Lessons from Primary Immunodeficiencies: Autoimmune Regulator and Autoimmune Polyendocrinopathy-Candidiasis–Ectodermal Dystrophy

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Summary:

The discovery of the Autoimmune Regulator (AIRE) protein and the delineation of its critical contributions in the establishment of central immune tolerance has significantly expanded our understanding of the immunological mechanisms that protect from the development of autoimmune disease. The parallel identification and characterization of patient cohorts with the monogenic disorder Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy (APECED), which is typically caused by biallelic *AIRE* mutations, has underscored the critical contribution of AIRE in fungal immune surveillance at mucosal surfaces and in prevention of multiorgan autoimmunity in humans. In this review, we synthesize the current clinical, genetic, molecular and immunological knowledge derived from basic studies in Aire-deficient animals and from APECED patient cohorts. We also outline major advances and research endeavors that show promise for informing improved diagnostic and therapeutic approaches for patients with APECED.

Keywords

AIRE; APECED; APS-1; thymic medullary epithelial cells; central tolerance; immunomodulation

Introduction

Immunological recognition of self and non-self is of paramount importance to the body's ability to harness the appropriate defense against invading pathogens while simultaneously avoiding a misdirected autoimmune response. The **A**uto**i**mmune **Re**gulator (AIRE) protein is a transcriptional regulator that plays a critical role in self-tolerance through orchestrating the promiscuous gene expression of tissue-specific antigens (TSAs) in a subset of medullary thymic epithelial cells (mTECs).[1] Naïve thymocytes that recognize these TSAs with high affinity undergo apoptosis in a process referred to as negative selection.[2–4] Dysfunction of

this process allows self-reactive T cells to escape into the periphery resulting in autoimmune destruction of certain tissues.[5] Consonant with the crucial role of AIRE in mediating central immune tolerance, loss-of-function mutations in the *AIRE* gene result in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED; Online Mendelian Inheritance in Man [OMIM] number, 240300), also known as autoimmune polyglandular syndrome type 1 (APS-1) or polyglandular autoimmune (PGA) syndrome type-1.

APECED/APS-1 is a rare monogenic disorder characterized by development of multiorgan autoimmunity that targets several endocrine and non-endocrine tissues, and susceptibility to a "signature" infectious disease, chronic mucocutaneous candidiasis (CMC), which manifests with chronic, recurrent and severe infections of the mucous membranes, skin and/or nails by the commensal yeast fungus *Candida*.[6–9] Since the initial clinical descriptions of APECED/APS-1 in the medical literature in between 1929 and 1943[10–12] and the subsequent discovery of its genetic etiology via heroic positional cloning efforts by the Finnish-German Consortium and Dr. Shimizu's research group in 1997[13,14], there have been significant advances in our understanding of this rare disease. In this review, we discuss the broad clinical implications of AIRE deficiency on autoimmunity, our understanding of the molecular and genetic mechanisms that play a role in the pathogenesis of APECED, and how these findings may guide therapeutic strategies based on the evaluation and treatment of ~120 APECED patients at the NIH Clinical Center in the past five years.

Prevalence

APECED exhibits its highest prevalence amongst certain historically isolated populations such as Persian Jews (1:9,000), Sardinians (1:14,000) and Finns (1:25,000)[14–18], which display enrichment for corresponding founder "signature" *AIRE* mutations; 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. The prevalence of APECED has also been estimated in other countries such as Slovenia (1:43,000), Norway (1:80,000) and Poland (1:129,000).[19–21] The estimated prevalence of APECED in most other countries is thought to be in the range 1:80,000–200,000, although a systematic effort to accurately calculate country-specific prevalence rates is missing thus far.

Diagnosis of the Suspected APECED Patient

The diagnosis of APECED is established by the presence of any two clinical manifestations from the classical triad of CMC, hypoparathyroidism and adrenal insufficiency (Addison's disease), although the diagnosis can be based on the development of only one of the classic triad manifestations in a child whose sibling already carries the diagnosis of APECED.[6,22,23] Nonetheless, recent work from our group and others has underscored the much broader and deeper clinical phenotype of APECED patients compared to that previously recognized.[7–9,18,24] Indeed, highlighting the vast phenotypic diversity of the disease, greater than 30 distinct manifestations can be seen, with more than 25 of those involving non-endocrine organs (Lionakis, unpublished data).[8,9] Specifically, beyond the classical triad components of APECED, patients may also exhibit varying frequencies of urticarial eruption, enamel

hypoplasia, intestinal malabsorption, autoimmune hepatitis, autoimmune pneumonitis, autoimmune gastritis, Sjogren's-like syndrome, B12 deficiency, vitiligo, keratoconjuctivits, alopecia, type-1 diabetes, tubulointerstitial nephritis and asplenia, amongst others (Table 1, Fig. 1).[7–9,18–20,25–30] Of interest, some of the above clinical manifestations, particularly those that target the skin, the lungs, the salivary glands, the stomach, the intestine and the liver appear to be significantly enriched among American APECED patients compared to their frequency reported in European patient cohorts. Whether this phenotypic discrepancy is explained by genetic, environmental, microbiome and/or other differences among the various patient cohorts or it is the result of the deeper systematic phenotyping that was recently performed in American APECED patients remains to be elucidated by future studies.

Unfortunately, the vast majority of APECED patients will develop several other disease components before reaching a classic diagnostic dyad, which would then raise suspicion for APECED. On average, patients suffer from more than five manifestations and up to 20 conditions have been reported per individual.[7,8,31] Given the heterogenous presentation, the disease often goes unrecognized leading to an unfortunate delay in the diagnosis. Within an American cohort that we recently evaluated and reported at the National Institutes of Health, the mean age of achieving a classic diagnostic dyad of APECED was over seven years of age.[8] Strikingly, only 20% of those patients developed their first two consecutive disease manifestations among the classic triad of manifestations. Instead, 80% of the patients within our cohort developed a median of three non-triad manifestations before developing a classic diagnostic dyad, which led to prolonged periods of diagnostic uncertainty and development of additional (often irreversible) autoimmune sequelae before a diagnostic dyad was reached and the APECED diagnosis was suspected and eventually established.

Among the early common manifestations that affected children in North and South America before the development of a classic diagnostic dyad, three manifestations were particularly prominent: urticarial eruption, enamel hypoplasia, and intestinal malabsorption. Less often, autoimmune pneumonitis, autoimmune hepatitis or keratoconjuctivitis were noted.[8] The urticarial eruption appeared as frequent as CMC as the first manifestation in the American APECED cohort; it is typically a non-pruritic maculopapular rash in the extremities and the torso (Fig. 2) that is not clearly associated with viral illness, vaccination or other inciting event, and is only infrequently accompanied by fever. The eruption is typically self-limited lasting days or weeks, and recurrences are frequent within the first 2–3 years of life. Histologically, the eruption features a characteristic picture of mixed myeloid and lymphoid cell infiltration, occasionally extending to the fat to cause panniculitis, but without vasculitis typically.

Importantly, recognition of these additional manifestations that appear early in the course of the disease may help to reduce diagnostic delay and allow for prompt institution of therapy (see below). Based on the above findings, we proposed expanded diagnostic criteria for American APECED patients by incorporation of the adjunct diagnostic triad of urticarial eruption, enamel hypoplasia and intestinal malabsorption to the classic triad criteria (Fig. 3). Upon such incorporation, a diagnostic dyad within these expanded diagnostic criteria would have been reached with a mean of four years earlier compared to reaching a diagnostic dyad

within the classic diagnostic criteria. This earlier diagnosis would have been reached before development of life-threatening Addisonian crisis or hypocalcemic seizures in approximately half of the affected patients.

We have now validated these expanded diagnostic criteria in other independent patient datasets enrolled consecutively in the National Institutes of Health natural history study from both North/South America and European countries. In these independent datasets, the expanded diagnostic criteria would have also fostered a much earlier diagnosis in both American and European APECED patients and would have assisted in reaching the APECED diagnosis before development of life-threatening endocrine complications in a significant proportion of the patients (Ferre *et al.*, manuscript in preparation). In agreement with the potential clinical utility of these expanded diagnostic criteria, Fierabracci *et al.*[32] recently retrospectively analyzed previously reported APECED cohorts from Finland, Sardinia and Turkey and showed that the application of these expanded diagnostic criteria would have also allowed for earlier recognition and diagnosis in these APECED patients. Collectively, these findings indicate that children with APECED are as likely or even more likely to be seen early in the course of their disease by dermatologists, allergists, dentists, hepatologists, pulmonologists, ophthalmologists and/or gastroenterologists than by endocrinologists. Therefore, the presence of any of these manifestations within the adjunct criteria even in the absence of endocrinopathies in a young child should alert physicians to consider the diagnosis of APECED.

Clinical suspicion for APECED should prompt a) genetic testing for *AIRE* mutations and/or deletions and b) screening for neutralizing autoantibodies (AAbs) against type I interferons such as interferon-omega (IFN-ω) and interferon-alpha (IFN-α) (Fig. 3). Regarding genetic testing, not all patients with clinical APECED are found to have biallelic *AIRE* mutations by Sanger sequencing. Some patients have large *AIRE* deletions, which require copy number variation analysis for establishing the genetic diagnosis, and some other patients have heterozygous *AIRE* mutations or have wild-type *AIRE* genotype, indicating that non-coding *AIRE* variants or non-*AIRE* coding variation may result in APECED in some patients (see below) (Fig. 3). AAbs against IFN-ω are highly sensitive and specific for APECED (>95%), even as early as the first few months of life before the appearance of the clinical manifestations and the other organ-specific AAbs (see below).[33–35] These type I interferon AAbs can also be seen in patients with secondary AIRE deficiency such as those with thymoma[36,37] or hypomorphic *RAG* mutations that present with immune dysregulation, particularly those with late-onset combined immune deficiency and granulomatous/autoimmune manifestations that present with severe viral infections[38], and in some patients with late-onset myasthenia gravis.[39] The later onset and differential clinical, genetic, laboratory and radiographic features of thymoma, RAG deficiency and myasthenia gravis make them discernible from APECED. Determination of type I interferon AAbs is not commercially available in the US, but the authors of this review can be contacted by physicians to perform the test from serum or plasma in their patients with suspected APECED.

Earlier diagnosis is critical for a number of reasons. First, several of the autoimmune manifestations in APECED are life-threatening including adrenal crisis, hypocalcemic

seizures, fulminant hepatitis, autoimmune lung disease and pneumococcal sepsis, which collectively account for the increased mortality in these patients.[7,9,18,40] Therefore, earlier diagnosis may allow for earlier screening and recognition of the aforementioned clinical complications. Second, earlier diagnosis may allow for the institution of prophylactic immunomodulation in children with APECED, which may result in prevention of the development of the full-blown syndrome and its irreversible autoimmune sequelae. To that end, a prospective clinical trial aiming at instituting prophylactic immunomodulation in children with APECED very early in the course of their disease is currently under development at the National Institutes of Health.

Clinical Manifestations of APECED Patients

APECED patients present with a characteristic combination of a) a single consistent infectious disease manifestation, CMC, and b) autoimmunity that affects several endocrine and non-endocrine tissues. There is significant patient to patient variability in the phenotypic expression of the syndrome and no clear correlation exists between the presence of a specific *AIRE* mutation or deletion and the individual patient's clinical phenotype, even among affected siblings within the same family (Table 1, Fig. 1). This indicates that there are complex interactions between genetic, epigenetic, and environmental factors that likely contribute to the development of organ-specific APECED manifestations in different patients. Most patients with APECED begin having symptoms in early childhood, with CMC and urticarial eruption being the most common initial manifestations of affected patients[8], although as noted earlier, the time between the onset of symptoms and the diagnosis of APECED is often long.

Infectious manifestations

Candida is a commensal yeast fungus that colonizes the oral and gastrointestinal mucosal surfaces, the skin and/or the female lower genital tract in up to 60–70% of healthy individuals. When perturbations in host immune responses and/or mucosal microbiome occur, *Candida* becomes an opportunistic pathogen and may cause mucosal or invasive infections.[41] Patients with APECED are at risk for mucosal *Candida* infections, which typically develop within the first 1–3 years of life, most often in the form of oral and/or esophageal candidiasis. CMC is the "signature" infection in >80% of all reported APECED patients associated with the presence of neutralizing AAbs against Th17 cytokines (see below), with the exception of the Persian Jew APECED population that carries the missense Y85C *AIRE* mutation and was reported to infrequently (<20%) develop CMC (Fig. 1).[15] The reasons for this cohort-specific variation in the prevalence of CMC remain unclear. Future studies are needed to examine whether Th17 AAbs are less prevalent within this patient population. Of note, chronic *Candida* infections of the oral and esophageal mucosal surfaces can lead to the development of oral or esophageal squamous cell carcinomas in ~5% of adult patients with long-standing mucosal candidiasis.[42,43] Carcinoma development in the context of monogenic disorders that manifest with CMC is not restricted to APECED but can also be seen in patients with *STAT1* gain-of-function mutations.[44,45] The mechanisms for this susceptibility to neoplasm development remain unclear, but may relate to enhanced EGFR signaling triggered by autoreactive CD4$^+$ T cells in the epithelial barrier.

[46] Candidiasis may also affect the intestinal or vaginal mucosal surfaces or the nails in APECED patients, albeit with much lower frequencies compared to the oral and esophageal mucosa, while involvement of the skin is very uncommon (Lionakis, unpublished observations). As opposed to the susceptibility to mucosal candidiasis, patients with APECED are not at heightened risk of developing invasive *Candida* infections, which depend on myeloid phagocytes for effective host defense (see below).[47–49]

Besides CMC, patients with APECED are not consistently susceptible to other infectious complications. A small proportion of patients (~10–15%) develop asplenia, which is not congenital but is most often acquired during childhood (Lionakis, unpublished observations), and as a result of that, they are at risk for development of invasive infections by encapsulated bacteria, such as *Streptococcus pneumoniae, Neisseria meningitidis* and *Capnocytophaga canimorsus*, including overwhelming sepsis. Those with asplenia exhibit Howell-Jolly and Pappenheimer bodies in their peripheral blood smear, have an atrophic or absent spleen on abdominal imaging, and require long-term prophylactic antibiotic therapy and vaccinations in order to prevent the development of life-threatening infections by these bacteria (see below).

A small proportion of APECED patients develop recurrent infections by herpetic viruses such as HSV and VZV (~15%, Lionakis, unpublished observations), without an obvious correlation between the titers or neutralizing potential of type I interferon AAbs or the levels of STAT1.[50,51] APECED patients have also sporadically been reported to develop *Giardia* intestinal infections, although the mechanisms for this susceptibility remain elusive.[52]

Endocrine autoimmune manifestations

Endocrine autoimmune disease is the hallmark of APECED syndrome, as the acronym indicates. Hypoparathyroidism, which typically develops earlier than any other endocrinopathy, and adrenal insufficiency are the most common endocrine manifestations. Additional endocrinopathies that develop with lower frequencies include hypothyroidism, primary ovarian or testicular failure, growth hormone deficiency and hypopituitarism. Some of these endocrine manifestations are associated with the presence of corresponding organ-specific AAbs, although these AAbs do not always predict the development of the corresponding endocrinopathy at the individual patient level (see below; Table 2). Of interest, type I diabetes is an uncommon complication of patients with APECED, which typically occurs later in adolescence or adulthood in ~5–10% of patients.

Non-endocrine autoimmune manifestations

As mentioned earlier, non-endocrine autoimmune manifestations have recently been recognized as common disease components in certain cohorts of patients with APECED. Urticarial eruption, as mentioned above, is a characteristic early common feature in American APECED patients (Fig. 2). Inflammation in the salivary and lacrimal glands (Sjogren's-like syndrome) and ocular inflammation in the forms of keratoconjuctivitis or, less often, retinitis, can occur, and the latter can lead to visual loss if left untreated (see below). Among the non-endocrine disease components, the pulmonary and intra-abdominal autoimmune manifestations are worthwhile expanding on further.

Although autoimmune pneumonitis had been reported in only ~2% of all reported APECED patients in the literature, we have recently identified lung autoimmunity in ~40–45% of American APECED patients (Ferre *et al.*, submitted manuscript).[8] Pneumonitis in APECED presents clinically with chronic cough and shortness of breath, and is most often misdiagnosed as asthma or recurrent bronchitis. Without immunosuppressive treatment (see below), repeated bouts of inflammation in the airways, the bronchioles and the lung parenchyma result in the development of bronchiectasis leading to recurrent bacterial respiratory infections and eventual hypoxemic respiratory failure. AAbs against BPIFB1 (BPI fold containing family B member 1) and the potassium channel KCNRG appear highly specific for lung autoimmunity in APECED patients (Table 2), but their sensitivity is ~65% and ~30%, respectively. This indicates that other yet-unknown lung autoantigens are the target of autoimmune attack in some patients with APECED pneumonitis. Therefore, because currently known AAbs do not capture all patients with pneumonitis, the best available screening modality for pneumonitis in APECED patients is computed tomography of the chest, which reveals ground-glass opacities and/or bronchiectasis, and also captures the ~5–10% of patients with early lung disease that are as-yet asymptomatic. Immunological analyses in the airways and lung parenchyma of *Aire*$^{-/-}$ mice and APECED pneumonitis patients have revealed characteristic compartmentalized immunopathology with accumulation of activated neutrophils in the airways and T > B lymphocyte infiltration in intraepithelial, submucosal, interstitial and peribronchiolar/bronchiolar tissue. B cell aggregates are commonly seen deeper in the lung parenchyma (Ferre *et al.*, submitted manuscript). Collectively, autoimmune pneumonitis is a common, early, severe, and often misdiagnosed manifestation of APECED patients, which features a characteristic regional immunopathology that has implications for immunomodulatory treatment (see below).

In addition, there are several intra-abdominal manifestations seen in APECED patients. Autoimmune gastritis features lymphoplasmacytic infiltration in the stomach tissue and can present with abdominal pain, nausea or vomiting. It may be accompanied by pernicious anemia caused by destruction of gastric cells that produce intrinsic factor, which is essential for absorption of vitamin B12 in the gut. Autoimmune gastritis, especially in the presence of intestinal metaplasia, and B12 deficiency both increase the risk for development of gastric adenocarcinoma in APECED patients. The most common intra-abdominal manifestation of APECED patients appears to be intestinal malabsorption of fat that manifests with chronic diarrhea, chronic constipation or an alternating pattern of both. The etiology of fat malabsorption is multifactorial and includes exocrine pancreatic insufficiency, autoimmune enteritis, and/or intestinal dysbiosis (Natarajan *et al.*, manuscript in preparation). Strikingly, autoimmune involvement of the colon is extremely uncommon in APECED patients, with frequency <2–3% (Lionakis, unpublished observations), highlighting the differential role of AIRE in protecting from autoimmune attack the different segments of the gastrointestinal tract.

Autoimmune hepatitis presents with transaminitis, but often is not accompanied by AAbs commonly seen in autoimmune hepatitis in non-APECED patients such as antinuclear antibodies (ANA), anti-mitochondrial antibodies (AMA), anti-smooth muscle antibodies (ASMA), or antibodies against the liver and kidney microsomal antigens (LKM-1) or soluble liver antigens (SLA). Some, but not all, patients with APECED-associated

autoimmune hepatitis feature AAbs against cytochrome P450 1A2 (CYP1A2), histidine decarboxylase (HDC) or aromatic L-amino acid decarboxylase (AADC) (Chascsa *et al.*, submitted manuscript).[53,54] This indicates that the pathogenesis of APECED-associated autoimmune hepatitis may be distinct from non-APECED autoimmune liver disease, and the full spectrum of liver autoantigens that are targeted by AIRE-deficient lymphocytes in APECED patients remains to be determined. Therefore, transaminitis in APECED patients warrants a liver biopsy and initiation of immunosuppressive treatment (see below).

A minority of APECED patients (<10%) develop autoimmunity in the kidneys that manifests with tubulointerstitial nephritis, with sparing of the glomeruli. The presence of autoimmune renal disease is critical to recognize in affected patients and to differentiate from nephrocalcinosis-induced kidney injury, which is more commonly seen and results from chronic oral and intravenous calcium supplementation for hypoparathyroidism, because it can lead to progressive renal failure.[55] Indeed, kidney transplantation has been reported in APECED patients with tubulointerstitial nephritis, with recurrence of tubulointerstitial nephritis and renal failure without effective immunomodulatory therapy post-transplantation (see below).[56]

Interestingly, APECED patients rarely develop certain other autoimmune conditions such as multiple sclerosis, glomerulonephritis, lupus, or rheumatoid arthritis. The underlying protective factors underpinning this finding are not yet fully understood, as some of the peripheral tissue antigens responsible for organ-specific autoimmunity are expressed in mTECs in an Aire-dependent manner. For example, myelin proteolipid protein (PLP), but not myelin oligodendrocyte glycopeptide peptide (MOG), which are autoantigens associated with multiple sclerosis depend differentially on Aire for expression in mTECs and protection from experimental autoimmune encephalomyelitis in mice; yet, APECED patients do not develop multiple sclerosis.[57] The immunological shadow of peripheral tissue antigens that Aire projects in the thymus is limited.[58] It is likely that other transcription factors expressed in the thymus, such as Fezf2 or others, may guide the expression of a complementary array of tissue specific antigens and may confer tolerance to specific tissues. Indeed, studies using Foxn1-Cre$^+$*Fezf2*$^{fl/fl}$ mice revealed a different phenotype than *Aire*$^{-/-}$ models with inflammatory infiltration of the lung, liver, kidney, stomach, small intestine, salivary gland, testis, and brain, some of which (i.e., kidney, brain) are not seen in Aire-deficient mice.[59]

Ectodermal dystrophy manifestations

Several manifestations of APECED patients have been attributed to ectodermal dystrophy, as the acronym implies. The most common such manifestation is enamel hypoplasia, which manifests early in the course of the disease and its recognition by pediatric dentists should trigger sequencing of the *AIRE* gene and testing for type I interferon AAbs, especially if accompanied by oral candidiasis or hypoparathyroidism. Other APECED-associated ectodermal dystrophy manifestations include vitiligo, alopecia, and nail dystrophy in the absence of fungal nail disease. The pathogenesis of these manifestations is poorly defined.

AIRE Structure

The *AIRE* gene is located in the long arm of chromosome 21 at position 22.3 (21q22.3), it contains 14 exons, and it encodes a 545 amino acid protein of 57,727 Da. AIRE is composed of four subunit domains, which are also found in other chromatin-associated proteins including the HSR/CARD (Caspase recruitment) domain in the N-terminal region of the protein, the SAND (Sp100, AIRE, nuclear phosphoprotein 41/75 or NucP41/75 region, and deformed epidermal autoregulatory factor 1 or DEAF1) domain, and two PHD (plant-homeodomain) zinc fingers (PHD-1, PHD-2) in the C-terminal region of the protein (Fig. 4). [60,61] The CARD domain is important for the specked nuclear localization of AIRE and the formation of AIRE homo-dimers and tetramers, which are essential to the functional viability of the AIRE protein. [62,63] The SAND domain of several family members binds DNA, but the AIRE SAND domain differs from other SAND domains as it is missing a critical DNA binding motif (KDWK). Indeed, it was recently discovered that AIRE SAND interacts with the ATF7ip (activating transcription factor 7-interacting protein 1)-MBD1 (methyl CpG binding protein 1) complex potentially in concert with PHD-1 to target TSA genes. [64] The two PHD zinc fingers suggest that AIRE regulates transcription epigenetically through interaction with unmethylated lysine 4 on histone H3 (H3K4me0). [65] Hence, PHD-1 is critical for the transcription-transactivation activity of AIRE and was shown to be essential for preventing autoimmunity in transgenic mouse models. [66–68] In addition to these four subunits, there are two NLS (nuclear localization signal) regions implicated in nuclear transport as well as four LXXLL motifs, which mediate interactions with other LXXLL-containing proteins and may play a role in the homo-oligomerization of AIRE. [61]

AIRE Expression and Function

mTECs and CD4+ T cells

Shortly following the discovery that biallelic mutations in the *AIRE* gene result in APECED in 1997 [13,14], Anderson and colleagues revealed in 2002 that Aire is predominately expressed within a subset of epithelial cells of the medullary thymus [58], although Aire expression has now been shown in extrathymic Aire-expressing cells (eTACs), and B cell and dendritic cell populations (see below). [69–72] These specialized cells, referred to as mTECs, facilitate ectopic expression of thousands of self-peptides where they are presented to developing thymocytes (T cells) via major histocompatibility complex (MHC) antigens. [61,73,74] T cells that bind to these self-peptide-antigens exceeding an appropriate affinity threshold undergo clonal deletion, thereby preventing their release into circulation. [4] In the absence of AIRE this process is defective, self-peptides are not expressed, allowing auto-reactive T cells to escape in the periphery and resulting in lymphocytic tissue infiltration and autoimmunity (Fig. 5). [75] This has been illustrated via adoptive transfer studies of Aire-deficient CD4+ T cells into a immunodeficient host, which is sufficient to recapitulate the autoimmune disease seen in Aire-deficient mice. Furthermore, depletion of the CD4+ T cell subset in Aire-deficient mice led to amelioration of the disease process. [76] This immunological shadow projected by Aire has been well illustrated through generation of *Aire*−/− mice in which the Aire-deficient thymic stroma was shown to be critical and sufficient for induction of autoimmunity and development of autoantibodies and lymphocytic infiltration involving

specific organs.[58,77] Though broadly involved in central tolerance, the immunological shadow that AIRE projects in the thymus is critical to the development of autoimmunity in specific organs as evidenced by disease in patients as well as murine models.[58] However, not all TSAs in the thymus require Aire for expression and induction of central immune tolerance. The transcription factor, Fezf2, which is regulated by the LTβR pathway but not the CD40/RANKL-Aire axis, was recently uncovered by Takaba *et al.*[59] to potentially regulate TSAs in mTECs in an Aire-independent manner. Specifically, Fezf2-deficient animals developed organ-specific autoantibodies and lymphocytic infiltration that were distinct from those seen in Aire-deficient mice. Based on these findings, it will be an important direction of future research to determine whether there are patients with loss-of-function *FEZF2* mutations who may present with monogenic organ-specific autoimmunity.

In addition to AIRE's role in negative selection of self-reactive effector T cells, AIRE has also been implicated in the positive selection of thymic FoxP3$^+$ regulatory T cells (Tregs) and a recent study demonstrated that a defect in the neonatal output of thymic Tregs (tTregs) in *Aire*$^{-/-}$ mice drives organ-specific autoimmunity.[78–82] In the presence of functional AIRE, T cell clones that recognize self-antigens with an intermediate affinity are positively selected and differentiate into the T regulatory subset. These specialized cells demonstrate immunosuppressive effects to halt autoreactive effector T cells in the periphery and have been shown to be important in maintenance of self-tolerance.[80] Which of these Aire-dependent functions is the predominant mechanism of tolerance responsible for disease development and/or whether these mechanisms operate in concert remains to be fully elucidated. Current evidence would suggest that the negative selection of effector T cells may play a more crucial role in pathogenesis over the positive selection of Tregs. For example, through performing double Aire-sufficient/Aire-deficient thymus transplant experiments in nude mice, Tregs derived from a functional Aire thymus were not sufficient to prevent autoimmunity.[3] In fact, these mouse data would suggest that transplantation of allogeneic cultured thymus alone without the concurrent surgical removal of endogenous AIRE$^{-/-}$ thymic tissue and the elimination of peripheral AIRE$^{-/-}$ lymphocytes is unlikely to be beneficial in APECED patients. In addition, genetic crossing of Aire-deficient and Foxp3-deficient mice to generate Aire-Foxp3 double-deficient animals results in much more severe autoimmune disease relative to each deficient mouse strain alone[83]. Moreover, in contrast to APECED patients, patients with *FOXP3* mutations who lack Tregs develop Immune Dysregulation, Polyendocrinopathy, Enteropathy X-linked (IPEX) syndrome, which is characterized by absent or defective Tregs and results in severe widespread autoimmunity early in life with organ specificity that is distinct from APECED patients such as the high prevalence of type I diabetes or glomerulonephritis.[84] The dichotomy in clinical presentation and disease onset and severity of these two monogenic diseases illustrates that although AIRE regulates thymic Treg generation through positive selection, the development of autoreactive CD4$^+$ effector T cells may play a primary role in the pathogenesis in APECED patients. Beyond the important role of these AIRE-dependent functions in induction of tolerance and prevention of autoimmunity, there is emerging literature on how therapeutic manipulation of the Aire-dependent thymic development of tumor-associated Tregs and of the Aire-dependent negative selection of tumor-specific effector T cells may have implications in designing novel anti-tumor therapeutic strategies.[85–87]

CD8$^+$ T cells and $\gamma\delta$ T cells

In addition to *AIRE$^{-/-}$* CD4$^+$ effector and/or regulatory T cells that drive the autoimmune disease, other lymphoid cell types likely play a role in modulating the disease development, as shown in the *Aire$^{-/-}$* mouse model. For example, adoptive transfer of *Aire$^{-/-}$* CD8$^+$ T cells into SCID (severe combined immunodeficiency) recipient mice was sufficient to drive autoimmune disease in peripheral nerve tissue, although to a lesser extent compared to adoptive transfer of *Aire$^{-/-}$* CD4$^+$ T cells.[88] How Aire may affect CD8$^+$ T cell development in the thymus is less defined relative to its role in CD4$^+$ T cell selection. In addition, recent evidence implicates $\gamma\delta$ T cells in the development of organ-specific autoimmune disease in the retina and lung of Aire-deficient mice. Specifically, Aire, via its regulation of IL-7 expression in mTECs, was shown to modulate the accumulation of thymic IL-17A$^+$ Vγ6$^+$ Vδ1$^+$ T cells.[89] Aire-deficient mice have expanded populations of thymic and peripheral perinatally generated IL-17A$^+$ Vγ6$^+$ Vδ1$^+$ T cells, and deletion of $\gamma\delta$ T cells in Aire-deficient mice ameliorates retinal and pulmonary autoimmune disease. A putative corresponding population of $\gamma\delta$ T cells in humans, IL-17A$^+$ Vγ9$^+$ Vδ2$^+$ T cells, are expanded in the peripheral blood of APECED patients, indicating that the role of these cells in mediating organ-specific autoimmune disease in humans deserves further investigation.

B cells and AAbs

Aire$^{-/-}$ auto-reactive B cells have also been suggested to be drivers of organ-specific autoimmunity in mice, predominantly in the lungs, although another study revealed a broader organ distribution for the role of Aire-deficient B cells in autoimmunity.[76,90] In fact, APECED patients exhibit a significant expansion in the autoreactive CD21^{lo} CD38$^+$ B cell subset from an early age, and *Aire$^{-/-}$* mice have increased peripheral accumulation of CD11b$^+$ CD11c$^+$ B cells, which are thought to functionally and phenotypically correspond to human CD21^{lo} CD38$^+$ B cells.[8] These findings may have translational implications with regard to the potential utility of anti-CD20-targeted immunotherapy in affected patients (see below). The mechanisms by which AIRE promotes central and/or peripheral tolerance of B cells, and whether these tolerance defects relate to B cell-intrinsic or B cell-extrinsic mechanisms remain unclear.[72]

In addition, the relative contribution of B cell-dependent production of AAbs over B cell-dependent T cell priming and expansion via antigen presentation in promoting autoimmunity in APECED patients is not fully understood (see below).[90] In fact, the extent to which AAbs play a direct role in pathogenesis is unclear, as transfer experiments of Aire-deficient serum have not resulted in development of autoimmune disease in mice. At the clinical level, the sensitivity and specificity of organ-specific AAbs is not 100%, and although positivity for certain AAbs may predict the development of certain disease components in certain cases, this is not a universal rule for all patients (Lionakis, unpublished observations).

However, the identification and characterization of a wide array of tissue-specific AAbs through the years has provided important information regarding organ-specific autoantigens that are targeted by autoimmunity in humans. Several AAbs have been identified in APECED and their role in autoimmunity has, in some instances, been extended beyond APECED. These include 21-OH (21-hydroxylase) for primary adrenal insufficiency[91,92],

NALP5 (NACHT, leucine-rich repeat, pyrin domain–containing protein 5) for hypoparathyroidism[93], TPH (tryptophan hydroxylase) for intestinal malabsorption[94], TGM4 (transglutaminase 4) for prostate-associated male infertility[95], TDRD6 (tudor domain containing protein 6) for hypopituitarism [96], BPIFB1 and KCNRG for autoimmune lung disease[26,97], and others (Table 2).[8,98–110] Future work will be needed to uncover other autoantigens that may provide explanation for the pathogenesis of the autoimmune attack in the spleen, salivary and lacrimal glands, nails and skin of APECED patients.

In addition to tissue-specific autoantibodies, as mentioned earlier, nearly all patients with APECED produce autoantibodies directed against type I interferons, which have early diagnostic utility.[33,35] Of note, the occurrence of AAbs against GAD65 (glutamic acid decarboxylase) in the majority of APECED patients[8] without the accompanying development of type I diabetes has led to investigations for the identification of potential protective factors that may prevent the development of type I diabetes in this patient population. To that end, Meyer and colleagues recently reported on the inverse correlation between the presence of high-affinity, neutralizing AAbs against type I interferons with type I diabetes in APECED patients, suggesting that these AAbs may harbor diabetes-ameliorating potential and, thus, may have therapeutic utility.[111] Studies in additional APECED cohorts will be needed to verify these interesting translational research findings.

The identification of neutralizing AAbs against IL-17A, IL-17F and IL-22, which are critical cytokines involved in the mucosal defense against *Candida* has been associated with the susceptibility to CMC in APECED patients.[112,113] How deficiency in a thymic transcription regulator leads to universal and isolated susceptibility to a single infectious disease, CMC, is indeed an intriguing question, and the Th17 cytokine AAbs suggest a potential autoimmune origin for CMC. However, in most reported studies, the correlation between the presence of Th17 cytokine AAbs and CMC is ~70%, indicating that other factors may also contribute to development of mucosal fungal disease in APECED.[8,112,113] Alterations in C-type lectin receptor signaling in macrophages and impaired production of antimicrobial factors in mucosal secretions have also been proposed as potential contributing factors.[114–116]

Thus far, the antifungal immune studies in APECED patients have focused on their systemic, but not their mucosal, immune responses. Because the immune response requirements for effective mucosal versus systemic host defense against *Candida* are strictly segregated, it is likely that yet-unknown defects at the mucosal level in these patients may also contribute to CMC susceptibility. To that end, we have established a mouse model of mucosal candidiasis in *Aire*^{−/−} deficient mice and have performed parallel studies of mucosal immunity in mucosal biopsy samples obtained from APECED patients. We have found that pathogenic T cells, not autoantibodies, drive fungal susceptibility at the mucosa via impairing the integrity of the mucosal barrier (Break et al, manuscript in preparation).

eTACs

Besides Aire-expressing mTECs, the identification of eTACs within secondary lymphoid organs of mice and humans has resulted in great interest in the potential extrathymic role of AIRE expression in induction of tolerance. The Anderson lab has shown that mouse eTACs are a MHCII^{hi}, EpCAM^{hi}, CD45^{lo}, CD80^{lo}, CD86^{lo} bone marrow-derived antigen

presenting cell population that expresses distinct self-antigens and is able to interact with and delete naïve autoreactive T cells through Treg-independent mechanisms that are still not fully characterized.[69,71]

AIRE-expressing cells that exhibit a dendritic cell-like morphology and express HLA-DR, CD11c, CD40, CD83, CD208 and CCR7 have also been reported in human peripheral lymphoid tissues such as lymph nodes (predominantly abdominal), tonsils and gut-associated lymphoid tissue (GALT), but not in spleen.[70] These cells, which appear to correspond to a subset of activated interdigitating dendritic cells, express TSAs such as insulin and "tolerogenic" molecules such as IL-10 or indoleamine 2,3-dioxygenase, raising the possibility of serving a potential tolerogenic function. Studies employing bone marrow replacement of eTACs or gene therapy within bone marrow-derived eTACs into Aire deficient and other studies using conditional Aire-knockout mice will be critical to directly examine the potential role of eTACs in organ-specific autoimmune disease and their ability to ameliorate disease. In fact, a preliminary report using conditional Aire-knockout mice revealed that Aire protects from infertility derived from autoimmune disease within the reproductive organs via expression in cells outside the thymic stromal or hematopoietic compartments, presumably within the gonads.[117] Future research will be required to determine whether different eTAC subsets exist in humans in order to examine their functional and regulatory roles, and compare whether their numbers and/or effector functions are defective in patients with APECED.

AIRE Partners and Regulation

AIRE appears to be tightly regulated with recent identification of important enhancer elements activated by nuclear factor-κB (NF-κB) signaling that are necessary for AIRE's expression. For example, the conserved non-coding sequence 1 (CNS1) proximal to the *Aire* promoter is a cis-regulatory element critically important for AIRE expression in mTECs. [118,119] Multiple additional regulators have also been recently reported to control AIRE expression and/or function. These include FBXO3 (F-box protein 3 E3), which ubiquitinates AIRE and regulates its transcriptional activity, SIRT1 (Sirtuin 1), which is abundantly found in mTECs where it acetylates Aire and regulates the expression of Aire-dependent TSA-encoding genes, and HIPK2 (homeodomain-interacting protein kinase 2), which phosphorylates AIRE and suppresses its coactivator activity, although its impact on the expression of Aire-dependent TSA-encoding genes in mTECs is modest.[120–122] HIPK2 was recently shown to also modulate the development of thymic tuft cells, which represent a subset of thymic epithelial cells that rely on the taste chemosensory molecule TRPM5 for their thymic function and pass through an Aire-dependent phase for their thymic development.[123] Moreover, the lysyl-hydroxylase Jmjd6 (Jumonji domain-containing protein 6) affects splicing of intron 2 of the *Aire* gene and is required for expression of mature Aire in mTECs, and Dgcr8 (DiGeorge syndrome critical region gene 8) is important for accumulation of Aire-expressive mTECs in the thymus.[124,125] Recent work from Herzig *et al*.[126] further demonstrated a very complex method of Aire expression regulation resulting from positive and negative mechanisms and the coordinated effort of multiple transcription factors, Irf4, Irf8, Tbx21, Tcf7 and Ctcfl. Whether patients with APECED-like clinical

disease with wild-type *AIRE* genotype have mutations in the aforementioned AIRE partners and regulators remains to be determined in future studies.

AIRE mutations in APECED patients

APECED follows an autosomal recessive pattern of inheritance with more than 100 *AIRE* mutations having been reported throughout the entire gene.[127] Depending on the patient cohort, homozygous or compound heterozygous *AIRE* mutations are seen more often. As mentioned earlier, homozygosity for the p.Y85C, p.R139X or p.R257X mutations is observed in the vast majority of Persian Jew, Sardinian or Finnish APECED patients, respectively. The 13-bp deletion 1094–1106del13 is enriched among North American, Norwegian, British and Irish APECED patients, and in North American patients, compound heterozygous *AIRE* mutations are most often seen, consonant with the greater genetic and ethnic diversity of the population.

A few important points are worthwhile highlighting about the genetics of APECED patients: first, as mentioned above, there is no clear genotype-phenotype correlation with organ-specific autoimmune manifestations and significant clinical variability is seen amongst patients (even siblings) with identical *AIRE* genetic variants.[127] These findings imply the presence of other disease-modifying genetic variants in APECED patients.[128] Indeed, this hypothesis was recently supported in mice with mutations in both *Aire* and the inhibitory protein tyrosine kinase *Lyn*, which developed autoimmune uveitis, underscoring the cooperation between central and peripheral tolerance checkpoints in the organ-specific phenotypic expression of autoimmune disease.[129] Similarly, mice with deficiency in both Aire and the T cell peripheral tolerance checkpoint Cbl-b (Casitas B-lineage lymphoma), which modulates CD28 co-stimulation, developed lethal exocrine pancreatic autoimmune disease.[130] Therefore, despite APECED being a monogenic disorder, these findings underscore the importance of other to be discovered genetic modifiers that may promote or ameliorate susceptibility of organ-specific manifestations of the disease. Investigation of such variants is an important direction of future research.

Second, patients with isolated hypoparathyroidism have been discovered with pathogenic biallelic *AIRE* mutations[131,132], raising the possibility of the existence of several unrecognized APECED patients with biallelic *AIRE* mutations who may develop isolated organ-specific autoimmune manifestations that are typical of APECED syndrome but have not developed a classic diagnostic dyad to raise awareness for *AIRE* genetic testing. Third, in the North American APECED population, we have witnessed a significant proportion (~15%) that does not have biallelic *AIRE* mutations or deletions and yet, the clinical presentation of the patients is indistinguishable from patients with biallelic *AIRE* mutations.[8] Whole exome and whole genome sequencing in these patients are a first step toward identifying potential digenic models of disease or non-*AIRE* coding variants that may phenocopy AIRE deficiency or non-coding variants that may affect AIRE expression and/or function.

Recently, dominant-negative *AIRE* mutations involving the SAND or PHD-1 domains have also been identified, some of which have a relatively high frequency in the general

population (up to 1 in 1000). These patients present with much less severe APECED-like disease, often with later onset, manifested by limited organ involvement such as pernicious anemia or vitiligo or an isolated endocrinopathy; AAbs are not always positive in these patients.[133–136] These findings indicate that such dominant-negative *AIRE* variants may modulate the phenotypic expression of common organ-specific autoimmune diseases. In that light, *AIRE* variants along with other risk loci were recently reported to be associated with Addison's disease.[137] Last, an intriguing recent report by the Matsumoto lab indicated that overexpression of human AIRE in mice resulted in the development of a paradoxical muscle-specific autoimmune disease, associated with impaired maturation of mTECs and defective expression of Aire-dependent TSAs.[138] These data both illustrate the importance for tight control of AIRE expression and function and raise the possibility that gain-of-function *AIRE* genetic variants may exist in humans, perhaps having a potential association with organ-specific autoimmune diseases such as polymyositis or dermatomyositis.

Management of APECED Patients

As the onset of symptoms, disease severity and progression, and number of disease manifestations are highly variable amongst patients the majority of patients are best served by a multidisciplinary team of providers at a tertiary care-level facility. At the initial visit, healthcare providers should perform a comprehensive evaluation with special attention to involvement of tissue sites reported.[8] The integration of specialists from immunology, infectious disease, endocrinology, pulmonology, hepatology, gastroenterology, dermatology, nephrology and dentistry provides the best possible evaluation for the individual patient. Subsequent visits over time should continue surveillance for the development of other disease manifestations and involvement of additional organ systems. The principles of evaluation and management of the APECED patients include treatment of CMC and other infectious complications, hormone and/or electrolyte replacement for irreversible endocrinopathies, and immunomodulation for non-endocrine end-organ disease.

CMC and other infectious complications

Management of CMC is challenging in APECED patients because a substantial proportion of them develops azole-resistant *Candida albicans* and *Candida glabrata* strains, thus limiting long-term oral treatment options.[139–141] In our experience, the use of oral swiss/swallow suspension of amphotericin B provides the best long-term preventive strategy in these patients with azole-resistance *Candida* strains. This topical therapeutic approach works best when it follows an induction treatment phase with a fungicidal parenteral drug such as an echinocandin in order to prevent recurrence of candidiasis rather than being effective as treatment of acute severe oral or esophageal candidiasis. Novel antifungal agents with remarkable *in vitro* and *in vivo* activity against azole-susceptible and -resistant *Candida* strains require future investigation in APECED patients with severe azole-resistant CMC[140,141], as does the Als3-based fungal vaccine that boosts IL-17-mediated anti-*Candida* immunity and is the first fungal vaccine shown to display efficacy in humans.[142,143] Routine screening for gastrointestinal cancers is a recommended practice for all patients. Patients should undergo routine oral examination in partnership with dental colleagues, and patients

with intestinal metaplasia or dysplastic features on gastric biopsies should be evaluated by gastric mapping.

Screening for asplenia should be performed in all patients via peripheral blood smear and abdominal ultrasound. In the subset of patients identified with asplenia, we initiate prophylactic oral penicillin VK or amoxicillin (or azithromycin in penicillin-allergic individuals). These individuals should be provided with a prescription of extended-spectrum fluoroquinolones (e.g., levofloxacin) to be available for initiation at the onset of fever. They should receive vaccination against pneumococcus (both 13- and 23-valent vaccines), meningococcus, and *Haemophilus influenzae* type b as well as annual vaccination for influenza.

Management of endocrinopathies

Close monitoring of hormone and electrolyte replacement is paramount to avoid acute Addisonian or hypocalcemic crises. Patients with adrenal insufficiency should carry an emergency card or medical alert bracelet and stress dose corticosteroids should be made available. Long-term calcium replacement results in nephrocalcinosis, which can lead to kidney stones and renal failure. The advent of recombinant PTH may help some APECED patients with hypoparathyroidism, especially in the setting of renal insufficiency and/or severe intestinal malabsorption.[144,145]

Management of non-endocrine end-organ disease

Immunosuppressive treatment is critical for the control of autoimmunity in the lungs, liver, small intestine, eye or kidney with a goal to prevent irreversible end-organ damage. The use of cyclosporine ophthalmic solution is efficacious in patients with autoimmune keratoconjunctivitis. Azathioprine has a long track record of success in the treatment of autoimmune hepatitis in patients with APECED with a favorable tolerability profile without hematological, hepatic or infectious complications (Chascsa et al, submitted manuscript). In APECED patients with *TPMT* (thiopurine S-methyltransferase) genotypes that predict poor metabolism of azathioprine, mycophenolate or mTOR inhibition are acceptable alternative options in order to avoid azathioprine-induced toxicity. We avoid cyclosporine or tacrolimus due to prevalent renal-associated toxicity in APECED patients. mTOR inhibitors appear particularly effective for biopsy-proven autoimmune enteritis. Less experience exists regarding the optimal treatment of tubulointerstitial nephritis, but azathioprine or mycophenolate appear to be effective in halting the progression of the disease, when started early. Monotherapy with rituximab appears ineffective in the small number of treated APECED patients with pneumonitis, nephritis or enteritis. Rituximab appears to be effective against APECED-associated autoimmune hepatitis, typically in combination with a T cell immunomodulator in the rare occasions where T cell-targeted monotherapy is ineffective (Lionakis, unpublished observations). In agreement with *Aire*−/− mouse studies of T and B cell depletion, autoimmune pneumonitis responds dramatically at the clinical, radiographic, and pulmonary function levels with the combination of azathioprine and rituximab. The majority of treated patients remain in remission on azathioprine monotherapy without the need for re-challenging with rituximab (Ferre *et al.*, submitted manuscript).

Currently, there is no consensus with regard to initiation of prophylactic immunomodulation in patients with APECED as an approach to prevent development of subsequent autoimmune manifestations. Future studies will be critical to examine the role of such a preventive immunosuppressive approach, especially in patients who are diagnosed very early in the course of their disease.

Conclusion and Future Prospects

The past 20 years have provided significant advances in our understanding of the clinical, genetic, molecular and immunological mechanisms that underpin the development of APECED. Description of the functional and regulatory mechanisms of AIRE has offered key information regarding mechanisms of self-tolerance and autoimmunity. The implications of dominant-negative heterozygous *AIRE* mutations and the collaboration of AIRE with other at-risk loci and peripheral tolerance checkpoints may shed light on the pathogenesis of other autoimmune disease states. Identification of patients early in the course of the disease is crucial and may allow for instituting disease-modifying interventions prior to the development of irreversible end-organ complications. The challenge moving forward will be to translate our improved understanding of the clinical, genetic, molecular and cellular features of AIRE deficiency into the development of novel strategies that will improve the diagnosis, risk stratification, immunotherapy, prognosis and quality of life of APECED patients.

Acknowledgments:

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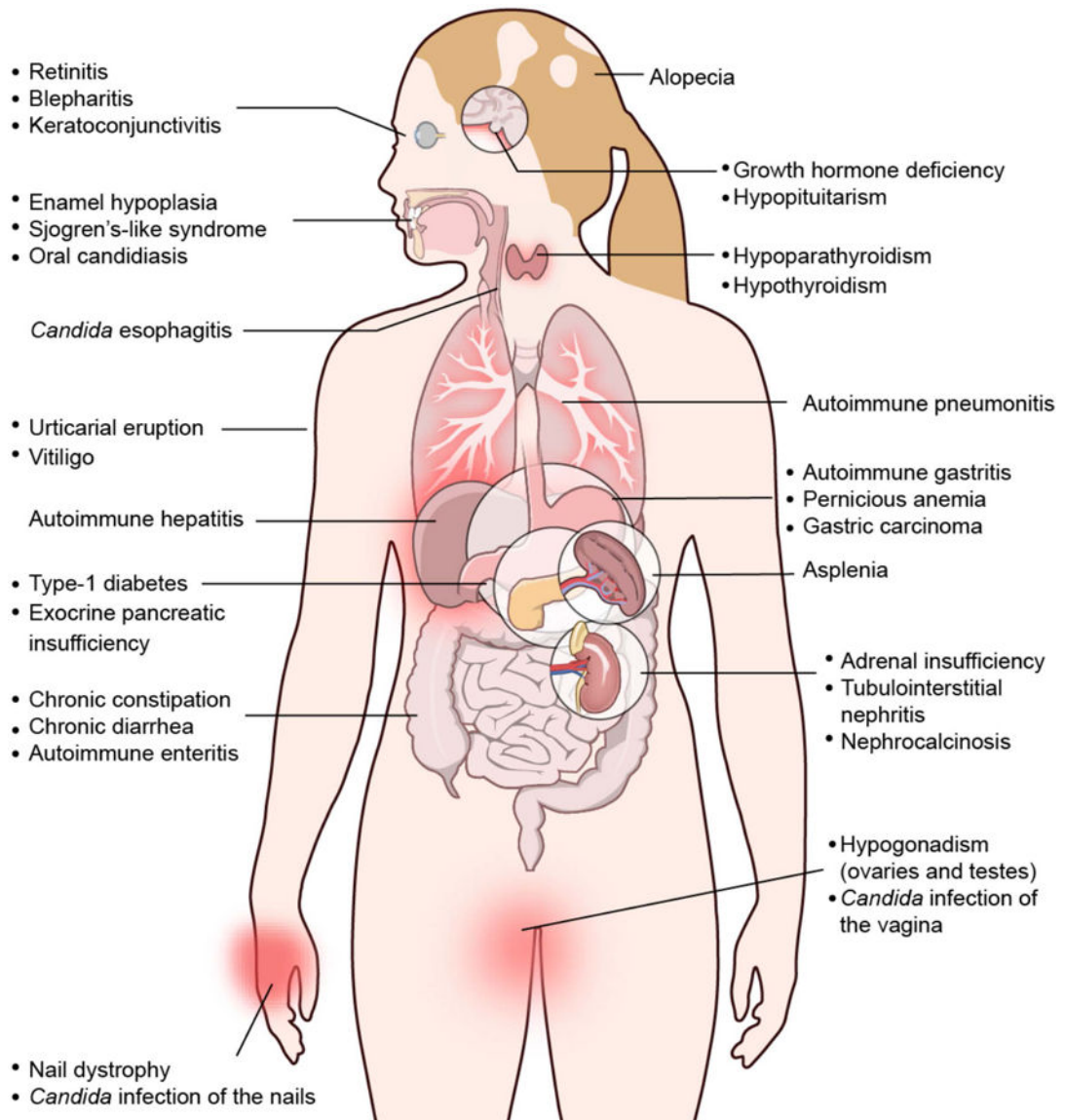
Fig. 1. Diagram depicting organ systems affected by APECED.

The presented organ-specific autoimmune manifestations affect APECED patients with varying frequencies (Table 1).
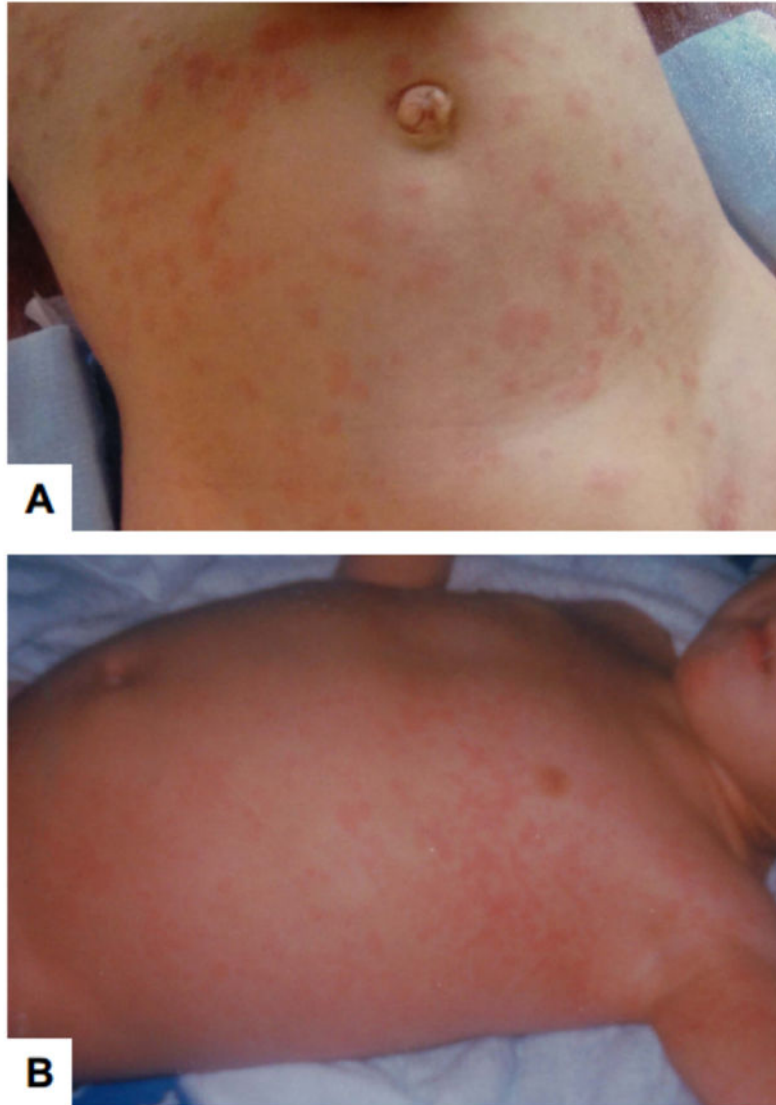
Fig. 2. Representative photographs of urticarial eruption in young children with APECED.
These images are derived from Ferre *et al.* (ref #8).

CLASSIC TRIAD:

ADJUNCT TRIAD:

CMC
Hypoparathyroidism
Adrenal insufficiency

+

Urticarial eruption
Intestinal dysfunction
Enamel hypoplasia

Any two manifestations within the combined classic and adjunct triads

↓

AIRE sequencing and copy number variation analysis
Type I interferon autoantibody testing

Fig. 3. Proposed diagnostic algorithm for capturing an earlier diagnosis in children with APECED.
Illustration of the combined classic diagnostic triad and adjunt triad comprised of urticarial eruption, intesntional dysfunction and enamel hypoplasia, which may lead to earlier time to diagnosis in children with APECED via triggering genetic testing for *AIRE* mutations and deletions/duplications and testing for type I interferon autoantibodies.
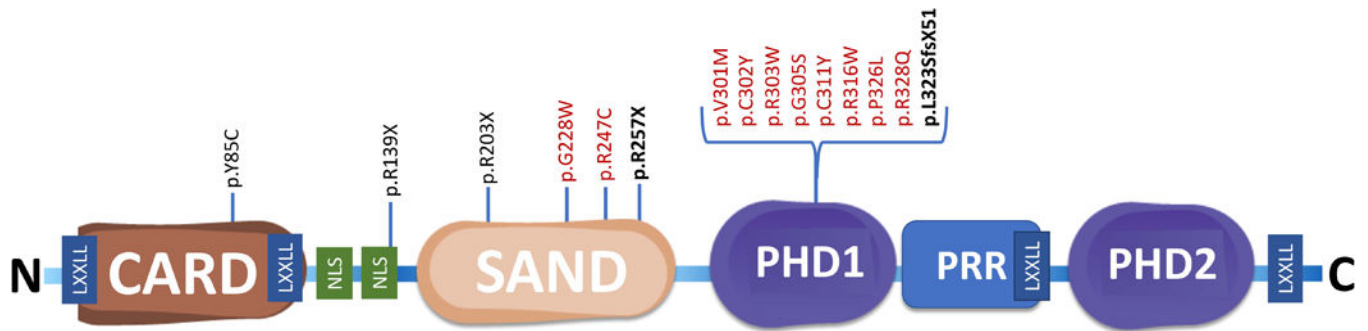
Fig. 4. Structure of the Autoimmune regulator (*AIRE*) gene and major mutations.
Comprised of four subunits including Caspase recruitment domain (CARD), Sp100, AIRE, nuclear phosphoprotein 41/75 or NucP41/75 region, and deformed epidermal autoregulatory factor 1 or Deaf1 domain (SAND) and two plant-homeodomain (PHD) zinc fingers. A proline rich region (PRR), two nuclear localization signal (NLS) regions and four LXXLL motifs are also displayed. Founder mutations p.Y85C (Persian Jews); p.R139X (Sardinians); p.R257X (Finns); and p.R203X (Sicilians) are displayed. The two most common mutations in our cohort R257X and 13bp mutation, p.L323SfsX51 are shown in bold. Biallelic (homozygous or compound heterozygous) mutations resulting in classical APECED (black). Dominant negative mutations in the SAND or PHD1 domains resulting in APECED-like organ-specific autoimmune manifestations (red). Select icons adapted from Reactome library (ref #148).
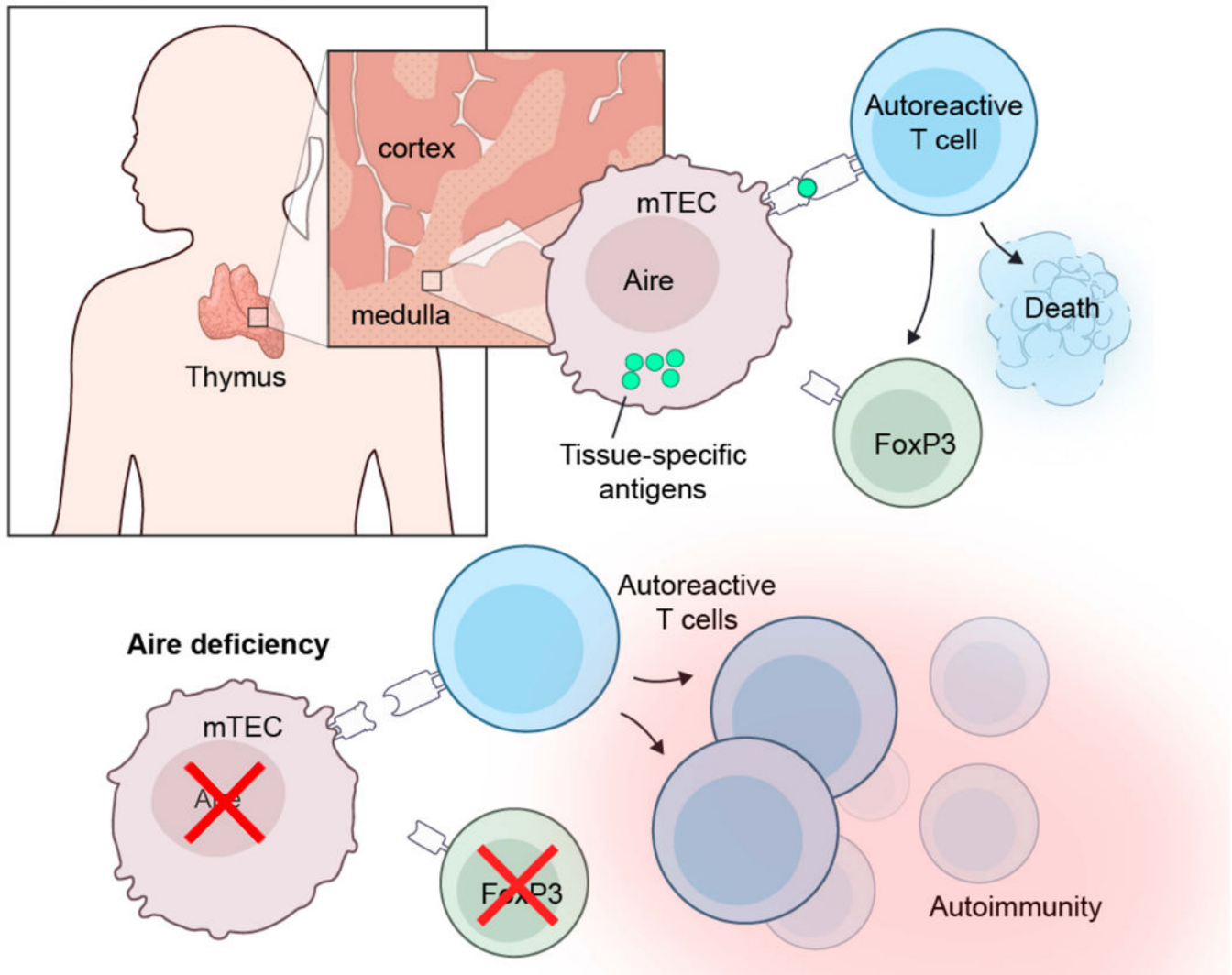
Fig. 5. Mechanisms of Aire-dependent induction of central immune tolerance in the thymus.
Aire is expressed in a subset of medullary thymic epithelial cells (mTECs) where it is
critical for the expression and surface display of peripheral tissue self-antigens (TSAs). Self-
reactive T cells with high affinity for TSAs either undergo deletion via apoptosis or become
regulatory T cells. In the absence of functional Aire, the expression and surface display of
TSAs on mTECs is impaired, which results in the escape of self-reactive T cells in the
periphery where they infiltrate tissues and cause the autoimmune manifestations observed in
APECED patients.

Table 1.

Prevalence (%) of Disease Manifestations in American APECED with Comparison International Cohorts

Disease manifestations	American (n=35)	Russian (n=112)	Finnish (n=91)	Norwegian (n=52)	Italian (n=41)	Irish (n=31)	Sardinian (n=22)	Iranian Jewish (n=23)	Turkish (n=23)	Indian (n=22)	French (n=19)	British (n=18)	Polish (n=16)	Sicilian (n=15)	Slovakian (n=4)
Classical triad															
CMC	86	75	100	77	83	100	95	18	82.6	96	89	100	87.5	80	0
Hypoparathyroidism	91	78	72	73	93	84	77	96	86	91	63	89	100	100	50
Adrenal insufficiency	83	67	72	63	73	68	68	22	56.5	55	79	83	50	67	100
Other endocrine															
Hypothyroidism	23	13	4	19	10	NR	0	4	21.7	4.5	5	NR	NR	13	50
Type 1 diabetes	11	9	16.5	8	2	10	4.5	4	8.7	4.5	5	28	NR	7	0
GH deficiency	17	NR	5.5	NR	NR	6	22	NR	4.3	NR	5	33	6.25	NR	0
Ovarian failure	38	48	25	33	36.5	61	61	38	8.6	60	71	NR	25	20	0
Testicular failure	21	NR	7.7	0	14.6	NR	0	33	13	0	0	NR	NR	NR	50
Intra-abdominal															
Intestinal dysfunction	80[*]	25	5	23	15	32	50	NR	8.7	27	26	NR	12.5	33	NR
Autoimmune hepatitis	43[*]	11	17.5	4	20	19	27	NR	13	9	11	11	37.5	20	NR
Autoimmune gastritis	49[*]	NR	19	NR	15	NR	32	9	8.7	NR	31.5	NR	NR	20	NR
B12 deficiency	29	8	31	15	15	10	32	9	17	21	21	22	6.25	20	NR
Interstitial nephritis	6	NR	9	3.8	NR	NR	NR	NR	NR	0	NR	NR	NR	NR	NR
Asplenia	9	2	19	16	11	NR	4.5	NR	0	4.5	0.5	NR	NR	NR	NR
Skin/Nail/Dental															
Urticarial eruption	66[*]	NR	14.3	NR	NR	NR	NR	NR	4.3	9	NR	NR	NR	NR	NR
Alopecia	17	34	35	31	37	19	45	13	39	27	53	22	18.75	14	25
Vitiligo	37	9	2	15	15	NR	22.7	NR	26	27	21	NR	NR	20	NR
Nail dystrophy	17	NR	84	13	NR	NR	55	NR	13	22	NR	NR	NR	27	NR
Enamel hypoplasia	86	28	77	72	NR	NR	45	NR	4.3	61	0	NR	NR	20	25
Other															
Pneumonitis	40[*]	NR	1	NR	NR	NR	NR	NR	0	4.5	NR	NR	NR	7	NR

Disease manifestations	American (n=35)	Russian (n=112)	Finnish (n=91)	Norwegian (n=52)	Italian (n=41)	Irish (n=31)	Sardinian (n=22)	Iranian Jewish (n=23)	Turkish (n=23)	Indian (n=22)	French (n=19)	British (n=18)	Polish (n=16)	Sicilian (n=15)	Slovakian (n=4)
Sjogren's-like syndrome	43[*]	NR	11	NR	12	NR	NR	NR	4.3	9	NR	NR	NR	NR	NR
Keratoconjunctivitis	29	NR	9	12	12	NR	36	0	4.3	9	31.5	11	25	13	NR
Early onset HTN	17	NR	15	NR	NR	NR	9	NR	NR	0	NR	NR	NR	NR	NR

Shown are approximate percentages of corresponding APECED manifestations pooled from various published studies comparing data from the American[8] or other international cohorts
9, 15, 18, 20, 24, 27–32, 40, 146, 147

[*]
Disease manifestations that appear more prevalent in American APECED patients relative to reported frequencies in European APECED patients.

CMC, chronic mucocutaneus candidiasis; GH, growth hormone; HTN, hypertension; NR, not reported.

Table 2.

Autoantigens in APECED patients and their associated disease manifestation.

Autoantigen	Tissue of origin	Associated clinical manifestation
IFN-ω	Cytokine	APECED, Thymoma, Myasthenia gravis, RAG deficiency
IFN-α	Cytokine	APECED (protection from type 1 diabetes?), Thymoma, Myasthenia gravis, RAG deficiency
IFN-β	Cytokine	Unclear
IFN-λ	Cytokine	Unclear
IL-6	Cytokine	Unclear
IL-17A	Cytokine	Chronic mucocutaneous candidiasis
IL-17F	Cytokine	Chronic mucocutaneous candidiasis
IL-22	Cytokine	Chronic mucocutaneous candidiasis
NACHT leucine-rich-repeat protein 5 (NALP5)	Parathyroid, ovary, breast, testis	Hypoparathyroidism
Calcium-sensing receptor (CaSR)	Parathyroid, pancreas, kidney	Hypoparathyroidism
21-hydroxylase (CYP21A2)	Adrenal gland	Primary adrenal insufficiency
17-α-hydroxylase (CYP17A1)	Adrenal gland	Primary adrenal insufficiency, ovarian failure
Thyroglobulin (TG)	Thyroid	Hypothyroidism
Thyroid peroxidase (TPO)	Thyroid	Hypothyroidism
Islet antigen-2 (IA-2)	Pancreas	Type I diabetes
Potassium channel-regulating protein (KCNRG)	Lung, cervix	Autoimmune pneumonitis
Bactericidal/permeability-increasing fold-containing B1 (BPIFB1)	Lung, stomach, esophagus, cervix	Autoimmune pneumonitis
Aromatic L-amino acid decarboxylase (AADC)	Kidney, intestine, brain, liver, pancreas	Autoimmune hepatitis, vitiligo
Cytochrome P450 1A2 (CYP1A2)	Liver	Autoimmune hepatitis
CYP2A6	Liver	Autoimmune hepatitis
Tryptophan hydroxylase (TPH)	Multiple	Intestinal dysfunction, autoimmune hepatitis, autoimmune gastritis
Hydrogen-Potassium ATPase	Stomach	Autoimmune gastritis

Autoantigen	Tissue of origin	Associated clinical manifestation
Intrinsic factor	Stomach	Pernicious anemia
Glutamic acid decarboxylase (GAD65)	Pancreas, brain	Intestinal dysfunction, vitiligo
Histidine decarboxylase (HDC)	Brain, stomach, lung	Intestinal dysfunction
Defensin, alpha 5 (DEFA5)	Paneth cells	Intestinal dysfunction
Side chain cleavage enzyme (CYP11A1)	Adrenal, ovary, testis	Primary adrenal insufficiency, ovarian failure, testicular failure
Transglutaminase 4	Prostate	Prostatitis (male infertility)
Testis-specific gene 10 protein (TSGA10)	Testis, brain	Unclear
Tudor domain containing protein 6 (TDRD6)	Testis, brain	Hypopituitarism (growth hormone deficiency)
Melanoma antigen family B 2 (MAGEB2)	Testis, female germ cells	Unclear
Protein disulfide isomerase-like testis (PDILT)	Testis	Unclear
Tyrosine hydroxylase (TH)	Brain, adrenal	Alopecia, enamel hypoplasia
SOX9/SOX10	Nervous system, breast	Vitiligo
Myelin protein 0	Nervous system	Neuropathy
Interphotoreceptor retinoid-binding protein	Retina	Retinitis pigmentosa