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CASE REPORT

# Early-onset hypoparathyroidism and chronic keratitis revealing APECED

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## Key Clinical Message

Early diagnosis of potentially life-threatening autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is crucial, but is often delayed due to the clinical heterogeneity of the disorder. Even in the absence of the classic disease triad of chronic mucocutaneous candidiasis, hypoparathyroidism, and adrenocortical insufficiency, a diagnosis of APECED should be considered in children who have hypoparathyroidism and chronic keratitis, with a past medical history showing a mild and transient *Candida* infection.

## Keywords

AIRE, APECED, hypocalcaemia, hypoparathyroidism, keratitis.

## Introduction

In children, the main causes of hypoparathyroidism include DiGeorge syndrome (22q11.2 deletion syndrome), parathyroid agenesis, mutations of the calcium-sensing receptor (*CASR*) or parathyroid hormone (*PTH*) genes, and pseudohypoparathyroidism. The disease is characterized by low parathyroid hormone (PTH) levels, hypocalcaemia, and hyperphosphatemia, and can be revealed by tetany, irritability, and seizure. Patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) also suffer from hypoparathyroidism along with chronic mucocutaneous candidiasis and Addison's disease (adrenocortical insufficiency), although the presence of two of this classic triad is diagnostic [1]. A spectrum of other autoimmune endocrine and ectodermal disorders such as type 1 diabetes mellitus, thyroid autoimmunity, hypogonadism, pernicious anemia, keratitis, and vitiligo may also manifest [1]. The disease is caused by loss-of-function mutations in the autoimmune regulator (*AIRE*) gene, which prevent the AIRE transcription factor from acting to ensure negative selection of autoreactive T cells in the thymus [2]. Due to the failure of immunological tolerance, affected organs are infiltrated by autoreactive T lymphocytes, and antibodies against tissue-specific proteins such as NACHT leucine-rich-repeat

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protein 5 (NALP5), calcium-sensing receptor (CaSR), and tyrosine hydroxylase can be present in patients' sera [3, 4]. These often associate with certain disease components [1, 3, 4].

The first disease components, most usually chronic mucocutaneous candidiasis and hypoparathyroidism, normally emerge in childhood, but the heterogeneity of APECED in terms of its initial clinical presentation can make it challenging to identify and, typically, the condition is diagnosed after the first decade of life [1, 5]. More recently, the presence of antibodies against interferon (IFN)-$\omega$ and IFN-$\alpha$2 has been shown to aid diagnosis due to their sensitivity and relative specificity for APECED [6, 7]. Anti-interleukin (IL)-22, anti-IL-17A, and anti-IL-17F antibodies are also common [8]. In addition, mutational analysis of *AIRE* enables more than 95% of cases to be identified [2]. Here, we report on an unusual clinical presentation of APECED in a young girl who had an early-onset of hypoparathyroidism and chronic keratitis, and who had experienced previously a mild and transient mucocutaneous candidiasis.

## Case History and Examination

A 3-year-old girl was admitted to our pediatric emergency unit after having repeated carpopedal spasms, dyskinesia, and disequilibrium. The girl's medical history showed that she had presented with her first episode of spasm with fever some months before experiencing a mild and transient mucocutaneous candidiasis at 1 year of age, and needed an ophthalmological examination at 2 years because of photophobia and lacrimation at which time she was diagnosed with bilateral keratoconjunctivitis of unknown etiology. She was born from healthy consanguineous parents without any neonatal problems, and had one healthy sibling. The patient's physical examination was unremarkable, but ocular assessment revealed chronic keratitis with progressive corneal scars (Fig. 1).

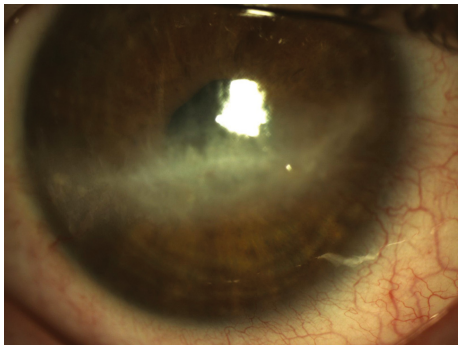

**Figure 1.** Ocular scars of recurrent chronic keratitis.

## Laboratory Evaluations

The patient's spasms were indicative of hypocalcaemia and, indeed, biochemical analyses revealed a severely low serum calcium level of 1.28 mmol/L (normal range, 2.2–2.7 mmol/L). This was coupled with a high serum phosphate level of 2.56 mmol/L (normal range, 1.3–1.85 mmol/L) indicating profound hyperphosphatemia. These evaluations, together with a low blood PTH concentration of <3 pg/mL (normal range, 10–80 pg/mL) and a decreased urine calcium: creatinine ratio of 0.06 mmol/L (normal range, 0.2–0.7 mmol/L), suggested that the patient had hypoparathyroidism. The serum level of 25-hydroxyvitamin D at 24 nmol/L (normal range, 80–250 nmol/L) was low. Creatinine at 34 $\mu$mol/L (normal range, 23–37 $\mu$mol/L) and magnesium at 0.83 mmol/L (normal range, 0.75–1.0 mmol/L) were at normal serum concentrations. Serum levels of thyroid-stimulating hormone (TSH) at 2.2 mIU/L (normal range, 0.4–3.1 mIU/L), and Free T4 (the active form of thyroxine) at 18.6 pmol/L (normal range, 11.1–20.6 pmol/L) were within the normal ranges, indicating unaffected thyroid function.

## Differential Diagnosis and Investigations

The absence of dysmorphy, cardiac malformation, and immunodeficiency ruled out the diagnosis of DiGeorge syndrome as a cause of the patient's hypoparathyroidism. Lack of mutations of the *CASR* gene also precluded this as the basis for the patient's severe hypocalcaemia. Ultrasonography examination also showed normal parathyroid glands. Despite the absence of the classic disease triad and lack of autoimmunity in her parents or sibling, we considered APECED as the possible diagnosis based on the association of hypoparathyroidism with a minor APECED component chronic keratitis, and a previous mild and transient mucocutaneous candidiasis.

After obtaining written consent, blood samples were collected from the child and her parents for genetic analysis of the *AIRE* gene using peripheral blood mononuclear cells (PBMCs) as a source of DNA. Subsequently, the diagnosis was confirmed by an *AIRE* gene frameshift mutation c.931delT (p.C311fsX376) affecting codon 311 of exon 8. This mutation leads to the production of a downstream stop codon at codon 376 and thus, a shortened and a nonfunctional AIRE protein lacking both plant homeodomain (PHD) 1 and 2 zinc fingers (Fig. 2). The patient was homozygous for this recessive mutation. Both parents were c.931delT heterozygotes.

Credence was added to the diagnosis by the detection of antibodies against IFN-$\alpha$2A (antibody index, 5.2; nor-


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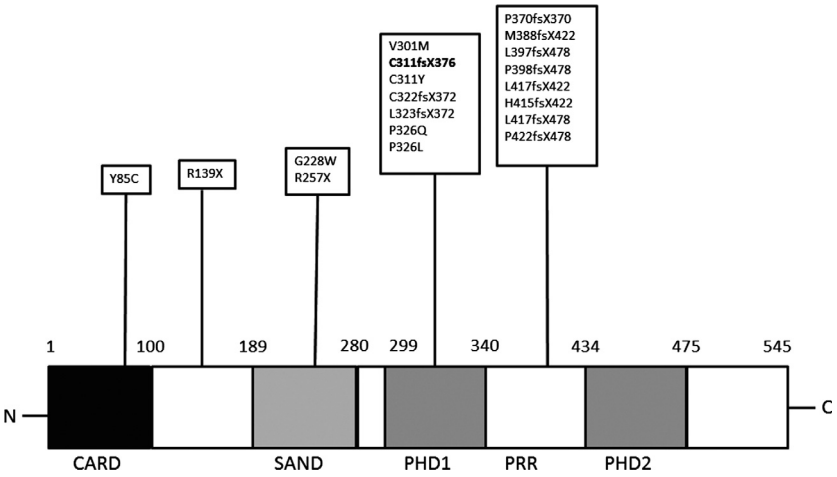


**Figure 2.** Schematic representation of the AIRE protein. Shown are selected mutations, including the C311fsX376 (c.931delT) variant; the caspase recruitment domain (CARD); the SP100, AIRE1, NucP41/P75, DEAF1 domain (SAND); two plant homeodomain (PHD) zinc finger motifs; and the proline-rich region (PRR). Amino acid residues of the AIRE protein are numbered from the amino (N) terminus to the carboxy (C) terminus.

mal range, 0.62–1.72), IFN-$\omega$ (antibody index, 2.79; normal range, 0.97–1.08), interleukin (IL)-22 (antibody index, 44.2; normal range, 0.87–1.39), IL-17F (antibody index, 16.2; normal range, 0.86–1.19). However, the patient was sero-negative for anti-IL-17A (antibody index, 0.97; normal range, 0.78–1.61), anti-IFN-$\lambda$ (antibody index, 0.89; normal range, 0.85–1.20), and anti-IFN-$\beta 1\alpha$ antibodies.

Further serological investigations indicated the patient was positive for antibodies against the CaSR (antibody index, 33.2; normal range, 0.97–1.22), but in CaSR functional assays, these did not activate the receptor. Anti-NALP5 antibodies were not detected (antibody index, 1.12; normal range, 0.79–1.19). The patient had no humoral autoimmune response against the adrenal glands: anti-21-hydroxylase antibodies that are markers for the prevalent APECED component Addison's disease were absent. Similarly, the patient was sero-negative for the major markers of type 1 diabetes mellitus, namely anti-islet cell, anti-glutamic acid decarboxylase (GAD65), and anti-tyrosine phosphatase-like protein IA2 antibodies. Thyroid autoimmunity was not apparent as indicated by normal function tests and an absence of anti-thyroid peroxidase and anti-thyroglobulin antibodies. Antibodies directed against actin, mitochondria, liver kidney microsomes, the nucleus, and dsDNA were not present.

## Treatment and Follow-up

Treatment of hypoparathyroidism consisted of combining calcium (0.5 g three times daily), 1,25-dihydroxyvitamin D3 (0.5 $\mu$g once daily) associated with 25-hydroxyvitamin D3 (100,000 IU every 3 months), and phosphate binders sevelamer hydrochloride, in order to alleviate spasms and return serum calcium and phosphate levels to 2.20 mmol/L and 1.66 mmol/L, respectively. However, the ocular lesions progressed and led to the loss of visual acuity and irreversible corneal scarring in spite of a topical treatment with steroids. A cornea graft is under consideration.

## Discussion

At the time of diagnosis, the 3-year-old patient did not have the classic APECED disease triad of chronic mucocutaneous candidiasis, hypoparathyroidism, and adrenocortical insufficiency, and familial evidence of the disorder was lacking. In addition, aside from hypoparathyroidism and chronic keratitis, none of the many other APECED-associated diseases were evident either clinically or immunologically. The diagnosis was therefore confirmed by detecting anti-IFN-$\omega$ and anti-IFN-$\alpha 2$ antibodies, and sequencing of the *AIRE* gene for potential mutations.

As part of the syndrome, chronic mucocutaneous candidiasis is apparent in 70% of cases by age 10 rising to 97% by age 30 and is the earliest and most common sign of APECED [1]. Prior to the diagnosis, our patient had suffered from only a single episode of mild and transient mucocutaneous candidiasis. The patient presented with hypoparathyroidism, the most frequent endocrine feature of APECED with a prevalence of 66% by age 10 and 85% by age 30 [1]. Like other endocrinopathies in APECED, the cause of hypoparathyroidism is considered to be autoimmune. Sporadic case reports of idiopathic

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hypoparathyroidism have documented lymphocytic and plasma cell infiltration of the parathyroid [9], as well as complement-mediated damage of the glands [10]. However, our patient had a normal parathyroid examination negating immunologically damaged glands as a cause of her hypoparathyroidism. Anti-CaSR antibodies which can have an etiological role in hypoparathyroidism by activating the CaSR and thus inhibiting PTH secretion, have been identified in a small number of cases of isolated hypoparathyroidism and APECED [11–13]. In our patient, although a humoral autoimmune response against the CaSR was detected, the antibodies did not appear to stimulate the receptor. Conceivably, anti-CaSR-activating antibodies may have been at too low level to be demonstrated with our assay. Interestingly, the patient was negative for anti-NALP5 antibodies which are reported markers for hypoparathyroidism in APECED [4]. However, these may develop in the patient over time.

Addison's disease appears most commonly following mucocutaneous candidiasis and hypoparathyroidism, and by age 10 in 40% of patients and by age 30 in 78% of patients [1]. There was no clinical or immunological evidence of adrenocortical insufficiency in the current case, but diagnosis of adrenal failure is crucial to avoid the risk of a fatal adrenal crisis. The potential development of Addison's disease will therefore be monitored as a diagnosis of APECED, meaning it is highly likely that this will occur later in the patient's lifetime given her young age.

The only nonendocrine disease identified in the patient at diagnosis was keratitis, the onset of which was rapid, probably before hypoparathyroidism. Keratitis is the first manifestation in only 3–14% of APECED cases, but is usually an early component and affects 25–50% of patients [1]. Symptoms include photophobia, blepharospasm, conjunctival redness, and decreased visual acuity, all of which were noted in our patient [14]. Although the pathophysiology of keratopathy is not fully understood, immune cells seem to play an essential role: in *AIRE*-deficient mice, corneal and conjunctival epitheliums, stroma and meibomian glands are infiltrated by CD4+ and CD8+ T cells [15]. Meibomian gland destruction and the loss of goblet cells result in the loss of tear film and may trigger inflammation [16], although the subsequent progression of epithelial edema and superficial opacity can be reversed under topical steroid therapy. Recurrences are frequent and lead to irreversible scarring (nodule and pannus formation), deep vascularisation, and blindness [14, 16].

Crucial to the diagnosis of APECED in this unusual case was the detection of typical anti-IFN-$\omega$ and anti-IFN-$\alpha$2 antibodies [6, 7]. Furthermore, antibodies against IL-22 and IL-17F were present [8]. The Th17 cell-associated cytokines, IL-17A, IL-17F, and IL-22, play a key role in protecting against *Candida* infection [17]. High levels of antibodies to the IL-17 family of cytokines are typically produced by patients with APECED and are suggested to predispose to candidiasis by virtue of their neutralizing activities [18]. Indeed, APECED patients with no or mild candidiasis have low or undetectable levels of antibodies against IL-17A, IL-17F, and IL-22 [18, 19]. Compatible with this theory, our patient was sero-negative for anti-IL-17A antibodies. However, she did have high levels of anti-IL-22 and anti-IL-17F antibodies, an observation analogous to a report of five APECED patients in which a lack of *Candida* infection correlated only with an absence of antibodies against IL-17A [19].

The final confirmation of APECED came with the identification of homozygous *AIRE* gene mutation c.del931T, a rare variant with only two previously documented cases [2, 20]. The first was described in a French patient analyzed as part of a series of 112 APECED patients from various ethnic backgrounds [2]. Although the exact clinical details of the patient were not given, the individual had at least two of the classic APECED triad of hypoparathyroidism, chronic mucocutaneous candidiasis, and adrenocortical insufficiency. A second case with this *AIRE* gene mutation was reported in a young male Sicilian [20]. Onset was at 6 years of age and, similar to our patient, he experienced candidiasis and hypoparathyroidism. In contrast, he suffered also from malabsorption, alopecia, enamel hypoplasia, and nephrocalcinosis, but not from keratitis. Overall, the c.del931T mutation does not appear to correlate with a specific clinical APECED phenotype.

Early diagnosis of APECED, a potentially life-threatening condition, is crucial. However, because of the clinical heterogeneity of the disorder, there is often a delay between the first symptoms and identification of the disease with a mean gap of 10.2 years [5]. As seen from this study, even in the absence of the main triad of disorders of chronic mucocutaneous candidiasis, hypoparathyroidism, and adrenocortical insufficiency, APECED should be considered in young patients who have hypoparathyroidism and chronic keratitis, and a past medical history showing a mild and transient *Candida* infection. An initial diagnosis can be confirmed quickly by serological testing for anti-IFN-$\omega$ and anti-IFN-$\alpha$2 antibodies, and sequencing of the *AIRE* gene where available. Long-term follow-up will be mandatory to screen for additional organ-specific autoimmunity which could develop over the following years and decades.

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## Conflict of Interest

None declared.

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