Diagnosis and Management of Polyendocrinopathy Syndromes

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KEYWORDS

• APS2 • APS1 • IPEX

The autoimmune polyendocrinopathy syndromes (APS) encompass a wide clinical spectrum of disease with monogenic and complex genetic etiologies. Their presentation is highly variable with the first manifestation occurring anywhere between infancy and late adult life. The commonest group of disorders seen, APS2 and its associated disorders, generally present in adulthood (**Table 1**) and are of complex genetic etiology. The rarer monogenic disorders, APS1 (see **Table 1**) and IPEX, tend to present in childhood or adolescence. In this article, we review the presentation, investigation, and management of these varied conditions.

AUTOIMMUNE POLYGLANDULAR SYNDROME TYPE 2 AND ASSOCIATED DISORDERS

APS 2

APS2 is defined by the presence of primary adrenocortical insufficiency with either autoimmune thyroid disease or type 1 diabetes in the same individual. An autoimmune origin of all the major components is necessary for the correct diagnosis of APS2. The association of autoimmune Addison's disease and autoimmune thyroid disease is known as Schmidt syndrome, and the association of Addison's disease with type 1 diabetes is also called Carpenter syndrome. Other endocrine and nonendocrine autoimmune disorders occur with increased frequency in these individuals and their families.[1]

Clinical features and course

APS2 is rare, with an estimated prevalence of 4 to 5/100,000.[2,3] Clinical presentation can be at any age but is most frequently in early adulthood, with a peak onset in the fourth decade. It affects both sexes, with a female–male ratio of 3:1.[2]

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Table 1
Comparison of the features of APS1 versus APS2

	APS1	APS2
Commonest age of onset	Childhood, 4–10 years	Adults,16–40 years
Female:male ratio	~1:1	~3:1
Main manifestations	Hypoparathyroidism Candidiasis Addison's disease	Addison's disease Hypothyroidism Type 1 diabetes
Prevalence	Rare, 3/million	Commoner, 1 in 15,000
Genetics	Autosomal recessive; *AIRE* gene	Complex; *HLA*, *CTLA4*, *PTPN22*, others

Major manifestations

By definition, Addison's disease is present in 100% of APS2 cases. Autoimmune thyroid disease occurs in 70% to 90% and type 1 diabetes in 20% to 50%.[1,4–6] Only about 10% have the complete triad.[4,6] Adrenal failure is the first endocrine abnormality in around 50%; however, type 1 diabetes already exists in around 20% and autoimmune thyroid disease in around 30%. Autoimmune thyroid disease encompasses a variety of thyroid disorders, including Hashimoto's thyroiditis, atrophic hypothyroidism, and less commonly Graves' disease and postpartum thyroiditis.

Delayed diagnosis and preventable deaths still occur in patients with undiagnosed adrenal failure. Signs and symptoms are often vague and nonspecific until an adrenal crisis ensues. Low morning serum cortisol concentrations and electrolyte abnormalities (hyponatremia and hyperkalemia) represent late changes, occurring at or just before the onset of clinical adrenal insufficiency. Hyperpigmentation may be observed and be a telltale feature.

In those who already have type 1 diabetes, deterioration of glycemic control with recurrent hypoglycemia and a decrease in total insulin requirements can be the presenting sign. The onset of autoimmune hyperthyroidism or thyroxine replacement for newly diagnosed hypothyroidism leads to enhanced cortisol clearance and can precipitate adrenal crisis in subjects with subclinical adrenocortical failure.[7] Clinicians should maintain a high degree of alertness for underlying adrenal failure before initiating thyroid hormone replacement. Conversely, cortisol inhibits thyrotrophin release, so thyroid-stimulating hormone (TSH) levels are often high at the initial diagnosis of adrenal insufficiency (typically 5–10 mU/L) but return to normal after initiation of glucocorticoid replacement in the absence of coexistent thyroid disease. An increasingly recognized component of APS2 is latent autoimmune diabetes in adults – LADA. By definition this is diabetes developing in adulthood with the presence of diabetes-associated autoantibodies but with relatively well-preserved islet cell function.[8] Thus, the clinician needs to remain vigilant for the development of other autoimmune conditions regardless of the age of the patient.

Minor manifestations

Minor manifestations are listed in **Table 2** together with their frequency. Primary hypogonadism is one of the commonest minor manifestations in APS2 females, with premature ovarian failure leading to secondary amenorrhea in around 10% of women younger than 40 years. Testicular failure is rare in APS2.[9] Pituitary involvement is occasionally seen with lymphocytic hypophysitis leading to empty sella syndrome, panhypopituitarism, or isolated failure of any of the anterior pituitary hormones.[10]

Table 2
Minor manifestations frequently associated with APS2

	Frequency, %
Minor manifestation	
Pernicious anemia	1–25
Gonadal failure	
Females	3.5–10
Males	1–2
Vitiligo	4–12
Alopecia	2–5
Autoimmune hepatitis	4
Malabsorption (including celiac disease)	1–2
Sjögren syndrome	1
Neoplasias	3
Rarer manifestations	
Endocrine	*Neurological*
Pituitary involvement	Myositis
Hypophysitis	Myasthenia gravis
Empty sella syndrome	Neuropathy
Late-onset hypoparathyroidism	Stiff man syndrome
Gastrointestinal	*Other*
Ulcerative colitis	Sarcoidosis
Primary biliary cirrhosis	Serositis
Dermatological	Selective IgA deficiency
Granuloma annulare	Idiopathic heart block
Dermatitis herpetiformis	Idiopathic thrombocytopenia purpura

Data from Refs. [9,40,41,44.]

In contrast to APS1, hypoparathyroidism is rare in APS2 (or APS3). If hypocalcemia does occur then celiac disease is the most likely reason, and the finding of an elevated parathyroid hormone (PTH) concentration in the latter will distinguish between the two.

Incomplete APS2

Patients with autoimmune thyroid disease or type 1 diabetes and adrenal autoantibodies in the serum or patients with Addison's disease and either thyroid and/or islet cell autoantibodies are sometimes classified as incomplete APS2.[4] Self-evidently, these patients may develop APS2 in the future, particularly those with evidence of subclinical disease such as an elevated TSH or impaired glucose tolerance. About 30% of subjects with positive adrenal antibodies progress to adrenal failure over a 6-year period.[11]

APS3

APS3 is defined as the association between autoimmune thyroid disease and an additional autoimmune disease other than Addison's disease.[4] Some authors use the term APS4 to encompass an association of autoimmune diseases not falling into the categories APS1 to 3.[4] This includes an extremely heterogeneous group of patients and it is generally more helpful to describe the individual components. Hashimoto's thyroiditis is the commonest form of autoimmune thyroid disease seen in APS3, although

Graves' disease and postpartum thyroiditis are also seen. Autoimmune thyroid disease is most commonly isolated, and polyglandular involvement in the form of APS3 or APS2 is rare (about 5%). Only 1% of patients with isolated autoimmune thyroid disease have adrenal autoantibodies (with risk of APS2), whereas 3% to 5% have evidence of pancreatic islet autoimmunity and/or clinical type 1 diabetes.[12]

Autoimmune thyroid disease is more commonly associated with pernicious anemia, vitiligo, alopecia, myasthenia gravis, and Sjögren syndrome, and autoimmune thyroid disease should be sought prospectively in patients with these conditions. Around 30% of subjects with vitiligo have another autoimmune disorder, with autoimmune thyroid disease and pernicious anemia being the most common. Many patients with vitiligo are asymptomatic, and other autoimmune diseases are diagnosed only by prospective screening, including evaluation of autoantibody status.[9,13] Up to 15% of patients with alopecia and nearly 30% of those with myasthenia gravis have autoimmune thyroid disease.

Genetics

APS2 is a genetically complex and multifactorial disease. It clusters in families and appears to show an autosomal-dominant pattern of inheritance with incomplete penetrance in some.[14] Susceptibility is determined by multiple genetic loci that interact with environmental factors. Only three genes have shown consistent association with APS2: *HLA*, *CTLA4,* and *PTPN22*. Of these, *HLA* appears to have the strongest gene effect.[1]

Many of the component disorders in APS2, including autoimmune thyroid disease, type 1 diabetes, Addison's disease, celiac disease, myasthenia gravis, selective IgA deficiency, and dermatitis herpetiformis, are associated with the same extended *HLA* haplotype: *HLA-A1*, *HLA-B8*, *HLA DR3*, *DQA1*0501*, *DQB1*0201* (*DQ2*). Thus, unsurprisingly, *HLA DR3*, *DQB1*0201* is associated with APS2.[4,15] Other HLA haplotype associations include *HLA DR4* with type 1 diabetes and, to a lesser extent, Addison's disease and *HLA DR5* in patients with a combination of Addison's disease and autoimmune hypothyroidism.[4] Other *HLA* haplotypes appear to be protective such as the *DR2* (*DRB1*1501*), *DQA1*0102*, *DQB1*0602* haplotype, which appears to provide dominant protection against type 1 diabetes, even in the presence of insulin autoantibodies.[14]

CTLA4 encodes an important negative regulator of T-cell activation that is expressed on the surface of activated T lymphocytes. Alleles of *CTLA4* have been linked primarily to autoimmune thyroid disease, both Graves' disease and Hashimoto thyroiditis,[16,17] but there is also an effect in type 1 diabetes.[18,19] Less consistent association has been shown in Addison's disease (either isolated or as part of APS2).[18,20]

The *PTPN22* gene encodes lymphoid tyrosine phosphatase (LYP), which plays a key role in early T-cell activation. Association with a functionally significant tryptophan for arginine variant in LYP has been found in a mixed UK cohort of AAD and APS2 subjects[21] and in Norwegian subjects.[22] A recent meta-analysis looking at results from five different European regions, including a German AAD cohort where association was not found, showed that overall there was a significant effect of PTPN22 in AAD.[23]

The association of the component disorders in APS2 is, therefore, in part related to the shared susceptibility alleles of *HLA*, *CTLA4,* and *PTPN22* conferring risk to the different diseases. It is also likely that there is a complex interaction between these variants, *CYP27B1* (see article by Husebye and Lovas elsewhere in this issue), and other as yet unidentified loci and environmental factors.

Autoantibodies and Pathogenesis

There are several hypotheses to explain why autoimmunity occurs against multiple organs in individuals with APS. It has been suggested that this may result from a shared epitope(s) between an environmental agent and a common antigen present in several endocrine tissues.[24] More likely, there is a subtle thymic defect of negative selection of autoreactive T cells, caused either by a defect in T-cell apoptosis or by a problem in presentation of self-antigens. Defects in $CD4^+CD25^+$ regulatory T-cell suppressor function[25] and impaired caspase-3 expression by peripheral T cells[26] have also been demonstrated. Thus, loss of peripheral suppression and/or defective peripheral apoptosis could be involved in the pathogenesis of this syndrome.[25,26]

At the onset of autoimmune adrenal failure, anti- 21-hydroxylase (21-OH; anti-P450c21) autoantibodies are detectable in more than 90% of patients with APS2,[3,4] declining to 60% with disease duration over 15 years. These 21-OH autoantibodies are highly specific, being found in only 0.5% of healthy subjects and those with other autoimmune diseases. Some 40% to 50% of patients with such adrenal autoantibodies have abnormal ACTH stimulation tests. Thus, 21-OH autoantibodies have a high predictive value for clinical Addison's disease.[3]

Other steroid-producing cell autoantibodies (SCA), such as those directed against 17α-hydroxylase and cholesterol side-chain cleavage enzyme, are present in 20% to 30% of patients with Addison's disease and are more frequent in females than males.[3,27] There is a strong association between the presence of these SCA and ovarian failure in women with APS2/3, but SCA are extremely rare in women with ovarian failure with no signs of adrenal autoimmunity.[3,27]

Autoimmune thyroid disease and type 1 diabetes are frequent components of APS2. In Hashimoto's thyroiditis, thyroid peroxidase autoantibodies are found in 90% to 100% and thyroglobulin autoantibodies in 60% to 70%. They are both also frequently found in Graves' disease, where TSH receptor autoantibodies are found in more than 90% of cases.[9] Many patients with thyroid autoantibodies but normal TSH progress very slowly to clinical disease (about 5% yearly).[28]

Islet cell autoantibodies are found in around 80% of new-onset type 1 diabetes patients.[3] The main islet autoantigens are insulin, GAD65, and the tyrosine phosphatase-related protein IA-2. Among recently diagnosed subjects with type 1 diabetes, the prevalence of antibodies to insulin and IA-2 is dependent on age, being most frequent in children and adolescents with type 1 diabetes, but less than 30% with adult onset or LADA.[8] The frequency of antibodies to GAD65 is 70% to 80% and is not influenced by age; this therefore gives the highest diagnostic sensitivity in LADA.[8,29]

Diagnosis and Follow-up

Once APS2/3 is suspected, a full assessment of endocrine function is needed. The number of disorders that will develop and the age at which they will present is unpredictable, so long-term follow-up is needed. Presymptomatic recognition of autoimmune disease minimizes associated morbidity and mortality. There is a clear link between the presence of organ-specific autoantibodies and the progression to disease, although there is often an asymptomatic latent period of months or years.

An autoimmune etiology should be sought in all subjects, but the presence of autoimmune disorders in family members is suggestive. In all patients with Addison's disease, there is a need to screen for other endocrine disorders, particularly autoimmune thyroid disease and type 1 diabetes. At diagnosis, screening for TPO and GAD65 autoantibodies is worthwhile. If negative, this should be repeated occasionally, perhaps every 2 to 3 years.

The determination of thyroid function should be carried out at least annually for early recognition of thyroid disease in all subjects with type 1 diabetes and Addison's disease. The determination of 17α-hydroxylase and cholesterol side-chain cleavage enzyme antibodies in females with Addison's disease and APS2 may identify subjects at high risk from primary hypogonadism before gonadotropins become elevated. Such subjects may be suitable for cryopreservation of ovarian material.

The determination of 21-OH autoantibodies should be performed in children presenting with type 1 diabetes, as positive adrenal autoantibodies are highly predictive of future adrenal insufficiency.[3] In subjects with 21-OH autoantibodies, an ACTH stimulation test, determination of electrolytes, and plasma renin activity enables identification of patients with preclinical adrenal dysfunction. If normal, the ACTH stimulation test should be repeated yearly with interval determination of postural blood pressure and electrolytes.

Screening for APS2-associated disorders should also be performed in women with primary or secondary amenorrhea or premature ovarian failure and young patients with vitiligo. As APS2 shows strong familial tendencies, family members should also be checked for features of associated endocrine conditions.

Management and Prognosis

Hormone replacement or other therapies for the component diseases of APS2 are similar whether the disease occurs in isolation or in association with other conditions, and disorders should be treated as they are diagnosed; however, certain combinations of diseases require specific attention. Thus, to avoid adrenal crisis, clinicians should maintain a high degree of suspicion for coexisting adrenal failure in subjects who are hypothyroid.[7] Hyperthyroidism increases cortisol clearance, so in patients with adrenal insufficiency who have unresolved hyperthyroidism, glucocorticoid replacement should be at least doubled until the patient is euthyroid. Decreasing insulin requirements or increasing occurrence of hypoglycemia in type 1 diabetes can be one of the earliest indications of adrenocortical failure. One of the most important aspects of managing these patients is to be continually alert to the possibility of the development of further endocrinopathies to ensure early diagnosis and treatment.

Mortality in patients with primary adrenal insufficiency is elevated approximately twofold compared with the background population.[30] Life expectancy is often reduced as a consequence of unrecognized adrenal crisis, but infectious disease, cardiovascular disease, and cancer also appear to be increased. Despite adequate hormonal replacement, quality of life is often impaired in these patients, with predominant complaints being unpredictable fatigue, lack of energy, depression, and anxiety (see article by Reisch and Arlt elsewhere in this issue). It has been shown that the number of patients receiving disability pensions is two- to threefold higher than the general population in certain countries.[31]

Summary

A high index of suspicion needs to be maintained whenever one organ-specific autoimmune disorder is diagnosed in order to prevent morbidity and mortality from the index disease as well as associated diseases. Further definition of susceptibility genes and autoantigens, as well as a better understanding of the pathogenesis, is required to improve the diagnosis and management of these patients.

AUTOIMMUNE POLYGLANDULAR SYNDROME TYPE I (APS1)

Definition

APS1, known as the autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy syndrome (APECED), is a rare and frequently debilitating disorder of childhood. It is inherited as an autosomal-recessive condition and the female–male ratio is close to 1. The clinical diagnosis of APS1 classically requires the presence of two of the three cardinal components: chronic mucocutaneous candidiasis, autoimmune hypoparathyroidism, and autoimmune adrenal failure.[1,32–36] Only one of these manifestations is required if a sibling has the syndrome.[1] There is a spectrum of associated minor components, which include endocrine and nonendocrine manifestations.

Although a rare disorder in most countries (about two or three cases per million in the United Kingdom),[37] it shows a founder effect leading to a much higher prevalence in certain populations: Finns 1:25,000,[32] Iranian Jews 1:9000,[33] and Sardinians 1:14,500.[38] There are also differences in the phenotype between different populations: for example, chronic mucocutaneous candidiasis and adrenal failure are among the commonest manifestations in most patients of European descent but are present in only about 20% of Iranian Jews.[33,36]

Clinical Features and Course

The first manifestation is typically mucocutaneous candidiasis, which develops in infancy or early childhood. Hypoparathyroidism characteristically develops around the age of 7 years and adrenocortical failure by the age of 13 years.[4,34,37] The complete evolution of the three cardinal features usually occurs in the first 20 years, with additional minor manifestations continuing to appear at least until the fifth decade.[1] Importantly, APS1 can present in other ways, either with one cardinal feature and several minor manifestations or with several minor manifestations and characteristic ectodermal dystrophy. This variability in the early clinical picture can make the diagnosis of APS1 challenging.

The cardinal triad occurs in around 60% of subjects, and the median number of disease components is four, with up to 10 manifestations in some subjects. Patients who present initially with adrenal insufficiency rather than candidiasis tend to develop fewer components than others.[32,36] It has also been reported that the earlier the first component presents, the more likely it is that multiple components will develop.[1,34] **Table 3** lists the cardinal and more common minor manifestations together with their frequency.

Cardinal Manifestations

Chronic mucocutaneous candidiasis (CMC)

Chronic mucocutaneous candidiasis is commonly the first manifestation of the syndrome, occurring as early as 1 month of age, but more typically in the first 2 years of life, and it should alert the clinician to the possibility of APS1. It is the most frequently occurring cardinal manifestation, present in 73% to 100% of patients,[1,32,34–36] and is usually mild or intermittent and responds well to periodic systemic anticandidal treatment. Oral candidiasis is the commonest presentation, but esophagitis is also found, causing substernal pain and odynophagia. Infection of the intestinal mucosa leads to abdominal discomfort and diarrhea. Candidal infection can also affect the vaginal mucosa, nails, and skin.

Hypoparathyroidism

This is frequently the first endocrine feature of APS1,[1,32–36,39] with a peak incidence between 2 and 11 years of age. Hypoparathyroidism occurs in around 75% to

Table 3
Frequencies of the major and main minor components of APS1

Disease	Frequency, %
Main manifestations	
Chronic mucocutaneous candidiasis	72–100
Autoimmune hypoparathyroidism	76–93
Autoimmune adrenal failure	73–100
Common minor manifestations	
Autoimmune endocrinopathies	
Hypergonadotrophic hypogonadism	17–69
Autoimmune thyroid disease	4–31
Type 1 diabetes mellitus	0–33
Pituitary defects	7
Gastrointestinal components	
Pernicious anemia	13–31
Malabsorption	10–22
Cholelithiasis	44
Chronic active hepatitis	5–31
Skin autoimmune diseases	
Vitiligo	8–31
Alopecia	29–40
Urticarial-like erythema with fever	15
Ectodermal dysplasia	
Nail dystrophy	10–52
Dental enamel hypoplasia	40–77
Tympanic membrane calcification	33
Other manifestations	
Keratoconjunctivitis	2–35
Hypo/Asplenia	15–40

Data from European and North American patients.[32,33,35–37,41] Iranian Jews have distinctly different frequencies from the other populations and have been excluded.

95%,[1,4,32,34–36] although there appears to be a slightly reduced penetrance, and later age of onset, in males.[40,41] Hypoparathyroidism may be asymptomatic but presents typically with tetany and grand mal seizures. Presentation may be precipitated by factors such as fasting or low calcium or high phosphate intake. The diagnosis is confirmed by a low or undetectable PTH level in the presence of hypocalcemia. Hyperphosphatemia and hypomagnesemia are common, with low urinary calcium excretion.

Adrenal failure

Autoimmune adrenal failure (Addison's disease) is typically the third of the cardinal manifestations to present in APS1, with a peak incidence around 13 years.[1,4,32–36] In most populations of APS1 patients, it occurs less frequently than the other major components (72% to 100%).[1,32,34–36] Destruction of the adrenal cortex may develop gradually, and deficiencies of cortisol and aldosterone can appear in either order up to 20 years apart.[36] Diagnosis of adrenal insufficiency is confirmed by a normal or low cortisol concentration with increased adrenocorticotrophic hormone (ACTH) and

a subnormal cortisol response to ACTH stimulation. Deficiency of aldosterone may be heralded by postural hypotension or salt craving, and is confirmed by a raised plasma renin activity even before the development of overt electrolyte disturbance.

Minor Manifestations

Autoimmune endocrinopathies

Primary hypogonadism is the commonest minor manifestation of APS1, occurring in 17% to 61% of cases.[1–4,36,42] It is almost invariably accompanied by adrenal failure. About half of APS1 females with hypogonadism present with primary amenorrhea, and the remainder have secondary amenorrhea. Male hypogonadism has been reported from puberty onward.[36] One male patient has been reported with azoospermia and possible antisperm autoimmunity.[36] Other autoimmune endocrinopathies are relatively infrequent and include type 1 diabetes mellitus and destructive autoimmune thyroid diseases (Hashimoto's thyroiditis or primary atrophic thyroiditis). Pituitary defects such as lymphocytic hypophysitis or autoimmune pituitary disease have occasionally been described ($\approx$ 5%) and can induce single or multiple hormonal defects.[4]

Gastrointestinal components

Chronic atrophic gastritis affects up to a third of patients with APS1 and can lead to a megaloblastic anemia due to vitamin B12 deficiency (pernicious anemia) or a microcytic anemia because of iron deficiency.[1,4,32–36] It can be a characteristic feature of an early ''atypical'' presentation of APS1 in the first year of life, being an initial manifestation in around 10%. Malabsorption occurs in 10% to 22% of cases [1,4,32–36] and presents with periodic or chronic diarrhea, usually with steatorrhea, but may be associated with constipation. It can be a result of a variety of causes including villous atrophy, exocrine pancreatic insufficiency, intestinal infections (*Giardia lamblia* or *Candida*), defective bile acid reabsorption, intestinal lymphangiectasia, and autoimmune destruction of the enterochromaffin cells of the small intestine.[34,36,43,44] There is a strong association with the hypocalcemia of hypoparathyroidism, as hypocalcemia impairs the secretion of cholecystokinin leading to a failure of normal gall bladder contraction and pancreatic enzyme secretion. Cholelithiasis is present in up to 40% on ultrasonography,[4] but is usually asymptomatic. Chronic active hepatitis develops in 5% to 30% of cases.[1,4,32–36] The clinical course varies from chronic but asymptomatic in most cases to the development of cirrhosis or fulminant hepatic failure with a potentially fatal outcome.[1,45] It may present in early childhood and can be the first manifestation of APS1; however, the risk of hepatitis is low after adolescence.

Skin autoimmune diseases

Vitiligo and alopecia are well recognized components that can appear at any age and are very variable in severity.[1,4,32–36] Recurrent urticaria with fever has been reported as an unusual manifestation in about 10% of patients during childhood.

Other Manifestations

Ectodermal dystrophy affects the nails and tooth enamel (**Fig. 1**). The pitted nails are unrelated to candidal infection and can be an important clue to the diagnosis of APS1. Dental enamel hypoplasia has been reported in 40% to 75% of patients,[1,4,32–36] although deciduous teeth are never affected. Keratoconjunctivitis may be the first manifestation of APS1 in some cases, and incidence varies from 10% to 40% between reports.[1,4,32–36,39] The initial symptoms are intense photophobia, blepharospasm, and lacrimation; permanent visual impairment and even blindness is not infrequent.[36] Asplenia or hyposplenism has been documented by ultrasonography or suggested by hematological parameters in up to 15% of APS1 cases.[36] It may be congenital or

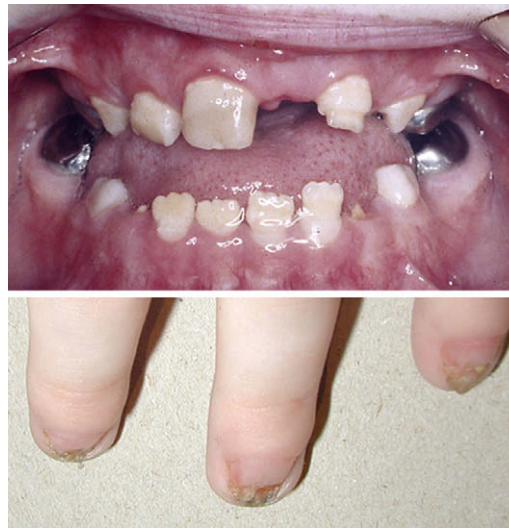

Fig. 1. Ectodermal features of APS1 illustrating the nail dystrophy and the dental enamel hypoplasia.

acquired, secondary to progressive autoimmune-mediated destruction or vascular insult to the spleen. It causes an additional secondary immunodeficiency, rendering subjects susceptible to pneumococcal sepsis.

Sudden death is well recognized in established APS1 patients, their siblings, and from postmortem studies of subjects in whom the diagnosis was not suspected.[32,35,37] It is presumed that these deaths are linked to undiagnosed adrenal failure, fulminant sepsis, or hypoparathyroidism.

Genetics

The gene defective in APS1 was identified by positional cloning in 1997 and is located on chromosome 21q22.3. It is named the autoimmune regulator or *AIRE* gene.[46,47] *AIRE* encodes a putative nuclear protein, containing several motifs suggestive of a transcription factor. It is expressed in a variety of tissues of the immune system but particularly in the medullary epithelial antigen-presenting cells in the thymus, where it is thought to play an important role in the central induction of self-tolerance (see article by Shikama, Nusspaumer, and Hollander, elsewhere in this issue). The molecular mechanism by which the AIRE protein induces central tolerance is still unexplained, however it is thought to be involved in the negative selective of potentially autoreactive thymocytes by regulating expression of self-antigens in the antigen-presenting cells of the thymus.[35,48]

Over 60 different disease-causing mutations have now been described in the *AIRE* gene.[32,34,35,38,46,47,49,50] These include point mutations, insertions, and deletions, and are spread through the whole coding region of the gene. Mutations affecting splice sites have also been reported. The most frequent *AIRE* mutations include the founder Finnish mutation in exon 6 (R257X) [46,47] and the common northern European mutation in exon 8 (964del13).[50] This 13-base pair (bp) deletion is seen frequently in Norwegian patients and in whites from the United States and United Kingdom,[50] where it accounts for more than 70% of all mutant *AIRE* alleles. Additional common mutations are found in isolated populations such as a mutation in exon 3 (R139X) found in

Sardinians[38] and a mutation in exon 2 (Y85C) in the Iranian Jewish population.[32] In several instances, only one mutant allele of the *AIRE* gene has been reported in typical APS1 patients, suggesting that the second mutation might be located in the regulatory regions of the gene.

It is possible that the specific manifestations that develop in a particular APS1 patient may depend on alleles at other loci such as human leukocyte antigens (*HLA*), because the same *AIRE* mutations are associated with varying phenotypes and clinical course even among affected siblings.[41,51] No consistent associations between APS1 manifestations and *HLA* alleles have been found, and no association between HLA type and autoantibodies in APS1 patients is seen.[52] No correlation between cytotoxic T lymphocyte antigen 4 (*CTLA4*) gene polymorphisms and APS1 have been found to date[4]; however, a negative correlation has been shown between an insulin gene polymorphism and the development of T1D in these subjects.[53] The factors determining an individual phenotype are not understood, and it is likely that there are several modifier loci involved.

Autoantibodies and Pathogenesis

The pathogenesis of many of the manifestations of APS1 is unclear, but autoimmunity is involved in the development of the endocrinopathies, and patients have circulating autoantibodies to a variety of antigens from other affected tissues. One recently identified autoantibody is directed against interferons, in particular interferon (IFN)-α and IFN-ω, and has almost 100% prevalence in APS1 subjects, regardless of the clinical picture or mutation type.[54] These anti-interferon autoantibodies have been found to be present at a very early stage and persist, being present after over 30 years of disease. They have not been found in any subjects with isolated AAD or APS2, so appear to be disease specific.[51,54] This clearly provides an excellent tool to aid in the diagnosis of APS1 in the prodromal stage or in atypical cases, and suggests the intriguing possibility that these autoantibodies may modulate the expression of immune responses directly.

Steroid 21-hydroxylase (P450c21) and cholesterol side-chain cleavage enzyme (P450scc) are the major adrenal autoantigens,[55] and were found in 81% of APS1 patients with and in 21% of those without adrenal failure. The presence of antibodies for at least one of these three enzymes correlates significantly with gonadal failure in female but not male patients.[34,35]

Autoantibodies to the extracellular domain of the calcium-sensing receptor have been reported in idiopathic hypoparathyroidism including up to 86% of subjects with APS1 (see article by Brown elsewhere in this issue).[56,57] This has not been replicated in several other studies,[40,55] and the prognostic significance and pathophysiological role of these autoantibodies remains undetermined. In addition, antibodies against a novel parathyroid-specific antigen, NALP5, have recently been found in about half of APS1 patients with hypoparathyroidism.[58]

Glutamic acid decarboxylase (GAD)-65 autoantibodies have been found in 75% of patients with diabetes up to 8 years before the onset, but these are nonspecific and are also found in 40% of nondiabetic APS1 patients.[59] Antibodies against the IA-2 tyrosine phosphatase-like protein and insulin are less common in these patients but have higher specificity (96% to 100%).[40] Circulating antithyroid antibodies have been found to be a poor marker for predicting hypothyroidism in APS1.[34]

The main autoantigens for hepatitis in APS1 appear to be cytochrome P450 1A2 (CYP1A2), P450 2A6 (CYP2A6), and aromatic L-amino acid decarboxylase (AADC).[60] Tryptophan hydroxylase autoantibodies have also been found to be a sensitive predictor of autoimmune hepatitis in APS1.[55] Although a rise in antibody titers to liver

antigens may predate biochemical evidence of liver disease, raised autoantibodies are not found in all APS1 patients with autoimmune hepatitis at biopsy.[45] Antiparietal cell and intrinsic factor autoantibodies precede parietal cell atrophy. Villous atrophy is associated with endomysial and/or tissue transglutaminase (TTG) autoantibodies.[34] Gastrointestinal dysfunction has been associated with autoantibodies to tryptophan hydroxylase (48% cases), histidine decarboxylase, and GAD-65.[55]

Measurement of autoantibodies may be of limited use in patients with APS1 in determining their risk of developing new components because the sensitivity of the antibody test may frequently be less than the patient's preexisting risk of the complication. There are, however, certain autoantibodies that are almost exclusive to APS1, particularly AADC, CYP1A2, tyrosine hydroxylase, tryptophan hydroxylase, IFN-α, and IFN-ω. This unique spectrum of autoantibodies can thus help to differentiate APS1 and other autoimmune diseases.[54,55]

Diagnosis of APS1

Perheentupa[36] found the classic criteria (two out of three cardinal manifestations) to be fulfilled by 5 years of age in only 22.0% cases, by 10 years in 67.0%, by 20 years in 89.0%, and by 30 years in 93.5%. Suspicion should be high in patients younger than 30 years with mucocutaneous candidiasis, hypoparathyroidism, adrenal failure, ectodermal dystrophy, keratoconjunctivitis, prolonged diarrhea, vitiligo, or noninfectious hepatitis. Such patients should be checked for other manifestations, particularly the sometimes subtle nail signs of ectodermal dystrophy, or oral or ophthalmic components. DNA screening for *AIRE* mutations and an autoantibody screen should be considered in subjects with an atypical presentation.

There is often no direct clinical value in DNA analysis in subjects with two or more cardinal features, but the molecular findings in a proband will be of value in counseling and for screening siblings. All patients with established APS1 and those with one or more suspicious features need close follow-up for the development of new components. Their siblings should also be examined, as one of the cardinal manifestations or a definite ectodermal component is diagnostic.

Diagnosis is often delayed, perhaps because of the long interval between development of the first and second manifestation. Up to two thirds of patients are not diagnosed until admission to hospital with acute adrenal insufficiency or hypocalcemic crisis, and nearly half of these already have one major component of APS1 present.[35] Increased awareness of APS1 is essential to prevent fatalities. Mutational analysis has aided the early diagnosis of APS1, but it must be remembered that there are a large number of possible mutations and, in the United Kingdom, only the commonest two are routinely screened. Thus, APS1 is not excluded by negative routine DNA analysis, and the presence of one abnormal allele in a child with a major or minor manifestation makes the diagnosis highly likely. The use of the recently identified anti-interferon autoantibodies may well play an important role in aiding diagnosis in the future.[54] The individual disease components of APS1 should be recognized by the standard endocrine surveillance methods.

Follow-up

The most important goal is the recognition of new disease components, which is essential, as some manifestations are life threatening. Regular review particularly for oral mucocutaneous candidiasis and signs of evolving adrenal insufficiency, such as postural change in blood pressure, is essential. Blood should be taken for basal hormone, hematological, and biochemical markers, and an occasional antibody

screen performed. This, together with a high index of clinical suspicion, allows earlier diagnosis and treatment of additional components as they develop.

The early diagnosis of Addison's disease is of particular importance and individuals at risk need an annual measurement of ACTH until adrenocortical failure develops.[32] Plasma renin activity should be measured at the same time. The patient, immediate family, and primary health care team must be made aware of the signs and symptoms of adrenal failure.[37]

Treatment

Treatment of the individual disorders is no different from treating patients with the isolated disorders, except that polypharmacy is the rule, and that malabsorption may complicate therapy. The different endocrine failures are managed by conventional hormonal replacement, which may be complex when a patient has several endocrine deficiencies. Immunosuppressive treatment with glucocorticoids (eg, for autoimmune hepatitis) can also complicate matters.

Mucocutaneous candidiasis is treated with local and/or systemic antifungal drugs, dental care, and oral hygiene, with expert oral surgical follow-up for refractory cases. Suppression of oral candidiasis is important because of the risk of oral carcinoma.

The serum calcium levels of APS1 patients appear to be labile compared with non-APS1 hypoparathyroidism. This is presumed to be a result of malabsorption, and the intermittent nature of this can lead to marked hypercalcemia with rapid onset of renal impairment. Standard treatment with vitamin D analogs often leads to hypercalciuria, so serum calcium levels need to be maintained at around the lower end of the normal range (2.0–2.2 mmol/L total serum calcium). The vicious cycle of hypocalcemia and malabsorption can usually be broken by an increased oral dose, but parenteral therapy may be required in severe situations. Hypomagnesemia may contribute to resistance and require treatment. In patients with adrenal insufficiency, alteration of the hydrocortisone dose will lead to an alteration in calcium absorption. Also of note is that unexplained hypercalcemia may be the first sign of the development of adrenal failure.

Autoimmune hepatitis is treated with immunosuppressive therapy, most experience being with the use of prednisolone and/or azathioprine. Liver transplantation has occasionally been reported in APS1-associated hepatitis.[45] Immunosuppressive therapy may increase the risk of *Candida*-related cancer and predispose the patient to generalized candidal infection.[34] Immunosuppressants are occasionally required for severe intestinal dysfunction with diarrhea, and there can be an associated improvement in control of serum calcium levels. Milder diarrhea has been found to respond to gut motility-reducing agents such as loperamide.

Live vaccines must be avoided in view of the underlying immunodeficiency[36] but, as splenic atrophy is a common component, all APS1 patients should receive polyvalent pneumococcal vaccine.[37]

Prognosis

Many patients feel chronically unwell, and the physical and psychological impact of the multiple problems should not be underestimated. Despite improved survival, mortality rates are still high at 10% to 20% and a recent review in Finland has found the average age of death to be 34 years (range 6.8 to 63 years).[41] Death is from a variety of causes including adrenal crisis, diabetic ketoacidosis, fulminant hepatic failure, oral carcinoma, septicemia, hypocalcemia, generalized candidal infection during immunosuppressive treatment, complications of kidney failure, and alcoholism.[32,34,36] Around 3% die before the diagnosis of APS1 has been made, with

adrenal failure the likely cause. Depression and suicide is high among this patient group, as the disease poses a great psychological burden, with the constant risk of developing life-threatening complications, disfiguring disease components, and the requirement for multiple medications. Working capacity may be maintained in subjects with a limited number of manifestations, but many are significantly incapacitated.[36,41]

Summary

The clinical presentation of APS1 is very variable. Diagnosis can be difficult initially when only one manifestation is present, and it often takes years for others to appear. Increased awareness of the condition, combined with analysis of specific autoantibodies and mutational analysis of the *AIRE* gene, should help to diagnose this condition earlier and prevent serious complications and fatalities.

IMMUNE DYSREGULATION, POLYENDOCRINOPATHY, AND ENTEROPATHY (X-LINKED) SYNDROME

Immune dysregulation, polyendocrinopathy, and enteropathy (X-linked) syndrome (IPEX) is a rare and devastating X-linked condition of male infants, affecting immune regulation and resulting in multiple autoimmune disorders. The first feature is commonly intractable diarrhea and failure to thrive because of autoimmune enteropathy occurring around 3 to 4 months of age. Type 1 diabetes and autoimmune hypothyroidism develop in the first year of life in around 90% and 50% of males respectively. Additional clinical features include eczema, autoimmune hemolytic anemia, autoimmune thrombocytopenia, recurrent infections, lymphadenopathy, membranous nephropathy, and striking growth retardation. Other autoimmune features are less frequent.[61] Sepsis may result from a primary defect in immune regulation but is exacerbated by autoimmune neutropenia, immunosuppressive drugs, malnutrition, enteropathy, and eczema.

The condition is heterogeneous in its presentation, with the occasional case not presenting until later childhood or adulthood.[62] Diabetes or eczema is a not infrequent initial presentation, but any of the disease components can present first. There are no estimates of incidence, but it is likely to be underdiagnosed because of the clinical variability in presentation and the presence of frequent new mutations. Diagnosis relies on the clinical presentation, family history, and elimination of other diagnoses with similar presentations. Genetic screening has proved useful in some cases. There is a high mortality in these infants, many succumbing to the untreatable diarrhea, malnutrition, and superimposed infections by 24 months of age. Survival into adolescence is occasionally seen with the use of aggressive immunosuppression and parenteral feeding, although symptoms are rarely entirely relieved.[61,63] There are increasing reports of the use of bone marrow transplantation in these infants, but experience is very limited.[61]

IPEX has been shown to be caused by mutations in the *FOXP3* gene, located at Xp11, encoding a transcription factor belonging to the forkhead/winged-helix family.[61] An increasing number of mutations have been reported, mainly in the coding region of *FOXP3*, although one mutation in the regulatory region has also been found.[61,63] *FOXP3* is specifically expressed in naturally arising $CD4^+CD25^+$ regulatory T cells and appears to convert naïve T cells to this regulatory phenotype (see article by Chatila elsewhere in this issue). Thus, *FOXP3* is a critical regulator of $CD4^+CD25^+$ T-cell development and function.[64] Severe autoimmunity in FOXP3 deficiency may in part therefore be because of aggressive helper T cells that develop from regulatory

T-cell precursors that, because of a lack of FOXP3, cannot mature.[65] In a few cases, no mutation has been identified. Although female carriers of *FOXP3* mutations appear to be healthy, a small number of cases of an IPEX-like syndrome have been reported recently in families with affected girls in whom no mutation was found.[63] It is likely that there may be an autosomal locus accounting for the problem in some families, and mutations in the interleukin (IL)-2 receptor subunit CD25 have been shown to cause a similar syndrome.[66] This genetic heterogeneity may explain some of the clinical variation seen in this syndrome but, as yet, no obvious genotype–phenotype relationship has been identified, and other modifying genes, such as *HLA*, as well as environmental factors, may influence the outcome.

SUMMARY

Most physicians will be made acutely aware of the diverse nature of the polyglandular syndromes at some point in their career. In the modern era, we should aim to use the powerful combination of clinical skills, autoantibody assays, and molecular genetic investigation, along with basal and dynamic endocrine testing, to institute early diagnosis and therapy for these clustering conditions. In the future, more accurate disease prediction may allow us to counsel individuals and families with greater certainty. Ultimately, when disease pathogenesis is more clearly understood, interventions to prevent an autoimmune endocrinopathy developing in those at high risk may become more than a theoretical possibility.

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