0021-972X/97/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 82, No. 3
Printed in U.S.A.

I. Adrenal Cortex and Steroid 21-Hydroxylase Autoantibodies in Adult Patients with Organ-Specific Autoimmune Diseases: Markers of Low Progression to Clinical Addison's Disease

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ABSTRACT

Adrenal cortex antibodies (ACA) were measured by immunofluorescence in 8840 adult patients with organ-specific autoimmune diseases without overt hypoadrenalism. Sixty-seven (0.8%) patients were ACA-positive, with the highest prevalence in those with premature ovarian failure (8.9%). Forty-eight ACA-positive and 20 ACA-negative individuals were enrolled into a prospective study. Antibodies to steroid 21-hydroxylase (21-OH), steroid 17α-hydroxylase (17α-OH) and cytochrome P450 side chain cleavage enzyme (P450scc) were measured by immunoprecipitation assay. Human leucocyte antigens D-related (HLA-DR) genotyping was also carried out and adrenal function assessed by ACTH test. On enrollment, 75% of ACA-positive patients had a normal adrenal function, while 25% revealed a subclinical hypoadrenalism. 21-OH antibodies were positive in 91% of ACA-positive sera. Eleven patients were positive for steroid-cell antibodies by immunofluorescence, and 9 revealed a positivity for antibodies to 17α-OH and/or P450scc. During the prospective study, overt Addison's disease developed in 21% and subclinical hypoadrenalism in 29% of ACA-positive patients, while 50% maintained normal adrenal function. Progression to Addison's disease was more frequent in patients with subclinical hypoadrenalism, high titers of ACA and higher levels of 21-OH antibodies, complement-fixing ACA and HLA-DR3 status. All 20 persistently ACA-negative patients were also negative for antibodies to 21-OH, 17α-OH, and P450scc, and all maintained normal adrenal function during follow-up. In conclusion, the detection of ACA/21-OH antibodies in adults is a marker of low progression toward clinical Addison's disease. (*J Clin Endocrinol Metab* **82:** 932–938, 1997).

ADRENAL cortex autoantibodies (ACA) are well-established markers of autoimmune Addison's disease, being detectable in about 90% of adult patients if determined closely with the onset of clinical symptoms of the disease, and they are absent in the other forms of adrenal insufficiency (1, 2).

In adult autoimmune disease patients without Addison's disease, the prevalence of ACA varies greatly, being 0.2–13.5% (3–6), while occurence is up to 5% in the general population (7). Follow-up studies of ACA-positive patients have suggested that only a few patients (3–6) or none (7) progress to overt hypoadrenalism. Furthermore, it has been reported that ACA can disappear spontaneously or after treatment with corticosteroids, with remission of a subclinical Addison's disease (6–8).

To clarify and extend these earlier observations we carried out a prospective study of 48 ACA-positive autoimmune diseases patients who did not have overt adrenal failure.

Received May 14, 1996. Revised November 18, 1996. Accepted November 25, 1996.

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Subjects and Methods

Subjects

Sera were obtained from 8840 adults with organ-specific autoimmune diseases but without overt hypoadrenalism (see Table 1 for details). Out of 40 patients with hypoparathyroidism, 30 developed the disease over 15 yr and 10 under 15 yr of age; the disease was isolated in 37 patients, and in the context of autoimmune polyendocrine syndrome (APS) Type 1 in 3 cases. In addition, sera were collected from 338 healthy subjects without personal or family history of autoimmune diseases.

Immunological study

Adrenal cortex (ACA) and steroid-producing cell autoantibodies (StCA). ACA of immunoglobulin (Ig) G class (ACA-IgG) were detected by the classical indirect immunofluorescence technique on human adrenal tissue, as previously described (4, 5). Titers of ACA-IgG were defined by doubling dilution up to the end point. ACA-IgA and -IgM were investigated by the same technique, using fluorescein-isothyocianate conjugated polyclonal antibodies against human IgA and IgM, respectively (Wellcome, Beckenham, U.K.). Complement-fixing ACA (CF-ACA) were detected by indirect immunofluorescence complement-fixation test (4, 5). The ability of ACA-positive sera to assemble the terminal complement com-

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

	Subjects		ACA-positive		StCA-positive		
	No.	F/M	No.	(%)	No.	(%)	Percentage of ACA-positive
Organ-specific autoimmune patients	8840	6540/2300	67	(0.8)	11	(0.1)	20
Chronic idiopathic hypoparathyroidism	40	22/18	1	(2.5)	1	(2.5)	100
Premature ovarian failure	45		4[a]	(8.9)	3	(6.6)	75
Thyroid autoimmune diseases	4353	3913/440	45	(1.0)	5	(0.1)	11
Insulin-dependent diabetes mellitus	3250	1720/1530	14	(0.4)	2	(0.06)	14
Vitiligo	1152	840/312	3	(0.3)	0	(0)	0
Normal controls	338	220/118	1	(0.3)	0	(0)	0

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

plex was evaluated by indirect complement fixation immunofluorescent test using normal human serum as source of complement, followed by IgG_2 mouse monoclonal antibodies directed against C9 (aE11) neoantigen (kindly supplied by T. E. Mollnes, Oslo, Norway), and by anti-mouse IgG rabbit FITC-serum (Sigma Co., St. Louis, MO). Steroid-producing cell autoantibodies (StCA) were tested on cryostat sections of human ovary and testis by indirect immunofluorescence complement-fixation test (9).

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 analyzed by SDS-PAGE, as previously described (10, 11). The immunoprecipitation assay (IPA) was carried out using the method of Colls (10). The results of 21-OH Abs were expressed as an index, and an index value of 2.6 or greater (based on a mean + 3 SD of 26 healthy blood donors) was considered to indicate the presence of 21-OH Abs. The results of 17α-OH and P450scc Abs were expressed as arbitrarily defined units (U/mL), as previously reported (11).

Functional study

Adrenal cortex function was evaluated by measuring basal plasma levels of cortisol (normal values 138–550 nmol/L), aldosterone (normal values 277–831 pmol/L), ACTH (normal values 4–22 pmol/L), and PRA (normal values 2–6 ng/L per 3 h). Plasma concentrations of cortisol were also measured 60 min after an iv bolus of 0.25 mg synthetic ACTH (rapid ACTH-test). Normally, cortisol increase is greater than 200 nmol/L with respect to basal values. Plasma ACTH was measured by a two-site immunoradiometric assay (Euro-Diagnostic, Apeldoorn, Holland). Plasma cortisol and aldosterone were detected by RIA (Diagnostic Products Corporation, Los Angeles, CA, and Sorin, Vercelli, Italy). PRA was determined according to Stockigt (12) with the omission of the boiling step. Functional tests were performed approximately once a year from entry into the study.

According to the values obtained, five distinct grades of adrenocortical dysfunction were recognized, as previously reported (5). Stage 0 was characterized by a normal adrenal function; Stage 1 by an increase in plasma renin activity, along with a normal or low serum aldosterone level; Stage 2 by a decreased plasma cortisol response to ACTH; Stage 3 by an increased basal plasma ACTH level; Stage 4 by a decreased plasma cortisol level with appearance of overt symptoms of adrenocortical failure.

Genetic study

Forty-three ACA-positive patients and 153 normal controls were typed for human leucocyte antigens (HLA) DRB1, DQA1, and DQB1 alleles, as previously described (13).

Statistical analysis

Actuarial survival rate was employed to estimate the likelihood of progression toward Addison's disease, according to the Cutler-Ederer method (14). All patients entered the life-table when ACA were first determined in our laboratory. The follow-up ended when overt hypoadrenalism occurred or when adrenal antibodies were last detected for unaffected individuals. The result of the survival analysis was expressed by plotting curves of cumulative risk of morbidity. The log-rank statistic was used to compare the estimates between the selected categories (15). The annual incidence of Addison's disease was evaluated by dividing the number of Addisonian patients by the patients' years of follow-up. Each patient contributed to the total sum of patients' years with a period ranging from the start of the observation until the disease was diagnosed, or until the observation period was ended. Sampling errors and 95% confidence intervals (c.i.) were calculated. Differences in the prevalence of ACA and HLA-DR antigens with respect to controls were evaluated by the Chi-square test. For HLA-DR the *P* value obtained was corrected multiplying by 9 the number of antigens tested.

Follow-up planning

Sixty-eight patients, 45 ACA-positive (43 females and 2 males) and 23 ACA-negative patients (matched for sex, age, and preexisting autoimmune diseases) were enrolled into a prospective study. The one ACA-positive subject from the normal control group was not followed-up. Informed consent was obtained, and the investigation was performed in accordance with the principles of the Declaration of Helsinki.

At the beginning of the study, all ACA-positive sera were characterized for ACA-IgG titers, CF-ACA, ACA-IgA, -IgM, and StCA. In addition, sera were tested for 21-OH, 17α-OH, and P450scc Abs. During follow-up, ACA titers and ACTH-tests were reevaluated at yearly intervals. The mean follow-up period of ACA-positive patients was 50 months (range 3–163). All ACA-negative patients were also periodically evaluated for ACA and by ACTH-test. The mean follow-up period was 45 months (range 6–100). In patients who developed overt Addison's disease, ACA were further tested.

Results

Immunological study

ACA-IgG were found to be positive in 67 of the 8840 (0.8%) adults with organ-specific autoimmune diseases and in one normal adult control (0.3%). The prevalence of ACA in the different groups of autoimmune patients varied from 0.3% in patients with vitiligo to 8.9% in those with premature ovarian failure (see Table 1), and only in this last group was the frequency of ACA significantly greater than in normal controls ($P < 0.0001$). StCA were found in 11 patients, all belonging to the ACA-positive group. The highest prevalence of StCA was found in premature ovarian failure (6.6%) (Table 1).

Follow-up study

Forty-five ACA-positive and 23 ACA-negative patients were available for the prospective study. Furthermore, 3

patients in the ACA-negative group became ACA-positive during the study and were moved to the ACA-positive group. Consequently, the ACA-positive group included 48 patients (Table 2).

The organ-specific autoimmune diseases in the 48 ACA-positive patients are listed in Table 2. Fifteen patients had Graves' disease (8 were receiving antithyroid drugs, 2 were in remission without therapy, while the other 5 were receiving replacement doses of L-thyroxine for spontaneous or surgical hypothyroidism). Eighteen patients had Hashimoto's thyroiditis (9 were under replacement doses of L-thyroxine). Thirteen patients had insulin-dependent diabetes mellitus and were on insulin therapy; 2 ICA-positive patients in this group had had gestational diabetes mellitus and, after the delivery, were controlled by oral hypoglycaemic agents. Four patients had premature ovarian failure (one was treated with estrogens and progestins). One patient with hypoparathyroidism received vitamin D and calcium. Eight had vitiligo, 1 chronic liver disease, and 1 ICA-positive patient had normal glucose tolerance. None of the patients were receiving corticosteroid therapy.

Overall in the ACA-positive group, low titers of ACA (<16) were found in 7 cases, medium titers (16–64) in 17, and high titers (>64) in 24 cases. Persistently positive CF-ACA were present in the sera of 39 patients (81%). CF-ACA showed association with high titers of ACA ($P < 0.05$, Chi-square test). Terminal complement complex ACA were found to be persistently positive in 20 of the 44 (46%) patients studied. Nine of the 48 ACA-positive patients also had ACA-IgA, and 1 patient had ACA-IgM. Eleven patients were also positive for StCA.

21-OH Abs were detected in 43 of 47 (91%) ACA-positive patients tested, with a mean index of 33 (range 9–121, Table 2); the mean 21-OH Abs index value did not show correlation with the titers of ACA. Of the 11 ACA-positive patients who were positive for StCA, all were also positive for 21-OH Abs, and 9 were positive for 17α-OH and/or P450scc Abs (Table 2).

Among the 20 patients persistently ACA-negative (2 males and 18 females), 12 had thyroid autoimmune diseases (6 with Graves' disease and 6 with Hashimoto's thyroiditis), 5 had insulin-dependent diabetes mellitus, 1 had both thyroid autoimmune disease and insulin-dependent diabetes mellitus, and 2 had idiopathic hypoparathyroidism. None of the ACA-negative patients were receiving corticosteroids.

The ACTH test upon entry to the study revealed that out of 48 ACA-positive patients, 36 (75%) were in Stage 0, and 12 (25%) in Stage 1, 2, or 3 (subclinical hypoadrenalism) (Table 2). During follow-up, of the 36 patients with normal adrenal function, 5 developed clinical disease and 7 subclinical adrenal insufficiency. Of the 12 patients with initial subclinical hypoadrenalism, 5 developed overt Addison's disease, and 7 maintained the subclinical hypoadrenalism. Overall, 10 patients (21%) developed clinical Addison's disease, 14 (29%) developed subclinical adrenal insufficiency, and 24 (50%) maintained a normal adrenal function (Fig. 1, Table 2). In the group of ACA-positive individuals who developed clinical or subclinical Addison's disease, the mean index value ±SD for 21-OH antibodies was 42 ± 26, compared with 24 ± 17 in patients who were maintaining normal adrenal function ($P = 0.01$, t test).

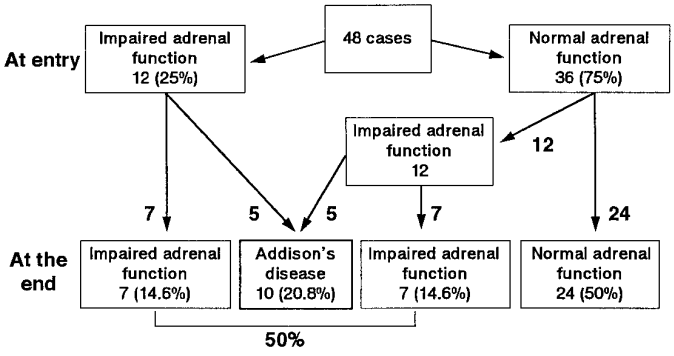

FIG. 1. Follow-up of 48 polyendocrine non-Addisonian adult patients with adrenal cortex antibodies.

Overt Addison's disease occurred after a mean latency period of 2.7 yr (range 4–71 months), but the mean time for a complete progression from normal adrenal function to clinical disease (*i.e.* from Stage 0 to Stage 4) was 5.2 yr, (range 23–71 months) (Table 2; no. 3a, 6a, 7a, 9a, 10a).

During follow-up none of the patients with ACA lost antibody reactivity or revealed any restoration in their functional adrenal status. Only one female, initially in Stage 1, showed first a progression up to Stage 3, but then she partially improved to Stage 2 (Table 2; no. 9b). One patient (Table 2; no. 6c) died of breast cancer after more than 9 yr of follow-up still maintaining normal adrenal function; no autopsy was performed. Another patient who had IDDM (Table 2; no. 12b) developed chronic renal failure and underwent transplantation of both kidney and pancreas. After transplantation she was able to stop the insulin therapy and was given immunosuppressive treatment with cyclosporin A, corticosteroids, and azathioprine. This patient was re-evaluated after 9 months of immunosuppression: ACA and 21-OH antibodies were still positive and the ACTH test performed 3 days after the withdrawal of corticosteroids revealed a normal adrenal function. Nine of the 10 patients (90%) followed after the onset of Addison's disease were still positive for ACA after a mean period of 51 months (range 3–120 months).

All of the 20 persistently ACA-negative patients were also negative for StCA, 21-OH, 17α-OH, and P450scc Abs and maintained normal adrenal function during the observation period.

The life estimates expressed as cumulative risks of morbidity, according to ACA status (Fig. 2a), ACA titers (Fig. 2b), CF-ACA (Fig. 2c), as well as the stage of adrenal function at entry (Fig. 2d) are plotted. Table 3 summarizes positive predictive values, annual incidences, and cumulative risks for Addison's disease according to the clinical and immunological features.

During follow-up, all 7 patients with StCA without hypergonadotropic hypogonadism (Table 2; no. 8a, 10a, 4–5b, 4c, 10c, 16c), maintained normal gonadal function.

TABLE 2. Clinical, immunological, and genetic features of 48 adult patients with adrenal cortex antibodies

a) Patients progressing toward overt Addison's disease

Pts. no.	Sex/Age	Diseases at entry	ACA		Antibodies to 21-OH	StCA	Antibodies to 17α-OH P450scc		HLA DR	Stages of adrenal function	Latency before Addison's disease	
			Titers	CF	(Index)		U/mL	U/mL			Yr	Months
1a	F/39	GD	>64	+	36.2	−	−	>32	3/5	2 → 4	2	11
2a	F/36	GD	16−64	+	23.4	−	−	−	4/5	1 → 4	1	6
3a	F/19	IDD, HT	>64	+	121.5	−	−	−	2/3	0 → 4	5	9
4a	F/33	HT	>64	+	39.0	−	−	−	2/5	3 → 4	0	4
5a	M/30	Vit	16−64	+	39.6	−	−	−	3/5	3 → 4	0	6
6a	F/51	HT, IDD	16−64	+	26.7	−	−	−	5/8	0 → 4	5	5
7a	F/30	HT	>64	+	47.3	−	4.1	−	n.t.	0 → 4	1	11
8a	F/27	GD	>64	+	102.0	+	33.8	−	5/5	3 → 4	0	8
9a	F/34	GD, Vit	16−64	+	39.8	−	−	−	4/8	0 → 4	5	11
10a	M/29	IHP, CMC	16−64	+	26.4	+	>64	−	w6/7	0 → 4	2	2

b) Patients with impaired adrenal function not progressing to overt Addison's disease

Pts. no.	Sex/Age	Diseases at entry	ACA		Antibodies to 21-OH	StCA	Antibodies to 17α-OH P450scc		HLA DR	Stages of adrenal function	Period of follow-up	
			Titers	CF	(Index)		U/mL	U/mL			Yr	Months
1b	F/50	GD	16−64	+	29.7	−	−	−	3/4	1 → 1	8	5
2b	F/26	Vit	>64	+	30.6	−	−	−	5/w6	1 → 1	9	1
3b	F/23	HT	>64	+	55.8	−	−	−	2/3	1 → 1	1	4
4b	F/31	GD	16−64	+	45.9	+	52.6	5.9	n.t.	1 → 1	9	1
5b	F/35	GD, Vit	>64	+	39.6	+	1.2	−	5/5	1 → 1	2	2
6b	F/20	IDD	>64	+	17.2	−	−	−	4/5	0 → 1	4	1
7b	F/23	POF, HT	>64	+	48.0	+	−	21.0	w6/w6	0 → 1	1	5
8b	F/29	POF	>64	+	36.5	+	−	9.1	3/5	3 → 3	1	1
9b	F/32	POF, HT	>64	+	82.2	+	−	>32	3/4	1 → 3 → 2	1	3
10b	F/26	CALD	>64	+	20.4	−	−	−	3/4	0 → 1	1	7
11b	F/47	HT	16−64	+	21.4	−	−	−	2/3	0 → 1	6	11
12b	F/31	IDD, CRF	>64[a]	+	29.7	−	−	−	3/4	0 → 1	7	1
13b	F/43	GD	16−64[a]	+	26.9	−	−	−	3/5	0 → 1	4	1
14b	F/64	GD	<16	−	9.2	−	−	−	1/3	0 → 1	7	1

c) Patients maintaining normal adrenal function

Pts. no.	Sex/Age	Diseases at entry	ACA		Antibodies to 21-OH	StCA	Antibodies to 17α-OH P450scc		HLA DR	Stages of adrenal function	Period of follow-up	
			Titers	CF	(Index)		U/mL	U/mL			Yr	Months
1c	F/45	IDD, GD	16−64	+	27.6	−	6.8	−	4/4	0 → 0	10	7
2c	F/34	Vit, GDM	>64	t	−	−	−	−	1/10	0 → 0	3	5
3c	F/56	HT, SSc	>64	+	36.8	−	−	−	1/4	0 → 0	2	4
4c	F/47	HT	>64	+	52.6	+	−	−	3/5	0 → 0	1	3
5c	F/23	IDD	<16	−	10.7	−	42.5	−	3/8	0 → 0	6	8
6c[b]	F/36	MG, IDD, HT	16−64[a]	+	39.4	−	−	−	3/7	0 → 0	9	8
7c	F/37	HT	16−64	+	26.9	−	−	−	1/2	0 → 0	13	7
8c	F/34	HT	>64	+	33.7	−	−	−	w6/w6	0 → 0	1	10
9c	F/41	GD	>64	+	52.1	−	−	2.2	2/3	0 → 0	0	11
10c	F/42	IDD	16−64	−	11.9	+	−	−	4/4	0 → 0	1	9
11c	F/34	HT, Vit	<16	+	30.0	−	−	−	2/7	0 → 0	7	1
12c	F/25	GD	16−64	+	27.8	−	−	−	1/5	0 → 0	8	10
13c	F/33	HT	>64	−	−	−	−	−	4/5	0 → 0	4	4
14c	F/52	GD, Vit	<16	t	13.6	−	−	−	5/5	0 → 0	6	5
15c	F/30	ICA	<16	+	11.9	−	−	−	5/5	0 → 0	6	3
16c	F/23	IDD	16−64	+	51.2	+	>64	−	3/4	0 → 0	6	9
17c	F/17	HT	16−64	−	n.t.	−	n.t.	n.t.	n.t.	0 → 0	1	1
18c	F/56	POF, HT, IDD	16−64	+	16.1	+	−	>32	2/3	0 → 0	5	4
19c	M/65	GD	<16	+	16.2	−	−	−	n.t.	0 → 0	6	3
20c	F/40	Vit	>64	+	29.3	−	−	−	4/5	0 → 0	1	6
21c	F/31	GD	>64	−	−	−	−	−	3/6	0 → 0	0	9
22c	F/27	IDD	>64	+	47.5	−	−	−	4/5	0 → 0	1	1
23c	F/34	GDM	<16	−	−	−	−	−	w6/w6	0 → 0	2	5
24c	F/69	HT	>64	+	19.3	−	−	1.5	n.t.	0 → 0	0	8

ACA, Adrenal cortex antibodies; 21-OH, 21-hydroxylase; StCA, steroid-producing cell antibodies; 17α OH, 17α-hydroxylase; P450scc, cytochrome P450 side-chain cleavage enzyme; HLA = human leukocyte antigens; For stages of adrenal function, see *Methods* section; n.t., not tested; t, transient; GD, Graves' disease; IDD, insulin-dependent diabetes; HT, Hashimoto's thyroiditis; Vit, vitiligo; POF, premature ovarian failure; CALD, chronic active liver disease; CRF, chronic renal failure; IHP, idiopathic hypoparathyroidism; GDM, gestational diabetes mellitus; SSc, systemic sclerosis; MG, myasthenia gravis; ICA, islet cells antibodies; CMC, chronic mucocutaneous candidiasis.

[a] Seroconverted for ACA.

[b] Deceased for breast cancer.

Genetic study

Prevalences of the HLA-DR antigens are summarized in Table 4. The frequency of HLA-DR3 and -DR4 was higher in ACA-positive patients than in controls, but a significantly increased prevalence of DR3 (corrected $P = 0.004$) was found only in the ACA-positive patients progressing to clinical or subclinical Addison's disease.

Discussion

In this study, which was carried out on a large number of adults with organ-specific autoimmune disease, the prevalence of ACA was from 0.3% (patients with vitiligo) to 9% (patients with premature ovarian failure). The prevalence in premature ovarian failure was significantly higher than in normal controls; prevalence in patients with other autoimmune diseases was similar to that of normal controls. During follow-up only 10 (21%) of ACA-positive adult patients progressed to overt adrenal failure, and a further 14 (29%) progressed to various stages of subclinical hypoadrenalism. All 24 ACA-positive patients who developed overt or subclinical adrenal failure were positive for 21-OH Abs, and the highest levels of ACA and 21-OH Abs were found in this group. These data confirm that 21-OH is a major component of ACA in adults with Addison's disease and extends our knowledge about the autoantigens recognized in those with potential disease (16).

The follow-up study also showed that, in addition to high levels of ACA/21-OH Abs, CF-ACA, initial impaired adrenal function, as well as HLA-DR3 status, were associated with the highest progression towards clinical Addison's disease in adults.

These results in adults are in contrast to our observations in children, where the majority of ACA-positive patients developed Addison's disease independently from HLA, levels of autoantibodies, stage of adrenal function, sex, and age (17). The reasons for these differences in progression to disease between adults and children are not clear at present and reflect our lack of understanding of the mechanisms involved in autoimmune destruction and failure of the adrenal cortex gland (2). Although sera from ACA-positive Addison's patients appear to react with different epitopes on the 21-OH

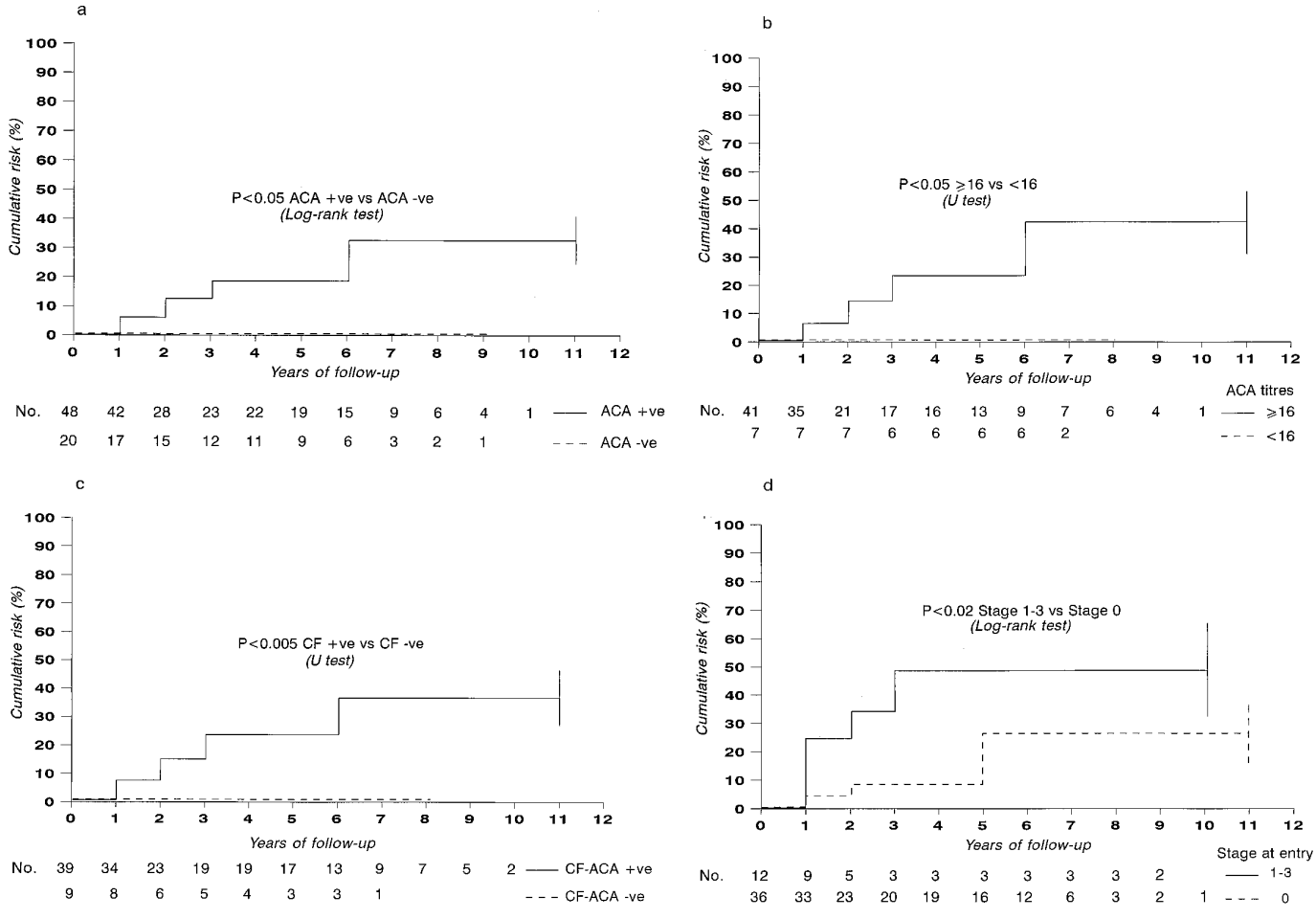

FIG. 2. Estimated probability of progression toward clinical Addison's disease according to: a, status of adrenal cortex antibodies (ACA); b, titers of ACA; c, complement-fixing ability of ACA; and d, stage of adrenal function at entry the follow-up. The cumulative morbidity rate for Addison's disease was significantly increased in ACA-positive patients, in subjects with mid to high *vs.* low titers of ACA, in complement-fixing positive (CF-ACA+ve) patients *vs.* negative (CF-ACA-ve), and in patients with latent hypoadrenalism (Stages 1–3) *vs.* those with normal adrenal function (Stage 0) at time of entry to the follow-up.

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

	Pts.	Progression to Addison's disease (no.)	Positive predictive value (%)	Annual incidence (%)	Cumulative risk (%) (95% c.i.)
ACA-positive					
All patients	48	10	20.8	4.9	31.6 (13.9–49.2)[a]
Males	3	2	66.6	22.5	*n.e.*
Females	45	8	17.8	4.1	24.4 (8.8–40.7)[a]
Low ACA titers (<16)	7	0	0	0	0[b]
Mid ACA titers (16–64)	17	5	29.4	4.9	36.6 (9.8–63.3)[c]
High ACA titers (>64)	24	5	20.8	8.6	50.5 (6.9–94.1)[a]
CF-ACA positive	39	10	25.6	5.9	36.1 (16.8–55.4)[a]
CF-ACA negative/transient	9	0	0	0	0[d]
Stage 0	36	5	13.9	3.0	25.7 (5.3–46.1)[a]
Stages 1–3	12	5	41.7	13.0	49.4 (15.6–83.2)[c]
Two or more OSAD	13	4	30.8	5.9	51.5 (14.9–88.2)[a]
One OSAD	35	6	17.1	4.4	21.6 (5.4–37.7)[a]
Persistently ACA-negative controls	20	0	0	0	0[e]

ACA, Adrenal-cortex antibodies; c.i., confidence interval; OSAD, organ-specific autoimmune disease; CF-ACA, complement-fixing adrenal-cortex antibodies; Stage 0, normal adrenal function; Stages 1–3, grades of subclinical adrenal insufficiency; *n.e.*, not estimated.
[a] estimates at 11 yr.
[b] at 7 yr.
[c] at 10 yr.
[d] at 8 yr.
[e] at 9 yr.

TABLE 4. HLA-DRB1 analysis in adult patients with adrenal cortex autoantibodies (ACA)

HLA-DR	All ACA+ patients (n = 43)	ACA+ patients developing clinical or subclinical AAD (n = 22)	ACA+ patients maintaining normal adrenal function (n = 21)	Normal controls (n = 153)
DR1	5 (12%)	1 (5%)	4 (19%)	25 (16%)
DR2	8 (19%)	4 (18%)	4 (19%)	34 (22%)
DR3	19 (44%)	12 (55%)[a]	7 (33%)	35 (23%)
DR4	14 (33%)	7 (32%)	7 (33%)	26 (17%)
DR5	18 (42%)	11 (50%)	7 (33%)	70 (46%)
DR6	6 (14%)	3 (14%)	3 (14%)	45 (29%)
DR7	3 (7%)	1 (5%)	2 (10%)	27 (18%)
DR8	3 (7%)	2 (9%)	1 (5%)	9 (6%)
DR10	1 (2%)	0	1 (5%)	6 (4%)

AAD, Autoimmune Addison's disease.
[a] corrected $P = 0.04$.

molecule, the majority of 21-OH Abs bind to central and C-terminal parts of the molecule (18, 19). The different progression to clinical Addison's disease in children and adults could be related to the heterogeneity of 21-OH Abs reactivity to 21-OH. Consequently, patients who develop antibodies to particular epitopes could go on to develop the clinical disease. These variations in humoral adrenal autoimmunity may reflect variations in cellular immune responses that are likely to be the prime pathogenetic factor (2).

Observation on the ability of 21-OH Abs to inhibit 21-OH enzyme activity *in vitro* (20) and association of ACA/21-OH Abs with the high progression to adrenal failure in children (17) could suggest a pathogenetic role of 21-OH Abs in Addison's disease. However, recent hormonal studies *in vivo* (21) and the present data on low progression to Addison's disease in ACA/21-OH Abs positive adults appear to indicate that such a mechanism is unlikely.

In our studies ACA/21-OH Abs remained positive in all patients over many years although some fluctuation in titers was observed. This is in apparent contrast to some previous studies on adult population, which have reported a tendency for ACA to disappear during long-term follow-up (6–8). These discrepancies might be related to differences in the methods employed and to the lack of standardization, rather than to a real seronegativization. International standardization and proficiency of a future program in the detection of ACA and 21-OH Abs will be necessary to correctly compare the results from various laboratories and to define a reference serum.

During our follow-up study the ACTH test was a specific and valuable method for assessing adrenal function and for identifying patients progressing to adrenal failure. As the shortest time period for progression from normal adrenal function to clinical disease in adult population was 23 months, we recommend that full assessment of adrenal function should be carried out on a yearly basis in ACA/21-OH Abs-positive patients, mainly in those with CF-ACA and HLA-DR3. Finally, we suggest the initiation

of substitutive therapy when the first stage of impaired adrenal function appears. In this way acute adrenal failure, in patients with ongoing autoimmune adrenalitis, can be prevented in the case of precipitating events such as stress or infections (22).

Note Added in Proof

Further two patients have recently progressed to clinical Addison's disease after 4 yr of follow-up (Table 2; no. 3b, 9b).

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The Third International Workshop On Resistance To Thyroid Hormone
October 12–14, 1997
Aspen, Colorado

The Third International Workshop on Resistance to Thyroid Hormone (RTH) will take place October 12–14, 1997, at The Given Institute and The Mountain Chalet in Aspen, Colorado prior to the 1997 Annual Meeting of the American Thyroid Association in Colorado Springs. The Workshop will focus on clinical and basic aspects of RTH, mechanism of thyroid hormone action, and animal models of RTH, with brief presentations and ample time for informal discussion and posters. Particular emphasis will be placed on participation by successful young investigators, junior faculty, and minorities. Land transportation from Aspen to Colorado Springs will be available on the morning of Tuesday, October 14.

For information, contact: The Third International Workshop on Thyroid Hormone Resistance, c/o Dr. Samuel Refetoff, The University of Chicago (MC3090), 5841 South Maryland Avenue, Chicago, IL 60637. Telephone (773) 702-6939. FAX (773) 702-6940.