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REVIEW

Update on autoimmune polyendocrine syndromes (APS)

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Abstract. Autoimmune Polyendocrine Syndromes (APS) were initially defined as a multiple endocrine gland insufficiency associated to an autoimmune disease in a patient. Neufeld & Blizzard (1980) suggested a classification of APS, based on clinical criteria only, describing four main types. APS-1 is characterized by presence of chronic candidiasis, chronic hypoparathyroidism, Addison's disease. It is a very rare syndrome interesting young subjects correlating to different mutations of AIRE (AutoImmuneRegulator) gene on chromosome 21. APS-2 is characterized by presence of Addison's disease (always present), autoimmune thyroid diseases and/or type 1 diabetes mellitus. It is a rare syndrome interesting particularly adult females and associated to a genetic pattern of HLA DR3/DR4. Autoimmune thyroid diseases associated to other autoimmune diseases (excluding Addison's disease and/or hypoparathyroidism), are the main characteristics of APS-3. The different clinical combinations of autoimmune diseases not included in the previous groups are characteristics of APS-4. In this paper criteria for defining a disease as autoimmune are presente. Furthermore, the classification, epidemiology, pathogenesis, genetic, animal models, clinical features, laboratory's tests, imaging, therapy, recent progresses in understanding the APS and a detailed analysis of large group of our patients affected by different types of APS are proposed and discussed.

Key words: autoimmunity, autoimmune polyglandular syndromes, autoimmune diseases, Addison's disease, APECED, Schmidt's syndrome

Classification and Characterization of APS

Autoimmune Polyendocrine Syndromes (APS) were initially defined as a multiple endocrine gland insufficiency associated to an autoimmune disease in a subject. Probably the first description of an APS dates back to 1855 when Thomas Addison described pernicious anemia and vitiligo, in a patient with idiopathic adrenal insufficiency (1). Subsequently the association between diseases in APS was noted not to be at random but in particular combinations and that some non-endocrine autoimmune diseases were also part of the syndromes. After a careful clinical observation of affected patients, Neufeld and Blizzard in 1980 suggested a classification of APS, based on clinical criteria only, and described four main types (Table 1).

Table 1. Classification of the APS according to Neufeld 1980 (2) (modified)

APS-1	Chronic candidiasis, chronic hypoparathyroidism, Addison's disease (at least two present)
APS-2	Addison's disease (always present) + autoimmune thyroid diseases and/or type 1 diabetes mellitus
APS-3	Autoimmune thyroid diseases associated with other autoimmune diseases (excluding Addison's disease and/or hypoparathyroidism)
APS-4	Combinations not included in the previous groups

Pathogenesis of APS

In 1908 Claude (3) first hypothesized a common pathogenesis of APS, however, it was not the time yet

for a correct interpretation of their pathogenesis. In fact, in 1912 Hashimoto described a mononuclear leukocyte infiltration that was defined as " struma lymphomatosa" in some patients with goitrous thyroid glands (4). Addison previously described a similar infiltration in the patient with adrenal insufficiency and idiopathic atrophy of the adrenal cortex (1). In 1940 a similar infiltration was described within pancreatic islets of patients with type 1 diabetes mellitus and was called "insulitis" (5). In 1954, Bloodworth et al. (6) suggested, for the first time, that the accumulation of antibodies in the thyroid gland in patients with hypothyroidism and adrenal insufficiency (Schimdt's Syndrome) might be subsequent to reduced levels and protection of adrenal cortex hormones. In 1956 some interesting discoveries were made, the first consisted in demonstration of autoantibodies in the sera of patients with Hashimoto's thyroiditis which reacted with thyroid autoantigens (7), the second in the possibility to reproduce experimentally in rabbits a thyroiditis similar to Hashimoto's thyroiditis by immunization using autologous thyroid tissue (8), the third in the discovery of a factor capable of stimulating the thyroid gland defined "long-acting thyroid stimulator (LATS)" in the sera of patients with Graves' disease (9) which was subsequently recognized as an autoantibody to the TSH receptor (10). Thanks to these observations in 1957, Witebsky and Rose (11) formulated the criteria to define an autoimmune disease (Table 2) and Hashimoto's thyroiditis was the first disease included. In the same year it was discovered also that patients with "idiopathic" Addison's disease (AD) had serum autoantibodies against cytoplasmatic antigens of the adrenal cortex, besides the lymphocytic infiltration (12). These data, determined the inclusion of AD among this group of diseases as well as Hashimoto's thyroiditis.

In the following years, based on these criteria, several diseases considered until then "idiopathic" found a collocation within the group of autoimmune diseases. To date over 60 human diseases belong to this group (13). In recent years, several hypotheses have been put forward to explain how an individual can lose its tolerance against self antigens (14). From these sudies emerge that autoimmune diseases can be determined, in genetically susceptible individuals, by the release of sequestered antigens, environment-induced alterations of host membrane proteins, cross-reactivity between environmental agents and host antigens. A defect in the cells regulating the immune response must be supposed to underly this immunological disorder. If on the one hand, these theories try to explain why some individuals develop an autoimmune disease, on the other they do not explain why others develop an APS. Recently, it has been suggested that an APS may be due to external agents sharing one or more epitopes with a common antigen in several endocrine tissues (14). An interesting hypothesis is that organs deriving from a same germ layer can express common germ-layer specific antigens that could serve as targets for the autoimmune responses in APS (15). According to this theory, APS type 2, in which the targets are the adrenal cortex (mesodermal layer), the thyroid gland and the pancreas (endodermic layer), would derive from the autoaggression of 2 different germ-layer specific antigens or of an antigen common to both mesoderm and endoderm.

Table 2. Criteria of definition of an autoimmune disease (11).

- Demonstration of serum autoantibodies and/or cell-mediated events
- Demonstration of a lympho-monocyte infiltration in the target organ
- Possibility of identifying and isolating autoantigens
- Possibility of inducing experimentally the disease in animals by immunization with autoantigens and to transfer the disease passively by serum or lymphocytes

Animal models of APS

The difficulty of understanding the pathogenesis of APS is due also to the few animal models available. In NOD mice and obese strain chickens, which develop spontaneously type 1 diabetes mellitus or Hashimoto's thyroiditis, APS never attain the clinical stage (16, 17). In other animal models APS develop only after important immune-manipulations or after viral infections (18-23), however, in human APS no important immunological alterations or viral infections have been shown to date.

Table 3. Percentage frequency of major and minor clinical features in APS-1 in different populations.

Clinical Disorders	USA n=71 Neufeld 1981 (2, 28)	Finland n=68 Ahonen 1990 (32)	Iran n=23 Zoglotora 1992 (34)	USA n=16 Wang 1998 (71)	S. Italy n=11 Perniola 2000 (72)	Norway n=20 Myhere 2001 (36)	N. Italy n=47 Betterle 2002 (33, 37)	Total n=256 range
Hypoparathyroidism	76	79	96	100	100	85	89	76-100
Candidiasis	73	100	18	75	100	85	83	18-100
Addison's Disease	100	72	22	93	82	80	77	22-100
Alopecia	32	72	13	50	nd	40	36	13-72
Hypogonadism	17	60	38	24	18	31	40	17-40
Keratitis	nd	35	0	nd	nd	10	6	0-35
Autoimmune Hepatitis	13	12	nd	31	27	5	19	5-31
Vitiligo	8	13	nd	12	0	25	15	0-25
Malabsorption	22	18	nd	6	18	10	11	6-22
Pernicious Anemia	13	13	9	nd	9	0	15	0-15
Chronic Thyroiditis	11	4	4	31	36	10	11	4-36
Type I Diabetes	4	nd	4	nd	0	0	4	0-12
Cancer	1	nd	nd	nd	nd	nd	7	1-7

nd = not determined

APS -1

a. Historical news

The first case of APS-1 was that of a child with chronic tetany, due to hypoparathyroidism associated with chronic candidiasis reported in 1929 (24). However, only in 1943 a case of a 12 year old girl with non tubercular AD, idiopathic hypoparathyroidism, chronic candidiasis and phlyctenular keratoconjuntivitis was described (25). In 1956, in consideration of other similar reports, Whitaker added AD to the description by Torpe (26). In the following years the reports of patients with different combinations of these 3 diseases (candidiasis, hypoparathyroidism and AD) became more frequent, and in 1958 Bronsky analyzed 50 cases reported in literature (27), in 1981 Neufeld 71 cases (28). The present review analysed the data of 256 cases (47 of which are from personal observations) derived from the 7 largest published studies (Table 3). To these data it may be important to add many other singular cases reported from some case reports.

The following major clinical features characterize APS-1: *chronic candidiasis (CC), chronic hypoparathyroidism (CH), Addison's disease (AD)*. This APS has been referred to as: Whitaker's syndrome (26), autoimmune polyendocrinopathy type 1 (APS-1) (2), and APECED (Autoimmune Poly-Endocrinopathy, Candidiasis, Ectodermic Dystrophy) (27a). In this review it will be continued to be called APS-1 as suggested by Neufeld (2, 28). Two of the three major features are sufficient to make the diagnosis of APS-1 (29, 30), however approximately half of these patients develop all three components (31-33). Exceptionally the diseases present simultaneously, and in general they follow a precise chronological order. Patients begin with CC, followed by CH and finally by AD. As to the major features, these usually develop within 20 years of age whereas the minor components continue to develop lifelong (2, 28, 31-33).

b. Epidemiology

APS-1 is very rare, however, in some geographical areas and communities it is relatively more frequent. The prevalence ranges from 1:9000 inhabitants among the Iranian Jewish community (34), 1:14,400 in Finland (32), 1:25,000 in Sardinia (35); 1:80,000 in Norway (36), and 1:200,000 in Northern Italy (Veneto) (37). The variable concentration of APS-1 among these populations is the consequence of the variable concentration of a constitutive gene (see genetic section). The female/male ratio varies in different series from 0.8-2.4 (2, 28, 32, 33, 36, 38).

c. Main clinical features

1. Chronic Candidiasis (CC)

CC is the first clinical manifestation to occur generally during the first months of life, even if it can present up to 21 years of age; for many years this manifestation can remain undiagnosed. The mean age of occurrence is anyway before 5 years. In our patients the mean age was 6.7 years. The age at presentation of CC in our patients is reported in Figure 1. The frequency of CC varies from 18-100% in the examined populations (Table 3). It is rare in Iranian Jews (18%), and very frequent in Italians and patients from Northern Europe. CC may affect nails, skin, tongue, and the mucous membranes. CC is due to a selective immunological deficiency of T cells towards *Candida albicans* (2, 28, 32, 33). Anyway, patients usually maintain a normal B cell response, which prevents the development of systemic candidiasis (39). The defect of T lymphocytes in patients with APS-1 is demonstrated by skin anergy to candida and tuberculin antigens (38). In some patients the infection spreads to the esophagus and with time can lead to chronic esophagitis with retrosternal pain and severe complications such as esophageal stricture with subsequent dysphagia (32, 33, 38). CC may lead in some patients to epithelial carcinoma of the oral mucosa, tongue or esophagus (33, 38). In some patients CC may give abdominal pain, meteorism, and diarrhea and the symptoms subside after systemic anti-fungal treatment (38). APS-1 in consideration of CC has been included among the immunodeficiency diseases by the WHO (40). A review of several medical centers pointed out that it is always useful to evaluate and follow-up carefully children with CC as this may be the first clinical presentation of an APS-1; in fact this study evidenced that about 50% of these children developed the other typical components of APS-1 with time (41). Treatment of CC has changed with years. In the past Amphotericin B associated with transfer factor yielded the best results (2). At present, periodical treatment with Itraconazole is useful, although this drug is more effective in patients with nail infections and does not seem effective in those with mucosal infections (33, 42).

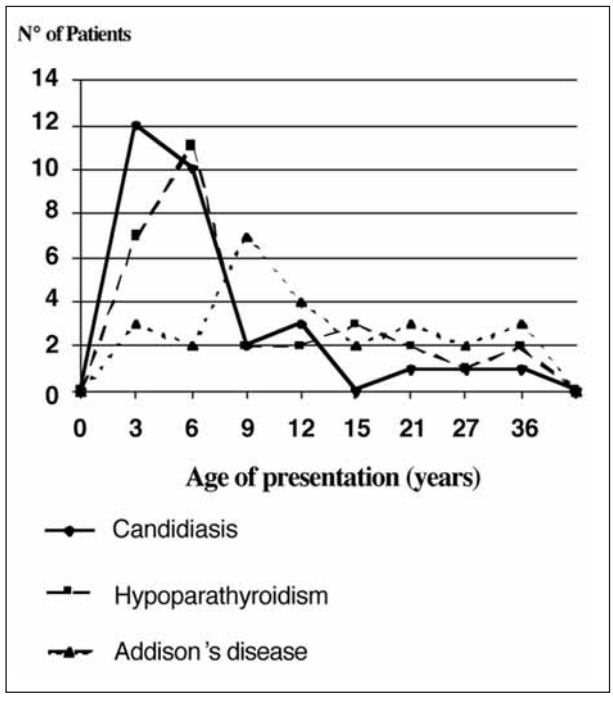

Figure 1. Age at presentation of the main diseases in APS-1 (personal series).

2. Chronic hypoparathyroidism (CH)

CH is the first endocrine disease to present usually after CC between 3 months and 44 years of age, although the presentation is usually before 15 years. In our series the mean age was 10.8 years and the features are reported in Figure 1. When CH presents in the neonate a differential diagnosis must be made with other genetic diseases as: a) Di George's syndrome (caused by a 22q11 gene deletion) (43, 44); b) Kenney-Caffey's disease (locus on chromosome 1q42-q43) (45); c) Barakat's syndrome (caused by haploinsufficiency of GATA3) (44, 46). Di George's syndrome is characterized clinically by a defect in the development of organs deriving from the neural crest and encounters cardiac defects in particular of the great vessels, hypocalcemic tetany due to failure of development of parathyroid tissue, and isolated T cell defect due to the absence of a normal thymus (47). Finally, hypoparathyroidism not associated with APS-1 can occur as an isolated disease with different patterns of inheritance (48, 49, 50).

a. Pathology

In the patients with APS-1 and CH in whom a post-mortem examination was possible an atrophy and infiltration of the parathyroid glands with mononuclear cells was shown. In some cases the atrophy was such that the parathyroid glands were undetectable (26, 38, 51).

b. Clinical Features

Clinically CH is characterized by: a) *paresthesias, b) neuro-muscular hypereccitability, c)hypotension, d) malabsorption and steatorrhea.*

The main sign is tetany (parossistic muscular contractions and generalized seizures). Contractions start from the periphery of the limbs and extend to the muscles of the trunk. The hands present a contraction in flexion of the thumb, which becomes opposed to the other hyperextended fingers (accoucher's hand). The upper limbs flex close to the trunk and the hands fold towards the forearms. The lower limbs are contracted and hyperextended with tarsal-podalic spasm. If the contractions involve the muscles of the face, the rims of the mouth assume the typical appearance of a carp fish mouth. In the worst cases the whole body is rigid. Contractures are painful but the patient never loses consciousness at variance with seizures. Sometimes there is a contraction also of the muscles of the larynx, which determines a stridulus laryngospasm. In some cases tetany is a medical emergency. In the mild forms tetany appears only if triggered (latent tetany) with the characteristic signs of *Chvostek* (contraction of the muscles innervated by the motor branch of the facial nerve, i.e. those at the corners of the mouth, base of the nose, eyelids, front) hammering the trunk of the facial nerve where it emerges, half-way between the corner of the lips and the external hearing meatus or of *Trousseau (*spasm of the hand muscles about 5 minutes after interrupting the afflux of blood). Latent tetany can be documented even by EMG during ischemia (ischemic garrot) (52).

Hypotension is the result hypotonia of the smooth muscles and is clearer during acute hypocalcaemia. Papilledema and calcification of the basal ganglia are other signs of chronic hypocalcaemia. Although cerebral calcifications are often very extended, only rarely they determine extrapiramidal dystonic syndromes or cerebellar syndromes. Subcapsular lens opacities and keratoconjunctivitis, sometimes with Sjögren's syndrome (see further down) can be typical. Other signs of hypocalcaemia are dry skin, thick hair and nail dystrophy. Defective enamel formation may also occur and is useful to date the beginning of hypocalcaemia back to childhood.

In some cases the correct diagnosis of CH is differed for months and the episodes of tetany are associated with alterations in the EEG dependent on low calcium levels; this leads to the incorrect diagnosis of epilepsy. Subsequently patients are treated for months with anti-epileptic drugs before the correct diagnosis of CH is made on the basis of low blood calcium levels or for the presentation of typical signs (36).

c. Laboratory tests

All the forms of CH are characterized by hypocalcaemia and hyperphosphoremia with low urine calcium and increased phosphorus. In the congenital and acquired forms and in Di George's Syndrome, PTH is low or undetectable whereas it is increased in pseudohypoparathyroidism (53).

Anti-parathyroid antibodies, identified in 11-38% of patients with CH by immunofluorescence on human parathyroids (54, 55) are not specific (56) as they recognize a human mitochondrial antigen of 46kD (57, 58). Thereafter, autoantibodies directed to the cell surface of the parathyroid cells have been identified (59) as cytotoxic in culture (60) but also these are non-specific as they are adsorbable by endothelial cells (61). Recently, in patients with APS-1 and CH, autoantibodies against calcium-sensing receptors have been reported (62), however, they have not been confirmed (38). Although cell-mediated immunity appears to play a role in CH, to date it is not possible to identify an antibody as a serological marker (63).

c. Treatment

Treatment is based on chronic administration of calcium and vitamin D. Acute hypocalcaemia must be treated administering calcium i.v.: in the adult 10-20

ml of 10% calcium gluconate (about 10 mg calcium/mL) in 10 minutes. In less severe cases the same dose may be given in 4-8 hours. Chronic treatment requires vitamin D and calcium per os in order to maintain blood calcium levels around 8-9 mg/dl. As PTH is missing, the renal enzyme 1-hydroxylase is inhibited, thus it is best to use vitamin D preparations already hydroxylated in position 1 as calcitriol, colecalciferol or dihydrotachysterol.

3. Addison's Disease (AD)

AD is the third main disease to present (the second endocrinopathy) in the natural history of APS-1 between 6 months and 40 years of age. In our patients the mean age at presentation was 14.6 years and the trend of presentation is shown in Figure 1. In the different populations studied, AD presented in 22-100% of cases (Table 3). Once again it is less frequent in Jewish and more frequent in Northern Europe (Table 3).

a. Pathology

The rare cases of APS-1 with AD in which the adrenal glands could be studied showed atrophy of the glands and lymphocyte infiltration (51, 64, C. Betterle, personal observation).

b. Diagnostic Imaging

Computerized Tomography (CT) scanning shows in patients with APS-1 and AD normal or atrophic adrenal glands.

c. Clinical Presentation

The signs and symptoms reported by patients with AD are due to the combined deficiency of glucocorticoids, mineralocorticoids and androgens (65, 66).

The main complaints are weakness, fatigue, malaise, apathy, weight loss and anorexia. Many symptoms are aspecific and usually present in a rather insidious manner. Weakness and fatigue are generalized, worsen with physical activity and improve with resting. Weight loss may vary from 2 to 15 Kg and is due to anorexia and dehydration and is visible in the advanced stages of adrenal insufficiency. Sometimes these patients feel the need to take suddenly some salts. In the undiagnosed cases the clinical conditions may worsen until the stage of adrenal crisis, which is a metabolic emergency and may lead to the exitus of the patient. Several factors may trigger an adrenal crisis such as infections, surgery or other stressful events. These subjects may be also extremely sensitive to drugs as narcotics and anesthetics and recover more slowly after illnesses and surgery.

Gastrointestinal symptoms are characterized by *nausea, vomiting, abdominal pain and diarrhea sometimes alternated with constipation.* Vomiting and abdominal pain may be early presenting features of an adrenal crisis. Cardiovascular symptoms are characterized by *hypotension,* mainly standing, which may determine syncopes. Metabolic symptoms are characterized by *hypoglycemia,* which presents after prolonged fasting or several hours after a meal rich in carbohydrates. This is common in children and is due to glucocorticoid deficiency combined with an increased peripheral utilization of glucose. Hypoglycemia is associated with increased insulin sensitivity, reduced gluconeogenesis and increased hepatic glycogen synthesis. In most cases *skin hyperpigmentation* is due to increased melanin synthesis. This is diffuse and is more intense in previously darker areas (areolae mammae, nevi, external genitalia), areas exposed to the sun or subjected to chronic micro traumas. Hyperpigmented spots may be seen on the mucosa of the mouth (cheeks, gengivas, rims of the tongue). The hyperpigmentation of the palmar folds and of scars formed during hypoadrenalism are typical, whereas previous scars remain white. Hyperpigmentation is due to increased levels of pro-opiomelanocortin, ACTH, β-lipotropin, melatonin or to a combined action of these peptides. Hair and nails become darker too. If vitiligo is also present the typical areas of depigmentation contrast more. If alopecia is coexistent areas of partial or complete loss of hair appear. Sexual dysfunction presents with primary or secondary amenorrhoea in 25% of females and is secondary to hypergonadotropic hypogonadism (see ahead). Thinning of pubic and axillary hair is secondary to reduced estrogen levels, reduced libido and sexual potency to adrenal androgen deficiency. Sometimes cases of asplenia have been reported (see ahead).

Neuro-psychological symptoms are characterized by *depression, psychosis, confusion, delirium, stupor, and pseudotumor cerebri*, which usually disappear with the correction of electrolytic disequilibria.

d. Laboratory tests

Full-blown AD is characterized by hyponatremia, hypochloremia and hyperkalemia with reduced plasmatic osmolality. Increased liver function tests and calcemia and reduced blood glucose levels may be present. The full blood count shows mild eosinophylia with lymphocytosis and micro- or macrocytic anemia. To diagnose AD, ACTH and cortisol must be measured at 8.00 a.m.: ACTH will be increased whereas cortisol reduced. Aldosterone and dehydroepiandrosterone are reduced and low aldosterone is associated with increased plasma renin activity. Testosterone levels are normal in males, while reduced in females as in these latter the adrenal gland is the main source.

In our patients with APS-1 and AD, antibodies against the adrenal cortex (ACA) determined by immunofluorescence, were positive in 86% of cases (Figure 2), however, at the onset of the disease they may be positive in up to 100% of cases. After 21-hydroxylase (21-OH) was recognized as the major antigen of ACA (67, 68, 69), laboratory tests to dose 21-OH antibodies were prepared. These antibodies were detected in 81% of our patients with APS-1 and AD (Figure 2) and at diagnosis may be positive in up to 100% of cases (37).

ACA/21-OHAbs may also be frequently detected (48%) in paediatric patients, which have the major components of APS-1 (CC and/or CH) without AD. Prospective studies have shown that all the positive children develop AD on average within 3 years of follow-up (70). Therefore, ACA/21-OHAbs must be assayed always in patients with APS-1 as they are useful diagnostic markers both in the presence and absence of clinical AD.

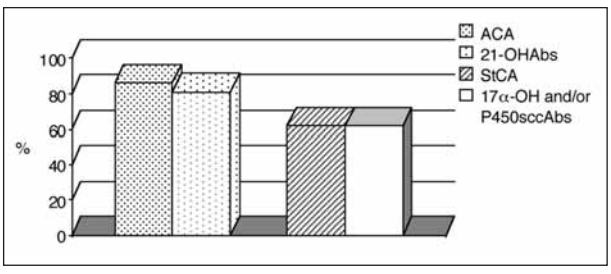

Figure 2. Frequency of ACA, 21-OH Abs, StCA, 17α-OHAbs and/or P450sccAbs in patients with AD and APS-1.

e. Treatment

Patients with AD must be instructed on the features of this endocrine deficiency, on the rational for replacement treatment, on the need to maintain treatment and modify it in the case of intercurrent diseases or stress, on when to consult a physician and when emergency parenteral treatment is required. Furthermore, one must explain that the treatment is a replacement treatment and as such if correct, does not have side effects, and must never be discontinued.

Patients with clinical AD must be started on 20-30 mg of hydrocortisone (in two or three doses) or 25-37.5 mg of cortone acetate. The aim is to use the minimum dose of drug to control clinical symptoms as higher doses than those necessary can determine undesired side effects as weight increase, hypertension, osteoporosis, and gastric ulcers. The determination of urine cortisol is useful to assess the appropriate dose of hydrocortisone (65).

Patients with primary AD must take also fludrocortisone in one dose of 50-100 mg, to replace aldosterone. Blood pressure levels, kalemia and plasmatic renin must be within the normal range and are the parameters necessary to regulate the adequate dose of drug (65).

All patients with AD should have a clinical record with themselves or a bracelet or a chain with the specific indications for their disease as is done usually in patients with cardiac disease (65). Patients must be advised to double or triplicate the dose of cortisone in the case of fever or disease and must always have some vials of cortisone to use in the case of vomiting (65).

d. Minor clinical manifestations

In patients with APS-1, other immune and non-immune mediated diseases have been described as 1) endocrinopathies: hypergonadotropic hypogonadism, autoimmune thyroid diseases, type 1 diabetes mellitus, lymphocytic hypophysitis; 2) autoimmune diseases of

the gastro-intestinal tract: chronic atrophic gastritis, pernicious anemia, coeliac disease; 3) malabsorption; 4) hepatic diseases: autoimmune hepatitis and cholelithiasis; 5) autoimmune diseases of the skin: vitiligo and alopecia areata; 6) autoimmune exocrinopathies as Sjögren's syndrome; 7) rheumatoid arthritis; 8) ectodermal dystrophy; 9) immunologic alterations: IgA deficiency, policlonal hypergammaglobulinemia; 10) asplenia; 11) malignant neoplasias; 12) calcification of basal ganglia, membranae tympani and sublenticular cataract; 13) vasculitis; 14) nephrocalcinosis (correlated with vitamin D therapy) (Table 3). It has been observed that the earlier was the first clinical presentation, greater will be the number of diseases that will develop during the life of a patient with APS-1 (28, 32, 33). Cancer of mucosae, usually develops in patients with long-standing diseases (Perheentupa 1999).

1. Hypergonadotropic hypogonadism

Hypergonadotropic hypogonadism is present in 24-60% of cases of APS-1 (Table 3) and is more frequent in females (60%) than in males (14%). In most cases hypogonadism presents after AD and usually as secondary amenorrhoea although in some cases as primary amenorrhoea (38, 73). In 60-80% of patients with APS-1, steroid-producing cell antibodies (StCA) have been detected (74-77). StCA are present in 60% of our patients (Figure 2). Generally, these antibodies are correlated with hypogonadism and their high frequency in APS-1 reflects the high incidence of hypogonadism in this APS (76-78). Recent studies have shown that the steroidogenic enzymes, 17α-hydroxylase (17α-OH) and P450 side chain cleavage (P450scc), are the main autoantigens recognized by StCA (77, 79, 80). RIA methods have been set to dose the antibodies against these enzymes (17α-OHAbs, P450sccAbs). The frequency of detection of these autoantibodies in our patients with APS-1 is summarized in Figure 2. A good correlation exists between StCA and 17α-OHAbs and/or P450sccAbs (37). Only in few patients with APS-1, hypogonadism and StCA it has been possible to analyze the hystology of the ovaries, which showed lymphocytic oophoritis (37, 76). Furthermore in most patients with lymphocytic oophoritis, StCA antibodies have been found in the circulation (76).

Generally, in lymphocytic oophoritis the primordial follicles are not inflammed while follicles in formation are infiltrated with lymphocytes and plasma cells (76). Immunohistochemistry has shown that the cells that infiltrate the ovaries are mostly T lymphocytes (CD4+ and CD8+) with few B-lymphocytes and many plasma cells, sometimes macrophages and NK cells (76).

Patients with APS-1 who are not hypogonadic may be StCA-positive; this occurs in 20% of cases and these patients have a high risk of developing clinical hypergonadotropic hypogonadism (73, 81).

2. Autoimmune thyroid diseases

Autoimmune thyroid diseases (ATD) occur in 4-36% of cases (Table 3), between 9-32 years of age and usually as chronic thyroiditis (33, 38). Idiopathic mixedema usually presents itself at a mean age lower than chronic thyroiditis (33). Graves' disease is exceptional in these patients. In our patients thyroid diseases presented in 11% of cases at an average age of 20.3 years. Anti-microsomial (anti-peroxidase) and/or anti-thyroglobulin antibodies are detected in most patients with autoimmune thyroid diseases (33, 38, 72). Anti-thyroid antibodies in the absence of clinical disease have been described in about 25% of cases with APS-1 (33, 38). Recently one of our patients, positive for thyroid antibodies for many years, developed hypothyroidism at 70 years of age, whereas another positive female patient developed Grave's disease. To our knowledge, this is the second case of APS-1 reported in the literature with this disease.

These data confirm that thyroid antibodies must be assayed in all patients with APS-1 and the positive cases must be carefully followed-up as they might develop autoimmune thyroid disease with clinical symptoms. With regard to clinical symptoms and signs, laboratory and radiological investigations in autoimmune thyroid diseases, please refer to two recent important reviews (82, 83).

3. Autoimmune Liver Diseases

Five-31% of patients with APS-1 presents with autoimmune hepatitis (Table 3) from 5-21 years of age.

The clinical history is very variable and includes subclinical diseases and fatal fulminating forms if not recognized immediately and treated with immunosuppressive drugs (32, 84). Autoimmune hepatitis correlates in APS-1 with positive antibodies against liver and kidney microsomes (anti-LKM) (72, 84). In these patients, these antibodies react with CYP-IA2 (85) and CYP-2A6 antigens (35). Anti-LKM antibodies are present even in 25% of our patients with APS-1 without alterations in their liver function tests. However, during follow-up only one of these subjects showed a transient alteration in liver function tests (33).

4. Alopecia Areata

Alopecia areata presents in 13-72% of cases (Table 3) from 3-30 years of age. Alopecia presents with patches on the scalp and then spreads to the entire scalp (total alopecia), and may expand further to eyelashes, eyebrows, axillary and pubic hair (universal alopecia). In some cases after a period of transient alopecia areata, hairs may regrow spontaneously (38). Autoantibodies against tyrosine hydroxylase have been detected in patients with alopecia areata (85a).

5. Vitiligo

Vitiligo presents in 0-25% of cases from the first month of life to 15 years (33). Complement-fixing melanocyte antibodies have been detected practically in all patients with vitiligo associated with APS-1 (33, 86, 87). These antibodies are not detected in patients with vitiligo and other APS (87). Recently it has been reported that 63% of patients with APS-1 and vitiligo have antibodies against transcription factors SOX9 and SOX10 (88). Complement-fixing melanocyte antibodies have been described in patients with APS-1 without vitiligo and in some cases preceded the presentation (89).

5. Type 1 Diabetes Mellitus

Diabetes Mellitus is very rare in APS-1 and presents in 0-12% of cases (Table 3). The age of presentation is 4-45 years (38). The disease is characterized generally by the presence of islet-cell antibodies (ICA), or autoantibodies to glutammic decarboxylase (GADAbs), to second islet antigen (IA-2Abs) and to insulin as in the classical form of the autoimmune type 1 diabetes (33, 38, 90, 91).

In patients with APS-1, autoantibodies against a 51kD antigen of the beta cells of the pancreatic islet-cell also have been described (92) and identified with the L-amino aromatic acid decarboxylase (93). However, this antibody is not correlated with type I diabetes mellitus but with autoimmune hepatitis and vitiligo (94). In patients with APS-1 without type I DM, autoantibodies against the endocrine pancreas are very frequent, in particular ICA are present in 18-30% of cases (33, 38, 72) and GADAbs in 41% (38). Despite the high prevalence of these autoantibodies, most patients with APS-1 do not develop type 1 DM. In one of our patients, who died at 18 years because of a cerebral hemorrhage and positive for ICA, GADAbs and 51kDAbs for many years, the immunohistochemistry of the pancreas did not show lymphocyte infiltration confirming that in these patients autoimmunity against the insulae was only serological (95). The hypothesis is that very few of these subjects with positive antibodies develop DM because they lack genetic predisposition or because autoantibodies can recognize different epitopes than those of patients with classical DM (33, 38).

6. Chronic atrophic gastritis with or without pernicious anemia

In patients with APS-1 chronic atrophic gastritis presents in 13-27% of cases and is associated with parietal cells antibodies. Pernicious anemia presents in 0-15% (Table 3), on average at 19 years (33). In this latter disease besides parietal cell autoantibodies even intrinsic factor antibodies are present (33, 72). Intrinsic factor antibodies have been shown even in 32% of patients with APS-1 without macrocytic anemia and some of these have developed pernicious anemia after a mean follow up of 7 years (33).

7. Malabsorption and alterations in the opening of the bowels

Malabsorption was first described in patients with

APS-1 in 1953 (96, 97). Malabsorption presents in 18-22% of cases (Table 3) and may be due to different causes. Coeliac disease associated to reticulin or endomisium antibodies can be one cause (33), cystic fibrosis (98), pancreatic insufficiency (99, 100), intestinal infections from Candida Albicans or Giardia Lamblia (100), intestinal lymphangectasia (101) could be other causes. In some patients with "idiopathic malabsorption" the symptom is well controlled with immunosuppressive treatment suggesting as possible cause a disorder of the immune system (102, 103). A recent study confirmed this hypothesis identifying in 48% of patients with APS-1 the tryptophan hydroxylase autoantibodies (TPH-Abs). These autoantibodies were correlated with gastrointestinal dysfunction resistant to all pharmachological treatments (104). The sera of patients with TPH-Abs reacted with normal human intestinal enterochromaffin cells, and cells containing serotonin were not found in intestinal biopsies from these patients as in the jejunum of normal subjects (104). TPH-Abs are absent in patients with gastrointestinal disorders but unaffected by APS-1 (104). Tryptophan-hydroxylase is an enzyme belonging to the group of pteridin-dependent enzymes, involved in the synthesis of neurotransmitters (105). The recent discovery of an idiopathic cholecystokinin deficiency in a patient with APS-1 with this complaint, confirms the multiplicity of causes of malabsorption (106).

9. Hypophysitis

Seven per cent of our cases with APS-1 present with lymphocytic hypophysitis with isolated or multiple tropin deficiencies. Rarely these patients have antipituitary autoantibodies. Autoantibodies against PRL-secreting cells have been described in patients with APS-1 who were not prolactin deficient and the clinical meaning of this finding remains uncertain (107).

10. Neoplasias

Patients with APS-1 can present with malignant neoplasias (squamous carcinoma of the mouth mucosa, the tongue, esophagus, adenocarcinomas of the stomach) (32, 33, 38). Four of our patients developed epithelial neoplasias (37).

11. Gallbladder Stones

Gallbladder stones were first reported in 1991 in 4/9 patients with APS-1 (108). The disease presents early with respect to the general population and could be secondary to malabsorption, which causes an alteration of the bile cycle and predisposition to deposit cholesterol stones. One of our patients developed stones at 28 years and the gallbladder was subsequently removed.

12. Ectodermic dystrophy

Ectodemic dystrophy presents in 10-52% of cases and is characterized by nail dystrophy, defects in the formation of tooth enamel, bad implant of teeth (32, 109a).

12. Asplenia

Asplenia has been very rarely described and may be congenital (Ivermark syndrome) or acquired. The acquired form seems to depend on a progressive immune-mediated destruction of the spleen or to vascular insults (108). Asplenia was first described in a patient with APS-1 in 1968 (109). The frequency of this finding is not established with certainty, infact it was reported by Parker in 2/3 patients (110), by Friedman in 4/9 (108), but in none of the 69 cases described by Ahonen (32) and 1 of 15 of our evaluated patients (33). Asplenia must be suspected whenever Howell-Jolly bodies, thrombocytosis, anysocytosis, poikilocytosis, target and burr cells are observed in the blood smear. The frequency of occurrence of major and minor diseases in patients with APS-1 are summarized in Table 3.

All these data evidence that patients with APS-1 tend to develop a large number of diseases during their lives, mainly autoimmune diseases. In Ahonen's study a total number of 231 diseases were documented in 69 patients (32). We documented 213 diseases in our 47 patients. Therefore, APS-1 surely represents the model of human autoimmune disease in which the most severe deficiency of immune tolerance, probably subsequent to the particular genetic condition (see ahead) occurs.

Table 4. APS-1: autoantibodies in minor diseases

Presentation	Autoantibodies to	Before the disease
Hypergonadotropic hypogonadism	steroid-producing cells (StCA)	Yes
	17α-OH	
	P450scc	Yes
Vitiligo	melanocyte (Complement-fixing)	Yes
	transcription factors (SOX9 and SOX10)	?
Autoimmune hepatitis	liver-kidney microsomal	Yes
	P450-IA2	?
	P450-2A6	?
Coeliac disease	reticolin	Yes
	endomisium	Yes
	transglutaminase	
Type I Diabetes Mellitus	islet-cell (ICA)	Yes
	GAD	Yes
	IA2	Yes
	51 kD	?
Autoimmune thyroid diseases	thyroid microsomal	Yes
	thyroglobulin	Yes
Chronic atrophic gastritis	parietal cells (PCA)	Yes
Pernicious anemia	PCA+ anti-intrinsic factor	Yes
Malabsorption	tryptophan hydroxylase	?
Alopecia areata	tyrosine hydroxylase	?
Adenohypophysitis	pituitary	?

13. Autoantibodies in the absence of disease

Patients with APS-1 often present with one or more positive autoantibodies in the absence of the corresponding clinical disease (Table 4). With the exception of ICA and/or GADAbs, which are associated with a low risk of developing type 1 DM, the other antibodies can precede the clinical disease. Therefore, we think it is useful and necessary to perform in patients with APS-1 a wide autoantibody screening and to repeat it periodically (every 1-2 yr).

Genetics

APS-1 presents both in a sporadic and familial form (31, 51, 111) with an autosomic recessive inheritance (27a, 112). Initially a correlation between class I major histocompatibility antigens (MHC) and APS-1 was described. HLA-A28 has been detected more commonly in patients with APS-1 with respect to the normal control populations, whereas HLA-A3 was present in patients with APS-1 and ovarian failure compared to patients with normal gonadal function (113). With regard to class II antigens an association with HLA-DR5 has been reported in Jewish Persians (114) and in Italians (33) with APS-1. The TCLA-4 (cytotoxic T lymphocyte antigen-4) gene has not been related to APS-1 in the different ethnic groups studied (115).

In 1994, investigating 14 Finnish families with APS-1, a relation was found with a gene on chromosome 21 (116). The responsible gene was subsequently isolated, identified, cloned and defined AIRE (Autoimmune Regulator) (117). The AIRE gene is made of 14 exons and codifies a 545 aminoacid protein (117, 118). Until the year 2001, 42 different mutations associated with APS-1 have been identified but only four seem to be the main (119) (Figure 3).

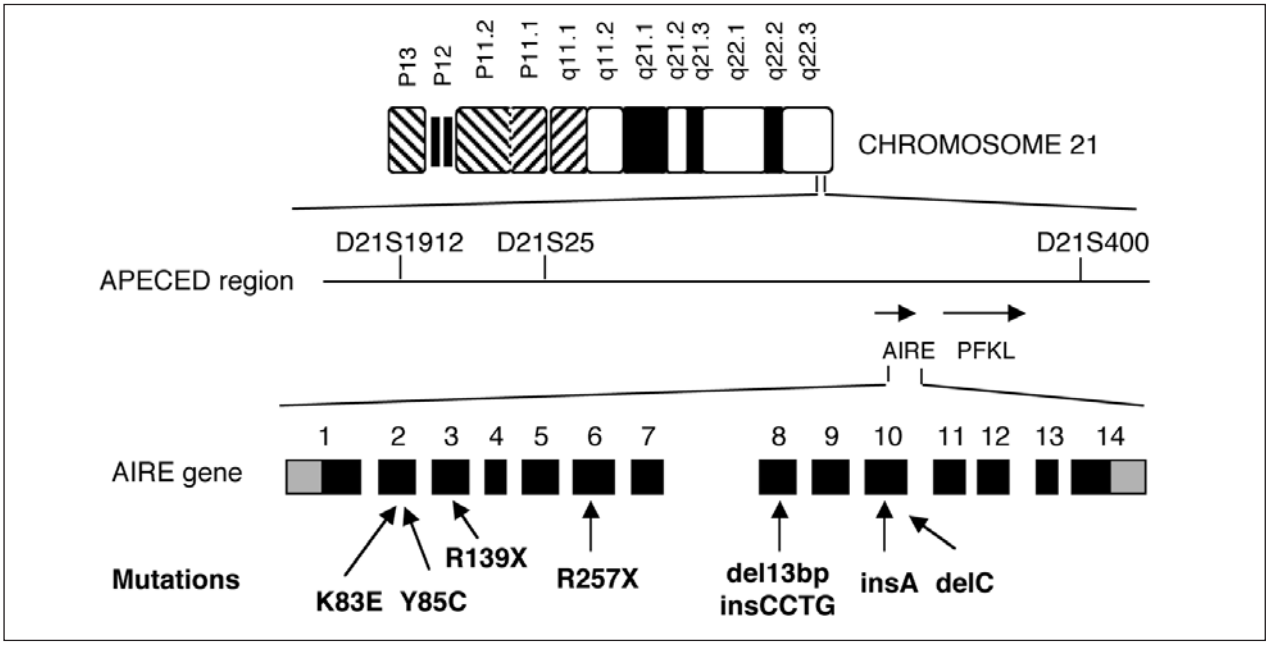

Figure 3. Schematic representation of chromosome 21 showing the map of the APECED region (at the top) and the structure of the AIRE gene with the main mutations associated with the presence of APS-1 (at the bottom).

The first and most important mutation discovered was the R257X mutation in exon 6 (117, 118-120). This is present in 82% of the alleles of the Finnish with APS-1, but is also the most frequent in other ethnic groups as in patients from Northern Italy, Switzerland, English, German, Newzealanders and Americans of Anglo-Saxon origin (71, 121). The *del13* in exon 8 (117-120) is the most common mutation in Caucasian American patients, in particular in those originating from Northern and Eastern Europe or in Anglo-Saxon patients, but also in Italians (36, 71, 118, 121-124). The R139X in exon 3 is the most common mutation in patients with APS-1 from Sardinia, being found in 18 of 20 alleles (123). The Y85C mutation is instead the only one identified in Jewish Persians (125). Other minor mutations occasionally described are: *insA*, 3 different *C mutations*, *k83E*, *Q173X*, *R203X*, *X546C*, *L28P*, *R15L* (103, 120, 126, 127).

These data show that APS-1 is the first autoimmune disease caused by the mutation of a single gene. However, the clinical expression of the disease depends on the presence of the mutation in both alleles of the AIRE gene. In fact the parents and siblings of patients with APS-1 who have just one mutated allele, generally do not present any sign of the syndrome. The mutations of the AIRE gene observed in APS-1 could be responsible for the alteration in the immunologic tolerance in humans (120). Subsequently the knowledge of the biological role of the AIRE gene could lead to new understanding of the mechanisms regulating tolerance and autoimmunity (38). The AIRE gene seems to be involved with negative selection and induction of self-reactive thymocyte anergy (128). The AIRE gene has a high concentration in the thymus (in epithelial cells and in the cells of the monocytic-dendritic line, which both are antigen-presenting cells) and a low concentration in the spleen, lymphonodes, pancreas, adrenal cortex and peripheral blood mononuclear cells (117, 118). Among the 47 Italian patients suffering from APS-1 we studied, 10 came from 5 different families and the other 37 with sporadic mutations had no family history of the disease. Seventeen came from the region Veneto, which has a population of approximately 3.5 milions. Considering these data we calculated the prevalence of APS-1 in this region, which was 1 case every 200,000 inhabitants. However, we observed that 9 of these 17

Table 5. Aplotypic analysis of the AIRE gene in 10 patients from the Veneto region

Patients	Mutations Allele1/Allele 2	Sex	Major Diseases	Age at presentation of APS-1
1. F.T.	R257X/R257X	F	CC+CH+AD	Adult
2. F.L.	R257X/R257X	F	CC+CH+AD	Young
3. A.E.	R257X/R257X	M	CC+CH+AD	Young
4. C.A.	R257X/R257X	M	CC+CH+AD	Young
5. C.G.	R257X/R257X	M	CC+CH+AD	Young
6. C.G.	del 13/R257X	F	CC+CH+AD	Young
7. C.E.	del 13/R257X	F	CC+CH+AD	Young
8. T.P.	del 13/del 13	F	CC+CH+AD	Adult
9. DG.F.	del 13/del 13	M	CC+CH+AD	Adult
10.S.M.	R139X/R139X	F	CC+CH+AD	Young

Patients 4 and 5 are brothers and patients 6 and 7 sisters. They belong to 2 different families.
CC: Chronic candidiasis; CH; Chronic hypoparathyroidism; AD: Addison's Disease

patients came from a limited area in the province of Vicenza. The high concentration of patients with APS-1 in this area is certainly due to a high concentration of mutations in the AIRE gene. In accordance with the preliminary data (121), the R257X mutation was confirmed to be the commonest in our regional population as it was found in 7/10 subjects (5 homozygotes and 2 heterozygotes for the del 13). Two patients were homozygotes for del13 (the most frequent in Anglo-Saxon patients). It is interesting to point out both patients homozygous for *del13* presented with APS-1 as adults. Furthermore, the tenth patient, a homozygous R139X mutation was found as described only in the population from Sardinia (Table 5). From a practical point of view, it is possible to date to perform a screening to identify the mutations of the AIRE gene in communities or areas at high risk of this disease or in unaffected relatives of patients with this syndrome. In particular it is useful to research the R257X and *del13*, but also R139X and Y85C, which are predominant in particular populations. These investigations will allow an early diagnosis of APS-1.

5. APS -2

a. History

The association between AD and chronic thyroiditis was first described in two patients in 1926 (129). In 1931 the first case of an association of AD, hyperthyroidism and diabetes mellitus (130) and in 1932 the first case of a patient with AD, hypothyroidism and diabetes mellitus were described. The last case died for ketoacidosis and the post mortem examination showed completely ialinized pancreatic islet with a lymphocyte infiltration, few cortical cells were visible in the adrenals but signs of chronic inflammation and fibrosis were present. In addition the thyroid showed a lymphocyte infiltration altering the normal structure of the gland (131). In the following years, APS-2 was progressively more frequently found and in 1964 Carpenter (in a review of the literature) described over 100 cases (132). He suggested this association should be called "Schmidt's syndrome" from the name of the Author who first described it. In 1981 Neufeld studied 224 cases (28). In the present review we examined 351 patients (Table 6).

b. Epidemiology

APS-2 (2) or Schmidt's syndrome (132) is characterized by AD associated with autoimmune thyroid diseases and/or type 1 DM (2). This APS is rather rare with an incidence of 1.4-4.5 cases every 100.000 inhabitants (127, 133). APS-2 affects mainly adult women, and is very rare in children (2, 28, 37, 75, 133). The mean age at presentation is 35 years (28, 132, 133). The F/M ratio is 2.7-3.7 (Table 6). Of the 107 cases we

Table 6. Diseases in APS-2

	Neufeld 1980 (2)	Papadopoulos 1990 (134)	Betterle 2002 (37)	Total
N° Patients	224	22	107	351
F/M	nd	2.7	3.7	2.7-3.7
Familiarity for APS 2	nd	nd	0	0
Main diseases	%	%	%	%
- Addison's Disease	100	100	100	100
- TAD	69	73	82	69-82
- DM type 1	52	41	30	30-52
Minor diseases	%	%	%	%
- Vitiligo	5	4.5	11	4.5-11
- Simple atrophic gastritis	nd	nd	11	11
- Hypergonadotropic Hypogonadism	4	9	7	4-9
- Autoimmune Hepatitis	nd	nd	4	4
- Alopecia	1	nd	4	1-4
- Neoplasias	nd	nd	2	2
- Pernicious anemia	<1	4.5	1	1-4.5
- Miastenia gravis	nd	nd	0	0
- Adenohypophysitis	nd	nd	0	0

nd = not determined

studied, 89% had AD associated with another main component of APS and only 11% had all three major components (29).

c. Major clinical manifestations

1. AD

One hundred percent of patients with APS-2 have AD being for convention the disease always present (2, 28, 37, 129, 133-135). In our 107 patients with APS-2, the mean age at presentation of AD was 36 years. In Figure 4 the trend of presentation of AD is reported.

The pathology, symptoms and clinical signs and the laboratory investigations of AD in APS-2 are identical to those reported in the previous chapter (please refer to) but for the age of presentation of the disease. Even in patients with APS-2 a CT or MRI scan of the adrenals shows normal or small glands, however in long-standing disease the glands are atrophic (37).

In patients with APS-2 of different duration, ACA are present in 89% of the cases while 21-OHAbs in 91% (Figure 5) (77), however if assayed at diagnosis of AD they can be positive in up to 100% of cases (37).

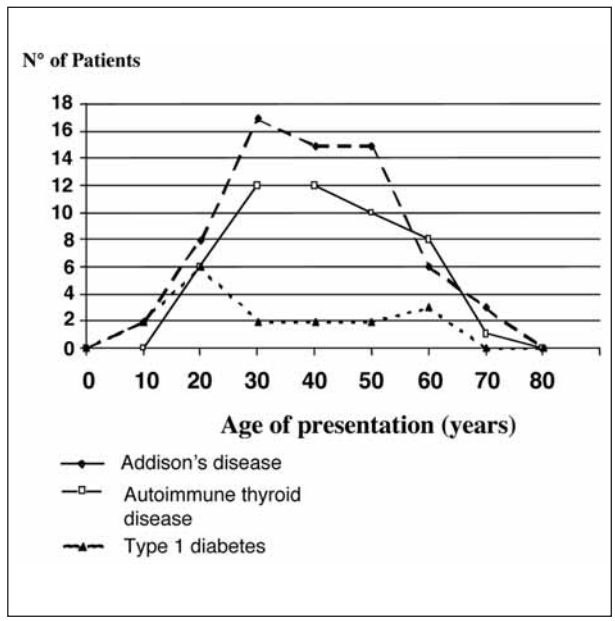

Figure 4. Age at presentation of the fundamental diseases in APS-2 (personal series).

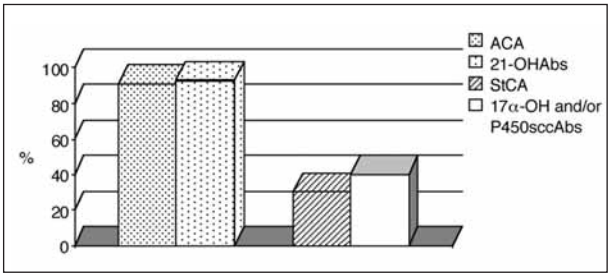

Figure 5. Frequency of ACA, 21-OHAbs, StCA, 17α-OHAbs and/or P450sccAbs in 55 patients with APS-2.

2. Autoimmune Thyroid Diseases (TAD)

In APS-2 TAD are reported to occur from 69-82% of cases, according to the series of patients (Table 6). In our patients, TAD occurred in 82% of cases: in particular, 50% had AD + chronic thyroiditis, 21% had AD + Graves disease and 11% had the complete triad AD + TAD + type 1 DM. The trend of presentation of TAD is summarized in Figure 4. Generally, Graves disease developed before AD at a mean age of 31 years, while chronic thyroiditis developed simultaneously or after AD at a mean age of 39.6 years (133, 134, 136). A recent review of literature will provide further knowledge on the diagnosis, clinical features and treatment of chronic thyroiditis and Graves disease (82, 83).

3. Type 1 Diabetes Mellitus

Type 1 Diabetes Mellitus (DM) occurs in 30-52% of patients with APS-2 (Table 6). In our series of patients with APS-2 type 1 DM presented in 30% of cases; in detail 18% had AD + DM, 12% had AD + TAD + DM. The mean age at presentation of DM was <28 years and the trend is summarized in Figure 4. Patients with DM in the context of APS-2 have a high frequency of positive ICA, GADAbs or IA2 Abs (137). DM may present acutely or have a non-acute onset, as in LADA (latent autoimmune diabetes of the adult).

d. Incomplete APS-2: a poorly known entity

Neufeld had established in his paper that a patient could be classified as having APS-2 if affected by TAD or type 1 DM and if in the family at least one member had APS-2 (2). We do not think this is correct; we think a patient should be considered to have incomplete APS-2 if besides AD, positive thyroid antibodies and/or ICA or GADAbs or if besides TAD and/or type 1 DM, ACA/21-OHAbs are positive independent of the family situation. With regard to this we would like to mention that the modern conception of autoimmune diseases considers chronic illnesses with a long period of latency mainly asymptomatic and that in the natural history of these diseases three distinct phases can be identified: a) potential; b) subclinical or latent, and; c) clinical. The first phase is characterized only by the detection of autoantibodies, markers of the disease with no functional alteration of the target organs; in the second phase appropriate functional tests show alterations and only in the third phase clear clinical manifestations present (13). Therefore, we have suggested classifying a subject with incomplete APS-2 as potential or subclinical, on the basis of the functional situation of the target organ. Patients with AD and ICA and normal glucose tolerance or patients with type 1 DM with ACA/21-OHAbs with normal adrenal function should be considered as having potential APS-2. Instead subjects with AD and thyroid autoantibodies with subclinical hypothyroidism or subclinical hyperthyroidism or patients with AD + ICA or GADAbs with impaired glucose tolerance after glucose challenge, or with type 1 DM + ACA/21-OHAbs and subclinical adrenal insufficiency, should be regarded as having subclinical APS-2 (138). The different combinations of incomplete APS-2 are summarized in Table 7.

This approach allows identifying incomplete APS-2 in the patients with only one constitutive disease of the syndrome (type 1 DM or TAD or AD). Patients with complete APS-2 are rather rare but represent only the " tip of the iceberg" while certainly many other cases of incomplete APS-2 exist and can be identified using only the approach we suggest. Once these subjects have been identified specific investigations must be carried out (fT3, fT4, TSH, oral glucose tolerance test, ACTH-test) to discriminate those with potential from subclinical APS-2. With regard to these latter, the onset of a specific treatment in a very early phase can be of great benefit and can prevent modifi-

Table 7. Possible combinations of incomplete APS-2

Chronic Diseases		Serology
Addison's Disease	+	Thyroid Abs and/or ICA and/or GADAbs
Thyroid autoimmune diseases (TAD)	+	ACA/21-OHAbs
Type 1 DM	+	ACA/21-OHAbs
TAD + Type 1 DM	+	ACA/21-OHAbs
None		ACA/21-OHAbs + thyroid Abs and/or ICA and/or GADAbs

ACA=Adrenal cortex antibodies; 21-OHAbs=21-hydroxylase autoantibodies; ICA=islet-cell autoantibodies; GADAbs=glutamic acid decarboxylase autoantibodies

cations in the well-being and sometimes be life-saving (37).

e. Minor autoimmune diseases

In APS-2 other minor autoimmune diseases can develop: for example hypergonadotropic hypogonadism (4-9%), vitiligo (4.5-11%), alopecia (1-4%), autoimmune hepatitis (4%), chronic atrophic gastritis, pernicious anemia (4.5-11%) and hypophysitis. These diseases, however, present with a lower frequency compared with APS-1 (Table 6). Generally, these minor diseases are associated with positive autoantibodies, serological markers of the illness, however sometimes these antibodies may precede the clinical onset of the corresponding disease (133).

Our 107 patients with APS-2, developed altogether 240 autoimmune diseases and this suggests that although in APS-2 the genetic susceptibility is completely different from that of APS-1 (see ahead), even in this disease an important alteration of immunologic tolerance occurs.

f. Genetics

APS-2 may present in several generations of a same family in an autosomic dominant fashion with incomplete penetrance (139, 140). HLA antigens play a role in conditioning T lymphocyte response to antigens and the association of different HLA alleles with many autoimmune disorders has been shown (141, 142). In patients with APS-2 an increased prevalence of HLA-DR3 and/or DR4 has been found (143). Several subsequent studies have confirmed the association with HLA-DR3 in patients with APS-2 and in particular with DRB1*0301, DQA1*0501, DQB1*0201 haplotype (133, 144-150), whereas the association with HLA-DR4 has not been confirmed (133, 145-149). Huang showed that the subtype HLA-DR3, DQB1*0201 was increased in American patients with APS-2 whereas HLA-DR4, DQB1*0302 was increased in the subjects who had associated type 1 DM (149). Recently an increased DR3-DQ2 and DR4-DQ8 independent of type 1 DM has been described in Norwegian patients with APS-2 (137). We have studied the HLA genetic arrangement in 40 patients with APS-2 and have found that DR3 and DR4 are significantly increased in APS-2 subjects with a relative risk of 2.74 and 3.19 respectively and that this increase is independent of type 1 DM. Furthermore we observed an inverse correlation with DR1, DR7, DR13 and DR14, which would give protection (Figure 6).

In patients with APS-2 other HLA-related genes have been studied, such as the TNF (Tumour necrosis factor) gene belonging to class III (148) and the MIC-A gene belonging to HLA class I (150), however because of a tight linkage disequilibrium among the genes of this region it is difficult to determine the independent role of each one.

The antigen 4 gene on cytotoxic T lymphocytes (CTLA-4) on chromosome 2 encodes a co-stimulatory molecule, which is an important negative regulator in the activation of T cells (151). This locus has been related to type 1 DM and to autoimmune thyroid diseases (Graves' disease and Hashimoto's thyroiditis) (152-154). Studies on German patients with APS-2 suggested that ala17 allele of CTLA-4 is significantly associated only in a subgroup of patients owning the HLA-DQA1*0501 allele (153). A study on APS-2 patients, from different European countries, showed that only in English there was an association with CTLA-4 (115, 155). Patients with APS-2 have been

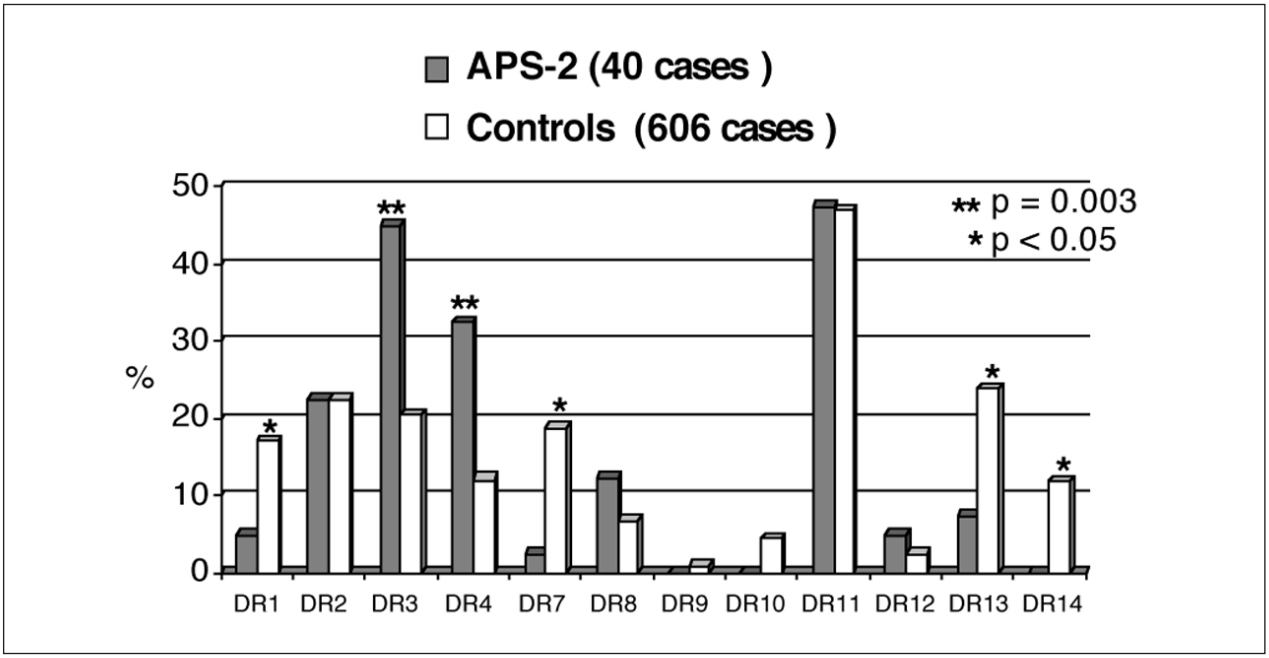

Figure 6. HLA-DR in patients with APS-2.

studied even for *del13* on the AIRE gene (typical of English with APS-1) but the frequency of this mutation was not different from that of the normal population (155).

C. APS-3: association between autoimmune thyroid diseases and other autoimmune diseases

1. APS-3 according to the classification by Neufeld and Blizzard

In the original classification by Neufeld, APS-3 was defined as the association between a clinical entity of TAD (Hashimoto's thyroiditis, idiopathic mixedema, asymptomatic thyroiditis, Graves' disease, endocrine ophtalmopathy, pretibial myxoedema) and another autoimmune disease as type 1 DM (Type 3a), chronic atrophic gastritis, pernicious anemia (Type 3b), vitiligo, alopecia, miastenia gravis (Type 3c). The authors also hypothesized a fourth group (Type 3d) which included other unspecified diseases (Figure 7). AD and/or chronic hypoparathyroidism were obviously categorically excluded from this APS (2).

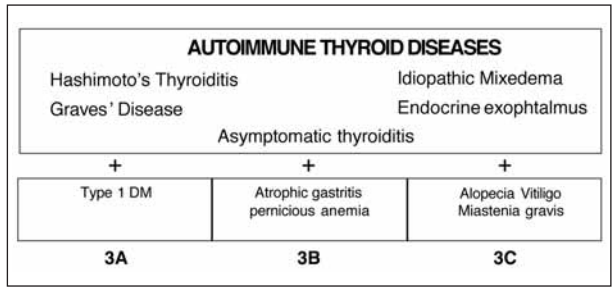

Figure 7. APS-3 and subgroups according to Neufeld (Neufeld 1980)

B. APS-3: proposal for a new classification

In the following years APS-3 appeared to be more complicated than initially reported by Neufeld. All these observations lead to a review of clinical, genetic and immunological aspects of this syndrome (156). However, even this review is incomplete and does not show the complex heterogeneity of this APS. For this reason we analyzed carefully the clinical and serological features of a series of 288 patients with TAD which came to our observation in the years

1997-99. The clinical analysis showed that 28% of the patients had another clinical autoimmune disease (and in many cases the observed combination could not be included in the classification suggested by Neufeld as for example TAD + coeliac disease, TAD + hypophysitis, TAD + autoimmune hemolytic anemia, TAD + multiple sclerosis, TAD + LES, TAD + Sjögren's syndrome). Furthermore, in patients with apparently isolated TAD, organ- and non organ-specific autoantibody screening showed a further 24% of patients with one or more of these autoantibodies, revealing an incomplete APS-3. On the basis of these data 52% of patients with TAD can be considered affected by APS-3 (complete or incomplete). Moreover, considering that TAD (82, 83) are the most frequent autoimmune diseases in the population being present on average in 7-8% of the general population (10% in women and 3% in males) it was calculated that 3.5-4% of the total population (5% of women and 1.5% of males) has a complete or incomplete APS-3. Moreover, considering that TAD are the most frequent diseases in patients with other autoimmune diseases in particular they are present from 10-30% of patients with DM type 1, pernicious anemia, coeliac disease, multiple sclerosis, vitiligo, alopecia, Sjögren's syndrome, autoimmune hemolytic anemia, biliary cirrhosis, LES, it is easy to understand why APS-3 is the most important and frequent among those described to date. Therefore we have suggested a revision of APS-3 evidencing 4 subgroups on the basis of the apparati or main systems implicated (Figure 8).

D. APS-4: Autoimmune diseases associated with other diseases not enclosable in the previous classifications

APS-4 is a rare syndrome, which includes all the clinical combinations, which cannot be allocated in one of the previously described APS (2). For example AD associated with hypogonadism, chronic gastritis, pernicious anemia, coeliac disease, miastenia gravis, vitiligo, alopecia, hypophysitis, etc. or the combinations of type 1 DM with hypogonadism, chronic gastritis, pernicious anemia, coeliac disease, miastenia gravis, vitiligo, alopecia, hypophysitis can be included in this specific APS.

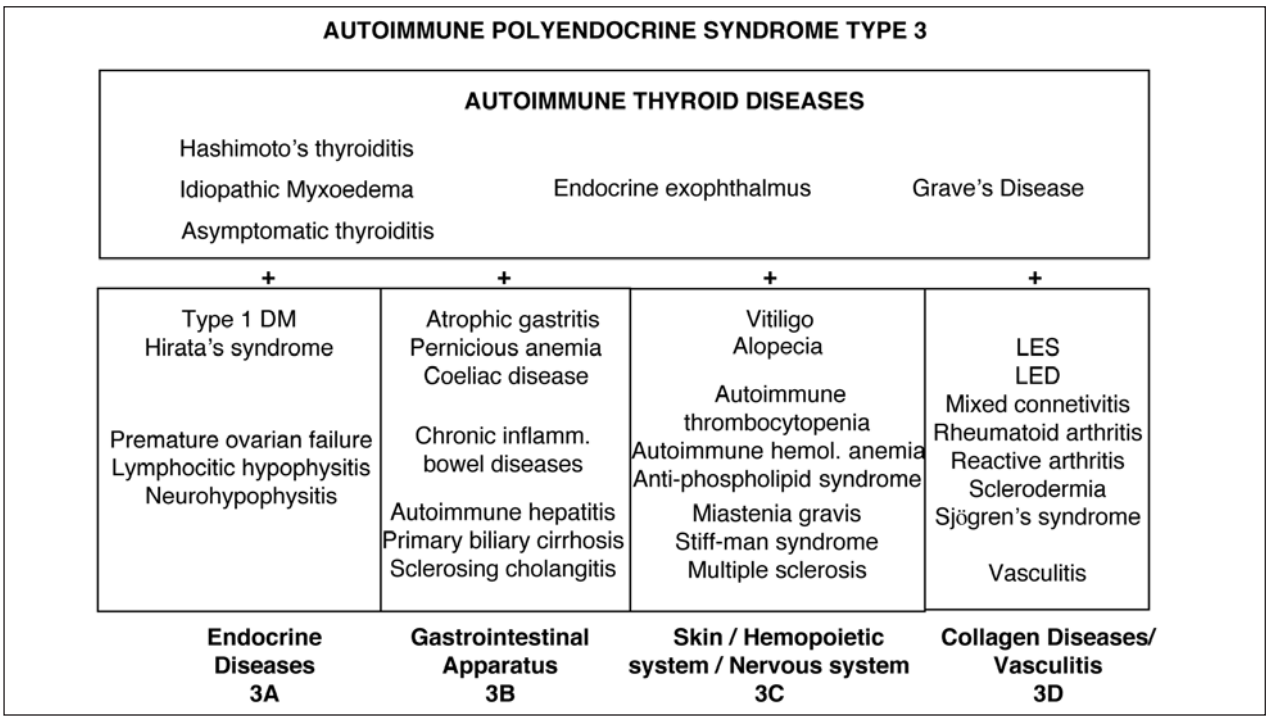

Figure 8. APS-3 and subgroups according to the new classification (Betterle 2001).

Conclusions

In recent years the study of APS has received a great impulse thanks to improved knowledge of autoimmune diseases and their natural history. This has allowed identifying patients with APS in the potential or subclinical phase and to start an early hormonal replacement treatment. The discovery of new autoantibodies correlated with clinical manifestations has allowed to understand the pathogenesis of such manifestations and to prepare new therapies. The identification of many autoantigens, targets of the autoimmune reactions, has allowed the preparation of new diagnostic tests very sensible and specific and to facilitate the diagnosis. The discovery of the genetic mutations in patients with APS-1 will allow us to perform screenings in high-risk populations or in the relatives of affected patients to identify the subjects at risk early. The study of the role of the AIRE gene and of its products on the immune responses will allow understanding better the mechanisms that regulate immunologic tolerance and subsequently those which allow autoimmunity.

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