

# Autoimmune polyglandular syndromes

---

Lavie, Or

Master's thesis / Diplomski rad

2023

Degree Grantor / Ustanova koja je dodijelila akademski / stručni stupanj: **University of Zagreb, School of Medicine / Sveučilište u Zagrebu, Medicinski fakultet**

Permanent link / Trajna poveznica: <https://um.nsk.hr/um:nbn:hr:105:034496>

Rights / Prava: [In copyright](#)/[Zaštićeno autorskim pravom.](#)

Download date / Datum preuzimanja: **2025-01-24**



Repository / Repozitorij:

[Dr Med - University of Zagreb School of Medicine Digital Repository](#)



**UNIVERSITY OF ZAGREB  
SCHOOL OF MEDICINE**

**Or Lavie**

**AUTOIMMUNE POLYGLANDULAR SYNDROME**

**Graduate thesis**



**Zagreb, 2023.**

According to the graduation requirements, the following thesis was completed at the University Hospital Center Zagreb (KBC Zagreb, Rebro), Department of Pediatrics endocrinology, under the mentorship of doc.dr. sc. Katja Dumić Kubat and was submitted for evaluation in the academic year 2022/2

## Contents

ABSTRACT .....	1
SAŽETAK .....	2
INTRODUCTION .....	3
CLASSIFICATION OF THE AUTOIMMUNE POLYGLANDULAR SYNDROMES .....	4
IMMUNOLOGICAL FACTORS IN PATHOGENESIS AUTOIMMUNE POLYGLANDULAR SYNDROMES .....	5
GENETICS OF AUTOIMMUNE POLYGLANDULAR SYNDROMES .....	12
ENVIRONMENTAL FACTORS IN PATHOGENESIS OF APS .....	14
CLINICAL APECTS OF AUTOIMMUNE POLYGLANDULAR SYNDROMES .....	15
AUTOIMMUNE POLYGLANDULAR SYNDROME TYPE 1 .....	15
AUTOIMMUNE POLYGLANDULAR SYNDROME TYPE 2 .....	20
IPEX SYNDROME .....	22
DIAGNOSTIC APPROACH AND FOLLOW-UP .....	23
AUTOANTIBODIES .....	26
NONORGAN-SPECIFIC AUTOANTIBODIES .....	26
ORGAN-SPECIFIC ANTIBODIES .....	27
TREATMENT .....	28
ACKNOWLEDGMENTS .....	30
REFERENCES .....	31
BIOGRAPHY .....	37

## ABBREVIATIONS

ACA - adrenal cytoplasmic autoantibodies  
ACTH - adrenocorticotrophic hormone  
AIRE - in autoimmune regulator  
APECED - autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy  
APS - autoimmune polyglandular syndromes  
APS-1 - autoimmune polyglandular syndrome type 1  
APS-2 - autoimmune polyglandular syndrome type 2  
ATD - autoimmune thyroid disease  
CARD - caspase recruitment domain  
CMC - chronic mucocutaneous candidiasis  
CTLA-4 - cytotoxic T-lymphocyte-associated protein 4  
FOXP3 - forkhead box P3  
GAD65 - glutamic acid decarboxylase 65  
HLA - human leukocyte antigen  
IL-10 - interleukin-10  
IPEX - immune dysfunction, polyendocrinopathy, X-linked  
LADA - latent autoimmune diabetes of adults  
MHC - major histocompatibility complex  
PD-1 - programmed cell death protein 1  
PHD - plant homeodomain  
POI - premature ovarian insufficiency  
PTPN22 - protein tyrosine phosphatase nonreceptor type 22  
SCA - steroidal cell/gonadal antibodies  
T1D - type 1A diabetes mellitus  
TCR - T cell receptor  
TGF- $\beta$  - transforming growth factor-beta  
TPO - thyroid peroxidase  
Treg - regulatory T cell

## ABSTRACT

The autoimmune polyglandular syndrome (APS) represents a group of complex disorders of immune dysregulation that leads to autoimmunity and development of at least two different endocrine or non-endocrine abnormalities. According to aetiology and pathogenesis, APS can present with three distinct phenotypes: APS type 1, APS type 2 and IPEX (immune dysfunction, polyendocrinopathy, enteropathy, X-linked) syndrome. Both, APS type 1 and IPEX syndrome are monogenic disorders that develop in infancy or early childhood, while APS type 2 is a complex polygenic disorder that usually presents later, in adolescence or adulthood. Clinical presentation can be acute such as in diabetes mellitus, symptomatic and slow-progressive such as in adrenocortical insufficiency or even asymptomatic such as in celiac disease. Autoantibodies precede tissue destruction, and measurement of these autoantibodies can be used to identify risk of subsequent tissue insufficiency.

The diagnosis is often challenging, mainly due to rarity of condition, variability of the early clinical picture and frequently insidious course of disease. Therefore, whenever one organ-specific autoimmune disorder is diagnosed important to always have a high index of suspicion for possible APS. Early diagnosis permits active screening for progressive tissue damage, while early initiation of treatment prevents acute and long-term complications.

**keywords:** *autoimmune polyglandular syndrome, APECED, IPEX, immune regulation, autoimmunity*

## SAŽETAK

Autoimuni poliglandularni sindrom (APS) spada u skupinu poremećaja imunosne regulacije koji dovode do pojave autoimunosti i razvoja najmanje dvije različite endokrine ili neendokrine bolesti. Prema etiologiji i patogenezi, APS se može prezentirati s tri različita fenotipa: APS tip 1, APS tip 2 i IPEX (imuna disfunkcija, poliendokrinopatija, enteropatija, X-vezani) sindrom. APS tip 1 i IPEX sindrom su monogeni poremećaji koji se prezentiraju u dojenčko doba ili ranom djetinjstvu, dok je APS tip 2 kompleksan poligeni poremećaj koji se obično razvija kasnije, odnosno u adolescenciji ili odrasloj dobi. Klinička prezentacija može biti akutna, kao što je slučaj u dijabetes melitusu, simptomatska i sporo progresivna, kao u adrenokortikalnoj insuficijenciji, ili pak asimptomatska, kao u celijakiji. Pojava autoantitijela prethodi razaranju tkiva, a mjerenje istih se može koristiti za detekciju i prepoznavanje rizika za razvoja moguće insuficijencije ciljnih tkiva.

Kako se radi o rijetkim bolestima s varijabilnom kliničkom prezentacijom te podmučnim tijekom, postavljanje točne dijagnoze predstavlja izazov. Iz tog razloga je u bolesnika koji boluju od jednog autoimunog poremećaja važno uvijek imati visok indeks sumnje da se radi o mogućem APS-u. Rana dijagnoza omogućuje aktivno traganje za pridruženim bolestima, a pravovremeno uvođenje terapije sprječava akutne i dugoročne komplikacije.

**ključne riječi:** *autoimuni poliglandularni sindrom, APECED, IPEX, imunosna regulacija, autoimunost*

## INTRODUCTION

The autoimmune polyglandular syndromes (APS) comprise a group of rare syndromes characterized by presence of combination of several endocrine and nonendocrine autoimmune diseases in a single individual (1). It has been reported for the first time almost 100 years ago by Schmidt, who described a patient with combination of hypothyroidism and primary adrenal insufficiency with lymphocytic infiltration of both the thyroid and adrenal glands (2).

It is a multisystemic disease of either monogenic or complex genetic aetiology with an insidious presentation, characterized by presence of circulating autoantibodies and lymphocytic infiltration of the affected tissues or organs, subsequently leading to organ failure. Hallmark of all APS is disturbed immune tolerance, meaning that immune system fails to differentiate accurately between self and non-self, leading to development of multiorgan autoimmune disorders (1,3). Although recent research proposed that the complex interactions between genetics and the environment play a major role in development aberrant immunological processes, exact reasons for the loss of self-tolerance remain to be elucidated (1,3,4).

Autoimmune polyglandular syndromes can present with three distinct phenotypes: autoimmune polyglandular syndrome type 1 (APS-1), syndrome IPEX syndrome (immune dysfunction, polyendocrinopathy, X-linked) and autoimmune polyglandular syndrome type 2 (APS-2). Both autoimmune polyglandular syndrome type 1 and IPEX syndrome are rare monogenic disorders that present in infancy and are caused by pathogenic variants in autoimmune regulator (*AIRE*) and the forkhead box P3 (*FOXP3*) gene, respectively. Autoimmune polyglandular syndrome type 2 is a polygenic disorder that is far more common than APS-1 and IPEX and usually presents in adolescence or adulthood (3,5).

Onset of symptoms in both APS-1 and APS-2 is gradual with wide variation in age of presentation and phenotype which makes the diagnosis challenging. Course of disease is most aggressive and progressive in patients with IPEX syndrome with invariable phenotype expression in neonatal age or early infancy (3,4).

Aim of this thesis is to review aetiology and pathogenesis of APS and discuss approaches for the appropriate diagnosis and treatment of patients affected with this



rare and still not completely understood condition.

## CLASSIFICATION OF THE AUTOIMMUNE POLYGLANDULAR SYNDROMES

According to aetiology and clinical presentation the autoimmune polyglandular syndromes can present with three distinct phenotypes:

1. Autoimmune polyglandular syndrome type 1
2. Autoimmune polyglandular syndrome type 2
3. IPEX syndrome (immune dysfunction, polyendocrinopathy, X-linked)

Autoimmune polyglandular syndrome type 1 is an autosomal recessive genetic disease caused by pathogenic variants in *AIRE* gene located on chromosome 21q22.3. It is also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). Diagnosis is established by presence of at least two of the following conditions: primary adrenocortical insufficiency (Addison's disease), primary hypoparathyroidism and/or chronic mucocutaneous candidiasis (6, 7).

Autoimmune polyglandular syndrome type 2 is a polygenic disorder. Major component of APS-2 is Addison's disease found in conjunction with autoimmune thyroid disease (ATD) - Schmidt syndrome or both ATD and type 1A diabetes mellitus (T1D) – Carpenter syndrome. Other non-endocrine autoimmune disorders that can be associated with APS-2 include: celiac disease, vitiligo, pernicious anaemia, myasthenia gravis, stiff man syndrome and alopecia. Additional two entities – APS-3 (ATD and T1D without adrenal dysfunction in association with other autoimmune disorders, such as pernicious anaemia, vitiligo, or alopecia) and APS-4 (vitiligo and alopecia, or T1D and celiac disease, or T1D and vitiligo) can be found in literature and are considered variations or expansions of APS-2 (8,9).

IPEX syndrome is an exceedingly rare X-linked recessive condition caused by pathogenic variants in *FOXP3* gene. Most commonly affected endocrine organ in patients with IPEX syndrome is exocrine pancreas with subsequent development of neonatal T1D. Other non-endocrine symptoms include: enteropathy, dermatitis,

autoimmune anaemia and thrombocytopenia (10, 11).

## IMMUNOLOGICAL FACTORS IN PATHOGENESIS AUTOIMMUNE POLYGLANDULAR SYNDROMES

Genetic factors, encompassing Human Leukocyte Antigen (HLA) associations and non-HLA genes, play a role in the development of APS, while immune dysfunctions disrupt both central and peripheral tolerance mechanisms.

The immune system serves as a remarkable defence mechanism that safeguards the body against harmful pathogens. However, in its powerful response, it must also maintain a delicate balance between recognizing and eliminating foreign invaders while avoiding destructive attacks on the body's own tissues. This delicate equilibrium is achieved through a process known as immune tolerance. Immune tolerance is a fundamental mechanism through which the immune system distinguishes self from non-self and prevents excessive immune responses against self-antigens (7). It is a critical regulatory mechanism that safeguards against autoimmune reactions, where the immune system erroneously targets healthy cells and tissues. Immune tolerance enables the immune system to differentiate between self and non-self, ensuring that self-reactive immune cells are either eliminated or suppressed. It involves maintaining immune homeostasis, enabling immune responses against pathogens while avoiding reactions against the body's own tissues. Central and peripheral tolerance mechanisms work in conjunction to achieve this balance (7).

Central tolerance is established during the development of immune cells in the thymus (T cells) and bone marrow (B cells). It entails a process called negative selection, wherein developing immune cells that recognize self-antigens with high affinity are eliminated (8). Negative selection eradicates T cells that exhibit high affinity for self-antigens presented by self-MHC molecules. This process, also referred to as clonal deletion, prevents the emergence of self-reactive T cells that could potentially trigger autoimmune responses. T cells with strong recognition of self-antigens undergo apoptosis and are effectively removed. This ensures that only immune cells with a diverse yet non-reactive repertoire are allowed to mature and enter the peripheral tissues (Figure 1). Thymic epithelial cells, including medullary thymic epithelial cells and dendritic cells, present tissue-specific antigens to developing T cells in the

thymus. Self-reactive T cells that exhibit high affinity for these tissue-specific antigens undergo negative selection. T cells that receive robust T cell receptor (TCR) signals upon interaction with self-antigens undergo apoptosis, eliminating self-reactive T cells from the developing T cell repertoire. Some self-reactive T cells that do not receive strong TCR signals undergo diversion toward the regulatory T cell (Treg) lineage. Tregs play a crucial role in suppressing autoimmune responses and maintaining immune tolerance (12).

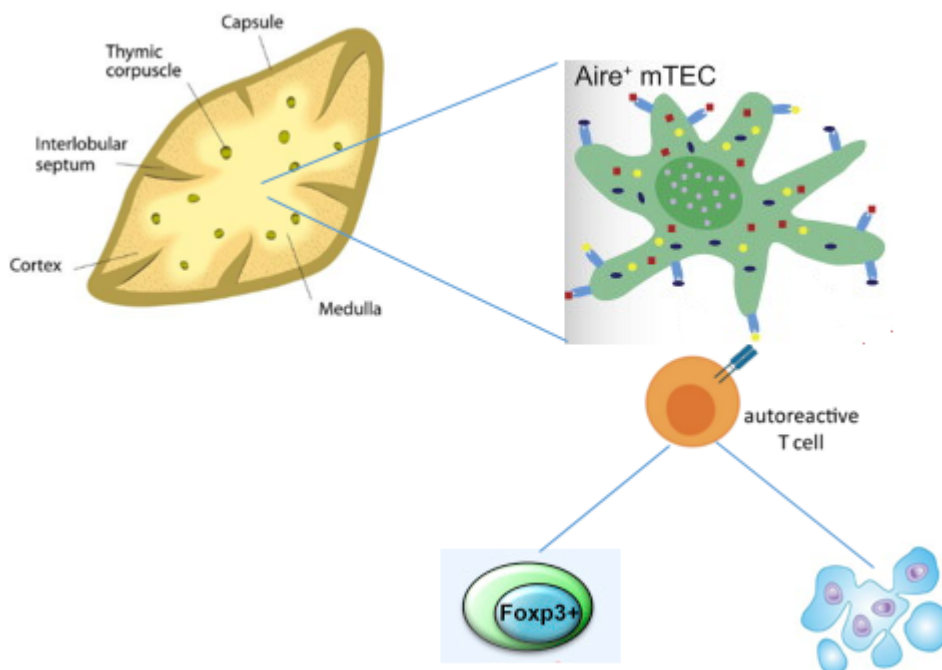


Figure 1 Normal central immune tolerance. The expression of the autoimmune regulator (*AIRE*) in medullary thymic epithelial cells (mTECs) plays a role in facilitating the presentation of tissue-specific antigens on the cell surface. This process allows for the elimination of autoreactive T cells that recognize self-proteins with high affinity, either through apoptosis or by promoting their differentiation into forkhead box P3 (FoxP3) expressing T regulatory cells (Tregs). Modified according to reference 1.

Central tolerance is established during the development of immune cells in the thymus (T cells) and bone marrow (B cells). It entails a process called negative selection, wherein developing immune cells that recognize self-antigens with high affinity are eliminated (8). Negative selection eradicates T cells that exhibit high affinity for self-antigens presented by self-MHC molecules. This process, also referred to as clonal deletion, prevents the emergence of self-reactive T cells that could potentially trigger

autoimmune responses. T cells with strong recognition of self-antigens undergo apoptosis and are effectively removed. This ensures that only immune cells with a diverse yet non-reactive repertoire are allowed to mature and enter the peripheral tissues. Thymic epithelial cells, including medullary thymic epithelial cells and dendritic cells, present tissue-specific antigens to developing T cells in the thymus. Self-reactive T cells that exhibit high affinity for these tissue-specific antigens undergo negative selection. T cells that receive robust T cell receptor (TCR) signals upon interaction with self-antigens undergo apoptosis, eliminating self-reactive T cells from the developing T cell repertoire. Some self-reactive T cells that do not receive strong TCR signals undergo diversion toward the regulatory T cell (Treg) lineage. Tregs play a crucial role in suppressing autoimmune responses and maintaining immune tolerance (12).

Positive selection takes place in the thymus and ensures the survival and maturation of T cells capable of recognizing self-major histocompatibility complex (MHC) molecules. This process guarantees the development of a diverse T cell repertoire capable of recognizing a wide range of foreign antigens.

Central immune tolerance serves as a critical protective mechanism against the onset of autoimmune diseases. Failures in central tolerance mechanisms can lead to the escape of self-reactive T cells, resulting in the breakdown of self-tolerance and the initiation of autoimmune responses (7).

In APS, deficiencies in central tolerance mechanisms allow autoreactive T cells, which recognize self-antigens, to evade into the peripheral tissues (13). Mutations in key genes like *AIRE* (APS-1) and *FOXP3* (APS-2) disrupt the negative selection of autoreactive T cells, enabling their survival and activation against self-antigens.

Peripheral tolerance mechanisms operate outside the primary lymphoid organs and act as checkpoints to uphold immune tolerance (7). These mechanisms involve immune suppression by Tregs and the induction of immune tolerance through various means such as anergy, deletion, and suppression of autoreactive immune cells. Tregs play a vital role in suppressing immune responses against self-antigens and promoting immune tolerance.

The *AIRE* gene is pivotal for central tolerance (14). It regulates the expression of tissue-specific antigens within the thymus, allowing developing T cells to encounter a diverse array of self-antigens (Figure 1). *AIRE* is predominantly expressed in medullary thymic epithelial cells in the thymus, which present a wide range of self-

antigens to developing T cells during their maturation process.

*AIRE* governs the expression of tissue-specific antigens that are typically limited to peripheral tissues (14). By facilitating the presentation of tissue-specific antigens in the thymus, *AIRE* aids in the elimination of self-reactive T cells. This process removes autoreactive T cells before they can trigger autoimmune responses.

The expression of *AIRE* in medullary thymic epithelial cells fosters the generation of a diverse and self-tolerant T cell repertoire by exposing developing T cells to a broad spectrum of self-antigens (15). This exposure assists in educating T cells to recognize and tolerate self-antigens, preventing autoimmune reactions.

*AIRE*-mediated tolerance induction involves a unique process called thymic epitope splicing. This mechanism enables the presentation of novel antigenic peptides resulting from the fusion of distinct tissue-specific antigens. Thymic epitope splicing expands the range of self-antigens encountered by developing T cells and enhances tolerance induction.

Furthermore, *AIRE* expression in medullary thymic epithelial cells promotes the differentiation of Tregs (15). Tregs are crucial in suppressing autoreactive T cells and maintaining immune tolerance. *AIRE*-driven Treg differentiation contributes to the control of autoimmune responses.

Hence, the expression of *AIRE* in medullary thymic epithelial cells primarily facilitates negative selection, allowing for the elimination of self-reactive T cells recognizing tissue-specific antigens within the thymus.

While central tolerance in the thymus eliminates self-reactive T cells during development, peripheral T-cell tolerance mechanisms operate outside the thymus to regulate and suppress autoreactive T-cell responses. *AIRE* also contributes to peripheral T-cell tolerance (Figure 2) by influencing the development and function of Tregs, thus controlling the equilibrium between effector and regulatory T-cell populations (16). *AIRE*'s impact on Tregs promotes immune tolerance in the periphery, ensuring the stability and suppressive activity of Tregs, which in turn control autoreactive T cells and maintain self-tolerance.

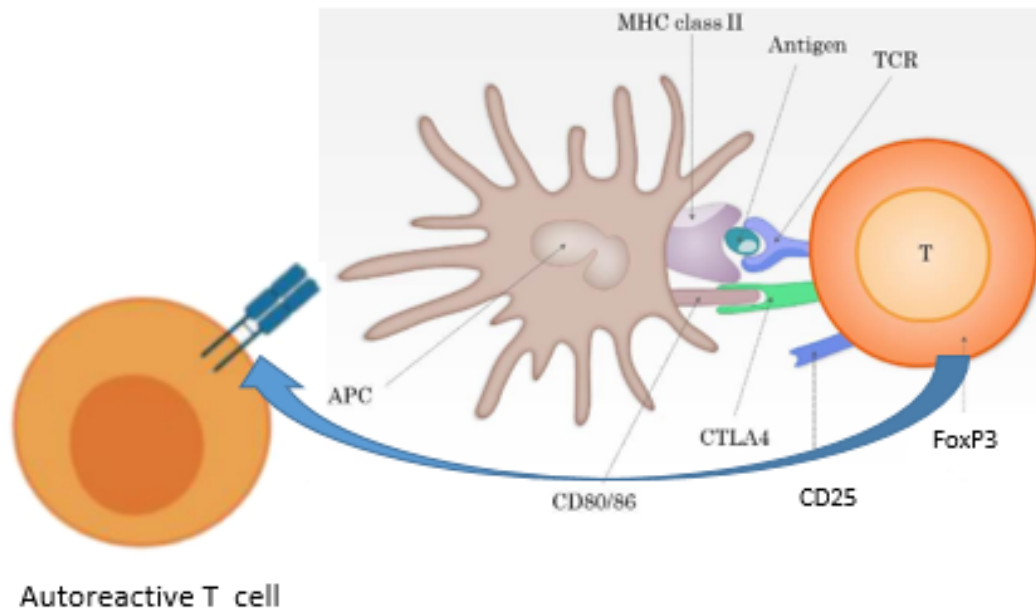


Figure 2 Peripheral T cell tolerance. It is illustrated how forkhead box P3 (FoxP3) expressing T regulatory cells (Tregs) effectively control autoreactive T cells through their interaction with antigen-presenting cells (APCs). Modified according to reference 1.

*AIRE*-expressing Tregs exert their suppressive effects through various mechanisms, including the secretion of immunosuppressive cytokines such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF- $\beta$ ). These cytokines inhibit the activation and proliferation of autoreactive T cells, preventing autoimmune responses.

*AIRE* also contributes to the induction of peripheral T-cell anergy, a state of functional unresponsiveness in T cells (14). Anergic T cells display reduced activation and effector functions, thus preventing autoimmune reactions.

In the periphery, *AIRE*-expressing cells can facilitate the deletion of autoreactive T cells through apoptosis. By selectively eliminating self-reactive T cells, *AIRE* helps maintain peripheral tolerance and prevents autoimmune pathologies.

*AIRE* can modulate TCR signaling in autoreactive T cells, leading to attenuated responses upon encountering self-antigens. This modulation helps prevent the activation and proliferation of autoreactive T cells, reinforcing peripheral tolerance.

While *AIRE* is primarily associated with the regulation of T-cell tolerance, emerging evidence suggests its involvement in the dysregulation of B cells, another crucial component of the immune system (17). *AIRE* is expressed not only in medullary thymic epithelial cells but also in a subset of B cells. Its expression in B cells suggests

a potential role in B-cell tolerance and regulation. *AIRE* may contribute to B-cell selection by influencing the negative selection of self-reactive B cells during their development. It promotes the removal of autoreactive B cells, preventing their maturation and activation. *AIRE*'s role in the thymus indirectly affects central B-cell tolerance by influencing T-cell selection and regulation. Through its impact on T-cell development and regulation, *AIRE* influences the development of T-dependent B-cell tolerance. The involvement of *AIRE* in peripheral B-cell tolerance is less understood but is currently receiving attention. Dysregulation of *AIRE* may contribute to the escape of autoreactive B cells from peripheral tolerance mechanisms, resulting in the production of autoantibodies and autoimmune responses (17).

*AIRE* dysregulation can lead to the breakdown of B-cell tolerance and the generation of autoantibodies targeting self-antigens. These autoantibodies contribute to tissue damage and inflammation observed in various autoimmune conditions. *AIRE* dysregulation can disrupt the balance between protective and pathogenic B-cell responses.

It may result in abnormal activation, proliferation, and survival of autoreactive B cells, thus exacerbating autoimmune processes.

*AIRE* dysfunction has been implicated in the development of other autoimmune diseases beyond APS-1, including autoimmune gastritis, vitiligo, and autoimmune hepatitis (18). Disruptions in *AIRE*-mediated tolerance mechanisms can contribute to the loss of self-tolerance and the onset of autoimmunity (Figure 3).

Multiple molecular mechanisms contribute to the breakdown of immune tolerance in APS (13). Dysregulation of immune checkpoints, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1), impairs the inhibitory signals necessary to control autoreactive T cells. This dysregulation can stem from genetic variations or acquired factors, further aggravating autoimmunity.

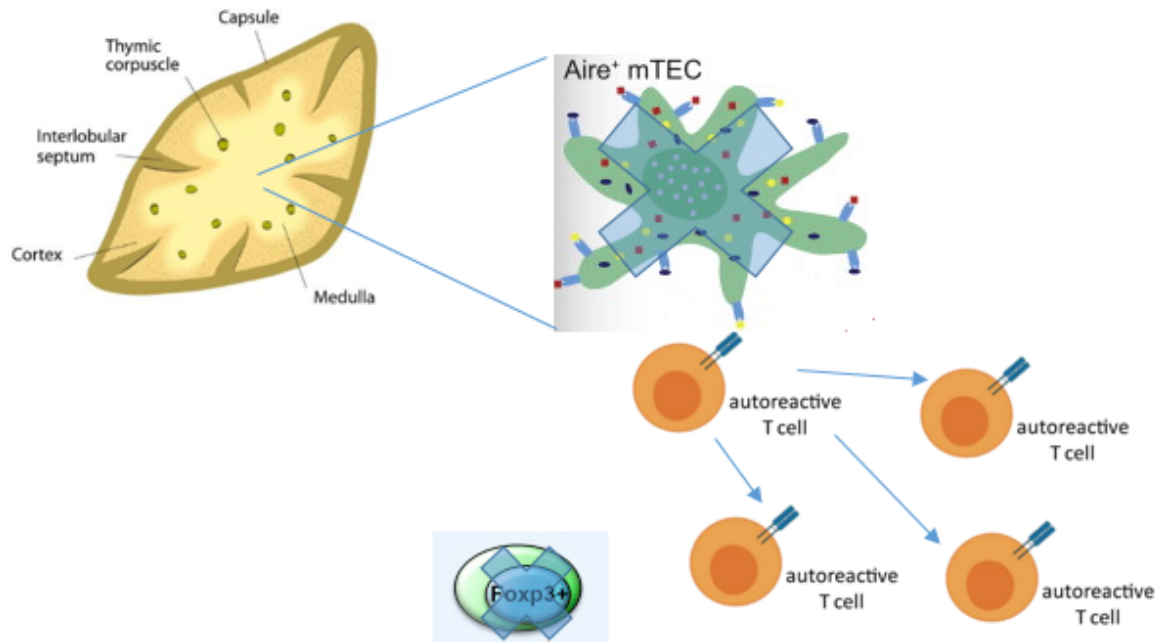


Figure 3 In the absence of the autoimmune regulator (*AIRE*), autoreactive T cells evade confinement within the thymus and migrate to the bloodstream and peripheral lymphoid organs. In these locations, they have the potential to initiate autoimmune reactions and trigger the development of APS-1. Additionally, the deficiency of T regulatory cells (Tregs) further contributes to the progression of autoimmunity. Modified according to reference 1.



## GENETICS OF AUTOIMMUNE POLYGLANDULAR SYNDROMES

Pathogenic variants in *AIRE* gene that codes for protein called the autoimmune regulator are responsible for development of APS-1 in 98% of patients. The mutated *AIRE* gene results in defect AIRE protein which causes autoimmune destruction of target organs by disturbing the immunological tolerance of the patients. The *AIRE* gene has 14 exons, encodes a 545-amino-acid protein of 57.5kDa which comprises four major domains (CARD - caspase recruitment domain, SAND - SP100, AIRE1, NucP41/ P75 and DEAF1, PHD1 - plant homeodomain 1, and PHD2 - plant homeodomain 1) that are be involved in nuclear transport, DNA binding, homomultimerization, and transcriptional activity (19).

*AIRE* gene is mostly expressed in epithelial antigen-presenting cells in the thymus, lymph nodes, foetal liver, pancreas, adrenal cortex, testes and peripheral blood cells (CD14-positive monocytes), but not in CD4-positive T-cells (19).

More than 100 different pathogenic variants of AIRE have been described so far (20). Clustering of same mutations has been observed in populations with higher frequency of APS-1 such as Sardinians (1:14 400), Finns (1:25 000), and Iranian Jews (1:6 000–9 000) (21, 22, 23). Most commonly reported *AIRE* mutation is R257X in exon 6 (24).

Even within the same family, APS-1 patients individuals with similar AIRE mutations have a wide array of clinical symptoms of varying severity. This shows that unexplained genetic variables may affect patient susceptibility to organ-specific symptoms of the disease. According to animal research, the immune checkpoint molecules Cbl-b and Lyn, in combination with AIRE, control autoimmune retinitis and exocrine pancreatitis, respectively.

In addition, the absence of the IL-2/STAT5 response regulatory element CNS0 in mice worsened autoimmune harm in various organs, including tissues that did not demonstrate autoimmunity when CNS0 or AIRE deficits were isolated (20, 24).

In addition, environmental variables may potentially play a role in the development of the clinical phenotype, as clinical characteristics differ across family members with the same mutation (24).

Although, APS-1 is classically inherited in autosomal recessive manner, recent advances suggest that certain heterozygous *AIRE* variants, particularly those in SAND and PHD1 domains, exert a dominant negative leading to nonclassical and

milder phenotype with comprising seemingly common forms of autoimmune diseases, including pernicious anaemia, vitiligo and autoimmune thyroid disease. These variants do not usually have the cytokine- and tissue antigen-targeted autoantibodies typical of traditional APS-1 patients. Given the relatively high minor allelic frequency of some of these mutations (e.g., p.V301M, p.R303Q) in the general population, genetic variation in AIRE may contribute more to the development of organ-specific autoimmune disorders than previously recognized (25).

Autoimmune endocrinopathy type 2, like other autoimmune illnesses, can be sporadic or familial. In many instances, various organ-specific manifestations of APS-2 exhibit shared genetic connections, with the bulk of these genes encoding crucial immune system regulatory components. Antigen presentation-involved MHC (HLA in humans) class II complex is the most significant gene related to APS-2. HLA-DR3 and/or DR4 haplotypes have been associated with Addison disease and type 1 diabetes, and latter research has shown that polymorphisms in DR3-DQ2 and DR4-DQ8 haplotypes confer an increased risk for type 1 diabetes, Addison disease, autoimmune thyroid disease, and celiac disease (26,27,28).

This indicates a shared immunogenetic etiopathogenesis and explains how a single individual might acquire several autoimmune illnesses. Graves' illness is associated with HLA-DR3, whereas Hashimoto's thyroiditis is associated with HLA-DR4 or DR5 (29,30).

Class, I molecules HLA-A and HLA-B have also been linked to type 1 diabetes risk (26). Although the relationship between APS II and certain HLA alleles is minimal, researchers have studied other susceptibility loci.

Genes encoding CTLA-4, the transcriptional regulator protein BACH2, protein tyrosine phosphatase nonreceptor type 22 (PTPN22), and CD25 (high-affinity IL-2 receptor) are also associated with APS II susceptibility (31).

Inactivating mutations of forkhead box P3 (*FOXP3*) gene lead to IPEX syndrome. It is a monogenic condition inherited in X-linked recessive manner. *FOXP3* gene provides the instruction for FOXP3, a transcription regulator that is crucial for the development and inhibitory function of regulatory T-cells (CD4+CD25+) important for the maintenance of tolerance to self tissue (10). To date more than 20 mutations in the *FOXP3* gene have been identified in patients with IPEX syndrome with no clear genotype-phenotype correlations. Impaired FOXP3 protein is unable to bind DNA in regulatory T cells leading to loss of immune suppressor function, immune

dysregulation and development of overwhelming autoimmunity in patients with IPEX syndrome (32).

## ENVIRONMENTAL FACTORS IN PATHOGENESIS OF APS

Viral infections, including enteroviruses and retroviruses, have been suggested as potential instigators of APS (33). Molecular mimicry and epitope spreading mechanisms can activate autoreactive immune responses, initiating or intensifying autoimmunity in individuals with genetic predisposition. Molecular mimicry refers to the resemblance between self-antigens and antigens derived from pathogens. In certain cases, infections can trigger an immune response that mistakenly targets self-antigens, leading to the development of autoimmune reactions. Molecular mimicry has been proposed as a potential mechanism contributing to the pathogenesis of APS, particularly in APS-2.

Various environmental factors, such as viral infections, hormonal fluctuations, and exposure to certain drugs or toxins, can act as triggers or modifiers in the development of APS (33). These factors have the potential to activate the immune system, modify immune responses, disrupt immune tolerance, or induce tissue damage, thereby contributing to the initiation or progression of autoimmune reactions in susceptible individuals.

The pathogenesis of APS is multifaceted, involving intricate interactions among genetic susceptibility, immune dysregulation, and environmental triggers. The specific combination of factors and their interactions likely differ among individuals, leading to the heterogeneity and diverse clinical manifestations observed in APS.

# CLINICAL APECTS OF AUTOIMMUNE POLYGLANDULAR SYNDROMES

## AUTOIMMUNE POLYGLANDULAR SYNDROME TYPE 1

In the conventional approach, the clinical diagnosis of APS-1 necessitates the presence of two out of the three fundamental components: chronic mucocutaneous candidiasis, autoimmune hypoparathyroidism, and autoimmune adrenal insufficiency (6,7).

Typically, mucocutaneous candidiasis manifests in infancy or early childhood as the initial sign, followed by the onset of hypoparathyroidism between the ages of 5 and 7, and finally, adrenocortical failure by the age of 13. The complete development of these cardinal features usually occurs within the first 25 years of life, while additional minor symptoms may persist until at least the fifth decade (7,34).

However, the clinical presentation in the early stages of APS-1 can exhibit variations, which can complicate the diagnostic process. Patients may present with a single cardinal feature along with several minor manifestations, or they may exhibit numerous minor manifestations along with ectodermal dystrophy. This variability further adds to the challenge of diagnosing APS-1, as affected individuals may seek medical attention for symptoms such as urticarial rashes, recurrent fever, abdominal distention, and growth failure (35).

### *1.1.1. Chronic mucocutaneous candidiasis*

**Chronic mucocutaneous candidiasis (CMC)** is frequently observed as the earliest indication of the condition, usually appearing within the first month of life or more commonly within the initial two years. This occurrence should serve as a warning sign to physicians regarding the likelihood of APS-1. In certain cases, CMC can manifest severely, characterized by persistent inflammation and the development of hyperkeratotic plaques. However, it often displays a moderate or intermittent nature and typically responds well to the periodic administration of systemic anti-fungal medications. Although some individuals may not experience CMC until adulthood, it remains the most prevalent cardinal symptom, affecting 73-100% of patients (7,36).

Candidal diaper rash is commonly identified during early childhood, while vulvovaginal candidiasis in females often emerges around the time of puberty. The colonization of *Candida* in the stomach can occasionally lead to abdominal discomfort and diarrhoea. A chronic nail infection caused by candidiasis can result in changes in nail colour, thickness, and erosion (36). If left untreated, chronic candidiasis can progress to squamous cell carcinoma, necessitating rigorous treatment.

An urticarial eruption, known as "APECED rash," along with CMC, represents the most common initial manifestations of the disease. This rash typically presents as a self-limited, non-itchy, and often recurring maculopapular rash with a distinct histological appearance characterized by a combination of neutrophilic and lymphocytic dermatosis. It is associated with NLRP3 inflammasome activation but does not involve eosinophilic infiltration or vasculitis. Peak incidence of this rash is between the ages of 2 and 11 years (6).

#### *1.1.2. Endocrine manifestations*

**Hypoparathyroidism.** Approximately 75 to 95 % of patients experience hypoparathyroidism, with men generally exhibiting a slightly lower prevalence and a later onset age (6, 7). Hypoparathyroidism manifests with symptoms such as tetany, paraesthesia, diarrhoea, and convulsions. The condition can be triggered by fasting, a calcium-deficient diet, or excessive phosphate intake. However, it typically arises after the onset of mucocutaneous candidiasis but before puberty. Roughly, 33% of patients with APS-1 develop hypoparathyroidism by the age of 5, 66% by the age of 10, and nearly 85 % by the age of 30 (7).

Seizures, carpopedal spasms, muscular twitching, and laryngospasm can serve as initial indicators of APS-1. However, these symptoms may be overshadowed by the relatively high levels of calcium associated with adrenal insufficiency. Probable explanation of this phenomenon is either reduced glomerular filtration caused by hypovolemia or increased activity of 1-hydroxylase (7,36).

**Primary adrenocortical failure** frequently presents as the third primary symptom of APS-1 typically occurring most frequently around the age of 13 (6,36).

Within most APS-1 patient groups, adrenal failure is less common compared to the other main symptoms. Development of adrenal insufficiency is gradually progressing and deficiencies in cortisol and aldosterone may appear at different times, with intervals of up to 20 years. Symptoms of this potentially life-threatening condition are unspecific and characterized by fatigue, weight loss, increased pigmentation of the

skin, low blood pressure, salt cravings, and abdominal discomfort.

Autopsies of affected individuals reveal atrophy of the adrenal glands, almost destruction of the adrenal cortex, and significant infiltration of inflammatory cells. Inadequate cortisol levels lead to increased production of proopiomelanocortin in the pituitary gland, which is cleaved into adrenocorticotrophic hormone (ACTH) and melanocyte-stimulating hormone. Diagnostic indicators of adrenal insufficiency include normal or low cortisol levels, elevated ACTH levels, and inadequate cortisol response to synthetic ACTH stimulation. The prevalence of adrenal insufficiency is 57 percent, with no gender bias (6,7).

Acute adrenal failure with development of adrenal crisis is a major cause of mortality (37). Among Finnish patients with APS1, who serves as a reference cohort due to their number, genetic homogeneity, centralized data, and regular updates, acute adrenal failure occurs in 84% of cases. To obtain this data, Finnish researchers estimate the onset of each APS1 component within predetermined age ranges, assuming all patients live up to the age of 50 (21).

Managing adrenal failure is challenging due to the inability of replacement therapy to replicate the normal hormone release patterns, thus posing the risk of inadequate or excessive dosage. In APS-1, this challenge is further compounded by the presence of additional hormonal deficiencies, particularly hypoparathyroidism and autoimmune thyroid disease (38).

Furthermore, the early manifestations of adrenal insufficiency may resemble psychiatric or gastrointestinal disorders, and patients may exhibit behavioural abnormalities, as well as symptoms such as nausea, vomiting, stomach discomfort, and diarrhoea (37,38).

Close examination often reveals hyperpigmentation (caused by elevated MSH levels) in areas not exposed to sunlight and postural hypotension over time. The presence of these symptoms, along with hypotonic dehydration, should raise suspicion of Addison's disease. If left undiagnosed and untreated, an adrenal crisis can occur, characterized by hyponatremia, hyperkalemia, acidosis, hypotension, and hypoglycemia, which can be fatal. Prompt and effective treatment with intravenous glucocorticoids and isotonic fluids is crucial in managing an adrenal crisis (6, 37).

**Primary hypogonadism.** Premature ovarian insufficiency (POI), previously known as premature ovarian failure or early menopause, refers to the loss of ovarian function before the age of 40. Clinical symptoms of POI, caused by estrogen deficiency, may

include the absence of menstruation (amenorrhea), infrequent menstruation (oligomenorrhea), vasomotor instability (hot flashes, night sweats), sleep disturbances, vulvovaginal atrophy, altered urinary frequency, painful sexual intercourse (dyspareunia), decreased libido, and fatigue (39).

The incidence of POI associated with APS varies depending on the specific type of APS, with APS-1 have the highest incidence of POI, exceeding 40%. In individuals with both Addison's disease and POI, Addison's disease often develops before the onset of POI. The coexistence of ovarian and adrenal failure in autoimmune polyglandular syndromes emphasizes the importance of evaluating for adrenal autoimmunity and evidence of adrenal insufficiency in all patients with POI, even in the absence of characteristic features indicative of APS (1,39).

**Type 1 diabetes mellitus.** Compared to other APS, T1D is less common in APS-1. Approximately 90% of individuals with APS-1 related T1D have at least one autoantibody associated with beta-cell autoimmunity (1,26). These autoantibodies tend to develop sequentially, with insulin autoantibodies typically being the first to appear, especially in young children.

The presence of GADA indicates susceptibility to widespread autoimmunity, while IA2A positivity may specifically indicate beta-cell destruction. Preclinical signs of beta-cell autoantibodies can be detected years before the actual diagnosis of diabetes (40). Individuals with Type 1 diabetes have an increased risk of developing secondary autoimmune diseases such as autoimmune thyroid disease, celiac disease, autoimmune gastritis, Addison's disease, and vitiligo, with the peak incidence occurring during middle age. Up to fifty percent of T1D patients with thyroid antibodies eventually develop clinical autoimmune thyroid disease.

It is advocated to detect antibodies and latent organ-specific dysfunction early on to alert clinicians and take proactive measures to prevent the progression of the disease. Patients and their family members should be educated on how to recognize the symptoms of underlying diseases.

At the time of clinical diagnosis of Type 1 diabetes, tests for TPO antibodies, PCA, EmA-IgA, and 21-OHAA should be performed. Starting one year after diagnosis, T1DM patients should undergo annual evaluations for thyroid dysfunction using TSH, T4, and TPO levels, as well as autoantibodies (40).

**Autoimmune thyroid disease** can be found in about 20% patients with APS-1 with peak incidence between 10 to 17 years. Type 1 diabetes and thyrogastric

autoimmunity (the descriptive term for the combination of autoimmune thyroid disease and atrophic gastritis) are associated with APS-1 but are substantially less common than in APS-2 (29).

### *1.1.3. Non-endocrine manifestations*

Non-endocrine symptoms of APS-1 include urticarial eruptions (66%), hepatitis (43%), gastritis (48%), intestinal dysfunction (80%), Sjogren-like syndrome (43%), and pneumonitis (40%) and are usually present in about 80% patients prior to satisfying the diagnostic dyad criteria for APS-1 (1,4,7). Interestingly, North and South American patients exhibit nonendocrine symptoms substantially earlier and more frequently than European cohorts. It is uncertain if genotype-phenotype relationships can explain the higher incidence of nonendocrine symptoms in the American population (4,7).

#### *1.1.3.1. Gastrointestinal components*

Although gastrointestinal symptoms are not a prominent feature of APS-1, they can have a significant impact on the well-being of children and are often overlooked due to the prevalence of other associated conditions (1,7).

Understanding the symptoms and underlying causes is limited, and these symptoms frequently coexist with other disorders that present similar manifestations, making diagnosis challenging.

**Chronic atrophic gastritis**, an autoimmune disease that specifically affects gastric parietal cells and intrinsic factor, is observed in up to one-third of APS-1 patients.

Its incidence peaks between the ages of 10 and 20. This condition can lead to megaloblastic anemia (pernicious anemia) or microcytic anemia due to vitamin B12 deficiency in early adulthood. Vitamin B12 deficiency can also result in peripheral neuropathy, spinal cord degeneration, and alterations in personality (1,6,7).

**Malabsorption** is believed to be caused by villus atrophy, exocrine pancreatic insufficiency, and intestinal infections. In some cases, it can manifest as an unusually early presentation of APS-1 during the first year of life, affecting approximately 10% of individuals, with chronic diarrhoea being the most common initial symptom.

**Cholelithiasis**, the presence of gallstones, is observed in up to 40% of ultrasound exams but is typically asymptomatic and is thought to be associated with abnormalities in the enterohepatic circulation (6).

**Chronic active hepatitis** affects approximately 5-30% of APS-1 patients. The clinical course varies, ranging from chronic liver disease without noticeable symptoms in most individuals to potentially life-threatening cirrhosis or fulminant hepatic failure (41). In



APS-1, the incidence of autoimmune hepatitis varies by ethnicity, with Finland having highest incidence. Liver failure or its treatment is a significant cause of death among APS-1 patients. In a relatively large study conducted by Perheentupa et al., three of sixteen patients with APS-1 related autoimmune hepatitis died from liver failure or sepsis following severe immunosuppression (42).

#### *1.1.3.2. Other non-endocrine manifestations*

**Ectodermal dystrophy** manifests as abnormalities in the fingernails and tooth enamel. The presence of pitted nails, which can be challenging to differentiate from fungal infections, is a frequent diagnostic feature for APS-1, independent of candida infection (1,4).

Hypoplastic dental enamel is observed in 40 to 75% of individuals affected by APS-1 (6). While initially believed to primarily affect permanent teeth, hypoplastic changes have also been observed in deciduous teeth. Enamel hypoplasia may occur before the onset of hypoparathyroidism and is not correlated with blood calcium levels.

Calcified plaques are present on the tympanic membranes of approximately one-third of the population, even in the absence of ear infections. Although the specific antibody target has not yet been identified, ectodermal dystrophy is presumed to have an autoimmune origin.

**Keratoconjunctivitis** occurs in 10 to 40% of individuals with APS-1 and may present as an early symptom of the condition. Common initial signs include severe photophobia, blepharospasm, and excessive tearing. Corneal scarring, permanent visual impairment, and even blindness can occur. After approximately 10 years from the onset of the disease, some individuals enter a quiescent phase. Ocular abnormalities in APS-1 do not correlate with systemic symptoms. **Autoimmune retinopathy**, characterized by degeneration of the photoreceptor cells and subsequent vision loss, has also been observed as part of the APS1 phenotype.

**Vitiligo**, a condition characterized by depigmented patches on the skin, can develop at any age but is mostly seen in childhood, affecting up to a quarter of individuals with APS-1. The severity of vitiligo can vary significantly and tends to worsen over time.

**Alopecia** affects around one-third of individuals and can affect all body regions to variable degrees, it can also progress swiftly at any age (1,4,6,37,42).

## AUTOIMMUNE POLYGLANDULAR SYNDROME TYPE 2

Autoimmune polyglandular syndrome type-2 is estimated to affect 4-5 individuals per 100 000 population. Clinical manifestations can occur at any age, with the highest prevalence observed in early adulthood, particularly in the fourth decade. It is less common among children and adolescents (1,26).

Among the various autoimmune polyendocrinopathies, APS-2 is the most frequent and affects both sexes, with a female-to-male ratio of 3:1 (excluding cases of type 1 diabetes and autoimmune thyroid disease). This predominance of females in APS-2 is primarily attributed to the higher incidence of autoimmune thyroid disease in females (4,26).

The three main diseases observed in APS-2 are Addison's disease, which is always present, autoimmune thyroid disease, which affects 70-90% of patients, and type 1 diabetes, prominent in 20-50% of cases. However, only approximately 10% of individuals with APS-2 exhibit all three characteristics. Around two-thirds of patients with autoimmune Addison's disease carry the genetic susceptibility for APS-2. While adrenal failure is the primary endocrine issue in nearly half of the patients, other minor components of APS-2 are typically present when adrenal failure is diagnosed (1,4).

Classic presentation of adrenal insufficiency in APS-2 includes symptomatic hypotension. Patients with Addison's disease also tend to have hyperpigmentation or vitiligo as well as a several-year history of intermittent, severe hypoglycaemia and fatigue. In those who already have T1D, recurrent hypoglycaemias and a decrease in total daily dose of insulin may point to evolving adrenal insufficiency. On the other hand side, the newly onset of hyperthyroidism or levothyroxine substitution in patients with ATD can lead to development of adrenal crisis in patients with Addison's disease (1,4,5).

Despite the high prevalence of thyroid autoantibodies, less than 20% of individuals with these antibodies demonstrate thyroid dysfunction, as indicated by an elevated concentration of thyroid-stimulating hormone. About 10 to 15% of APS patients, incorrectly diagnosed with type 2 diabetes owing to the onset of diabetes after age 40, have progressively increasing autoimmune diabetes (also referred to as latent autoimmune diabetes of adults or LADA) (1,4).

Primary hypogonadism leading to premature ovarian failure and secondary amenorrhoea develops in about 10% females with APS-2 under age of 40 years. Testes are rarely affected. Anterior pituitary failure due to lymphocytic hypophysitis

can also be observed (43). While pathognomonic in patients with APS-1, hypoparathyroidism is very rare but described in APS-2 patients. Hypocalcaemia is usually seen as part of vitamin D deficiency in patients with coeliac disease (4,39).

Between 20% and 40% of vitiligo patients exhibit another component of APS II, with thyrogastric autoimmunity being the most prevalent. Asymptomatic individuals with vitiligo require autoantibody screening to diagnose concurrent autoimmunity. Segmental vitiligo with dermatomal involvement is not associated with autoimmunity (44).

Up to 15% of individuals with alopecia (areata, totalis, universalis) and 5% of their first-degree relatives experience thyroid dysfunction. Although cutaneous manifestations like vitiligo or alopecia are more common in APS-1 than APS-2, the majority of patients presenting with either manifestation plus another autoimmune disease are classified as having incomplete APS-2 (4).

Around 30% of individuals with myasthenia gravis, an autoimmune disease characterized by the presence of anti-acetylcholine receptor autoantibodies and muscle weakness worsening during muscle contraction, also develop ATD. Both Hashimoto thyroiditis and Graves' disease can coexist with myasthenia gravis. Patients with myasthenia gravis and ATD tend to have a lower incidence of thymic abnormalities, acetylcholine receptor chain autoantibodies, and milder myasthenia symptoms. The occurrence of ocular myasthenia is higher in individuals with Graves' disease (45).

## IPEX SYNDROME

The disease presents very early in life and affected infants show overwhelming and almost fulminant autoimmunity. The first symptom is usually intractable diarrhoea due to autoimmune enteropathy leading to malabsorption, malnutrition failure to thrive and high mortality in these patients. The most consistent pathological finding is total villous atrophy of the small intestine, with inflammatory cell infiltration of the lamina propria. Dermatitis is a very common feature of IPEX syndrome and it may be eczematiform, ichthyosiform or psoriasiform. Type 1 diabetes mellitus can develop as early as first week of life. More than 90% patients with IPEX will develop T1D by the end of the first year of life, and about 50% will develop ATD. Other associated diseases include autoimmune haemolytic anaemia and thrombocytopenia, membranous nephropathy and facial myopathy. Prognosis is poor with only small number of patients survive into

childhood or adolescence, usually those with successful bone marrow transplantation (10, 11).

## DIAGNOSTIC APPROACH AND FOLLOW-UP

The approach to diagnosing polyglandular syndromes is three- fold:

- autoantibody screening to verify the autoimmune nature of the suspected endocrinopathy and test for the involvement of other organs and tissues
- full assessment of endocrine function in patients with confirmed autoantibodies and autoantibody-negative subjects in whom disease is suspected clinically
- molecular analysis to confirm the diagnosis, and screen siblings and other relatives for their potential carrier status.

To limit related morbidity and possible severe complications, it is essential to diagnose multiorgan autoimmune disorders before their onset of symptoms. Maintaining a high degree of suspicion while doing a comprehensive history and physical examination is vital. In addition, the presence of a family history of multiorgan autoimmune illness should increase the possibility of polyglandular disorders.

In patients with APS-1 it may take a substantial length of time for the traditional trio of symptoms to manifest, resulting in widespread misdiagnosis. To achieve an early and accurate diagnosis, healthcare professionals must maintain a high level of suspicion, especially in patients under 30 years old who present with mucocutaneous candidiasis, hypoparathyroidism, primary adrenal failure, ectodermal dystrophy, keratoconjunctivitis, prolonged diarrhoea, vitiligo, or autoimmune hepatitis. Patients with ectodermal dystrophy should have a complete screening for additional indications, giving specific attention to oral or ocular manifestations, as well as the often-inconspicuous nail abnormalities.

It is vital to be watchful for all symptoms, major and small, linked with APS-1 and to initiate genetic and autoantibody screening with a low threshold. Patients often see dermatologists, gastroenterologists, dentists, or ophthalmologists before exhibiting the characteristic endocrine problems.

Chronic candidiasis affects numerous sites, such as the skin, nails, tongue, and mucous membranes, and is diagnosed based on clinical examination findings and

Candida species-positive cultures. If left untreated, candidiasis may cause oesophageal strictures and contribute to the development of squamous cell carcinomas of the oral mucosa and/or oesophagus (7, 8). Patients with APS-1 must thus get therapy for acute mucosal fungal infections. Acute bouts of mucosal candidiasis often respond well to four-week induction treatment. This therapy aims to reduce the chance of infection recurrence after treatment has been discontinued, and it often includes the administration of a triazole antifungal medicine such as fluconazole (1,7,8). Hypoparathyroidism presents predominantly via symptoms induced by hypocalcaemia, which results from insufficient parathyroid hormone secretion. Patients may have tetany, convulsions, altered mental state, congestive heart failure, or stridor. Examining Chvostek's sign and Trousseau's sign should be part of the physical examination (3).

Diagnosing adrenal insufficiency may be difficult due to the slow onset of symptoms. The clinical manifestations of adrenal insufficiency vary from asymptomatic to life-threatening, generally developing after stressful events such as illness, surgery, or accidents. Chronic tiredness, muscular weakness, lack of appetite, weight loss, nausea, vomiting, diarrhoea, darkening of the skin, and low blood pressure are among the classic symptoms (4,7).

Not only do many children present with many minor symptoms instead of one of the three main components, but there is also a substantial time gap between the onset of the first and second manifestation, which contributes to delayed diagnosis. Up to two-thirds of patients are not diagnosed until they are hospitalized with acute adrenal insufficiency or hypocalcaemic crisis, and approximately half of these patients already display at least one severe APS-1 symptom.

Addison's disease, a kind of adrenal insufficiency, must be diagnosed as soon as possible. All patients with APS-1 are at risk and should have yearly ACTH and renin activity testing. Rapid adrenal insufficiency may develop in APS1, and yearly evaluations may not be adequate to avoid acute manifestations. If adrenal antibody testing is positive, electrolytes, early morning cortisol, ACTH concentrations, and renin activity should be monitored every six months, and a Synacthen test should be conducted if clinical or biochemical concerns exist (7).

When **APS-2** is suspected, a comprehensive evaluation of endocrine function is necessary. Long-term surveillance is required due to the unpredictability of the course and timing of the development of numerous diseases within APS-2. It is necessary to

maintain a high level of clinical suspicion, especially in individuals who have not yet shown adrenal insufficiency or diabetes. To decrease associated morbidity and mortality, presymptomatic autoimmunity must be detected promptly. Even though there is often a latent period of months or even years in which no symptoms are apparent, the formation of organ-specific autoantibodies is strongly linked to disease progression (1,3,7). A lack of autoantibodies does not rule out the potential for disease. The presence of P450c21 autoantibodies in individuals with clinical and biochemical signs of adrenal insufficiency is indicative of the autoimmunity of the disease.

When diagnosing type 1 diabetes, it is recommended to test for thyroid peroxidase (TPO) and glutamic acid decarboxylase 65 (GAD65) autoantibodies. If the first screening is negative, periodic reevaluation should be considered, maybe every two to three years (7).

The detection of insulin and IA-2 autoantibodies is a sensitive predictor T1D in children and adolescents with Addison's disease, especially when both autoantibodies are present. In these situations, a fasting blood glucose test, an HbA1c test, and in certain instances a glucose tolerance test should be done (40).

To facilitate early diagnosis of thyroid disease, the thyroid function of all individuals with T1D and Addison's disease should be examined at least once a year.

Before an increase in gonadotropin levels, females with Addison's disease and APS2 may be identified as high-risk for primary hypogonadism. These individuals may be suitable candidates for the cryopreservation of ovarian tissue (39).

Additionally, young patients with vitiligo and young women with primary or secondary amenorrhea or premature ovarian failure should be screened for APS2-associated illnesses.

Children with T1D should be tested for P450c21 autoantibodies since positive adrenal autoantibodies are a strong predictor of subsequent adrenal insufficiency. Using an ACTH stimulation test, electrolyte analysis, and plasma renin activity test, patients with preclinical adrenal insufficiency may be diagnosed in individuals with P450c21 autoantibodies. If the results are normal, the ACTH stimulation test must be conducted annually, along with periodic postural blood pressure and electrolyte checks (47).

Children with T1D had a greater rate of IgG-TTG antibodies linked with malabsorption than adults with Addison's disease or T1D, which is equivalent to the general population (40).

Due to their great incidence in healthy first-degree relatives and the general population, gastric parietal cell or intrinsic factor autoantibodies have little predictive value for autoimmune gastritis and pernicious anaemia (around 5-10 %) (1, 4).

Due to the significant familial propensity seen in APS-2, family members should also be screened for endocrine abnormalities.

## AUTOANTIBODIES

Autoantibodies in APS are mostly IgG antibodies that bind to self-antigens. As discussed before, the detection of autoantibodies in APS serves a variety of important functions. Firstly, it permits the creation of an accurate autoimmune diagnosis. Secondly, the finding of autoantibodies in asymptomatic individuals implies an increased risk of developing clinical illness in the future, necessitating greater surveillance of these patients. Thirdly, the existence of an autoantibody or a single autoimmune illness in a person may imply an increased risk for additional autoimmune disorders.

## NONORGAN-SPECIFIC AUTOANTIBODIES

- Antiinterferon Autoantibodies - IFN- $\alpha$  and IFN- $\omega$  are essentially uniformly present in APS-1 individuals, irrespective of clinical presentation, mutation type, illness duration, gender, or ethnicity. Autoantibodies against interferons have been found during the first year of life, even before the development of symptoms. These autoantibodies persist throughout the disease's progression, becoming detectable for over 30 years. They have not been discovered in individuals with isolated autoimmune Addison's disease or APS-2, nor in unaffected heterozygotes, showing that they are illness-specific to APS-1 alone. This finding provides a significant diagnostic tool for diagnosing APS-1 at the prodromal stage or in atypical instances since anti-interferon autoantibodies have a sensitivity, specificity, and predictive value of more than 98%. In addition, it presents the fascinating potential that these autoantibodies may directly affect the expression of the immune response. The early development of these antibodies shows that the autoimmune process begins soon after or possibly before birth. Surprisingly, all 60 Finnish APS I patients and all 16 Norwegian APS-1 patients studied had substantial levels of interferon-specific autoantibodies (48).

- Autoantibodies to IL-17A, IL-17F, and IL-22- A research including 33 individuals diagnosed with APS-1 and mucocutaneous candidiasis indicated the presence of significant levels of IL-17A, IL-17F, and IL-22-targeting autoantibodies. In contrast, none of the 37 control persons or 103 patients with isolated autoimmunity tested positive for these antibodies. In the following cohort studies, these results have been verified further (49).

### ORGAN-SPECIFIC ANTIBODIES

- Adrenal cytoplasmic autoantibodies - ACA are found in all forms of autoimmune Addison's disease, including isolated Addison's disease, APS-1, and APS-1. Within three years, around fifty percent of asymptomatic individuals who test positive for ACA develop Addison's disease. In addition, ACA is observed in 75% to 100% of individuals who have recently had Addison's disease or are on the verge of developing the condition (4, 50).

- Adrenal enzyme autoantibodies- The autoantigen 21-Hydroxylase (P450c21) is a major target for autoantibodies in the sera of Addison's disease patients. P450c21 autoantibodies are an even more sensitive sign of the illness than anti-cortical autoantibodies.

Approximately 75% of individuals with APS-1 and APS-2 exhibit positive P450c21 autoantibodies. A combination of elevated ACA and P450c21 autoantibodies is linked with a more severe impairment of adrenocortical function and an increased risk of developing Addison's disease. Typically, clinical criteria or, in asymptomatic individuals, the presence of autoantibodies associated with APS-2 are used to differentiate between APS-1 and APS-2 (46, 50).

- Steroidal cell/ gonadal autoantibodies- - Certain individuals with ACA have sera antibodies that are cross-reactive with tissues involved in the reproductive-steroid synthesis, namely the theca interna of the Graafian follicle, Leydig cells of the testis, and/or the syncytiotrophoblastic layer of the placenta. When these sera detect antigens in both adrenal and reproductive steroid-producing tissues and cannot be absorbed by adrenal extracts, they are known as steroidal cell/gonadal antibodies (SCA). The presence of SCA is associated with an increased chance of developing primary autoimmune gonadal failure in asymptomatic individuals. In women, this often appears as primary amenorrhea or early menopause, although in men, no symptoms are typically present. In addition, SCA has been reported to predict gonadal failure in women with APS (autoimmune polyglandular syndrome) who had regular menstrual



cycles originally. In short research including 11 female patients with APS-1, all 11 (100%) who tested positive for SCA throughout a 12-year follow-up period developed primary ovarian failure. When ACA is present but SCA is absent, gonadal failure is uncommon. Like other organ-specific autoimmune disorders, autoimmune ovarian failure is histologically defined by the infiltration of inflammatory cells into the ovaries (51, 52).

- Autoantibodies in hypoparathyroidism - The existence of NALP5 autoantibodies in patients with idiopathic hypoparathyroidism is unusual (0.69 %), and their capacity to effectively identify APS I-associated hypoparathyroidism is only around 50%, with a sensitivity of 26% (53). In contrast, individuals with autoimmune hypoparathyroidism have CaSR-activating autoantibodies. CaSR autoantibodies have a better sensitivity (83%) for identifying autoimmune hypoparathyroidism than NALP autoantibodies. The sensitivity of CaSR autoantibodies, however, remains modest at 39% (54, 55).

## TREATMENT

The treatment approaches for the component disorders of APS-1, APS-2 or IPEX syndrome, such as adrenal insufficiency, hypothyroidism, type 1 diabetes, enteropathy, iron-deficiency anaemia, and pernicious anaemia, remain consistent whether these conditions occur independently or in conjunction with other ailments.

Given the diverse range of pathologies that can manifest in individuals with APS, a multidisciplinary team is essential for their evaluation and management.

The treatment of APS-1 patients with several individual illnesses is identical to the treatment of patients with isolated disorders, with the difference that polypharmacy is routinely adopted and therapy may be exacerbated by malabsorption (6). Especially among adolescents, ensuring patient adherence to therapy may be a difficult task. Immunosuppressive therapy with glucocorticoids may further complicate matters, and in more severe situations, such as fulminant hepatic failure, immunosuppressant combinations are typically required. Numerous individuals need psychological

treatment, as they typically exhibit high rates of despair, social isolation, alcoholism, and drug abuse, especially during the transition to adulthood (7).

Specific treatments for endocrine diseases including hypoparathyroidism and Addison's disease entail hormone replacement. In addition, many major non-endocrine symptoms of APS-1, such as autoimmune hepatitis and severe intestinal dysfunction, need immunosuppressive therapy (7).

Modern immunosuppressants are typically well tolerated and may result in a dramatic improvement in several features of APS-1, including autoimmune hepatitis and intestinal dysfunction. An increasing amount of research suggests that early immunosuppression, along with adequate monitoring, is suitable for seriously afflicted individuals (1,4,6,7).

Individuals affected by APS-1 may exhibit asplenia or functional hyposplenism, leaving them susceptible to severe infections. Therefore, prompt administration of vaccinations and, if required, prophylactic antibiotics should be included in the treatment plan for these individuals (47).

Regardless of whether the illness occurs alone or in combination with other ailments, the administration of hormone replacement therapy and other therapies for the numerous diseases included by APS2 stays the same. Such drugs should be commenced upon diagnosis; however, some illness combinations need special consideration. In individuals with untreated and undiagnosed adrenal insufficiency, levothyroxine medication for hypothyroidism might provoke a life-threatening adrenal crisis (8). To avoid an adrenal crisis, physicians must maintain a high degree of suspicion for concomitant adrenal insufficiency in hypothyroid patients. In individuals with adrenal insufficiency and persistent hyperthyroidism, hyperthyroidism enhances cortisol clearance. Therefore, glucocorticoid replacement treatment should be at least increased until euthyroidism is achieved. For individuals with type 1 diabetes, hyperthyroidism is related to a loss in insulin sensitivity and a worsening of glycemic management in T1D patients. In addition, in T1D patients, a decrease in insulin needs or an increase in hypoglycemia incidence may act as early markers of adrenocortical failure (8,9).

The primary therapeutic approach to the most severe form of APS - IPEX syndrome hematopoietic stem cell transplantation leading to better long-term survival (56). These patients need continuous supportive and often inpatient care especially in early

ages (10). Those experiencing severe enteropathy since early infancy are not able to thrive without total parenteral nutrition or elemental enteral nutrition. Low-carbohydrate-containing formula and fluids are used in infants with neonatal T1D. L-thyroxin is necessary for patients with hypothyroidism due to AITD. Other replacement therapies with glucocorticoids and platelet transfusion are used in thrombocytopenia. Autoimmune granulocytopenia and pancytopenia need special hematologic procedures. Eczema is treated with antiallergic ointments, balms, and steroids (10, 11, 56).

## ACKNOWLEDGMENTS

I am incredibly grateful to all those who have played a part in the successful completion of this project. I would like to express my deepest appreciation to everyone involved.

First and foremost, I want to convey my sincere gratitude to my supervisor, Dr. sc. Katja Dumić Kubat, for their unwavering guidance and expertise. Their mentorship and valuable feedback have played a pivotal role in shaping this project .

Furthermore, I would like to acknowledge the support and assistance provided by the faculty and staff at the University of Zagreb.

To my friends and colleagues who have accompanied me on this journey, I extend my sincere appreciation.

Finally, I would like to express my gratitude to my family and my partner, Ophir, for their unwavering support during challenging times and for being instrumental in my personal and professional growth, helping me become the person and doctor I am today.

## REFERENCES

1. Husebye ES, Anderson MS, Kämpe O. Autoimmune Polyendocrine Syndromes. *N Engl J Med*. 2018;378(12):1132-41.
2. Schmidt MB. Eine biglandulare Erkrankung (Nebennieren und Schilddrüse) bei Morbus Addisonii. *Verh Dtsch Ges Pathol*. 1926;21:212-21.
3. Paparella R, Menghi M, Micangeli G, Leonardi L, Profeta G, Tarani F, et al. Autoimmune Polyendocrine Syndromes in the Pediatric Age. *Children*. 2023; 10(3):588. doi: 10.3390/children10030588.
4. Michels AW, Gottlieb PA. Autoimmune polyglandular syndromes. *Nat Rev Endocrinol*. 2010;6:270-7.
5. Frommer L, Kahaly GJ. Autoimmune Polyendocrinopathy. *J Clin Endocrinol Metab*. 2019;104(10):4769-82.
6. Ferré EMN, Schmitt MM, Lionakis MS. Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy. *Front Pediatr*. 2021;9:723532. doi: 10.1016/j.isci.2023.106818.
7. Bjørklund G, Pivin M, Hangan T, Yurkovskaya O, Pivina L. Autoimmune polyendocrine syndrome type 1: Clinical manifestations, pathogenetic features, and management approach. *Autoimmun Rev*. 2022;21(8):103135.
8. Singh G, Jialal I. Polyglandular Autoimmune Syndrome Type II. [Updated 2023 Jan 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK525992/>
9. Betterle C, Lazzarotto F, Presotto F. Autoimmune polyglandular syndrome Type 2: the tip of an iceberg? *Clin Exp Immunol*. 2004;137(2):225-33.
10. Ben-Skowronek I. IPEX Syndrome: Genetics and Treatment Options. *Genes (Basel)*. 2021;12(3):323.
11. Park JH, Lee KH, Jeon B, Ochs HD, Lee JS, Gee HY, et al. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome: A systematic review. *Autoimmun Rev*. 2020;19(6):102526.

12. Amendt T, Jumaa H. Adaptive tolerance: Protection through self-recognition. *Bioessays*. 2022;44(3):e2100236. doi: 10.1002/bies.202100236
13. Halonen M, Eskelin P, Myhre A-G, Perheentupa J, Husebye ES, Kämpe O, Rorsman F, Peltonen L, Ulmanen I, Partanen J. AIRE Mutations and Human Leukocyte Antigen Genotypes as Determinants of the Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy Phenotype. *J Clin Endocrinol Metab*. 2002;87:2568-74.
14. Chan AY, Anderson MS. Central tolerance to self revealed by the autoimmune regulator. *Ann N Y Acad Sci*. 2015;1356(1):80-9.
15. Anderson MS, Venanzi ES, Chen Z, Berzins SP, Benoist C, Mathis D. The cellular mechanism of Aire control of T cell tolerance. *Immunity*. 2005;23:227-39.
16. Gardner JM, Devoss JJ, Friedman RS, Wong DJ, Tan YX, Zhou X, Johannes KP, Su MA, Chang HY, Krummel MF, Anderson MS. Deletional tolerance mediated by extrathymic Aire-expressing cells. *Science*. 2008;321(5890):843-7.
17. Yamano T, Nedjic J, Hinterberger M, Steinert M, Koser S, Pinto S, Gerdes N, Lutgens E, Ishimaru N, Busslinger M, Brors B, Kyewski B, Klein L. Thymic B Cells Are Licensed to Present Self Antigens for Central T Cell Tolerance Induction. *Immunity*. 2015;42(6):1048-61.
18. Bruserud Ø, Oftedal BE, Wolff AB, Husebye ES. AIRE-mutations and autoimmune disease. *Curr Opin Immunol*. 2016;43:8-15.
19. Björnses P, Halonen M, Palvimo JJ, Kolmer M, Aaltonen J, Ellonen P, Perheentupa J, Ulmanen I, Peltonen L. Mutations in the AIRE Gene: Effects on Subcellular Location and Transactivation Function of the Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy Protein. *Am J Hum Genet*. 2000;66:378-92.
20. Sharifinejad N, Zaki-Dizaji M, Tebyanian S, Zainaldain H, Jamee M, Rizvi FS, et al. Clinical, immunological, and genetic features in 938 patients with autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED): a systematic review. *Expert Rev Clin Immunol*. 2021;17(8):807-17.
21. Ahonen P, Myllärniemi S, Sipilä I, Perheentupa J. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. *N Engl J Med*. 1990;322:1829-36.

22. Meloni A, Willcox N, Meager A, Atzeni M, Wolff AS, Husebye ES, et al. Autoimmune polyendocrine syndrome type 1: an extensive longitudinal study in Sardinian patients. *J Clin Endocrinol Metab.* 2012;97(4):1114-24.
23. Zlotogora J, Shapiro MS. Polyglandular autoimmune syndrome type I among Iranian Jews. *J Med Genet.* 1992; 29:824–26.
24. Orlova EM, Sozaeva LS, Kareva MA, Oftedal BE, Wolff ASB, Breivik L, et al. Expanding the Phenotypic and Genotypic Landscape of Autoimmune Polyendocrine Syndrome Type 1. *J Clin Endocrinol Metab.* 2017;102(9):3546-56.
25. Oftedal BE, Assing K, Baris S, Safgren SL, Johansen IS, Jakobsen MA, et al. Dominant-negative heterozygous mutations in AIRE confer diverse autoimmune phenotypes. *iScience.* 2023;26(6):106818. doi: 10.1016/j.isci.2023.106818
26. Weinstock C, Matheis N, Barkia S, Haager MC, Janson A, Markovic A, et al. Autoimmune polyglandular syndrome type 2 shows the same HLA class II pattern as type 1 diabetes. *Tissue Antigens.* 2011;77:317–32.
27. Maclaren NK, Riley WJ. Inherited susceptibility to autoimmune Addison's disease is linked to human leukocyte antigens-DR3 and/or DR4, except when associated with type I autoimmune polyglandular syndrome. *J Clin Endocrinol Metab.* 1986;62:455–59.
28. Betterle C, Dal Pra C, Mantero F, Zanchetta R. Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: autoantibodies, autoantigens, and their applicability in diagnosis and disease prediction. *Endocr Rev.* 2002;23(3):327–64.
29. Hunt PJ, Marshall SE, Weetman AP, Bunce M, Bell JI, Wass JAH, Welsh KI. Histocompatibility leucocyte antigens and closely linked immunomodulatory genes in autoimmune thyroid disease. *Clin Endocrinol.* 2001;55:491–9.
30. Levin L, Ban Y, Concepcion E, Davies TF, Greenberg DA, Tomer Y. Analysis of HLA genes in families with autoimmune diabetes and thyroiditis. *Hum Immunol.* 2004;65:640–7.
31. Eriksson D, Bianchi M, Landegren N, Nordin J, Dalin F, Mathioudaki A, et al. Extended exome sequencing identifies BACH2 as a novel major risk locus for Addison's disease. *J Intern Med.* 2016;280:595–608.

32. Verbsky J, Chatila TA. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) and IPEX-related disorders. *Curr. Opin. Pediatr.* 2013;25:708–14.
33. Dittmar M, Kahaly GJ. Polyglandular autoimmune syndromes: immunogenetics and long-term follow-up. *J Clin Endocrinol Metab.* 2003;88(7):2983-92.
34. Perheentupa J. Autoimmune polyendocrinopathy-candidiasisectodermal dystrophy. *J Clin Endocrinol Metab.* 2006;91(8):2843–50.
35. Buzi F, Badolato R, Mazza C, Giliani S, Notarangelo LD, Radetti G, et al. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome: time to review diagnostic criteria? *J Clin Endocrinol Metab.* 2003;88(7):3146–8.
36. Ahonen P, Myllärniemi S, Sipilä I, Perheentupa J. Clinical variation of autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) in a series of 68 patients. *N Engl J Med.* 1990;322:1829–36.
37. Bruserud Ø, Oftedal BE, Landegren N. A longitudinal follow-up of autoimmune polyendocrine syndrome type 1. *J. Clin Endocrinol Metab.* 2016; 101(8):2975–83.
38. Perniola R, Fierabracci A, Falorni A. Autoimmune Addison's Disease as Part of the Autoimmune Polyglandular Syndrome Type 1: Historical Overview and Current Evidence. *Front Immunol.* 2021; 26;12:606860. doi: 10.3389/fimmu.2021.606860
39. Szeliga A, Calik-Ksepka A, Maciejewska-Jeske M, Grymowicz M, Smolarczyk K, Kostrzak A, Smolarczyk R, et al. Autoimmune Diseases in Patients with Premature Ovarian Insufficiency-Our Current State of Knowledge. *Int J Mol Sci.* 2021;22(5):2594.
40. Van den Driessche A, Eenkhoorn V, Van Gaal L, De Block C. Type 1 diabetes and autoimmune polyglandular syndrome: a clinical review. *Neth J Med.* 2009;67(11):376-87.
41. Lankisch TO, Jaeckel E, Strassburg CP. The autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy or autoimmune polyglandular syndrome type 1. *Semin Liver Dis.* 2009;29(3):307-14.
42. Perheentupa J. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J Clin Endocrinol Metab.* 2006;91(8):2843-50.
43. Cemeroglu AP, Bober E, Dundar B, Buyukgebiz A. Autoimmune polyglandular endocrinopathy and anterior hypophysitis in a 14 year-old girl presenting with

- delayed puberty. *J Pediatr Endocrinol Metab.* 2001;14(7):909–14.
44. Alkhateeb A, Fain PR, Thody A, Bennett DC, Spritz RA. Epidemiology of vitiligo and associated autoimmune diseases in Caucasian probands and their families. *Pigment Cell Res.* 2003;16(3):208–14.
45. Tsao CY, Mendell JR, Lo WD, Luquette M, Rennebohm R. Myasthenia gravis and associated autoimmune diseases in children. *J Child Neurol.* 2000;15(11):767–9.
46. Coco G, Dal Pra C, Presotto F, Albergoni MP, Canova C, Pedini B, et al. Estimated risk for developing autoimmune Addison's disease in patients with adrenal cortex autoantibodies. *J Clin Endocrinol Metab.* 2006;91(5):1637–1645.
47. Pollak U, Bar-Sever Z, Hoffer V, Marcus N, Scheuerman O, Garty BZ. Asplenia and functional hyposplenism in autoimmune polyglandular syndrome type 1. *Eur J Pediatr.* 2009;168:233–5.
48. Meloni A, Furcas M, Cetani F, Marcocci C, Falorni A, Perniola R, et al. Autoantibodies against type I interferons as an additional diagnostic criterion for autoimmune polyendocrine syndrome type I. *J Clin Endocrinol Metab.* 2008;93(11):4389–97.
49. Puel A, Doffinger R, Natividad A, Chrabieh M, BarcenasMorales G, Picard C, et al. Autoantibodies against IL-17A, IL17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. *J Exp Med.* 2010;207(2):291–7.
50. Browne SK, Holland SM. Immunodeficiency secondary to anticytokine autoantibodies. *Curr Opin Allergy Clin Immunol.* 2010;10(6):534–41.
51. Falorni A, Laureti S, Candeloro P, Perrino S, Coronella C, Bizzarro A, et al. Steroid-cell autoantibodies are preferentially expressed in women with premature ovarian failure who have adrenal autoimmunity. *Fertil Steril.* 2002;78(2):270–9.
52. Ahonen P, Miettinen A, Perheentupa J. Adrenal and steroidal cell antibodies in patients with autoimmune polyglandular disease type I and risk of adrenocortical and ovarian failure. *J Clin Endocrinol Metab.* 1987;64(3):494–500.
53. Tomar N, Kaushal E, Das M, Gupta N, Betterle C, Goswami R. Prevalence and significance of NALP5 autoantibodies in patients with idiopathic



- hypoparathyroidism. *J Clin Endocrinol Metab.* 2012;97(4):1219–26.
54. Gavalas NG, Kemp EH, Krohn KJ, Brown EM, Watson PF, Weetman AP. The calcium-sensing receptor is a target of autoantibodies in patients with autoimmune polyendocrine syndrome type 1. *J Clin Endocrinol Metab.* 2007;92(6):2107–14.
55. Kemp EH, Habibullah M, Kluger N, Ranki A, Sandhu HK, et al. Prevalence and clinical associations of calcium-sensing receptor and NALP5 autoantibodies in Finnish APECED patients. *J Clin Endocrinol Metab.* 2014;99:1064–71.
56. Barzaghi F, Amaya Hernandez LC, Neven B, Ricci S, Kucuk ZY, Bleesing JJ, et al. Long-term follow-up of IPEX syndrome patients after different therapeutic strategies: An international multicenter retrospective study. *J Allergy Clin Immunol.* 2018;141(3):1036-1049.e5. doi: 10.1016/j.jaci.2017.10.041.

## BIOGRAPHY

- Or Lavie was born on June 8th, 1992, in Kfar-Saba, Israel.
- Or pursued his medical education and training between 2017 and 2023 at the University of Zagreb in the international English program. During this time, Or successfully completed steps 1 and 2 of the USMLE exam and actively participated in CROSS – Croatian Student Summit, a one-month international scientific congress for students and young scientists, with a focus on neurology and technology in the modern world. Additionally, Or obtained the Professional EPALS (European Pediatric Advanced Life Support) Certification.
- Or attended Hadassim High School in Even Yehuda, Israel from 2007 to 2010.
- From 2011 to 2016, Or served as a Combat Officer in the Artillery Corps of the Israeli Defense Forces, holding the rank of Second Lieutenant. In this role, Or led and trained combat soldiers and new recruits, took part in multiple combat operations, and gained extensive experience working with artillery units and equipment.