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COMMENTS

Diagnosis of Glucocorticoid-Remediable Aldosteronism in Primary Aldosteronism: Aldosterone Response to Dexamethasone and Long Polymerase Chain Reaction for Chimeric Gene

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ABSTRACT

Aldosterone suppression by dexamethasone, and high 18-hydroxycortisol and 18-oxocortisol levels are used to differentiate glucocorticoid-remediable aldosteronism (GRA) from other forms of primary aldosteronism. These methods are time consuming, expensive, and impractical for large studies. Moreover, diagnosis of GRA requires a confirmatory genetic test. We evaluated 117 patients with primary aldosteronism referred to our centers by the use of a long PCR technique to reveal the chimeric gene of GRA. In 60 of 117 patients, the response of aldosterone to dexamethasone (2 mg/day for 4 days) was also assessed. None of our patients, including 2 pairs of siblings, was positive for the chimeric gene. The results of long PCR were confirmed by Southern blotting. Despite a negative genetic test, 6 patients (1 with aldosterone-producing adenoma and 5 with idiopathic hyperaldosteronism) had plasma aldosterone suppressed by dexamethasone (*i.e.* ≤2 ng/dL). Of 117 patients, 43 were identified as having aldosterone-producing adenoma and 74 as having idiopathic hyperaldosteronism. In our experience, the long PCR technique is a reliable and simple test to at least exclude GRA in patients with primary aldosteronism. A short term dexamethasone suppression test of aldosterone can be misleading in identifying GRA. The prevalence of GRA in primary aldosteronism remains to be established. (*J Clin Endocrinol Metab* **83:** 2573–2575, 1998)

GLUCOCORTICOID-REMEDIABLE aldosteronism (GRA) is a rare form of inherited primary aldosteronism in which aldosterone secretion is solely regulated by ACTH (1, 2). Although presentation of GRA is variable, with a number of subjects having normotension and normokalemia (3), evidence for a mineralocorticoid excess state remains the first indication to investigate the possibility of this disease. Indeed, a family history of hypertension in presumedly affected subjects (4) is common in different varieties of primary aldosteronism (5). Barely detectable aldosterone levels after a short dexamethasone trial and abnormally high secretion of two steroids, 18-hydroxycortisol and 18-oxocortisol, have been recognized as biochemical markers specific for GRA (2, 4). However, there are several pitfalls in the use of dexamethasone suppression testing (4, 6), and the two steroids are also elevated in patients with aldosterone-producing adenoma (APA) (7, 8). Definitive diagnosis can only be reached by genetic tests showing a hybrid gene originating from a fusion of the genes encoding steroid 11β-hydroxylase and aldosterone synthase (9). Recently, a PCR technique allowing rapid demonstration of a chimeric gene in GRA patients has been introduced (10). We evaluated a large population of patients with primary aldosteronism in whom both PCR and dexamethasone suppression testing were employed.

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Subjects and Methods

One hundred and seventeen consecutive patients (61 males and 56 females, aged 28–67 yr), including 2 pairs of siblings, referred since 1994 to our centers were studied. All were hypertensive, and the slight majority (64 of 117 = 54.7%) had spontaneous hypokalemia of varying degrees. At the time of the study, subjects were all hospitalized, had been consuming a daily diet containing 120–150 mmol sodium and 60 mmol potassium for at least 2 weeks, and had been off all medications for at least 1 month. In most patients, PRA was suppressed and unresponsive to stimuli such as upright posture and captopril, and plasma aldosterone was high on several occasions. Diagnosis was ascertained by demonstration of a supine plasma aldosterone (nanograms per dL)/PRA (nanograms per mL/h) ratio of more than 50 and failure of plasma aldosterone to decrease below 5 ng/dL after 2-L 0.9% sodium chloride iv infusion over 4 h (11, 12).

In all patients, the chimeric 11β-hydroxylase/aldosterone synthase gene of GRA was studied in leukocyte DNA extracted from 20–30 mL peripheral venous blood using the long PCR technique of Jonsson *et al.* (10). For each patient, the isolated DNA was subjected to 2 concurrent amplification reactions with sense primers specific for the 5′-untranslated regions of the aldosterone synthase and 11β-hydroxylase genes. Antisense primer was specific for the intron E region of the aldosterone synthase gene. Amplification reactions were carried out using the XL PCR kit from Perkin-Elmer (Branchburg, NJ) (10). In all patients the long PCR results were confirmed by Southern blotting, as previously described (13). Five patients already demonstrated to be genetically GRA

by either PCR or Southern blotting were used as positive controls (14, 15). PCR detection of chimeric genes was also performed in 30 healthy volunteers, all of whom were negative.

In a subset of patients with primary aldosteronism, the results of genetic tests, which are 100% sensitive and specific for the diagnosis of GRA, were prospectively compared with those of a traditional clinical test, *i.e.* the aldosterone response to dexamethasone. For this test, supine plasma aldosterone and cortisol were measured under baseline conditions and after 4 days of dexamethasone (2 mg/day, orally; 0.5 mg every 6 h). Blood samples were taken on the fifth day, 2 h after the morning dose of dexamethasone (at 0600 h). Suppression of aldosterone by dexamethasone was arbitrarily defined as plasma levels of 2 ng/dL or less; this is a strict criterion, being the lower limit of normal range for supine position. Plasma cortisol suppression (*i.e.* <5 μg/dL) was assumed as an index of the dexamethasone effect. PCR tests were performed without prior knowledge of the aldosterone response to dexamethasone administration. After initial diagnosis (16, 17), three of the previous five patients with genetically proven GRA underwent the same dexamethasone suppression protocol of the present study, and all showed a decrease in aldosterone to below 2 ng/dL.

The differential diagnosis of APA or idiopathic hyperaldosteronism (IHA) was made by documenting lateralization in APA (computerized axial tomography, adrenal scintiscan with ^{75}Se-labeled cholesterol after dexamethasone administration, or adrenal venography with aldosterone measurements in adrenal venous blood). Informed consent for the study was obtained from all subjects.

Plasma aldosterone and PRA were determined by RIA with kits purchased from Sorin Biomedical Diagnostics (Vercelli, Italy). The intra- and interassay coefficients of variation (CVs) for aldosterone were 7.9% and 9.6%, respectively; the normal range is 2–12 ng/dL supine and 5–30 ng/dL upright. The intra- and interassay CVs for PRA were 5.4% and 9.1%, respectively; the normal range is 0.4–3 ng/mL·h supine and 1.5–6 ng/mL·h upright. Plasma cortisol was measured by a RIA kit from Diagnostic Products Corp. (Los Angeles, CA); the normal range at 0800 h is 5–20 μg/dL, with intra- and interassay CVs of 4.1% and 5.0%, respectively.

The statistical significance of differences between groups was assessed by Student's t test for paired or unpaired data or by χ^2 test corrected for continuity, as appropriate. $P < 0.05$ was considered significant. Results are expressed as the mean ± SD.

Results

None of our 117 cases with primary aldosteronism were positive for the chimeric gene of GRA. Negative results of long PCR were confirmed in all patients by Southern blotting. Clinical, biochemical, and hormonal data of the patients with the diagnostic classification of APA or IHA are shown in Table 1. The group of IHA (63.3%) was prevalent over the group of APA (36.7%), and family history of hypertension was more frequent in IHA than in APA patients. Serum K and upright PRA were significantly higher in IHA than in APA patients, whereas supine and upright aldosterone levels were higher and the aldosterone/PRA ratio was greater in APA than in IHA patients.

Of 60 subjects given 4-day dexamethasone treatment, 23 were classified as APA and 37 as IHA. In both groups aldosterone significantly declined after dexamethasone treatment, from 44.0 ± 11.2 to 29.8 ± 18.9 ng/dL ($P < 0.001$) in APA and from 27.3 ± 7.6 to 19.1 ± 11.2 ng/dL ($P < 0.001$) in IHA. In the presence of a negative genetic test, 1 patient with APA (4.3%) and 5 patients with IHA (13.5%) had aldosterone suppressed (*i.e.* ≤2 ng/dL) by 4-day dexamethasone trial (Fig. 1). These 6 patients showed no peculiar characteristics compared to the other APA or IHA patients for all parameters, in particular supine and upright aldosterone (26.7 ± 7 and 40.9 ± 19.5 ng/dL, respectively), PRA (0.27 ± 0.24 and 0.37 ± 0.36 ng/mL·h, respectively), and serum K (3.3 ± 0.3 mmol/L). Referring to the genetic data, the specificity of the dexamethasone suppression test with the above aldosterone cut-off was, therefore, 90% overall. In all patients, correct glucocorticoid intake was confirmed by suppression of cortisol levels. The 2 pairs of siblings did not show a significant fall in aldosterone after dexamethasone treatment. All patients who fulfilled the criteria for APA had unilateral adrenalectomy and showed restoration of normal electrolyte and hormonal patterns at the last follow-up (range, 2–36 months).

TABLE 1. Clinical, biochemical, and hormonal data of patients
The patients were divided into APA and IHA groups.

	APA	IHA
Patient no.	43	74
Age (yr)	51.3 ± 9.4	50.1 ± 8.5
Sex (M/F)	23/20	38/36
Fam. hypertension	17	51[a]
Systolic BP (mm Hg)	203.7 ± 23.7	195.4 ± 28.5
Diastolic BP	115.4 ± 11.1	117.9 ± 16.2
K (mmol/L)	3.2 ± 0.5	3.7 ± 0.4[a]
Supine PRA (ng/mL · h)	0.13 ± 0.06	0.16 ± 0.11
Upright PRA	0.18 ± 0.12	0.29 ± 0.2[a]
Supine ALDO (ng/dL)	39.4 ± 12.4	24.9 ± 8.6[a]
Upright ALDO	47.8 ± 16.8	39.3 ± 15.1[a]
Supine ALDO/PRA ratio	335 ± 154	176 ± 64[a]

Fam. hypertension, Family history of hypertension; BP, blood pressure; K, serum potassium; ALDO, plasma aldosterone levels. Values are the mean ± SD.

[a] $P < 0.01$ IHA *vs.* APA patients.

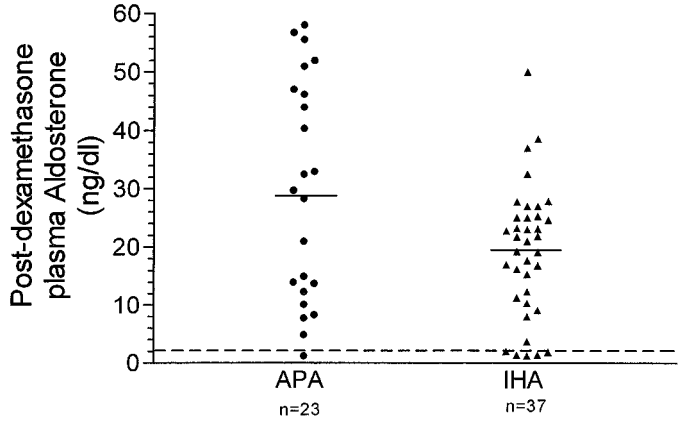

FIG. 1. Supine plasma aldosterone levels after 4 days of dexamethasone treatment (2 mg/day) in patients with APA and IHA who were negative at genetic screening for glucocorticoid-remediable aldosteronism. The *dotted line* indicates the cut-off for aldosterone suppression of 2 ng/dL.

Discussion

Our population was composed by subjects with primary aldosteronism ascertained by hormonal and morphological findings. A family history of hypertension was fairly frequent (68 of 117 = 58.1%), resulting in a nonspecific indication for GRA. In addition to clinical and biochemical evaluation, the collection of a single blood sample allowed us to rapidly look for the molecular abnormality characteristic of GRA. Diagnostic screening by molecular techniques has been employed to identify affected relatives of genetically proven GRA index cases (15, 18–20). Scarce data are available on the

prevalence of GRA in other clinical settings, *i.e.* GRA unrelated patients (20). This is possibly due to the need for genetic screening techniques, such as Southern blotting, which are unpractical and expensive when used in a large number of subjects. Analysis of DNA by long PCR, a faster and less expensive methodology, has been now validated in patients with GRA (21), and our own results confirm full correspondence with those obtained by Southern blotting. Negative results obtained by long PCR excluded this disorder in our population, and this was confirmed by Southern blotting in all cases. It cannot be excluded, however, that other molecular mechanisms, *i.e.* gene conversions or point mutation, undetectable by long PCR or Southern blotting techniques may be the cause of a different genetic type of GRA (22, 23). Based on genetic data, the prevalence of GRA in primary aldosteronism remains to be established, although it can be reasonably predicted to be lower than that suggested in early clinical studies (5, 24, 25).

Our study indicates that a short term dexamethasone course can be misleading in identifying GRA among patients with primary aldosteronism, as 6 of 60 cases tested by us showed aldosterone suppression. In agreement with data reported by others using a higher aldosterone cut-off in a small population (4), the low specificity of the dexamethasone suppression test in primary aldosteronism is not surprising, as a transient ACTH dependency of aldosterone secretion has been described in patients with either APA or IHA (26–29). Also in our subjects, there was no evidence that differences in baseline aldosterone, PRA, or serum K was involved in the abnormal responsiveness to dexamethasone. An increased expression of ACTH receptor messenger ribonucleic acid in adrenal tissues, as recently found in some APAs (30), might explain this phenomenon. The underlying mechanisms, however, are still unclear. The low specificity of dexamethasone suppression testing should also be considered to avoid the risks of giving dexamethasone for a long period of time to patients with primary aldosteronism (31) awaiting confirmation of GRA by genetic test. Long PCR results were negative in two pairs of siblings, in whom no suppression of aldosterone was detected after dexamethasone treatment, suggesting that they could belong to a type of familial hyperaldosteronism other than GRA (19).

In conclusion, in our experience the long PCR technique is a reliable and simple test at least to exclude GRA among patients with primary aldosteronism. Such a direct genetic approach for the diagnosis of GRA may overcome the inconvenience and low specificity of dexamethasone testing. The prevalence of GRA in this clinical setting remains to be established.

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