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Polyglandular Autoimmune Syndromes

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Abstract

The polyglandular autoimmune syndromes (PAS) comprise a wide spectrum of autoimmune disorders and are divided in a very rare juvenile (PAS type I) and a relatively common adult type with (PAS II) or without adrenal failure (PAS III). First clinical manifestation of PAS I usually occurs in childhood whereas PAS II mostly occurs during the third and fourth decades. PAS I is caused by mutations in the autoimmune regulatory (*AIRE*) gene on chromosome 21 and is inherited in an autosomal recessive manner. Mutations in the *AIRE* gene result in defect proteins which cause autoimmune destruction of target organs by disturbing the immunological tolerance of the patients. Genetic testing may identify patients with PAS I, but not those with PAS II/III. For PAS II/III, susceptibility genes are known which increase the risk for developing autoimmune disorders, but must not be causative. These are certain human leukocyte antigen genes, the cytotoxic T lymphocyte antigen gene, and the protein tyrosine phosphatase non-receptor type 22 gene on chromosomes 6, 2, and 1, respectively. Actual diagnosis of PAS involves serological measurement of organ-specific autoantibodies and subsequent functional testing. Management of patients with PAS including their family relatives is best performed in centres with special expertise in autoimmune endocrine disorders.

Definition and epidemiology

The polyglandular autoimmune syndromes (PAS) form different clusters of autoimmune disorders and are rare endocrinopathies characterized by the coexistence of at least two glandular autoimmune mediated diseases (1). Two major subtypes of PAS, types I and II are distinguished according to age of presentation, characteristic patterns of disease combinations, and different modes of inheritance (2-3). The coexistence of adrenal failure with either autoimmune thyroid disease (AITD) and/or type 1 diabetes (T1D) is defined as Schmidt's or Carpenter's syndrome. A third subtype has been described in adults that, contrary to types I and II, does not involve the adrenal cortex. Since apart from absence of adrenal failure no clinical differences between types II and III have been described, PAS may be divided in a very rare, foremost juvenile type I and a more common adult type II.

PAS I, also known as APECED (autoimmune polyendocrinopathy, candidiasis and ectodermal dystrophy) or MEDAC (multiple endocrine deficiency autoimmune candidiasis syndrome), usually

manifests in infancy or childhood at age three to five years or in early adolescence and, therefore, is also called juvenile autoimmune polyendocrinopathy (4,5). It is defined by a persistent fungal infection (chronic mucocutaneous candidiasis), the presence of acquired hypoparathyroidism, and Addison's disease. In most patients, mucocutaneous candidiasis precedes the other immune disorders, usually followed by hypoparathyroidism (table 1 and fig. 1). The female-to-male ratio varies from 0.8:1 to 2.4:1. The highest prevalences of PAS I have been found in populations characterized by high degree of consanguinity or descendant of small founder populations, particularly in Iranian Jews (1: 600-9,000) and Finns (1: 25,000). In the general population, PAS I is a very uncommon disease in contrast to what is observed in selected populations; however, genetic screening of first-degree relatives of affected subjects may unravel a higher prevalence than expected due to the detection of clinically milder or atypical cases.

In contrast, prevalence of PAS II is 1:20000 (6). It is more frequently encountered in women, and the male-to-female ratio is 1:3 (7). This syndrome has a peak incidence at ages 20-60 years, mostly in the third or fourth decade, and it is common for multiple generations to be affected by one or more component diseases. There is familial clustering and family members of patients are often affected (2). While there is some correlation between the ages of onset of one PAS illness with another, many years may separate the onset of different diseases (7). All the disorders resulting in tissue destruction appear to have a prolonged phase of cellular loss preceding overt autoimmune glandular disease.

Pathogenesis

Cell-mediated immune processes play an important role in the immunopathogenesis of PAS. Naive CD4+ T cells, upon encountering their cognate antigens presented on professional antigen-presenting cells, differentiate into effector cells (TH1, TH2, TH17, regulatory or iTreg) that are characterized by their cytokine production profiles and immune regulatory functions. TH1 cells produce interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) and regulate antigen presentation and immunity against intracellular pathogens, whereas TH2 cells, which produce interleukin (IL)-4, IL-5, and IL-13, mediate humoral responses and immunity against parasites, and are important mediators of allergic diseases. TH17 cells express IL-17, IL-17F, IL-21, and IL-22 (and IL-26 in humans) and

participate in inflammation and autoimmunity processes. iTregs express Forkhead box P3 (Foxp3) transcription factor and mediate immune suppression by secretion of transforming growth factor-β (TGF-β) and IL-10 and by contact-dependent mechanisms (8-9). TH17 cells develop via an independent lineage from TH1 or TH2 cells. Differentiation of TH cells is steered by the innate immune system, which provides T cell receptor (TCR), and co-stimulatory signals as well as an appropriate cytokine microenvironment that ultimately leads to the preferential induction of one specific cell lineage over the other. IFN-γ, IL-12, or IL-4, which are important for TH1 and TH2 differentiation, have been shown to be dispensable for TH17 cell differentiation *in vitro* and *in vivo*, providing one of the first clues that TH17 cells are indeed an independent lineage from TH1 or TH2 cells. Finally, IL-6 and TGF-β potently initiate TH17 differentiation (9).

Lymphocyte infiltrations of the various glands are associated with functional loss of epithelial cells with scarring. The cellular defect in PAS may be associated with abnormal balances in cytokine production by T cells. A polarized Th2 response is associated with Graves' disease (GD) and Th1 with T1D. In contrast, PAS I results from biased Th2 immune responses to self-antigens and defective protective Th1 responses against invasion of yeast Candida albicans (10-11). A dominance of T-helper cells and a deficiency of T suppressor cells have been demonstrated for endocrine autoimmunity (12). Also, a hypothesis for the pathogenesis of PAS has been recently presented (13). Accordingly, a genetically predisposed person might develop an autoimmune process after initiation by an infectious agent. This might initiate *via* cross reactivity in the area of antigen presenting MHC molecules, a TH2-dependent immune process which is primarily of humoral origin and partly local. This immune process probably chronifies due to deficient T suppressor cell activity.

In PAS several organ-specific autoimmune diseases are clustered. Although PAS I is caused by loss of central tolerance (1), the precise aetiology of PAS II is unknown. Further evidence refers to the Tregs which are implicated in self-tolerance and autoimmunity. As previously stated, the adaptive immune system does not only consist of immune-stimulatory CD4+ helper or effector T cells and cytotoxic CD8+ T cells but is also regulated by immunosuppressive T cells. Three classes of immunosuppressive CD4+ T cells are known to date: induced Th3, Tr1 cells, and the naturally present CD4+CD25+FoxP3+ Tregs (14). Tregs are generated in the thymus and are present in all healthy

animals and humans. Because the FOXP3-encoded scurfin protein interferes with IL-2 gene activation, Tregs do not secrete IL-2 and do not proliferate upon TCR-stimulation. Treg suppress the activation of CD4+ and CD8+ cells *in vitro* and *in vivo*. They thereby prevent autoimmune disease, although the exact mechanism of suppression is unknown, and cell-contact is crucial at least *in vitro*. Depletion of Treg leads to autoimmunity in mice (15) and dysfunction of Tregs has been linked to autoimmune diseases (16). Defects in survival of suppressive function of Tregs may contribute to uncontrolled expansion of auto aggressive lymphocytes. Reduced numbers of Tregs are observed in myasthenia gravis (17) and a reduction of suppressive Treg function was reported in multiple sclerosis (18).

In a murine model, depletion of thymically derived CD4 (+) CD25 (+) Tregs, which exert suppression in a contact-dependent manner, resulted in a syndrome which is similar to human PAS with multiple endocrinopathies (19). Hence, loss of active suppression in the periphery could be a hallmark of PAS. Tregs from peripheral blood of both patients with PAS, as well as control patients with single autoimmune endocrinopathies, and normal healthy donors showed no differences in quantity, except for patients with isolated autoimmune diseases, in functionally important surface markers, or in apoptosis induced by growth factor withdrawal (16). Strikingly, PAS Tregs were defective in their suppressive capacity. The defect was persistent and not due to responder cell resistance, whereas overt quantitative or phenotypic abnormalities in Tregs from these patients were not observed. Also samples of FOXP3 messenger RNA from PAS Tregs were semi-quantified and showed similar levels of FOXP3 transcripts compared with normal donor Tregs despite defective suppressor function, indicating that true Tregs were dysfunctional. Defective Treg function in humans with PAS has wide implications for autoimmunity in general. The results indicate that once molecular mechanisms of suppressor function are better delineated, manipulations of human Tregs may allow improvement of immunomodulatory strategies in diseases with impaired suppressor function. Another category of immunosuppressive cells are the Tregs Tr1/Th3, and knowledge of development and functions of such immunoregulatory cells may elucidate the aetiology for developing autoimmunity (20).

Innate immunity and the enzyme deoxyribonuclease 1

Another important aspect is related to the activity of the enzyme deoxyribonuclease 1 (DNase 1). This glycoprotein is ubiquitously expressed in human tissues and plays a role in the regulation of apoptosis. It catalyzes DNA hydrolysis by cleaving double-stranded DNA. The activity of this enzyme was lowered in patients with PAS compared to healthy subjects (21-22). Such a deficiency in DNase 1 may result in reduced or delayed removal of DNA from nuclear antigens and, thereby, may promote disease susceptibility to autoimmune disorders. Antigen complexes containing DNA can initiate the manifestation of endocrine autoimmunity by stimulation of an innate immune signalling pathway via Toll-like receptors (TLRs). The innate immune response is activated by the recognition of a set of highly conserved molecular structures found in most microorganisms. These pathogen associated molecular patterns are detected by a set of ten TLRs that are mainly expressed on macrophages, B cells and dendritic cells (23-24). TLR9 is a receptor for unmethylated CpG motifs that are common in bacterial DNA and are also present in mammalian DNA. The activation of TLR-9 promotes the production of IL-12 and a shift towards a Th1 immune response, activities that are accompanied by the breakdown of tolerance. Antigen complexes containing DNA can initiate the production of autoantibodies (Abs) by their ability to activate B cells without a second signal from T helper cells. This “single handed” B cell activation is mediated by antigen recognition with cell-surface antigen receptors and the additional co stimulatory activation of TLR9 by antigen-associated self DNA (25). Decreased serum DNase activity with elevated levels of circulating DNA and resulting activation of the TLR9 immunstimulating pathway can also trigger organ-specific autoimmune disease. Most of the natural insulin Abs are polyreactive and bind in addition to insulin one or more antigens including thyroglobulin, IgG, and DNA (21). Complexes containing DNA between polyreactive natural Abs, i. e. insulin, anti-insulin and circulating DNA may initiate an autoimmune process by TRL9 co-activation. Thus, susceptibility for immune co-activation by autoantigen receptor activation and TLR9 stimulation may be increased in individuals with reduced serum DNase activity.

Genetics

PAS type II

The inheritance of PAS II is complex, with genes on chromosome 6 playing a predominant role. In man this chromosome contains the major histocompatibility loci. Within some families, autoimmune endocrine disease susceptibility appears to be inherited as an autosomal dominant form associated with a specific HLA haplotype. Nevertheless, family members may manifest different diseases, though the more common the disease in the general population, the higher its prevalence in affected families.

While genetic factors determine disease susceptibility, there is less than 100% concordance in monozygotic twins for the respective diseases. This suggests that environmental factors may be involved in disease pathogenesis and that together with genetic factors they may contribute to the loss of immune self tolerance. Based on a genetic predisposition, epigenetic external factors, like viral or bacterial infections (26), and psychosocial factors might induce an autoimmune cascade. Environmental factors may have an important influence on the development of autoimmune diseases, but the exposure to environmental pathogens does not always lead to disease. With respect to genetics, PAS II is supposed to be a polygenic disease with autosomal dominant inheritance and incomplete penetrance. Also, familial clustering provided evidence for a genetic predisposition.

Many of the PAS II component diseases are associated with HLA alleles B8 and DR3. The DR antigen has two glycoprotein chains coded for by the DR loci of chromosome 6. The alleles HLA-B8 and DR3, which are coded for by separate genes, are more often found together on the same chromosome than one would predict from their frequency in the general population. Such associations are common for alleles of different genes in the histocompatibility region (linkage disequilibrium). The allele DR3 is most closely associated with autoimmune endocrine disease. PAS II is associated with HLA DR3 and DR4 antigens, and interestingly frequencies for DQA1*0301 and *0501 were increased in patients with PAS II compared to controls (27). The genotype DR3/4, DQ2/DQ8 with DRB1*0404 has been found to be the highest HLA genotype risk for Addison's disease, either as a single disease or within PAS II (28). An association of PAS II with HLA-B8 has been observed in three generations of a family, whereas ten unaffected subjects did not show B8 (29). HLA associations have also been described for PAS II component diseases T1D (DR4-DQB1*0302) and GD (B8). T1D locus 1

contains the MHC region (6p21) and, in whites revealed a positive association with HLA DRB1*04-DQA1*0301-DQB1*0302 (DR4-DQ8) or DRB1*03-DQA1*0501-DQB1*0201 (DR3-DQ2) and a negative association with DRB1*15-DQA1*0102-DQB1*0602 (30-31). A significant association between Hashimoto thyroiditis (HT) and HLA DR3 was identified (32), whereas an increase in HLA-DR5 in affected family members with HT was found (33). A whole genome linkage study on a data set of 56 multiplex, multigenerational AITD families, using 387 microsatellite markers was performed (34). Only one locus on chromosome 6 was linked with both GD and HT. This locus was close to, but distinct from, the HLA region.

Furthermore, there is evidence that MHC class III genes are associated with PAS II, most specifically the gene encoding tumor necrosis factor alpha (TNF-α), a multifunctional proinflammatory cytokine, which mediates inflammatory and immune functions (35). Within the TNF-α gene, the -308*A allele of an A/G single nucleotide dimorphism occurred more frequently in patients with PAS II than in healthy controls. PAS patients with AITD and the TNF-α -308 AA genotype showed the highest prevalence of Abs against thyroid peroxidase (TPO) and thyroglobulin (Tg). Compared with patients carrying the wild GG genotype, those with the AA genotype showed a four- or twofold increase in Tg and TPO Abs, respectively. Since TNF-α inhibits the expression of thyroid specific genes such as TPO and Tg and thereby, thyroid function, these findings may partially explain the decrease in serum thyroid hormone concentrations in HT. Therefore, an inhibition of the TNF-α by TNF-α inhibitors might be useful in diagnosis and in therapy in both AITD and PAS.

In patients with PAS, presence of the TNF-α -308*A allele was significantly related to the presence of the HLA-DRB1*03 allele, suggesting that HLA-DRB1*03 and TNF-α -308*A alleles are associated. The TNF-α gene lies within the MHC class III region, telomeric to the class II region. Thus, PAS is associated with the TNF-α -308*A allele in adults. TNF-α -308*A allele might directly confer susceptibility to PAS by affecting TNF-α cytokine production, as outlined above. However, another closely linked gene, particularly the HLA-DRB1*03 allele, may primarily determines disease susceptibility to PAS. Hence, the observed association of TNF-α -308*A with PAS could be secondary due to linkage disequilibrium of TNF-α -308*A with HLA-DRB1*03. Linkage disequilibrium describes the non-random association of alleles at linked loci during meiosis as well as

the resultant co-segregating allele combinations that are observed in the patients. TNF-α might be a silent polymorphic locus which is in linkage disequilibrium with the causative HLA-DRB1*03 allele, and could be used as a surrogative genetic marker. Also, an interaction between the protein products of the TNF-α and HLA alleles may follow since TNF-α both mobilizes immune cells as well as induces the aberrant expression of MHC class II antigens. As a consequence, an increased expression of TNF-α protein, being observed for the TNF-α -308 AA genotype, might cause inflammation leading to tissue damage. Finally, heterozygosis for TNF-α increased the risk for T1D in DQA1*0501-DQB1*0201/DQA1*0301-DQB1*0302 positive individuals, most probably due to a linkage disequilibrium with HLA class 2 genes (36).

The MHC class I related gene A (MICA), located on chromosome 6 within the HLA region, is an additional locus associated with PAS. The MICA exon 5 microsatellite is of special interest, because this trinucleotide (GCT) repeat lies in the transmembrane region encoding alanine residues. It is characterized by five common alleles (MIC-A4, -A5, -A5.1, A6 and A9); allele A5.1 shows an additional 1-bp insertion (GGCT). The MICA molecule is a membrane-bound glycoprotein. It binds to NKG2D which is an activating receptor of natural killer (NK) cells (37). The G-insertion in the MIC A5.1 allele results in a frame shift mutation accompanied by a premature stop codon. It codes for a protein with a shortened transmembrane segment lacking its cytoplasmic tail. Compared to healthy controls, frequency of the MICA 5.1 allele and the A5.1/A5.1 genotype was increased in patients with PAS II (38), indicating that the MICA microsatellite polymorphism may contribute to genetic susceptibility to PAS. Frequencies of the MIC A5.1 allele were not significantly different in HLA-DRB1*03-carriers and -non-carriers, suggesting that the risk conferred by MICA association might be independent of HLA-DRB1*03 risk. In another study, combination of MIC-A5 and HLA-DR3-DQ2 and/or HLA-DR4-DQ8 represented a strong genetic marker for T1D with PAS (39).

Further susceptible genes encompass the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) gene, a strong inhibitor of T-cells (40). CTLA-4 is a T-lymphocyte surface antigen that interacts with B7-1 (CD80) and B7-2 (CD86) of antigen presenting cells. It inhibits T-cell activation by reducing IL-2 production and IL-2 receptor expression and by arresting T cells at the G1 phase of the cell cycle (41-43). An A/G single nucleotide polymorphism (designated as CT60) downstream the CTLA-4 gene has

been associated recently with GD, HT with odds ratios of 1.5 and 1.6 respectively (42-43). It has been reported that the disease susceptible genotype G/G induces a reduced mRNA expression of the soluble form of CTLA-4 which might lead to a less efficient T-cell inhibition. This could explain why autoimmune processes can be induced more easily in CT60 G/G carriers. An association between patients with both AITD and T1D (PAS) and the CTLA-4 CT60 SNP was also detected and was even stronger than the association of CT60 with AITD alone. This is supported by the odds ratio of 2.0 that is larger than those reported for GD or HT alone. Therefore, the CTLA-4 CT60 SNP seems to play a role in the joint susceptibility for both diseases.

Of further interest is the recent finding that the protein tyrosine phosphatase nonreceptor 22 (PTPN22) gene might contribute to susceptibility to PAS II (44). This gene encodes an intracellular phosphatase with negative regulatory effects on T-cell activation. A functional single nucleotide polymorphism (C1858T) in the PTPN22 gene is associated with several autoimmune diseases (45-48). Due to the higher prevalence, previous studies investigated associations of *PTPN22* C1858T with AITD and T1D as single diseases. With respect to T1D and AITD as separate entities, for each a less than twofold increased risk (OR) for *PTPN22* T allele carriers was reported (49). When examining patients with both diseases, we noted a fourfold increased risk for T allele carriers to develop PAS, indicating that *PTPN22* plays a more important role in the overall genetic risk for PAS than it does for AITD or T1D alone and that *PTPN22* C1858T plays a role in this obvious cumulative and concurrent presence of both diseases (50-51). Also, these findings do not suggest the existence of a separate set of susceptibility genes for the disease combination, but rather indicate the presence of combined susceptibility genes. The underlying pathophysiology is a key factor for understanding autoimmune processes. LYP encoded by *PTPN22* is expressed in lymphocytes where it binds to the protein kinase (Csk), a suppressor of the lymphocyte-specific protein tyrosine kinase (Lck) and the protein tyrosine kinase p59fyn (Fyn), which are associated with the TCR signalling pathway (44,49). The C→T exchange at position 1858 at codon 620 leads to the substitution of arginine (CGG) by tryptophan (TGG). Paradoxically, the disease predisposing T allele for tryptophan is a gain of function variant that leads to an even higher T-cell suppression (44,51). It is speculated that autoreactive T-cells escape thymic deletion more easily due to a loss in TCR signalling and thus remain in the periphery. The key

status of T-cells in AITD and T1D might explain why *PTPN22* 1858T is associated with the joint susceptibility for both diseases. In our recent study (50), patients with adult PAS who carried the *PTPN22* 1858 T allele, were significantly more frequent carriers of DRB1*03 than T non-carriers, showing that the combination of *PTPN22* 1858T and DRB1*03 provides patients with a higher risk than expected.

PAS type I

PAS I is a monogenic disease with autosomal recessive inheritance (52-60). Mutations of a single gene that is termed the autoimmune regulator (*AIRE*) gene play a crucial role. The *AIRE* gene is assigned to chromosome 21q22.3 and has been cloned by two independent research groups (52,57). The *AIRE* gene is expressed in tissues which are involved in the maturation of the immune system such as thymus, lymph nodes, and peripheral blood cells (CD14-positive monocytes), but not in CD4-positive T cells. It is mainly expressed in epithelial antigen-presenting cells in the thymus where it is possibly involved in the central induction of self-tolerance. Thus, the *AIRE* gene is an important mediator of central tolerance. *AIRE* may regulate negative selection of organ-specific T cells. The *AIRE* gene encodes a 545-amino-acid protein of 57.5 kDA which comprises several domains which may be involved in nuclear transport, DNA binding, homomultimerization, and transcriptional activity. It shows several motifs indicative of a transcription factor. It includes two zinc fingers. It upregulates the transcription of organ-specific self-antigens in medullar thymic epithelial cells. Also, it plays a role in the negative selection of organ-specific thymocytes. At least three splice variant mRNAs products have been described including one which results in a premature stop codon in the *AIRE* protein and a transcript predicted to be a candidate for nuclear-mediated decay (NMD). The mutated *AIRE* gene results in defect *AIRE* proteins which cause autoimmune destruction of target organs by disturbing the immunological tolerance of the patients (59). Many *AIRE* mutations alter the nucleus-cytoplasm distribution of *AIRE*, thereby disturbing its association with nuclear dots and cytoplasmic filaments. In the coding region of the AIRE gene, 45 different mutations have been reported. Mutations comprise nonsense and missense mutations, deletions, and small insertions (5,61). The R257X mutation results in a stop codon instead of CGA (coding for arginine), and therefore, leading to a truncated regulator

protein. Mutational analysis of AIRE helps identify patients with atypical phenotypes resembling to PAS I, e.g. the AIRE mutation R257X was responsible for 82 % of PAS I alleles in a Finn population. In contrast, the AIRE R257x and 13bpdel mutations were never observed in our investigated population with PAS II (35).

Associations with HLA class II alleles, e.g. DR3, were also reported in PAS I. In a study comprising patients with PAS I from 12 different countries, Addison's disease was found to be significantly and positively associated with the HLA-DRB1*03 allele (relative risk RR 8.8). Here, only one of 19 patients with HLA-DRB1*03, in contrast to 28 of 85 patients without this allele, had not developed Addison's disease (4). In these patients, the component disease alopecia was significantly associated with HLA-DRB1*04 (RR 4.8) and DQB1*0302 (RR 6.6). In contrast, the most common protective alleles for T1D (DRB1*15 and DQB1*0602) were similarly protective in PAS I patients, as indicated by significant negative correlations (1,28).

Clinical spectrum

First clinical manifestation of the very rare PAS I usually occurs in childhood. Nevertheless, the main component diseases of type I develop in the first 20 years of life, and further associated diseases may not evolve until the fifth decade or later. In comparison, PAS II mostly occurs in adulthood during the third and fourth decades. PAS II presenting in childhood is extremely rare; a case with hypothyroidism, followed by diabetic ketoacidosis, and adrenal insufficiency has recently been reported (62). In adults, the presence of one autoimmune endocrine disease is associated with an increased risk of developing autoimmunity to other tissues. Each of these disorders is characterized by several stages beginning with active autoimmunity and followed by metabolic abnormalities with overt disease. T1D is a very frequent component disorder of PAS II and is often its first symptom. At our institution, screening of 471 patients with T1D showed in 127 cases (27%, 85/127 females) multiple glandular involvement. Additionally, 19 (4%) and 8 (2%) had vitiligo and immune gastritis, respectively. Subsequent screening of 15,000 consecutive subjects with endocrine disorders revealed a PAS prevalence of 1% (n=151, 75% female). These 151 subjects have been followed since then (63). There is often a long time interval between the manifestation of the first and second component

disease of PAS II which often comprises years to decades. Most frequent disease combinations are T1D / AITD (41%), followed by AITD / Addison's disease (14.6%), T1D / vitiligo (9.9%), AITD / vitiligo (9.9%), T1D / AITD / pernicious anaemia (5.3%), hypogonadism / alopecia (5.3%), and T1D / Addison's disease (3.3%).

The simultaneous occurrence of immune hypothyroidism and T1D is often accompanied by hypoglycaemia due to decreased insulin need and increased insulin sensitivity. Hypothyroid children show growth disorders caused by chronic hypoglycaemia and decreased food intake. Nevertheless, growth disorders of hypothyroid children are essentially due to hypothyroidism itself; if properly treated, growth is normal as well as the metabolic status, including insulin requirements. Substitution therapy with levothyroxine leads to increased insulin dosage. Similarly, concomitant presence of Addison's disease and T1D also leads to frequent hypoglycaemia due to decreased gluconeogenesis and increased insulin sensitivity. In contrast, hyperthyroidism is accompanied in 50% of the cases by glucose intolerance and in 3% of the cases by overt diabetes. Impaired glucose tolerance is due to decreased insulin sensitivity and hepatic storage of glycogen, whereas both secretion of glucagon and intestinal glucose absorption are enhanced. In diabetic children with concomitant Graves' disease, and its risk of fluctuating thyroid functional status, treatment of T1D is much more difficult which might call for a decision of definitive treatment, especially when remission is not obtained after an antithyroid drug course of reasonable duration.

Other disorders like immune gastritis, pernicious anemia, and alopecia areata may occur in PAS. Immune gastritis, eventually leading to pernicious anemia, is an organ-specific autoimmune disease characterized by pathological lesions, affecting the fundus and body of stomach, that are typified by gastric mucosal atrophy, selective loss of parietal cells from the gastric mucosa, submucosal lymphocyte infiltration, as well as circulating gastric parietal cell Abs. Subsequently, pernicious anemia, which is considered to be the most common cause of vitamin B_{12} deficiency, may develop. In this case, additionally Abs to the intrinsic factor, itself a secretory product of gastric chief cells, are found in the circulation and in gastric secretions.

Diagnosis, screening, and treatment

Actual *diagnosis* of PAS involves serological measurement of organ-specific Abs and subsequent functional testing (63-68). These special diagnostic approaches and the management of patients with PAS including their family relatives are best performed in centres with special expertise in autoimmune endocrine diseases. Although the clinical recommendations are not evidence based and a cost-benefit analysis that has shown the following is useful is lacking, the following suggestions rely on an approximately twenty year, long term and extensive personal experience with more than 350 patients with PAS and their corresponding families, kindred's, siblings, first and second degree relatives. Therefore, they may be viewed as expert recommendations reflecting the author's own individual practice.

Detecting organ-specific Abs verifies etiology of the disease and identifies patients who may develop autoimmune polyendocrinopathies. Circulating organ-specific Abs are present in each of the component diseases of PAS (table 2). Occasionally, as in antigonadal and antiadrenal Abs, a given group of Abs will cross react with more than one gland (steroid-producing cells). Abs may bind to a cell surface without functional effects, or may be blocking or stimulatory. Examples of blocking Abs include those directed at the acetylcholine receptor in myasthenia gravis. Prevalence of organ-specific Abs is high in healthy relatives of PAS patients and is associated with the number of involved glands. These "silent" Abs (e.g. anti-islet Abs) may precede clinical disease by years and are predictive for the development of future autoimmune endocrine disorders.

In adrenal autoimmunity, Abs are directed against antigens identified as P450 cytochromes, i.e. enzymes involved in the synthesis of steroid hormones. These are 21-hydroxylase (OH), 17-alpha-OH, and the side chain cleavage enzyme. The 21-OH has been identified as major target antigen in Addison's disease and has been detected in 85 % of patients with PAS and adrenal failure (65). Abs against steroidal enzymes (21-OH) are of high prognostic value and will help identify patients at risk for developing Addison' disease. This might prevent delayed diagnosis of adrenal failure. In contrast, thyroid Abs without clinical disease manifestation are noted in nearly 50% of the PAS patients, and the time interval between the detection of Abs and presentation of disease is longer for AITD than for

other immunopathies. Thus, high titers of Abs against thyroid peroxidase and thyroglobulin do not automatically precede the development of clinical AITD.

With respect to T1D, it has been demonstrated that antiidiotope reagents were able to distinguish between childhood-onset T1D and adult-onset T1D with polyendocrine susceptibility (69). Childhood-onset T1D-islet cell Abs differed from adult-onset T1D-islet cell Abs in their specifity for human insulin and from their antiidiotope amino acid sequence. Based on improved immunogenetic understanding and ab screening assays, T1D is now predictable (1,28). Finally, genetic investigation is especially useful for PAS I, less for type II, because one would likely identify the alleles associated with disease that is already identified, e.g. DR3/DR4 in patients with T1D (70).

Screening: Approximately one in seven first degree relatives of patients with PAS have an unrecognized endocrine disorder, usually the relatively common immune thyroiditis. Most specifically, in subjects with either monoglandular T1D or the relatively rare immune adrenal failure, organ-specific Ab screening and functional testing will help identify both patients at risk for developing PAS as well as an already present subclinical polyglandular syndrome. In the presence of a patient with clinical and biochemical signs of primary adrenal failure, the determination of 21OH Abs enables the unequivocal demonstration of the autoimmune origin of the disease. In subjects with Addison's disease, screening for other endocrine disorders is recommended, given the frequent association of autoimmune adrenal insufficiency with AITD, T1D or other immune-mediated diseases. Thus, in any patient with Addison's disease, determination of TPO, Tg, GAD 65, and islet cell Abs is recommended and if negative, repeated every 2-3 years. Since most PAS II patients are adults, determination of insulin or IA2 Abs is not strictly necessary, given the low diagnostic sensitivity of these markers for adult-onset T1D (71-72). In the case of positivity for GAD65 and islet cell Abs., an oral glucose tolerance is needed to demonstrate a glucose intolerance not revealed by fasting blood glucose. Although T1D develops frequently before Addison's disease, GAD65 Abs are detected in 5-7% Addison patients without T1D and a proper follow-up should also be performed in those islet cell Ab positive patients. The determination of 17OH and P450scc Abs will enable the identification of subjects at high risk for autoimmune primary hypogonadism, with a high positive predictive value in

women. Furthermore, determination of transglutaminase abs could be included in the screening of PAS children with T1D. Also, determination of 21OH Abs may be performed in patients with T1D and AITD, as the identification of subjects positive for adrenal Abs is highly predictive for future adrenal insufficiency. In subjects with 21OH abs and normal cortisol levels, an ACTH stimulation test, will enable the identification of subjects with pre-clinical adrenal dysfunction. Subjects with normal cortisol response could simply be followed-up, with re-evaluation of adrenal Ab levels, basal and ACTH-stimulated cortisol on a yearly basis (table 3).

Treatment: Many of the endocrine disorders of PAS are adequately treated with hormonal replacement therapy if the disease is recognized early. Subjects with pathological ACTH test and increased levels of basal plasma ACTH require close clinical follow-up with repetition of the test every 6 months. A replacement therapy with hydrocortisone or cortisone acetate should be considered in the case of undercurrent stressful events. Hypoglycaemic episodes and a decreasing insulin requirement in a type 1 diabetic can be one of the earliest signs of the development of adrenal failure. Replacement of levothyroxin without simultaneous adrenal steroid replacement in a hypothyroid patient with Addison's disease can precipitate an adrenal crisis (73). Replacement of levothyroxin increases the cortisol turnover rate in the liver, and this may tax a failing adrenal gland.

Concluding remarks

In PAS II, genetic associations with HLA haplotypes and polymorphisms of genes encoding immunologically relevant gene products have been reported. The associations of autoimmune endocrinopathies with polymorphisms of these particular genes support the hypotheses that they may function to influence general predisposition, increase susceptibility, or influence the clinical presentation of autoimmune diseases. Other factors such as environmental triggers and yet unidentified genetic loci may modulate disease or target tissue phenotype. Therefore, future research is required to identify the other players in the process of regulating immune tolerance and the process of defining the tissue targets of autoimmune diseases. Since actual data are scarce, further studies are necessary to elucidate the specific and general immunologic mechanisms which underlie the

development of PAS. Nowadays, genetic screening is useful for the monogenic PAS I, but not for the polygenic type II. Continuing research is warranted to further clarify the genetic background of PAS II, to identify susceptibility genes, and to understand their interactions. Potential susceptibility genes to PAS II are – besides the HLA genes – the CTLA-4 and cytokine-related genes TNF-α and/or PTPN22 (74). Additional knowledge regarding these genes will allow genetic screening of patients at risk. Advances in genetics and in pathogenesis of PAS II may be valuable in the prevention of morbidity and mortality of the subjects involved. Future research should also focus on family studies with a large number of samples from different population groups. This might offer further knowledge on the inheritance of PAS II as well as on the familial risk to develop this syndrome.

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Declaration of interest

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Legend of figure one

Prevalence of most frequent autoimmune endocrine component diseases in PAS type I (white colums) and PAS type II (grey columns). *Abbreviations*: parathyr., hypoparathyroidism; adrenal, Addison's disease; gonads, hypogonadism; pancreas, type 1 diabetes mellitus or T1D; thyroid, autoimmune thyroid disease or AITD.

EJE-09-0044-Figure one

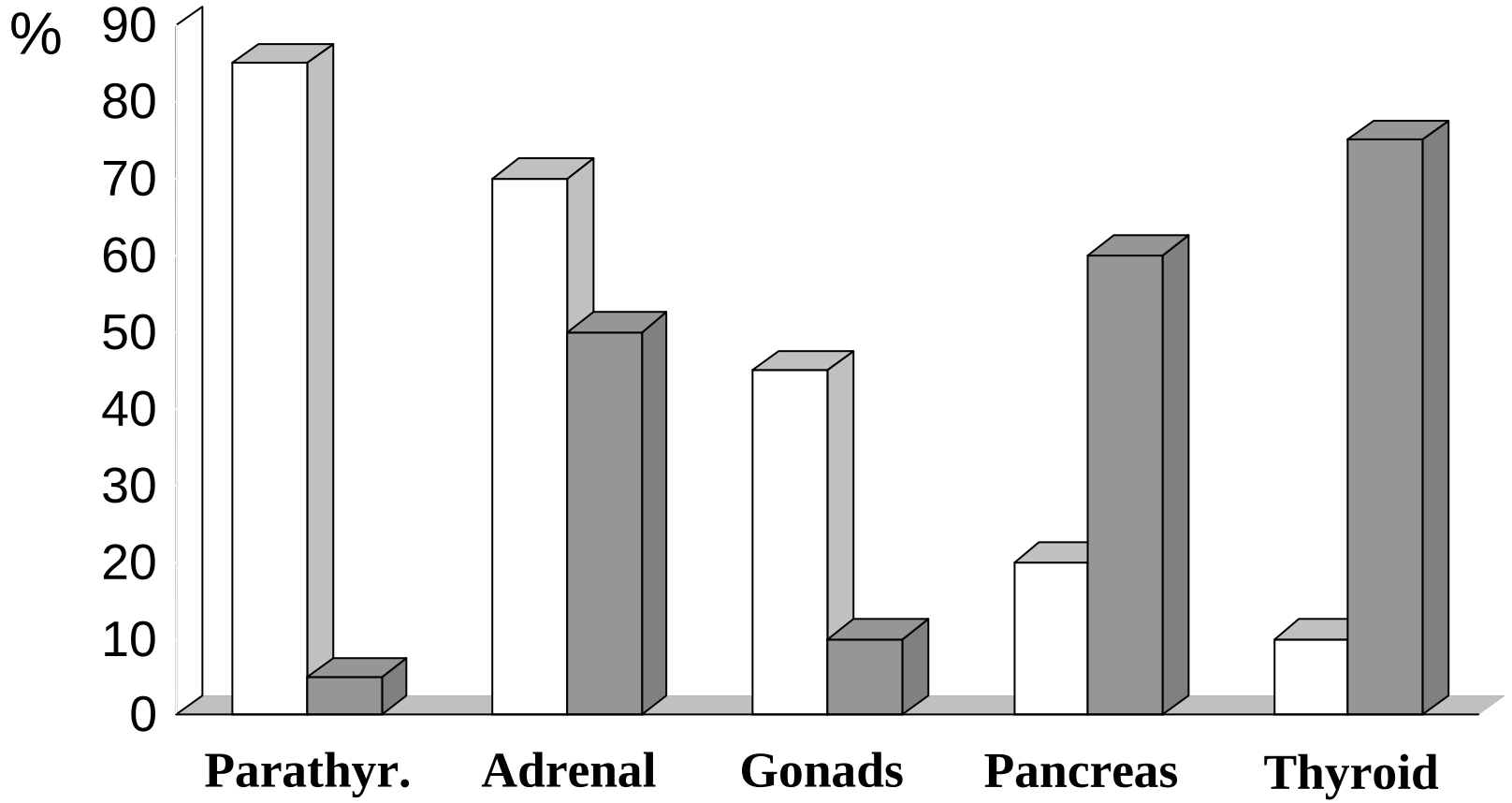

TABLE 1 Characteristics of the polyglandular autoimmune syndromes (PAS)

	PAS type I	**PAS type II**
Prevalence	very rare	relatively common
Incidence	< 1: 100,000 / year	1-2: 10.000 / year
M/f ratio	3: 4	1: 3
Onset	childhood	childhood through adulthood
Inheritance	monogenic (Aire gene)	polygenic
Autoimmune endocrine diseases	hypoparathyroidism (80-85%) Addison's disease (60-70%) type 1 diabetes (< 20%) hypogonadism (12%) thyroid disease (10%)	thyroid disease (70-75%) type 1 diabetes (50-60%) Addison's disease (40%) hypoparathyroidism (3%) hypopituitarism (0-2%)
Concomitant disease	Mucocutaneous candidiasis (70-80 %)	no candidiasis
Non-endocrine diseases	Immune gastritis, pernicious anemia, celiac disease, immune hepatitis, vitiligo, alopecia areata, Sjögren's syndrome, systemic lupus erythematodus, rheumatoid arthritis, myasthenia gravis	

Table 2: Localisation of the different autoantigen(s) and the corresponding diseases

Disease	Autoantigen	Tissue / Cells
Type 1 diabetes	GAD_{65}, IA-2, islet cells, insulin	ß-cells
Graves‘ disease	TSH receptor	Thyrocytes
Hashimoto‘s thyroiditis	TPO / Tg	Enzyme / Protein
Hypoparathyroidism	Ca^{2+} sensitive receptor	Parathyroid
Addison‘s disease	21-OH, 17-OH, P450scc	Enzyme
Hypogonadism	17-OH, CYP450scc	Leydig- / Theca cells
Immune gastritis	H^+, K^+-ATPase	Gastric parietal cells
Pernicious anaemia (stomach)	Intrinsic factor	Chief cells
Celiac disease	Transglutaminase, Gliadin	Small intestine
Immune hepatitis	P450D6, 2C9, P4501A2	Liver
Alopecia areata	Tyrosinhydroxylase	Hair follicles
Vitiligo	Tyrosinase	Melanocytes

GAD: glutamic acid decarboxylase; IA2: protein tyrosine phosphatase; 17-OH and 21-OH: 17- and 21-alpha-hydroxylase (steroidogenic P450 enzymes); SCC: side chain cleavage enzyme (steroidogenic P450 enzyme); Tg: thyroglobulin; TPO: thyroid peroxidase

TABLE 3: Diagnostic investigation for polyglandular autoimmune syndromes

Autoantibodies to

- Islet cells, GAD, (optional IA2)
- TPO, TSH receptor
- Cytochrome P450 enzymes (especially 21-Hydroxylase)
- H^+-K^+-ATPase of the parietal cells, intrinsic factor
- Transglutaminase, (optional gliadin)

Functional testing

- TSH, FSH, LH, fT_4, testosterone, estradiol, glucose, fasting morning cortisol
- ACTH stimulation test (when adrenal autoantibodies are present)
- Serum Na^+, K^+, Ca^+, blood cell count

Molecular analysis of AIRE gene for PAS type I, only

HLA-typing and subtyping (optional and for scientific purpose)

Abbreviations: AIRE gene, autoimmune regulator gene; GAD, glutamic acid decarboxylase; IA2, protein tyrosine phosphatase; TPO, thyroid peroxidase