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II. Adrenal Cortex and Steroid 21-Hydroxylase Autoantibodies in Children with Organ-Specific Autoimmune Diseases: Markers of High Progression to Clinical Addison's Disease

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ABSTRACT

Adrenal cortex autoantibodies (ACA) were measured by immunofluorescence in 808 children with organ-specific autoimmune diseases without adrenal insufficiency. ACA were found in 14 children (1.7%), mostly in hypoparathyroidism (48%). Ten ACA-positive and 12 ACA-negative children were followed up for a maximum of 10 yr by evaluation of adrenocortical function (ACTH test) and autoantibody status. In all patients steroid-producing cell autoantibodies were assessed by immunofluorescence and autoantibodies to steroid 21-hydroxylase, 17α-hydroxylase, and cytochrome P450 side-chain cleavage enzyme by immunoprecipitation assay. All 10 ACA-positive patients were positive for 21-hydroxylase autoantibodies. Six were positive for steroid-producing cell autoantibodies and 5 also for autoantibodies to 17α-hydroxylase and/or P450 side-chain cleavage enzyme. Overt Addison's disease developed in 9 (90%) ACA/21-OH-antibody-positive children after 3–121 months, and 1 remaining child had subclinical hypoadrenalism. By contrast, all ACA/21-OH antibody-negative children maintained normal adrenal function. Adrenal failure was not related to ACA titres, sex, adrenal function, type of preexisting autoimmune disorder, or human leucocyte antigens D-related status. In conclusion, in children with autoimmune endocrine diseases, ACA/21-hydroxylase autoantibodies are important predictive markers for the development of Addison's disease. (*J Clin Endocrinol Metab* **82:** 939–942, 1997)

ADRENAL cortex autoantibodies (ACA) are well-established markers of autoimmune Addison's disease; in fact, they are found in more than 90% of children at the clinical onset of the disease (1). In contrast, ACA are rarely found in children without overt adrenal failure, being detectable in around 1% of those with insulin-dependent diabetes mellitus (2). A few follow-up studies, performed on ACA-positive children, have revealed variable progression to clinical Addison's disease ranging from 20–92% (3–5). However, one study indicated that in some cases the disease can develop without ACA (4). In an attempt to obtain further information about prevalence, change over time, predictive value of ACA, and the nature of the autoantigens involved, we screened a large group of young patients with nonadrenal organ-specific autoimmune diseases, and 22 of them (10 ACA-positive and 12 ACA-negative) were recruited into a prospective study.

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Subjects and Methods

Subjects

We studied 808 children (496 females and 312 males) less than 15 yr of age (range 5–12, mean 8.3 yr) affected by organ-specific autoimmune disease (OSAD) but without clinical Addison's disease (Table 1). One hundred healthy normal controls, matched for sex and age, were evaluated for ACA and 22 also for 21-steroid hydroxylase autoantibodies (21-OH Abs).

Immunological study

Adrenal cortex (ACA) and steroid-producing cell autoantibodies (StCA). Immunoglobulin classes of ACA and complement-fixing (CF) ACA were detected by the classical indirect immunofluorescence test on human adrenal tissue, as reported in the preceeding paper (6). StCA were tested on cryostat sections of human ovary and testis by indirect complement-fixation immunofluorescence as reported (6).

Autoantibodies to steroid 21-hydroxylase (21-OH Abs), 17α-hydroxylase (17α-OH Abs), and to P450 side chain cleavage (P450scc Abs). ^{35}S-21-OH, ^{35}S-17α-OH, and ^{35}S-P450scc were prepared using an *in vitro* transcription/translation system, and the respective autoantibodies were tested by immunoprecipitation assay (IPA) as reported (6).

Follow-up planning

Twenty-two children, 9 ACA-positive and 13 ACA-negative, were initially enrolled into the prospective study. The main preexisting organ-specific autoimmune diseases in patients with ACA are summarized in

TABLE 1. Prevalence of adrenal cortex (ACA-IgG) and steroid-producing cell antibodies (StCA) in children with organ-specific autoimmune diseases

Diseases	Subjects		ACA-positive		StCA-positive		
	No.	F/M	No.	(%)	No.	(%)	% in ACA-positive
Chronic idiopathic hypoparathyroidism	21	10/11	10[a]	(47.6)	6	(28.6)	60
Thyroid autoimmune diseases	207	160/47	0	0	0	0	0
Insulin-dependent diabetes mellitus	430	220/210	4	(0.9)	0	(0)	0
Vitiligo/alopecia/others	150	106/44	0	0	0	0	0
Total cases	808	496/312	14	(1.7)	6	(0.7)	43
Normal controls	100	71/29	0	(0)	0	(0)	0

[a] $P < 0.0001$ *vs.* normal controls (Chi-square test).

TABLE 2. Clinical and immunological features of children with ACA who entered the follow-up study

Patient no.	Sex	Age	Diseases at entry	ACA		Antibodies to 21-OH index	StCA	Antibodies to 17α-OH P450scc		HLA DR	Stages of adrenal function	Latency before Addison's disease	
				Titers	CF			μ/mL	μ/mL			Yr	Months
1	F	7	IHP, CMC	>64	+	2.6	+	64.0	11.1	2/5	1→4	2	1
2	M	12	IDDM	>64	+	16.4	−	−	−	1/2	1→4	2	1
3	M	6	IHP, CMC	0/<16[a]	f	−/5.8[a]	− /−	−/1.6	−/−	3/5	0→4	3	8
4	M	5	IHP, CMC	>64	+	18.2	+	1.7	>32	2/5	3→4	0	3
5[b]	M	10	IHP, CMC	16–64	+	18.2	+	−	−	5/7	0→4	1	10
6	F	10	IHP, CMC	16–64	+	54.7	+	>64	20	7/w6	0→4	10	1
7	F	6	IHP	16–64	+	6.4	−	−	−	n.t.	0→4	1	2
8	M	12	IDDM	16–64	f	45.7	−	−	−	3/4	3→4	1	8
9	F	8	IHP	16–64	f	36.5	+	−	2.4	3/8	3→4	1	1
10	F	12	IHP	>64	+	20.9	+	>64	>32	n.t.	2→2	[c]	

ACA, Adrenal cortex antibodies; 21-OH, steroid 21-hydroxylase; StCA, steroid-producing cell antibodies; 17α-OH, steroid 17α-hydroxylase; P450 scc, cytochrome p450 side-chain cleavage enzyme; HLA-DR, human leukocyte antigens; D-related; Stages of adrenal function, see *Materials and Methods* section (Stage 4, overt adrenocortical failure); IHP, idiopathic hypoparathyroidism; CMC, chronic mucocutaneous candidiasis; IDDM, insulin-dependent diabetes mellitus; n.t., not tested; f, fluctuating; [a] Seroconverted for ACA during follow-up; [b] Deceased; [c] Observation period 24 months without clinical Addison's disease.

Table 2. Out of the 13 initially ACA-negative patients with organ-specific autoimmune diseases (6 females and 7 males), 9 had idiopathic hypoparathyroidism with or without candidiasis, and 4 had insulin-dependent diabetes mellitus (IDDM).

The mean follow-up period in ACA-positive and ACA-negative patients was 2.6 yr (range 3–121 months) and 2.9 yr (range 6–100 months) respectively.

Informed consent was obtained from the children's parents in accordance with the principles of the Helsinki Declaration.

Positive and negative patients were periodically evaluated for immunological parameters and adrenal function by an ACTH-test (6). None of the followed patients were receiving corticosteroid therapy. Statistical analysis was carried out as described (6).

Other investigations

Adrenal function was evaluated by ACTH-test as described (6). Eight ACA-positive patients and 153 normal controls were typed for human leucocyte antigens (HLA) DRB1, DQA1, and DQB1 alleles as described (6).

Results

Immunological study

Fourteen children out of 808 (1.7%) with organ-specific autoimmune diseases, and none of the normal controls, were found to be positive for ACA of immunoglobulin (Ig) G class (ACA-IgG) (P = n.s.). The prevalence of ACA was significantly increased in patients with idiopathic hypoparathyroidism (47.6%) ($P < 0.0001$ *vs.* normal controls) (Table 1).

StCA were found in 6 of 808 (0.7%) children with organ-specific autoimmune diseases, all belonging to the ACA-positive group. The highest prevalence of StCA was found in patients with hypoparathyroidism (28.5%) (Table 1).

Follow-up study

All the 9 initially ACA-positive children maintained their positivity during follow-up. One of the patients initially recruited as ACA-negative acquired ACA-IgG after 36 months of observation and was included in the group of ACA-positive children (Table 2, no. 3). All 10 ACA-positive patients were found to have 21-OH Abs, with a mean index value of 22.5 (range 2.6–54.7) (Table 2). The mean index value in the 22 normal children was 0.3 (mean + 3 SD). Six patients were also positive for StCA, and all but one had 17α-OH and/or P450scc Abs (Table 2).

At the beginning of the follow-up out of 10 ACA/21-OH Abs positive children, the ACTH-test revealed a Stage 0 (normal adrenal function) in 4, a Stage 1 (increased plasma renin activity) in 2, a Stage 2 (no cortisol response to ACTH) in 1, and a Stage 3 (increased basal levels of ACTH and low of cortisol) in 3 patients.

Clinical Addison's disease developed in 9 of the 10 ACA/21-OH Abs positive children after a mean latency period of 2.7 yr (range 3–121 months) (Table 2, no. 1–9). One of these patients was the "seroconverted" child who developed clinical disease 8 months after the first discovery of ACA in his serum (Table 2, no. 3). One female patient is still disease-free after more than 2 yr of observation (Table 2, no. 10); however she maintained Stage 2 of adrenocortical dysfunction

through the follow-up. None of the other patients with initially impaired adrenal function revealed any improvement of their status. In four patients (Table 2, no. 3, 5–7), a complete progression from Stage 0 to Stage 4 was documented over a period of at least 14 months. The last patient in this group remained at Stage 2 through the follow-up.

Two ACA/21-OH Abs positive patients were brothers (Table 2, no. 3, 5), both with Type 1 autoimmune polyendocrine syndrome. One of them (no. 5) died, by the age of 18 yr, of renal failure caused by nephrocalcinosis, six yr after the onset of clinical Addison's disease. At autopsy, his adrenal glands revealed atrophy with the presence of lymphocytic infiltrates.

None of the 12 persistently ACA-negative sera were positive for 21-OH Abs, 17α-OH Abs or P450scc Abs. None of the 10 persistently ACA/21-OH Abs negative patients developed either clinical Addison's disease or biochemical signs of impairment in adrenal function.

The estimated probability of progression to Addison's disease in ACA/21-OH Abs-positive compared with ACA/21-OH Abs-negative children is plotted in Fig. 1.

Table 3 summarizes positive predictive values, annual incidences, and cumulative risks for Addison's disease according to ACA/21-OH Abs status, sex, and preexisting endocrine autoimmune diseases.

Out of the 6 patients with StCA (4 females and 2 males), 2 females (no. 1, 6) developed premature ovarian failure at the ages of 16 and 36 respectively, one (no. 10) had normal menses at the age of 14, one female (no. 9) and one male (no. 4) were still prepuberal; the male no. 5 did not reveal any infiltration of the gonads at autopsy.

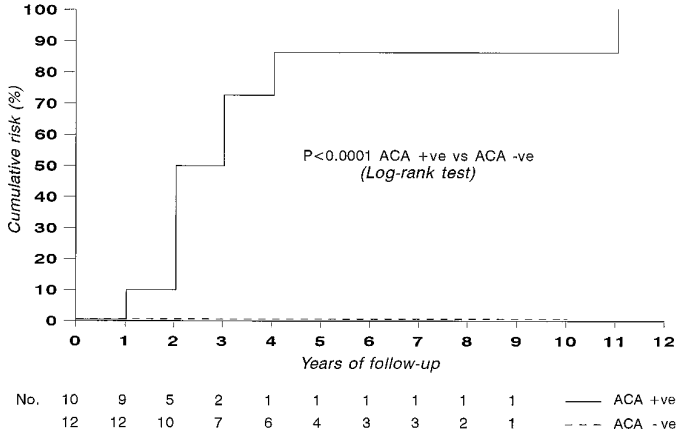

FIG. 1. Estimated probability of progression to overt Addison's disease in children according to adrenal-cortex antibodies.

Genetic study

HLA genotypes of the 8 ACA-positive children are shown in Table 2. No significant association was found between any human leucocyte antigen (HLA) genotype and presence of ACA with respect to the general population.

Discussion

In the present study we demonstrated that the prevalence of ACA in the children with organ-specific autoimmune diseases without overt Addison's disease varied between 1% in sera from children with IDDM to 48% in those with hypoparathyroidism. Moreover, ACA are virtually absent in children with other autoimmune diseases.

Our follow-up study indicates, for the first time, that the ACA-positive children have a high risk of developing Addison's disease and that the risk appears to be unaffected by sex, antibody titers, adrenal function, preexisting autoimmune diseases, or HLA-DR status. In contrast to a previous study (4), no impairment of adrenal function was found in our persistently ACA-negative children with autoimmune diseases.

While ACA-positive children appear to progress with very high frequency towards overt Addison's disease, only a much smaller proportion (21%) of ACA-positive adults show a similar phenomenon (6), and the reasons for this difference are not clear at present.

The main mechanism of autoimmune damage of the adrenal cortex in autoimmune Addison's disease is not known, although cell-mediated immunomechanisms seem most likely to be involved (7). ACA are a serological marker that appears to be closely associated with the ongoing cell-mediated autoimmune attack on the adrenal cortex (7).

The different progression towards the disease between ACA-positive children and adults may be caused by possible age-related differences in cellular autoimmune responses. For example, in the case of IDDM, the presence of islet-cell antibodies in unaffected first-degree relatives confers more risk for the onset of IDDM in young persons than in adults (8).

The current study indicates that 21-OH is the major autoantigen in ACA-positive children before development of overt Addison's disease, all ACA-positive sera were also 21-OH Abs-positive, and that 17α-OH or P450scc are the major antigens of StCA. These data confirm and extend earlier studies demonstrating that in children with clinical Addison's disease the major adrenal autoantigen is 21-OH (9–11) and are in contrast to a report by Krohn *et al.* (12), which

TABLE 3. Positive predictive value, annual incidence, and cumulative risk for Addison's disease in children with organ-specific autoimmune diseases

	Pts.	Progression to Addison's disease (no.)	Positive predictive value (%)	Annual incidence (%)	Cumulative risk (%) (95% c.i.)
ACA-positive	10	9	90	34.6	100
Males	5	5	100	52.6	100
Females	5	4	80	24.2	80 (44.2–100)
IHP	8	7	87.5	31.4	100
IDDM	2	2	100	53.3	100
Persistently ACA-negative	12	0	0	0	0

ACA, Adrenal-cortex antibodies; c.i., confidence interval; IHP, idiopathic hypoparathyroidism; IDDM, insulin-dependent diabetes mellitus.

suggested that 17α-OH was the major autoantigen in children with APS Type 1.

In addition, our study provides further information about the latency period before development of overt Addison's disease, which, although quite variable, usually required at least 1 yr for a complete progression from normal adrenal function to clinical Addison's disease. On the basis of these results we suggest that children with organ-specific autoimmune diseases, particularly those with hypoparathyroidism and IDDM, should be screened for the presence of ACA/21-OH Abs, and those positive should undergo evaluation of adrenal function at least every 6 months.

Finally, we suggest that substitutive therapy be initiated at the first stage of this potentially life-threatening condition (13).

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