CLINICAL STUDY

Mutational analysis of the autoimmune regulator (AIRE) gene in sporadic autoimmune Addison's disease can reveal patients with unidentified autoimmune polyendocrine syndrome type I

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Abstract

Objective: To investigate whether patients with Addison's disease and polyendocrine syndromes have undiagnosed autoimmune polyendocrine syndrome type I (APS I).

Materials and methods: Forty patients with clinical manifestations resembling APS I and with autoantibodies typical of this condition were screened for Norwegian autoimmune regulator (AIRE) gene mutations.

Results: A 30-year old man who had developed Addison's disease at the age of 12, but had no other components of APS I, was homozygous for the 1094–1106 deletion mutation in exon 8 of the AIRE gene, the most common mutation found in Norway.

Conclusions: APS I patients with milder and atypical phenotypes are difficult to diagnose on clinical grounds. Autoantibody analysis and mutational analysis of AIRE may therefore be helpful modalities for identifying these individuals.

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Introduction

Autoimmune adrenocortical failure (Addison's disease) can be observed as an isolated entity or as part of an autoimmune polyendocrine syndrome (APS) (1, 2). APS I is an autosomal recessive disease which is directly associated with mutations in the autoimmune regulator (AIRE) gene (3, 4). The APS I phenotype is diagnosed from the presence of two of the triad of main components, namely Addison's disease, hypoparathyroidism and chronic mucocutaneous candidiasis. Patients often have several additional autoimmune manifestations and ectodermal dystrophies (2, 5, 6). Clinical symptoms typically appear in childhood and additional manifestations can develop throughout life (5, 6). A number of circulating autoantibodies against cytochromes involved in the biosynthesis of steroid hormones and against enzymes involved in neurotransmitter biosynthesis are found in patients (7–9). Autoantibodies against aromatic L-amino acid decarboxylase (AADC), side-chain cleavage enzyme (SCC) and 17 α -hydroxylase (17OH) are relatively specific for APS I patients (10–12).

The clinical presentation of APS I varies in terms of both the number of components and the age at presentation, even within the same family (5, 6). In a study on 20 Norwegian patients, the age at diagnosis of the first clinical manifestation ranged from 1 to 20 years and the length of time before these patients were subsequently identified as having APS I varied from 2 to 49 years. Patients may have only one of the major components or only some of the other minor components and therefore do not fulfil the diagnostic criteria of APS I. Mutational analysis of the AIRE gene provides a definitive diagnosis of APS I. Using DNA analysis of the common Norwegian AIRE mutations (6) and assays of autoantibodies, we investigated patients with APS I-like clinical manifestations for the presence of undiagnosed APS I.

Materials and methods

Subjects

From our register of patients with Addison's disease ($n = 94$), 40 patients were selected on the basis of one of the following criteria: Addison's disease

Table 1 Characteristics of the patients screened for mutations in autoimmune regulator (AIRE).

Sex	Age (years)	Age at diagnosis of Addison's disease (years)	Manifestations	Autoantibodies
M	49	11	A	21OH
F	76	56	A, T, D, G	21OH
M	53	6	A, T, G	Negative
F	39	4	A, D, G	Negative
F	74	68	A, T	21OH, SCC, 17OH
M	29	12	A	SCC
M	19	13	A	21OH
M	35	3	A, D, G	21OH
F	47	—	T, P, G	21OH, SCC
F	26	—	T, HP	Negative
M	35	12	A	21OH
M	60	12	A, G	17OH
F	17	12	A, D, coeliac	21OH
F	40	36	A, T, D, G	21OH
F	30	19	A, T	21OH, SCC
M	50	3	A, T	21OH
F	56	41	A, T, G	21OH, SCC
F	80	34	A, T, G	21OH
F	61	36	A, T, G	21OH
F	55	26	A, T, G, V	21OH, 17OH
F	46	32	A, G	21OH, SCC
F	80	—	IF adrenal positive	Negative
F	36	27	A, P, candidiasis	21OH, 17OH
F	36	11	A, T, Turner syndrome	21OH
F	27	29	A, T, candidiasis	21OH, 17OH
M	52	—	V	Negative
F	44	28	A, D	21OH, 17OH
F	36	—	D, IF testis positive	Negative
M	49	13	A, T, G, D	21OH
F	28	26	A, G	21OH, SCC, 17OH
M	59	20	A	21OH, AADC
F	46	36	A	AADC
F	47	22	A, P	21OH, 17OH, AADC
M	22	—	Candidiasis	Negative
F	70	44	A	AADC
F	51	28	A, D	21OH, AADC
F	10	2	A, T	AADC
M	13	9	A, D, T	21OH
F	8	—	Candidiasis	Negative
M	11	8	A	21OH

A, Addison's disease; T, autoimmune thyroid disease; D, diabetes mellitus; G, gonadal failure; coeliac, coeliac disease; HP, hypoparathyroidism; IF, immunofluorescence; 21OH, 21-hydroxylase; SCC, side-chain cleavage enzyme, 17OH, 17 α -hydroxylase; AADC, aromatic L-amino acid decarboxylase; P, pernicious anemia; V, vitiligo.

diagnosed at <20 years of age, autoantibodies against AADC, SCC or 17OH and the presence of clinical manifestations resembling APS I. Clinical details are summarized in Table 1.

Mutational analysis of AIRE

Mutational analysis of exons 6 and 8 was performed as described previously (13). Exon 10 was amplified and sequenced using primers and conditions also described by others (13).

Other methods

In vitro transcription and translation followed by immunoprecipitation assays were used to determine the presence of autoantibodies as described previously (12).

Results

We identified one new APS I patient who was homozygous for the 13 bp deletion mutation in exon 8 (1094–1106 del), the most common AIRE mutation in Norway (Fig. 1). The deletion was also confirmed by sequence analysis on two separate blood samples. In addition, both parents were heterozygous for this mutation (Fig. 1). We were unable to amplify exon 10 of the AIRE gene from three of the patients. From two of these three we could not collect enough DNA to perform the analysis of exon 10, whereas PCR analysis of one of these subjects revealed no amplicon of this region. We cannot therefore rule out the possibility of mutations in exon 10.

The patient was a 30-year-old man who was diagnosed with Addison's disease at the age of 12. He

Figure 1 Agarose gel electrophoresis analysis of the 1094–1106 del mutation in exon 8 of the AIRE gene in the family with one affected member with APS I. Lane 1, molecular weight markers; lane 2, APS I patient homozygous for the 13 bp deletion; lane 3, parent 1 who is heterozygous for the 13 bp deletion; lane 4, parent 2 who is heterozygous for the 13 bp deletion; lane 5, healthy control.

reported none of the other endocrinopathies or ectodermal manifestations associated with APS I. After the diagnosis was made by DNA analysis of AIRE, the patient was again thoroughly examined by us and by an experienced dermatologist and by a dentist at the Faculty of Dentistry. No signs of other manifestations of APS I were found. He has two older sisters and an older brother who are healthy. His oldest sister died at the age of 4 during an acute varicella infection.

Discussion

The clinical presentation in APS I patients is very heterogeneous in terms of age at presentation, number of components and the time span between presentation of new manifestations (5, 6). Great variability is seen even among members of the same family (14). Therefore, we used DNA analysis of Norwegian mutations in AIRE (6) and assays of autoantibodies in an attempt to find previously unidentified APS I patients with milder and possibly atypical disease.

We found one such patient who was homozygous for the most common Norwegian mutation, the 13 bp deletion in exon 8 (1094–1106 del) (6). This patient, a 30-year-old man, had had isolated Addison's disease since the age of 12, but had no other components of APS I. He had autoantibodies against SCC and glutamic acid decarboxylase (GAD), autoantibodies which are found relatively commonly among these patients (6, 10), but are also seen in other autoimmune diseases (8).

In a similar study, British Addison patients were screened for the most common British AIRE mutation (1094–1106 del). One of 90 Addison patients and one of 576 healthy controls were found to be heterozygous (carriers) for the 1094–1106 del mutation in AIRE, but none were homozygous (15). Based on analysis of 817 individuals, another group concluded that the R257X and the 1094–1106 del mutations

of AIRE, respectively, are so rare in the general population that they do not contribute to susceptibility for the more isolated autoimmune endocrine disorders like Addison's disease, insulin-dependent diabetes mellitus, Graves' disease and Hashimoto's thyroiditis (16).

Patients with a clear clinical picture of APS I, but with no mutations in exons of AIRE, have been reported (17). These individuals probably have mutations in introns or in promotor regions of this gene or may have large deletions, since the penetrance of APS I is thought to be 100% (18). No studies have reported disease-causing mutations on both alleles of AIRE in healthy patients (13, 16, 19). However, a recent report has described an Italian APS I family with a novel mutation in exon 6 and evidence of a dominant mode of inheritance (20).

It is important to make the diagnosis of APS I. These patients should be followed-up more closely than other patients with Addison's disease or APS II, since several life-threatening complications can occur such as autoimmune hepatitis, hypoparathyroidism and squamous cell carcinoma of the oral mucosa. Assay of autoantibodies (i.e. SCC, 17OH, AADC and GAD, and possibly also tryptophan hydroxylase (21) and tyrosine hydroxylase (22)) should be considered in patients who develop Addison's disease before the age of 20 and in patients with an APS I-like phenotype. Analysis of the hot-spot AIRE mutations should also be considered. We cannot exclude the possibility that the death of a girl in acute varicella infection was due to adrenocortical failure as a part of undiagnosed APS I.

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