased to 26-30 breaths a minute.

Immunology tests at this stage, showed the absolute number of T-cells had decreased due to lymphopenia, although relative numbers of the majority of T-cell subpopulations were



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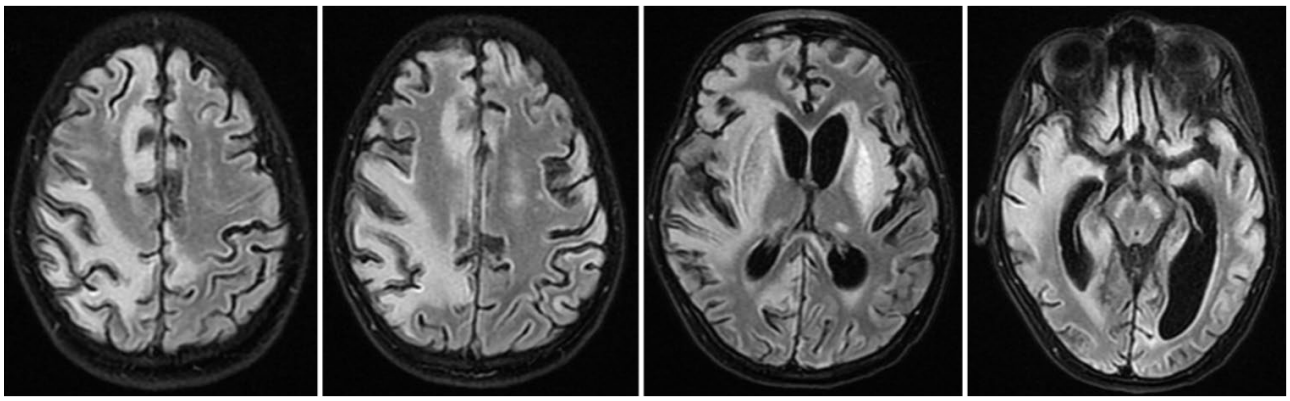

b

Figure 1. Brain damage of a patient with APECED: Multifocal lesion of the cortex and subcortical parts of the white matter in the cerebral hemispheres and cerebellum are visualized in the upper frontal gyri, parietal and occipital-temporal parts bilaterally, as well as in the cerebellar hemispheres (March 15, 2019); b: Diffuse expansion of the process, atrophy of brain parenchyma, severe necrotic-type thinning of the cortex, with areas of cystic mollities in the putamen part of the brain (mainly on the right side), diffuse leukopathy with lesions in cerebral hemispheres, cerebellum, basal ganglia, corticospinal tract, brain stem (more severe on the right side) (June 13, 2019).

normal, except for CM CD4+ T-cells. Suddenly, the absolute and relative numbers of regulatory T-cells were normal. The patient had profound B-cell lymphopenia, and more than half of B-cells were CD21low. NK-cells, lymphopenia with the complete absence of NKT were also detected (**Table 3**).

Although her general condition after one month of treatment, temporarily improved, she gradually started to develop behavioral disorders: anxiety, depression, and suicidal thoughts, which were at first regarded as the natural aftermath of a long-lasting hospital stay and numerous medical interventions. However, at week six of inpatient treatment, overt neurological symptoms became evident: partial ptosis, photophobia, a decrease in muscle strength, hyperesthesia, and one episode of seizures. An MRI of the brain showed radiographic features of PRES syndrome. Despite adequate therapy aimed at normalizing hydration and electrolyte balance, her neurological symptoms progressed: muscle twitching (fasciculations) of the left arm appeared, accompanied by myalgia, hemi-type sensitivity disorders, hemiplegia, sensory-neural deafness and bell's palsy on the left side. She continued to be depressed and had sleep disorders. The condition progressed rapidly with the development of neurologic deficiency, with generalized tonic-clonic seizures, loss of coughing and swallowing reflexes, anisocoria, and finally, the development of a coma.

Differentiation was performed between viral and autoimmune encephalitis. The Cerebrospinal Fluid (CSF) appeared to be normal. No viruses or other pathogenic microorganisms in CSF were found. Brain structure autoantibodies, available for determination in our country, were studied, in particular: the amphiphysin (AMP), CV (CV2) antigen, PNMA2 / Ta (Ma2 / Ta) antigen, the antigen, type-2 (Ri) neuron nucleus, the Purkinje cell antigen (Yo), and the antigen of the type-1 (Hu) neuron nucleus. All were within normal values. A second brain MRI was done which found multifocal areas of damage in the cortex and subcortical parts of the white matter hemispheres of the brain (in the upper frontal gyri, parietal and occipital-temporal parts, bilaterally) and the cerebellum (**Figure 1a**).

Despite intensive treatment with systemic antibacterial, antiviral, and antifungal medicines, our patient's condition did not improve. The immunosuppressive treatment was further augmented with high doses of steroids (500 mg/day), IVIG 2 g/kg, followed by rituximab, as a treatment of choice for autoimmune encephalitis. However, there was no favorable effects. The child continued to be in a coma, breathing through a tracheostomy tube, and was fed through a nasogastric tube.

The next brain MRI was performed in three months, it found a marked worsening due to a more diffuse expansion of the process, atrophy of the brain parenchyma, severe necrotic-type thinning of the cortex, with areas of cystic mollities in the putamen part of the brain (mainly on the right side), diffuse leukopathy, with lesions in cerebral hemispheres, the cerebellum, basal ganglia, the corticospinal tract, the brain stem (more severely on the right side) (**Figure 1b**). Intensive therapy was stopped. From this point on the patient received only substitutive and palliative therapy. Eight months after the first appearance of the symptoms of the brain damage, the girl died due to multiple organ failure.

Discussion

APECED, comprises a wide spectrum of clinical signs, normally manifesting in early childhood with autoimmune endocrinopathies and CMC. Hypoparathyroidism (HP), is the most common endocrine manifestation of APECED, affecting the majority of patients (64% of Finnish patients) (21). The prevalence of HP in our group of patients was also high (75%), and in all cases, it developed in early childhood as the first manifestation of an endocrinopathy. It usually occurs before the age of 10, but can also be diagnosed during adulthood. HP manifests with paranesthesia of the face and fingers, muscle cramps, or tonic seizures in the case of overt hypocalcaemia (21, 22). In all of our patients with HP, marked signs of hypocalcaemia, including seizures, were the main reason for seeking medical help. Diagnosis is confirmed based on routine laboratory findings: hypocalcaemia, hyperphosphatemia, in the absence of renal failure and low parathormone (PTH) levels. Brain calcifications located in the brain structures associated with neurological complaints have been reported (25). One of our patients also had

multiple calcifications in the brain, as well as nephrocalcinosis. Idiopathic HP is rarely seen in the general population. Therefore in a case of HP, APECED should always be considered. The mainstay of therapy includes vitamin D derivatives, plus oral calcium and magnesium. Regular monitoring of plasma calcium, at least every 2 months, along with the periodic control of plasma magnesium, phosphate and 24-hour urinary calcium is recommended (21).

Adrenal insufficiency (AI) is seen in 50 to 100% of the APECED patients. It is the result of an autoimmune adrenalitis with the destruction of no less than 90% of the adrenal cortex. It was the first endocrine manifestation in up to 30% of our APECED cases (21). While eighty-five percent of our patients had AI, in only one case it was the first manifestation of the disease, preceding HP. AI usually manifests with both a glucocorticoid and mineralocorticoid deficiency, but one or the other can precede (26). Typical symptoms include fatigue, nausea, anorexia, salt craving, hypotension, weight loss, and pigmentation of the skin, and gingiva. Hypotension, hyponatraemia and hyperkalaemia are signs of a mineralocorticoid deficiency, while hypoglycaemia, and more rarely anaemia, lymphocytosis, and eosinophilia may signal a glucocorticoid deficiency. An adrenal crisis, usually triggered by stress, such as infection or surgery, manifesting with vomiting, abdominal pain and hypotension up to hypovolemic shock can be potentially lethal (26). Severe adrenal crises were present in two of our patients, one case being fatal due to the delay in seeking medical help. Replacement therapy with oral hydrocortisone with or without a fludrocortisone is a mainstay of therapy (26, 22).

Thyroid involvement is reported in up to 50% of the cases. It occurs mainly during adulthood (the mean age being 26.5 yrs.) (21). In our cohort, only one child developed hypothyroidism at the age of 9.5 years and received substitution therapy with levothyroxine. The clinical presentation and pathology are similar to APECED-non-related hypothyroidism. Antibodies to thyroglobulin, microsomes and thyroid peroxidase are found. As the occurrence of thyroid dysfunction is unpredictable, patients should be monitored regularly for TSH levels.

Type 1 diabetes is a rare feature of APECED, reported in 2 to 13% of patients in the cohorts, usually at 30 to 40 years of age (21). However, in our case the patient had already developed diabetes at the age of 6 and needed lifelong substitution of insulin therapy.

Other manifestations such as hypopituitarism, hypogonadotropic hypogonadism, corticotropic hormone deficiency and diabetes insipidus have been reported in rare cases. Multiple central deficiencies are also possible (1, 21). These were not seen in our patients.

A fraction of the patients, have autoimmune affections of the skin and skin appendages – alopecia areata, vitiligo, etc. However we have not seen such changes in our patients. One of the frequent symptoms is nail dystrophy, the aetiopathogenesis of which is far from clear. However, nail infection may be the most common cause. Twenty-two to 50% of the Finnish APECED patients, have had nail pitting without positive candidiasis on fungal culture (21). Mosaic damage of nails of varied severity was seen in four out of seven patients of our cohort, and only two of them had a good response to antifungal therapy.

Gastrointestinal symptoms in APECED and their underlying pathogenesis are poorly understood. The prevalence of GI symptoms (excluding hepatitis) is roughly estimated at up to 25% of the cases. Review articles usually describe GI manifestations as "diarrhea", "constipation" and "malabsorption". According to published case reports, intestinal involvement in children can be severe, leading to malabsorption, multiple nutritional deficiencies, growth impairment and possible death (21, 23, 27). Studies of the GI function with intestinal biopsies are rare. Among our patients two children (28.6%) had chronic diarrhea, one of them with marked symptoms of growth failure and malabsorption, accompanied by autoimmune hepatitis. Upon biopsy of the small intestine, shrunken and flattened villi were found as well as sporadically hyperplastic crypts and lymphocyte infiltration of the intestinal lining (Marsh III). The genesis of enteropathy was difficult to interpret, due to the absence of distinct criteria for IBD and serology markers of celiac disease. However, in both patients, improvement was obtained by augmenting the immunosuppressive therapy, designed for other organ-specific autoimmune symptoms of

APECED.

Autoimmune hepatitis (AIH), affects 8 to 27% of the APECED patients (20, 23, 28). Its spectrum ranges from asymptomatic and/or self-limited cytolysis that may fluctuate over time to fulminant hepatitis with liver failure. Fifty-seven percent of the patients in our cohort, had liver involvement in the form of autoimmune hepatitis. Notably, in two children from one family, AIH was the first symptom of APECED. In all patients, the disease responded well to routine therapy with corticosteroids and azathioprine.

Renal diseases associated with APECED, includes specific autoimmune tubulointerstitial nephritis (TIN), that can manifest by renal tubular acidosis, nephrocalcinosis, and chronic renal failure requiring kidney transplantation. It is estimated that up to 9% of patients develop TIN (21, 29). The suspected pathogenic mechanism is immunity against tubular cells. Nephrocalcinosis may also be related to hypoparathyroidism. One of our patients aged 11, developed nephrocalcinosis, with simultaneous calcifications of the brain. Therefore, we linked this process with HP and its treatment. In one other patient, chronic urinary infection developed against the background of serious immunosuppressive therapy.

Respiratory-related symptoms are uncommon in APECED. In a large series of 110 European patients, only 7 were found with respiratory symptoms (6.4%) (30). Those symptoms included recurrent lower respiratory infections, obstructive respiratory asthma-like symptoms, chronic cough, dyspnea, thoracic pain and hyperresponsiveness of the airways (30, 31). There are sporadic reports of lymphocytic bronchiolitis with interstitial pneumonitis (6, 31). One of our patients also had marked signs of pneumonitis that clinically resembled fibrosing alveolitis with a progressive course of the disease. In the presented case, the development of lung damage with a non-distinct clinical cough and breathlessness, and the formation of granulomas shown in the CT data, may be evidences of lung involvement, in the form of granulomatous and lymphocytic interstitial lung disease (GLILD). However, progressive worsening of the patient's condition with the development of encephalitis, did not allow us to perform a lung biopsy to confirm this hypothesis.

Defective dental enamel formation of the permanent teeth is frequent, and affects from 25% to 82% of the patients (21). The occurrence of enamel hypoplasia is independent of hypocalcaemia and hypoparathyroidism, and is considered as one of the "ectodermal' manifestations of APECED (21). Ocular manifestations are also one of the most disabling, as they can lead to severe visual impairment and even complete blindness (32). Virtually, any of the ocular structures can be affected, from the eyelashes, the sclera and the cornea to the retina and even the optic nerve. Two of our patients also had eye lesions, one of them suffered from vision loss.

In adult patients with APECED, we found very few descriptions of autoimmune encephalitis with clinical presentation of limbic encephalitis, accompanied by seizures (33, 34). However, all of these cases refer to the slow process of damaging the central nervous system by antibodies, Auto Abs to GAD, TH, and 5-HT neurons, which unfortunately we were not able to determine. The patient described in this report, had a rapidly progressing course of encephalitis that developed simultaneously with autoimmune lung damage and enteropathy, against a background of existing polyendocrinopathies and autoimmune hepatitis. The process was accompanied by fever and rash at the peak of fever. Administration of the first and second line of immunosuppressive therapy, according to existing recommendations for treatment of autoimmune encephalitis (35), brought about the remission of systemic signs, pulmonitis and enteropathy. However, this therapy did not prevent the progressive and massive brain damage.

Conclusion

APECED is a severe multiorgan disease with a progressive course, and the gradual development of new symptoms, complicates the diagnosis at an early stage of the disease, and offers a poor prognosis. The most prevalent mutation of the AIRE gene in Western Ukraine, is the R257X (c.769C>T) mutation. This disorder needs a multidisciplinary approach in diagnosis and therapy (endocrinologist, immunologist, rheumatologist, etc.); and may require immediate help with life-threatening events: adrenal crises,

hypocalcaemia, encephalitis, etc. The treatment for polyglandular autoimmune syndrome, type I, is targeted at whatever organ is affected, but it does not affect the original course of the disease.

Conflict of interest

The authors declare no conflicts of interest regarding this study.

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