Autoimmune Diseases and Diagnostic Approaches

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TABLE OF CONTENTS

PREFACE

Autoimmunity is the impairment of immunological tolerance and an immune response against one's own antigens. Autoimmune diseases occur when a person's immune system attacks the person's own body incorrectly. Autoimmune diseases are still not fully understood for its reasons and treatment possibilities. New studies on autoimmunity are constantly being carried out, and new diagnostic and therapeutic protocols are emerging. With new discoveries in the field of autoimmune diseases day by day, innovations in the diagnosis and treatment of diseases have gained more importance in recent years. For this reason, it is important to follow and compile current studies. This collection presents a multi-disciplinary collection of leading and emerging scholars. This book will examine current, emerging, and cutting edge approaches to autoimmune diseases and their diagnostic methods in the broadest sense by experts in the field.

The Editors

CHAPTER 1

AUTOIMMUNE ENDOCRINE DISEASES IN CHILDREN

FATİH KILIÇBAY

Introduction

Autoimmune endocrine diseases in children are serious disorders that cause long-term use of health care resources and enormous morbidity. They include Addison's Disease, type 1 diabetes mellitus, thyroiditis, Graves' Disease, and polyglandular syndromes. Multiple autoimmune endocrine diseases can coexist in the same individual or cluster in families. In recent studies, the basis of autoimmune diseases has been shown to develop due to genetic predisposition and environmental factors. The main basis of the treatment in these diseases is to replace the hormones produced by the damaged endocrine organ, to reveal the disorder that causes the damage, and to apply specific treatment for it.

Autoimmune polyglandular syndromes

Autoimmune polyglandular syndromes (APSs) are entities whose classifications are still being discussed, and can be changed. According to the latest classification, today, APSs are separated into Types 1, 2, 3, and 4, with alopecia being found in all types; however, APS Type 3 is the most common one. For autoimmune polyglandular syndrome Type 1, it is required that there are at least two autoimmune Addison’s Diseases such as chronic candidiasis and chronic hypoparathyroidism; and for APS Type 2 there must be autoimmune adrenal insufficiency (AD) and in addition, Type 1 autoimmune diabetes mellitus (T1DM) and/or autoimmune thyroid disease must be present (Table 1.1). Other autoimmune diseases that accompany APS Type 1 and Type 2 may also be found (Obermayer-Straub and Manns 1998; Majeroni and Patel 2007).

APS1 occurs as a result of the inability to eliminate the antibodies created against receptors by T cells (Perheentupa 2006). Normally, T cells are prevented from entering the peripheral circulation because of the release of antigens only in tissues. For example, for insulin, the expressed T cell and its receptor will be able to initiate apoptosis by connecting to the insulin antigen expressed in the thymus (Figure 1.1) (Sperling and Yau 2017). This ectopic release of the antigens in the thymus is mediated by the autoimmune regulator gene (AIRE) in chromosome 21, and was identified as the gene responsible for APS1 in 1997 (Finnish-German APECED Consortium 1997). This gene plays roles in the adaptive immune response; and mutations are seen more than in APS1 syndromes (Kisand and Peterson 2015). Although the mutation of this gene is detected in a small group, it is found to be increased in Trizomy-21 cases. This may be because of the impaired function of the gene. It was understood that the mutations in AIRE might have autosomal dominant effects and appear as autoimmune disorders later in life in different models compared to classic APS1 (Kisand and Peterson 2015). The rate of such mutations can rise up to 1/1000. Although it is seen especially in Iranian Jews (1/9000), in Sardinia (1/14500), and in Finland (1/25000), it can also be seen as a result of dysfunction of regulatory T cells (Kisand and Peterson 2015; Sperling and Yau 2017).

Abnormalities are detected in B and T cells in all forms of APSs. The antibodies are detected against circulating hormonal, connective tissue, and protein antigens (for thyroid and steroid synthesis).

APS2 is characterized with Type 1 diabetes mellitus, adrenal insufficiency and hypothyroidism. The pancreatic beta cell appears, depending on antibodies for the synthesis of adrenal and thyroid tissues. APS3 is the same as APS2 except for adrenocortical insufficiency. Some authors identified APS2a or the second as APS2b based on this similarity. On the contrary, APS2a is rare, and APS2b is common. A total of 20% of Type 1 DM patients have Hashimoto's Disease; and anti-TPO and anti-TG antibodies are detected in these patients. The responsible genes are in the DQ and DR regions of the HLA complex in the short arm of chromosome 6 in APS2 and APS3. As a result of the mutations in these genes, antigens are presented to dendritic and macrophage cells with HLA. The easy presentation of its own tissue antigens causes the initiation of autoimmunity by causing the reduction in regulatory T cells (Sperling and Yau 2017).

It is absolutely necessary that autoimmune thyroid disease is present in addition to Addison's Disease and/or hypoparathyroidism for autoimmune polyglandular syndrome Type 3. APS Type 3 was classified by Betterle et al. in 2001; and was divided into 4 sub-groups as Types 3A, 3B, 3C, and 3D (Betterle and Zanchetta 2003; Aung 2019).

In addition to autoimmune thyroid disease, the presence of alopecia is classified as APS Type 3C. Also, all the other autoimmune polyglandular involvement combinations that do not meet the definition of APS Type 1, 2, or 3 are classified as APS Type 4 (Betterle and Zanchetta 2003).

Autoimmune thyroid disease is the most common autoimmune disease in society; and is seen in 7-8% of the general population. Previous studies showed that 52% of people with autoimmune thyroid disease have other autoimmune diseases (complete) or organ-specific antibodies (incomplete) even if the clinical autoimmune disease does not exist. In this respect, 3.5-4% of the total population has complete or incomplete APS Type 3 (Betterle and Zanchetta 2003).

Autoimmune Polyglandular Syndrome Type 3 is more common in middle-aged women; however, it can also be seen in all other age groups. The etiology of APS Type 3 includes autoimmune, environmental and genetic factors. The heredity model of the Autoimmune Polyglandular Syndrome Type 3 has not been defined well; and is considered to show heterogeneous transition. The relations among HLA DR1, HLA DQA1, HLA DQB1, and HLA DR B1 from HLA Class 2 genes were shown; however, non-HLA genes were not identified (Erdoğan et al. 2010). Also, the fact that there are family cases suggests that there may be an autosomal dominant transition with incomplete penetration. However, because of this incomplete penetration, first-degree relatives of patients might not have any autoimmune diseases (Aung 2019).

It was shown that the risk of developing alopecia totalis and alopecia universalis increases in patients with HLA-DQ 7 and HLA DR 4 positivity (Colombe et al. 1999).

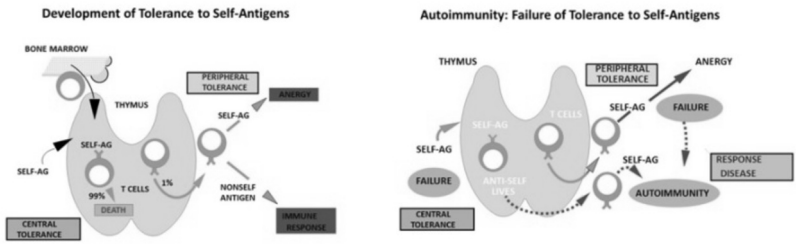


Figure 1.1: Left: The developing T cell migrates from the bone marrow to the T cell, causing apoptosis and self-tolerance of the T cell against antigens that are mediated by the AIRE gene and its own antigens. **Right:** Because of the failure of self-tolerance with inactivating mutations of the AIRE gene, inactivity of self-antigens, failure of central tolerance and lack of recognition of its own antigens occur, leading to an autoimmune response (Sperling and Yau 2017)

Table 1.1: APS Classification (Erdoğan et al. 2010)

APS Type 1	**MAJOR CRITERIA** (Diagnostic) *Chronic Candidiasis *Chronic Hypoparathyroidism *Autoimmune Addison's Disease (there must be at least 2 involvements) Other autoimmune diseases that can accompany
APS Type 2	**MAJOR CRITERIA** (Diagnostic) *Autoimmune Adrenal Insufficiency (100%) (definitely required) In addition, *Type 1 Autoimmune DM and/or Autoimmune Thyroid Disease Other autoimmune diseases that can accompany
APS Type 3	**MAJOR CRITERIA** (Diagnostic) * Autoimmune Thyroid Disease (definitely required) In addition, * The presence of an autoimmune disease other than Addison's Disease and/or Hypoparathyroidism
APS Type 4	Autoimmune Polyglandular Involvement, which does not meet the definition of APS Type 1, 2, 3 is classified as APS Type 4.

Autoimmune polyglandular syndrome 1 (APS1 apeced)

APS1 is characterized with 3 classic features; mucocutaneous candidiasis, hypoparathyroidism and adrenal insufficiency (Addison's Disease with cortisol deficiency), occasional aldosterone deficiency, and significant elevation in adreno cortico trophic hormone (ACTH) are defined.

Among the clinical findings are hyperpigmentation, abdominal pain, vomiting, weight loss and electrolyte disorders and hypoglycemia. There must be at least 2 or 3 clinical characteristics to diagnose APS1. Other symptoms include periodic fever, keratoconjunctivitis, chronic diarrhea, gonadal insomnia, Hashimoto's Thyroiditis, Vitamin B12 deficiency,

chronic active hepatitis, and ectodermal dystrophy. These clinical manifestations are called APECED (Autoimmune Poly Endocrinopathy, Candidiasis, Ectodermal Dystrophy) (Kisand and Peterson 2015).

The characteristics of ectodermal dystrophy are defined as visible changes in the tympanic membranes that are characterized with enamel hypoplasia affecting permanent teeth and hollow nail dystrophy which have nothing to do with the candidiasis of nails, and calcium accumulation (Perheentupa 2006).

Many affected patients show symptoms before the age of 5. Non-endocrine symptoms appear before endocrine symptoms in approximately 75% of cases. Mucocutaneous candidiasis occurs as the first finding in approximately 60%, and approximately 5% of patients experience vitiligo, alopecia, hepatitis, and keratopathy (Table 1.2). Mucocutaneous candida is the most common non-endocrine clinical finding; and it occurs because of the inability of candida to be included in cells by macrophages because of defective receptors (Brännström et al. 2006).

Endocrine disorder occurs mostly with hypoparathyroidism. Addison's Disease occurs in 60% of patients. The first finding in Addison's Disease is hypoparathyroidism in about 30% of patients. Ovarian insufficiency is detected in 60% of the affected patients, and testicular insufficiency is detected in 15% of patients. Atrophic gastritis due to gastric parietal cell atrophy, B12 deficiency, and Type 1 DM are detected in 12% of patients. Type 1 DM occurs as a late complication following the hypoparathyroidism and adrenal insufficiency in the early period (Sperling and Yau 2017). Although anti-thyroid antibodies are seen more, hypothyroidism is detected in fewer patients. Rarely, diabetes insipidus, pituitary, secondary growth hormone deficiency, and infertility because of sperm antibodies in men, and ovarian failure in women are also detected (Figure 1.2). (Frommer and Kahaly 2019; Perheentupa 2006).

Parathyroid, adrenal and thyroid glands, and antibodies that are in the circulation against pancreatic islet cells, cause the disease by affecting cell B function as well as T cells. Although lymphocytic infiltration of parathyroid glands is also common, the NACHT Leucine-Rich-Repeat Protein 5 (NALP5) Protein, which acts as an antigen, was discovered in 2008 (Alimohammadi et al. 2008). This protein was found to be higher especially in patients with APS1 hypoparathyroidism.

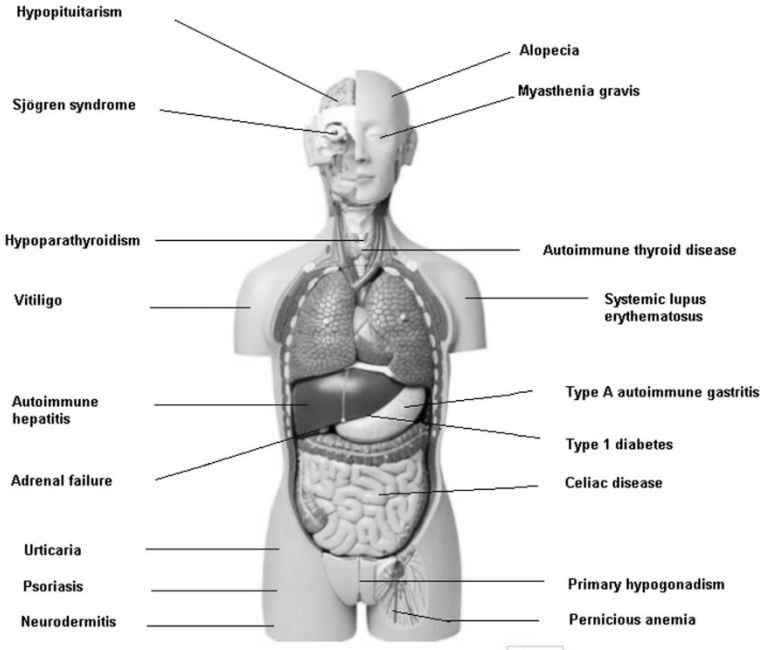


Figure 12.2: Distribution of Autoimmune Polyendocrine Disorders (Frommer and Kahaly 2019)

Counter bodies of Cyp21, Cyp17 and Cyp11A1, which are enzymes of adrenal cytochrome P450, are present in many patients; however, they are reduced by glucocorticoid treatment. Antibodies against liver microsomal proteins and parietal cells (a & b subunits of H+/K + ATPase), and against intrinsic factor were also reported. Antibodies can be neutralized by Type 1 interferon. Mostly, interferon-1α and -1ω appear to be specific to these antibodies; and therefore have a clinical diagnostic use (Meager 2006).

Patients who have AIRE mutations also have autoantibodies that improve high-affinity disease, which can explain the low incidence and late appearance of T1 diabetes in APS1 patients (Meyer et al. 2016). The cause of this autoimmunity is inactivating mutations in the autoimmune regulatory gene (AIRE) on chromosome 21q22.3 (Kisand and Peterson 2015). This condition is an autosomal recessive hereditary disease. Spot mutations that result in an autosomal dominant form were reported. However, although this autosomal dominant form appears to be less severe than classical autosomal recession, it suggests that this genetic disorder may be more

common in various immune disorders not because of AIRE (Sahoo et al. 2016).

Treatment of APS1

Treatment algorithms were proposed for this syndrome. The suppression of the immune system (glucocorticoids such as prednisone, cyclosporine, the calcineurin inhibitors tacrolimus and sirolimus, methotrexate, mycophenolate mofetil and rituximab, a CD20 inhibitor), hormonal treatment (Vitamin D, B12), calcium phosphorus, adrenal hormones, and thyroid hormones are recommended to be replaced with measurements (Kisand and Peterson 2015).

Affected patients should be examined in terms of ALT and AST values for HbA1c, fasting glucose values, and liver function tests with 6 month to 1 year intervals. When hypoparathyroidism and mucocutaneous candidiasis are detected, patients must be examined for adrenal insufficiency. When ketoconazole is used as an anti-candida agent, there might occur false positives in patients because steroids can inhibit hormone synthesis. Cortisol should be started from a stress dose when adrenal insufficiency is diagnosed.

Table 12.2: Clinical Characteristics of APS1 (Sperling 2017)

Disease	**% Ratio**
Mucocutaneous Candidiasis	80%
Hypoparathyroidism	70-80%
Adrenal Insufficiency	60%
Type 1 Diabetes Mellitus	12%
Hypothyroidism	4%
Ovarian Failure in Affected Females	60%
Testicular Failure in Affected Males	14%
Gastric Parietal Cell Failure	15%
Hepatitis	13%
Ectodermal Dysplasia	33%
Keratopathy	22%
Alopecia	27%
Vitiligo	13%

Autoimmune polyglandular syndrome 2 (APS2a; Schmidt syndrome)

APS2 is characterized by the triad of T1DM, Addison's Disease, autoimmune hypothyroidism, hyperthyroidism or Hashimoto's Disease. Type 1 DM and Addison's Disease must definitely be found. Autoimmune hypothyroidism or other autoimmune diseases can exist together, and these are celiac disease, vitiligo, alopecia, myasthenia gravis, pernicious anemia, IgA deficiency, hepatitis and hypogonadism. The peak prevalence of the syndrome is between 20 and 40 years of age. The syndrome is more common in women on an autoimmune basis, and is associated with specific HLA DR3i DR4 haploids, and class II HLA alleles DQ2 and DQ8, which are strongly associated with celiac disease.

Islet cell antibodies (GAD65, IA2, ZnT8), thyroid (anti thyroidglobulin TG, anti-thyroid peroxidase TPO), adrenal and gliadin-bound antibodies leading to Addison's Disease (Anti-21-hydroxylase or anti-17-hydroxylase), and celiac disease (tissue transglutaminase) exist widely, and should be examined periodically in those with Addison's Disease, and one or more autoimmune endocrinopathies such as T1DM (Figure 1.3) (Sperling 2017).

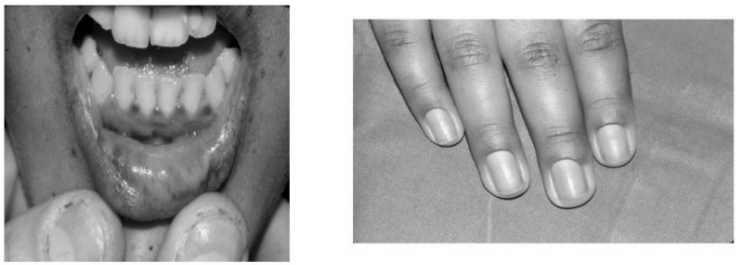


Figure 12.3: A 16-year-old patient who was diagnosed with APS2 Syndrome, Addison's Disease and Type 1 DM as a result of recurrent hypoglycemia attacks (Sperling 2017)

Autoimmune polyglandular syndrome 3 (APS2b)

APS3, which is also known as APS2b, is sometimes known as Carpenter Syndrome. Although it is based on the same autoimmune basis as APS2, it does not involve Addison's Disease (Table 1.3). Approximately 20% of patients with T1DM have thyroid globulin (TG) and thyroid peroxidase (TPO) antibodies; however, only very few patients have clinical or biochemical hypothyroidism (Giménez-Barcons et al. 2014).

Table 1.3: Clinical characteristics of APS2 and APS3 (Sperling 2017)

APS2	**APS3**
Type 1 Diabetes Mellitus	Type 1 Diabetes Mellitus
Thyroid autoimmunity	Thyroid autoimmunity
Adrenal Insufficiency	

Autoimmune adrenal insufficiency

Thomas Addison identified the disease in 1885 for the first time in patients with anemia, weakness, heart failure, irritability, stomach irritation, and changes in the skin at the hospital where he worked (Addison 1855). In the postmortem examination of 11 patients with the disease, he detected tuberculosis in 6 patients, adrenal malignancy in 3 patients, hemorrhage in 1 patient, and fibrosis in 1 patient. Addison identified idiopathic adrenal fibrosis. He also identified vitiligo in these patients. In this way, vitiligo was defined as a skin finding of autoimmune disorders, and as an autoimmune disease (Ortonne et al. 1983).

The autoimmune adrenalitis case identified by Addison was most likely the first reported case of autoimmune polyendocrine syndromes. After this initial report, Trousseau identified this adrenocortical insufficiency as "Addison's Disease" in 1856 (Trousseau 1856).

Prevalence and etiology

Adrenocortical Insufficiency occurs secondary to primary damage of the adrenal cortex, pituitary or hypothalamus diseases (Oelkers 1996). AI occurs acutely or chronically, and the patient dies if the diagnosis is not made in both cases. Primary adrenocortical ınsufficiency is rare, and the frequency varies between 0.45/100000 and 11/100000 (Betterle et al. 2002). The most common cause before chemotherapy treatment was tuberculosis infection. Recently, however, especially in the study which analyzed 1240 patients, Autoimmune adrenocortical ınsufficiency was detected 45-94% more frequently (Betterle et al. 2002). Although a decrease is expected in adrenocortical ınsufficiency disease due to decreased prevalence of tuberculosis, the incidence has remained constant over the years (Orth et al. 1992).

Chronic adrenal damage is caused by autoimmunity, tuberculosis, infectious fungal diseases (e.g., coccidioidomycosis and histoplasmosis) or viral infections (e.g., cytomegalovirus and HIV) (Opocher and Mantero 1994). It is known that malignant tumors cause metastasis (breast, lung,

stomach, lymphomas and melanoma) or primary tumors cause chronic adrenal insufficiency (Orth et al. 1992). Similarly, it can cause adrenal bleeding due to anticoagulation therapy with dicumarol or heparin, or acute adrenal insufficiency in Waterhouse-Friderichsen Syndrome. Waterhouse-Friderichsen Syndrome is defined as septic shock caused by bacteria, such as *Neisseria meningitidis, Hemophilus influenzae, Pseudomonas aeruginosa, Escherichia coli, pneumococci*, and acute adrenal bleeding due to infection. It is known that mitotane, aminoglutethimide, metopyrone, trilostane, ketoconazole, rifampin, etomidat, and siproteron acetate cause adrenal insufficiencies among the drugs that disrupt adrenals (Betterle et al. 2002). Metabolic disorders, amyloidosis, hemochromatosis, and sarcoidosis may cause Chronic Adrenal Insufficiency. Among the rare causes, there are gland hypoplasia, enzyme deficiencies in the cortisol synthesis pathway, adrenal hemorrhage due to birth traumas, or Cushing's Disease in the mother (Kannan 1988).

Adrenocortical Insufficiency due to genetic diseases is rare. Adrenoleukodystrophy is a genetic disease, the progressive demyelination disease of the Central Nervous System, and it is also known as Schilder's Disease. This syndrome is caused by the mutations of a gene encoding a structural protein in the terminal segment of chromosome X. Elevated long-chain fatty acids are detected in the bloodstream in this disease. AD develops due to the build-up of long-chain fatty acids in the organs and adrenal gland. Symmetric demyelination is detected in the parieto-occipital region in cranial MRI (Moser 1997).

Congenital Adrenal Hypoplasia is an X-based recessive disorder, defined as delayed puberty due to hypogonadotropic hypogonadism based on abnormal gonadotropin secretion at hypothalamic and pituitary levels and adrenal insufficiency due to adrenal cortex developmental disorder. This disease is associated with the dose-dependent mutation of a nuclear receptor in the DAX-1 gene on the X chromosome, or the Steroidogenic Factor (SF-1) gene controlled by the 9th chromosome (Vaidya et al. 2000). These two nuclear receptors can act jointly for normal gonadal, adrenal, and hypothalamic development, and function as necessary regulators.

The Kerns-Sayre Syndrome, which is defined by progressive external ophthalmoplegia, cardiac transmission defect, retinal pigment degeneration, and deafness mitochondrial cytopathy may be associated with adrenal insufficiency caused by various deletions of mitochondrial DNA (Clark 1993). AD can also coexist with GH deficiency, thyroid diseases, hyperaldosteronism, hypogonadism, Diabetes Mellitus, and hypoparathyroidism (Betterle et al. 2002).

Other genetic defects causing Adrenal Insufficiency are ACTH resistance syndromes, such as Familial Glucocorticoid Deficiency and Triple A Syndrome. It is a rare autosomal disorder that is characterized with Familial Glucocorticoid Deficiency, growth deficiency, recurrent hypoglycemia, pigmentation, and recurrent infection. Elevated ACTH and low cortisol levels are available in laboratory findings. Mutations of the ACTH receptor gene associated with G protein were detected in approximately 40% of patients; however, specific genetic mutations have not yet been found in about 60% of patients (Vaidya et al. 2000).

Triple A Syndrome is an autosomal recessive disorder associated with mutations of a gene on chromosome 12, which is characterized by a trio of adrenocortical insufficiency, achalasia, and alacrima because of ACTH resistance, also known as Allgrove Syndrome (Vaidya et al. 2000).

Congenital Adrenal Hyperplasia is the most common cause of adrenal crisis because of 21-hydroxylase deficiency, which also causes salt loss in the first 2 weeks of life. Affected females have ambiguous and virilised genitals, and are usually diagnosed at birth. However, males are not diagnosed because of salt loss 2-3 weeks after birth, and they are diagnosed with acute adrenal crisis.

Deficiency of 3-hydroxisteroid dehydrogenase or P450 SCC enzyme can occur with adrenal insufficiency during the neonatal period. Affected boys can admit with ambiguous genital structures, or pherotypically female. There are no sexual development defects in Congenital Adrenal Hyperplasia due to aldosterone synthesis defect (Ten et al. 2001).

Smith-Lemli-Opitz Syndrome is caused by the mutation in sterol 7-reductase enzyme, which catalyzes the last step in cholesterol biosynthesis. It causes Primary Adrenal Insufficiency in this case. The syndrome is characterized with mental retardation, microcephaly, congenital cardiac anomalies, syndactyly, and a not-fully-developed male genital system (Ten et al. 2001).

Adrenal Insufficiency was reported in several patients, especially in pituitary or hypothalamic space-occupying diseases. Other hormonal axes may be affected and accompanied by neurological and ophthalmological symptoms. The frequent Secondary Adrenal Insufficiency occurs with CRH suppression following glucocorticoid therapy (Oelkers 1996).

Table 12.4: Causes of primary adrenal insufficiency (Zelissen et al. 1995)

Autoimmune Adrenalitis
Isolated Primary Adrenal Insufficiency
Autoimmune Polyglandular Syndrome Type I
Autoimmune Polyglandular Syndrome Type II
Infectious Adrenalitis
Tuberculosis
Common Fungal Infection
HIV Infection and AIDS
Syphilis
African Trypanosomiasis
Metastatic Cancer
Primer lung, breast, gastric, colon cancer, and lymphoma
Adrenal hemorrhage or infarct
Medications
Ketoconazole
Fluconazole
Rifampin
Phenytoin
Barbiturates
Megestrol acetate
Others (aminoglutetimid, etomidat, metirapone, suramin, mitotane)
Others
Adrenoleukodystrophy and adrenomyeloneuropathy
Congenital adrenal hypoplasia
Familial glucocorticoid deficiency
Familial glucocorticoid resistance
Defective cholesterol metabolism

Clinical and laboratory findings

Most of the symptoms appear silently in primary and secondary adrenal insufficiency, and are in the form of fatigue, weakness, orthostatic hypotension, weight loss, and anorexia (Oelkers 1996; Ten 2001). Most patients may admit with gastrointestinal symptoms, such as vomiting, abdominal cramp, and diarrhea. The most specific symptom of Adrenal Insufficiency is the hyperpigmentation of the skin and mucosal surfaces as a result of plasma corticotrophin concentrations that are elevated due to decreased cortisol levels (Betterle et al. 2002). Thinning of the axillary and

pubic hair is detected in patients with secondary adrenal insufficiency disease, which is generally not detected in patients with isolated corticotrophin deficiency. Decreased libido is seen in primary and secondary adrenal insufficiency. Orthostatic hypotension is more common because of hypovolemia as a result of aldosterone deficiency in secondary insufficiency. Hyponatremia, hyperpotassemia, hypoglycemia, normocytic anemia, lymphocytosis, and eosinophilia are seen in patients with adrenal insufficiency (Oelkers 1996). Although hyponatremia is seen in both disorders, its mechanism is different. It appears with sodium loss due to aldosterone deficiency in the primary disorder. The secondary insufficiency occurs due to water retention as a result of increased vasopressin based on cortisol deficiency (Betterle et al. 2002).

The measurement of plasma ACTH can be used to make the differential diagnosis of primary and secondary adrenal insufficiency. In patients with primary adrenal insufficiency, although plasma corticotrophin concentrations always exceed 100 pg/ml (22 pmol/liter), plasma cortisol levels are detected to be within the normal range. Although PRA increases because of sodium loss in primary adrenocortical insufficiency, basal plasma aldosterone concentrations are low or at the lower limit of normal values.

If adrenal insufficiency is suspected and previous measurements are normal, a 250 μg synthetic ACTH test can be done for the patient.

Idiopathic AD as an autoimmune disease

Autoimmune AD deficiency was first identified in 1957 when the antibodies were shown against adrenal cortex hormones (Oelkers 1996). In subsequent studies, many authors showed that idiopathic AD can be autoimmune by nature. In histopathological findings, mononuclear cell infiltration, which causes the atrophy of the adrenal cortex, showing cell-mediated immunity to adrenal cortex antigens, inducing the disease with adrenal cortex extracts in animal models, identifying steroidogenic enzymes expressed as their own antigens in adrenals, in relation with other autoimmune diseases, together with major histocompatibility antigens, is defined as autoimmune AD (Betterle et al. 2002).

Histopathology of adrenals in autoimmune AD

The infiltration of lymphocytes, plasma and macrophage cells is observed in the active phase of the disease in autoimmune adrenal insufficiency. Losses and necrosis are detected in the cells in the 3-layer structure of the adrenal cortex. Usually, the residual tissue remains, but the cortex is

destroyed and replaced by fibrosis tissue. At the end of the adrenal cortex destruction, normal cells remain in the medulla region. Sometimes, the absence of adrenals was also reported, but this mostly occurs as a result of ischemic damage (McNicol 1994).

Cellular immunity in autoimmune AD

Antigen-specific T lymphocyte response AD was shown in previous studies (Nerup and Bendixen 1969). Also, a nonspecific decrease was reported in suppressive T lymphocyte function in patients with AD. It was speculated in another study that the percentage of activated T lymphocytes in peripheral blood increased in patients in early periods compared to patients with prolonged autoimmune AD (Rabinowe et al. 1984). A proliferative T cell response was shown against an adrenal specific protein fraction with a molecular mass of 18-24 kDa in patients with autoimmune AD.

Classification and characterization of APS

Multiple Endocrine Organ Failure has often been identified together with other autoimmune and non-autoimmune diseases. It was found that various autoimmune diseases occur with certain combinations. Neufeld and Blizzard identified these clusters of diseases as Polyglandular Autoimmune Diseases in 1980 (Neufeld and Blizzard 1980), organized and classified them into four main types, and also called them Autoimmune Polyendocrine Syndromes (APS). Autoimmune AD is the main element of these disease groups, and is dealt with in 4 types.

APS-1: This is known as APECED Syndrome (Autoimmune Polyendocrinopathy, Candidiasis, and Ectodermal Dystrophy). Two symptoms being together are diagnostic. Chronic mucocutaneous candidiasis is the first symptom. The second finding is mostly hypoparathyroidism. Addison's appears at the age of 10-12. It can be accompanied by gonadal inadequacy, vitiligo, alopecia, nail dystrophy, dental hypoplasia, intestinal malabsorption, chronic active hepatitis, etc. Hypothyroidism and Type 1 Diabetes are less common (<10%). Family members should be monitored closely, because it can also develop in siblings. Addison-related sudden death may develop. The AIRE-1 gene in the 21st chromosome (q22.3) is responsible. This gene encodes the transcription factor, which plays an important role in immune response. It is inherited autosomal-dominant or recessively (Nagamine et al. 1997).

APS-2: In this Polyendocrinopathy, Type 1 Diabetes (Carpenter Syndrome) or Autoimmune Thyroid Disease (Schmidt's Syndrome) accompanies Addison's Disease. Vitiligo, gonadal insufficiency, alopecia, chronic atrophic gastritis, and pernicious anemia may also be seen. It appears in the second decade. The incidence of middle-aged women within the same family is high. Tissue group HLA DR3-DR4 frequency is increased. It passes as autosomal dominant. Its relations with HLA antigens DQA1*0301, DQA1*0501, DQB1*0201, DQB1*0302, DRB1*0404, and DRB1*0301 APS-2 were detected (Yu et al. 1999).

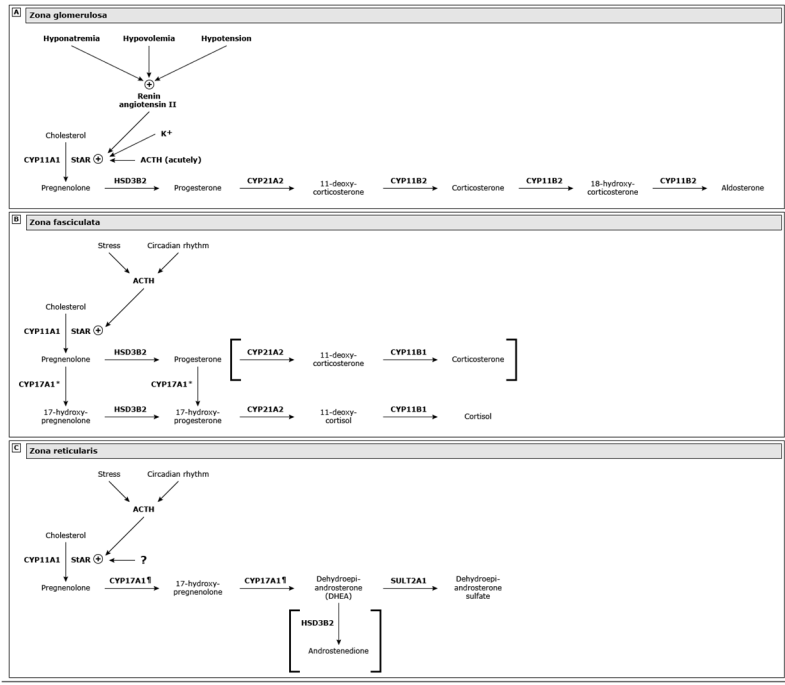
Isolated autoimmune AD

Clinical features: Isolated AD represents the 4th group of diseases that do not have an autoimmune component. Isolated AD patients should be investigated in terms of autoimmunity when they are detected as normal or atrophic in negative ACA and imaging. Screening should be done for organ-specific HLA-DR antinuclear and antiphospholipid antibodies. When the reason cannot be revealed, it is often described as idiopathic (Oelkers 1996). *Isolated AD as incomplete APS:* These patients should be screened in terms of autoimmunity every 2-3 years after being diagnosed with Isolated AD. This strategy will be beneficial in diagnosing the autoimmune diseases that may accompany it or appear later. Due to the dynamic nature of autoantibody positivity, and since it is the onset of different autoimmune diseases, patients may need to be reclassified with Isolated Autoimmune AD when detected (Oelkers 1996).

Genetic pattern: It was found that HLA-DR3 was increased in most patients with AD (Böehm 1991). Also, the G allele CTLA-4 was found to increase in British patients with isolated AD; however, there was no significant correlation (Oelkers 1996).

Serological markers of autoimmune AD

The most important characteristic of autoimmune diseases is that they have antibodies that recognize their own antigens. The autoantibodies that cause Adrenal Autoimmunity are ACA steroid antibodies, 21-OH antibodies, and steroid-producing cell antibodies. Antibodies to other components of the adrenal cortex are 1-Adrenal surface autoantibodies, 2-ACTH receptor antibodies, and 3-Anticorticosteroid hormone antibodies.



(A) Zona glomerulosa (ZG): Controlled primarily by the renin-angiotensin system through angiotensin 2 as well as potassium ions and ACTH (acutely), renin secretion is stimulated by hyponatremia, plasma volume

(B) Zona fasciculata (ZF): Cortisol is the major product because CYP17A1 (17-hydroxylase activity*) predominates. The square brackets ([ ]) indicate that corticosterone synthesized from progesterone by CYP21A2 and then CYP11B1 is usually a minor pathway (except in CYP17A1 (17-hydroxylase*)) deficiency

(C) Zona Retikularis (ZR): The question mark (?) indicates that there are other as yet defined factors involved in the control of steroidogenesis in the ZR. The square brackets ([ ]) indicate that HSD3B2 production of androstenedione is a minor pathway in the ZR. However, not shown is that DHEA from the ZR is converted to androstenedione (and then testosterone) in peripheral tissues
ACTH: Corticotrophin
* CYP17A1 has high 17-hiydroxylase activity in the zona fasciculata, but minimal 17,20-lyase activity because of low expression of a cofactor (cytochrome b_5) necessary for full 17,20- lyase activity. Therefore, the ZF is not a source of significant adrenal androgen precursors as in the ZR (C)

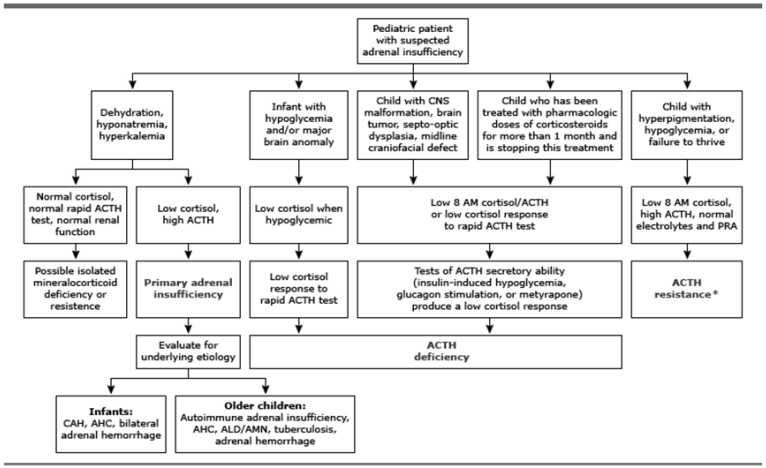
Figure 1.4: Normal Steroid Hormone Synthesis (Brozzetti et al. 2010)

ACA/21-OH Abs and autoantigens

ACAs are organ-specific autoantibodies reacting with all three layers of the adrenal cortex, and they produce homogeneous cells. Some rare sera react with only one or two, of the three cortical layers. ACAs are usually the IgG1, IgG2, and IgG4 subclasses (Dean et al. 1983). Autoimmune Adrenalitis is characterized with the presence of serum antibodies against P450scc, P450c17, and P450c21 (Song et al. 1994). These enzymes play roles in the hydroxylation of steroids and side chain reactions (Figure 1.4). P 450 SCC (CYP21A2) is from the sub-group of counter antibodies IgG1 and IgG2a.

Confirming adrenal insufficiency

The first step in the definition of Adrenal Insufficiency is to measure the plasma cortisol and ACTH levels early in the morning. If the serum cortisol level is low, the ACTH stimulation test should be done (Bornstein et al. 2016). If the plasma cortisol level is low and the electrolytes are normal, the ACTH Warning Test should be done. If the ACTH level is low, central adrenal insufficiency should be considered (Figure 1.5).



CNS: central nervous system; ACTH: adrenocorticotropic hormone; PRA: plasma renin activity; CAH: congenital adrenal hyperplasia; AHC: adrenal hypoplasia congenital; ALD: adrenoleukodystrophy; AMN: adrenomyeloneuropathy
*ACTH resistance is a characteristic of familial glucocorticoid deficiency (usually caused by a mutation in the melanocortin 2 receptor [*MC2R*] and of "triple A syndrome" (Allgrove syndrome)

Figure 1.5: Diagnosis Algorithm in Adrenal Insufficiency (Bornstein et al. 2016)

Treatment in primary adrenal inadequacy

Urgent treatment is important. Initially, blood samples are taken, and the ACTH, cortisol, electrolytes, complete blood count, blood glucose, aldosterone and PRA are examined, or the serum is separated. The degree of dehydration of the patient is calculated at around 10-15% according to the characteristics, the loss is found, and the daily need for fluid is added to it. The serum speed is adjusted so that half of the calculated liquid is administered in the first 8 hours.

If there is hypoglycemia, an IV bolus can be administered as dextstrosis of 10% to 1 mL/kg. The electrolyte levels of the patient are checked at an interval of 6-8 hours. After the 8th hour, the Na content of the liquid can be reduced by 2/3 and then by 1/3 according to the condition of the patient (Swerdlow et al. 1998).

If the potassium level is above 7 mEq/L, calcium gluconate can be administered as slow infusion at 0.5-1 mL/kg. Attention should be paid to cardiac arrhythmia. If potassium continues to be high, insulin infusion may be initiated. Kayexelate, which is potassium-binding resin, may be initiated in hyperpotassemia.

Hydrocortisone should also be started with fluid treatment. It is administered in the form of 10 mg in newborns, 25 mg in nurslings, 50 mg in children in the gaming period, 75 mg in schoolchildren, and 100 mg in adolescents as bolus hydrocortisone sodium succinate (melted in water). Perfusion is continued to be administered with 6-hour breaks as 100 mg/m2/24 hour. The dose is reduced by 25% every day to a physiological dose in 4-5 days (Root and Shulmann 2004).

After the acute crisis is over, hydrocortisone treatment is initiated. This dose is 10 mg/m2/24 hour. Depending on the difference in cortisol sensitivity, the dose may vary between 8 and 15 mg/m2/24 hour. It is preferred to divide it into three doses, because the half-time of the hydrocortisone is approximately 6 hours. Hydrocortisone is preferred because of its shorter effect. After the growth cartilage closes, the equivalent prednisolone can be initiated at a single dose a day. In cases of stress (infection, trauma, minor surgical intervention), the dose should be increased to three times. A specific protocol is prepared for major surgical interventions, and the dose is increased 8-10-fold. High doses of hydrocortisone cause obesity, strias, hypertension, osteoporosis, and growth cessation.

Type 1 diabetes

Diabetes is a metabolic disease, which is defined by hyperglycemia stemming from insulin secretion or inadequacy in the insulin effect, affecting protein and fat metabolism as well as carbohydrate metabolism (Chiang et al. 2014). The etiological classification that was developed by the World Health Organization (WHO) and the American Diabetes Association (ADA) is shown in Table 1.5 (American Diabetes Association 2019).

Type 1 diabetes (T1DM)

Type 1 Diabetes (T1DM) occurs due to insulin deficiency caused by damage to pancreas beta cells (Chiang et al. 2014; American Diabetes Association 2019). Although 90-95% of cases have autoimmune destruction of beta cells, 5-10% have beta cell destruction due to unknown causes. There is a pronounced lack of insulin in both cases, and exogenous insulin treatment is required. Although immune-mediated T1DM (Type 1A) was called “insulin-dependent diabetes” or “juvenile onset diabetes” in previous years, this terminology is not used today (American Diabetes Association 2019).

Epidemiology

Although T1DM accounts for approximately 10% of all diabetes cases in all ages, it accounts for more than 85% of patients diagnosed with diabetes under the age of twenty. In general terms, its incidence increases parallel to the increase in age, and peaks between the ages of 5 and 7 and in adolescence (Kliegman and Robert 2016). The first peak is associated with increased exposure to infections during school start-up, and the increase in the puberty period is considered to be due to increased sex steroid hormones, increased growth hormone, and psychological stress levels (Alemzadeh 2004). The incidence of T1DM changes between 0.7 and 40 per 100 thousand on a worldwide scale, differing in various ethnic groups among communities and according to regions in the same community (Sperling et al. 2014).

Etiopathogenesis

Genetic, environmental and autoimmune factors play roles in the emergence of T1DM. Environmental factors play triggering roles in individuals who

have a genetic predisposition, initiating the autoimmune process, and progressive beta cell damage causes insulin deficiency. Clinical findings occur when insulin deficiency becomes obvious (Skyler et al. 2017).

Table 1.5: Etiological classification of diabetes (American Diabetes Association 2019)

<table>
<tr><td colspan="2">I. Type 1 diabetes (Beta cell destruction causing insulin deficiency)
A. (Type 1a) Immune mechanism-mediated
B. (Type 1b) Idiopathic</td></tr>
<tr><td colspan="2">II. Type 2 diabetes (Insulin resistance and relative insulin deficiency)</td></tr>
<tr><td>III. Other specific types
A. Genetic defects in beta-cell function (Monogenic diabetes)
i. Neonatal diabetes
ii. Maturity Onset Diabetes of the Young (MODY)
iii. Mitochondrial DNA mutations
iv. Wolfram syndrome-DIDMOAD
v. Thiamine-respondent megaloblastic anemia and diabetes
B. Genetic defects in insulin function
i. Type A insulin resistance
ii. Leprechaunism (Donohue syndrome)
iii. Rapson Mendenhall syndrome
iv. Lipoatrophic diabetes syndrome
C. Other genetic syndromes that co-exist with diabetes
i. Down syndrome
ii. Turner syndrome
iii. Klinefelter syndrome
iv. Prader Willi syndrome
v. Bardet Biedl syndrome
vi. Alström syndrome
vii. Werner syndrome</td><td>E. Diabetes due to drugs or toxic agents
i. Vacor
ii. Pentamidin
iii. Nicotinic acid
iv. Glucocorticoids
v. Thyroid hormones
vi. Diazoxide
vii. Beta adrenergic agonists
viii. Thiazides
ix. Phenytoin (Dilantin)
x. Alfa-interferon
xi. Others
F. Exocrine pancreas diseases
i. Pancreatitis/ionized radiation
ii. Trauma, pancreatectomy
iii. Neoplasia
iv. Cystic fibrosis
v. Hemochromatosis
vi. Fibrocalculous pancreopathy
vii. Transfusion-related iron load
viii. Others
G. Infections
i. Congenital rubella
ii. Cytomegalovirus
iii. Others
H. Diabetes-associated endocrinopathies
i. Acromegaly
ii. Cushing’s Disease
iii. Glucagonoma</td></tr>
</table>

D. Immune mechanism and other rare forms of diabetes i. IPEX ii. Autoimmune polyendocrinopathy syndrome (APS) iii. "Stiff-man" syndrome	iv. Phaeochromocytoma v. Hyperthyroidism vi. Somatostatinoma vii. Aldosteronemia viii. Others
IV. Gestational Diabetes	

Genetic predisposition

Multiple genes determine the predisposition to T1DM. The genes causing predisposition for T1DM are the Major Histocompatibility Complex (MHC) and non-MHC-related genes. MHC Class II molecules are localized on the surface of antigen-presenting cells, mediating the destruction of the cells to which the antigen is bound by presenting it to macrophages. The genes encoding MHC Class II molecules are localized on the 6th chromosome. These genes encode the proteins, which constitute Human Leukocyte Antigens (HLA). It was shown that various HLA gene alleles are associated with the predisposition to or protection from T1DM (Haller et al. 2005).

The HLA genotype is associated with T1DM risk in the Caucasian race. Specific combinations of HLA DR and DQ alleles determine genetic sensitivity (Nguyen et al. 2013). The haplotypes with the highest risk are DRB1*03:01, DQA1*05:01, DQB1*02:01 and DRB1*0405, DQA1*03:01, DQB1*03:02. The odds rate for individuals with the two highest risk HLA haplotype heterozygotes for islet autoimmunity and T1DM development is 30. Less than 10% of those with HLA genes that create predisposition to diabetes show clinical disease findings. A homozygous lack of aspartic acid at position 57 of the beta chain of HLA-DQ increases T1DM risk by 100 times (Haller et al. 2005). The haplotypes that protect from T1DM are DR 2 haplotype (DRB1*15:01, DQA1*01:02, DQB1*06:02), DRB1*14:01, DQA1*01:01, DQB*05:03 and DRB1*07:01, DQA1*02:01, DQB1*03:03 (Mayer-Davis et al. 2018).

Having an individual with T1DM in the family increases T1DM risk. The risk of T1DM increases 3-4 times with T1DM in the mother, 5-6 times with T1DM in the father, 30 times with T1DM in the mother and father, 5 times with a sibling diagnosed with T1DM, 6-10 times with a dizygotic twin diagnosed with T1DM, and 30-65 times with a T1DM monozygotic twin (Guo and Tuomilehto 2002; Kliegman and Robert 2016). Since the risk of dizygotic twins is higher than the sibling risk, it suggests that other factors aside from the shared genotypes (e.g., the shared intrauterine environment)

may play roles in increasing the risk in dizygotic twins. There is no family history of T1DM in 85% of newly diagnosed patients (Guo and Tuomilehto 2002; Kliegman and Robert 2016).

Environmental factors

Epidemiological studies show that environmental factors trigger the development of immune-mediated T1DM in individuals with genetic predisposition. It is not yet fully understood how environmental factors start beta cell damage in the pancreas. The autoimmune process usually starts months and years before the appearance of clinical symptoms (Mayer-Davis et al. 2018).

The importance of environmental factors in T1DM development was emphasized in migration studies and studies conducted in twins. Studies conducted in monozygotic twins show only a 13-33% parallelism for T1DM. The geographical difference in the incidence of T1DM in children and adolescents varies too much to be explained by genetic factors. The significant increase in the incidence of T1DM in children and adolescents in recent years cannot be the result of increased genetic disease predisposition in the society alone; most likely, the changes in lifestyles and environmental factors are also effective in this respect. Migration studies show that the incidence of T1DM increases in population groups that switch from a low incidence area to a high incidence area (Knip 2011).

Viruses, foods taken with diets, and chemicals are the most important and possible known environmental factors (Kliegman and Robert 2016). Enteroviral infections in pregnancy, infancy, childhood and adulthood were associated with islet autoimmunity and the development of T1DM in many populations. Congenital Rubella Syndrome was associated with the development of T1DM. The data supporting the role of other viruses, such as CMV, Mumps, Influenza, Rotavirus and H1N1 in T1DM development, are inadequate (Mayer-Davis et al. 2018). In 39% of newly diagnosed Type 1 diabetes children, Coxsackie B virus-specific IgM was detected. This rate is 6% in normal children (King et al. 1983).

Autoimmunity

Genetic and environmental factors play triggering roles in the onset of the autoimmunity process with which a decrease occurs in the level of insulin hormone secreted from the pancreas (Haller et al. 2005). When the damage increases to 80-90% in the pancreas beta cell, the clinical characteristics of diabetes appear.