0021-972X/00/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 85, No. 5
Printed in U.S.A.

Primary Hyperaldosteronism in Essential Hypertensives: Prevalence, Biochemical Profile, and Molecular Biology*

CARLOS E. FARDELLA, LORENA MOSSO, CELSO GÓMEZ-SÁNCHEZ, PAOLA CORTÉS, JULIA SOTO, LUIS GÓMEZ, MAURICIO PINTO, ALVARO HUETE, EVELINE OESTREICHER, A. FORADORI, AND JOAQUÍN MONTERO

Departments of Endocrinology (C.E.F., L.M., P.C., L.G., M.P., E.O.), Internal Medicine (J.M.), Radiology (A.H.), and The Associated Unit of Clinical Laboratories (J.S., A.F.), Faculty of Medicine, Catholic University of Chile, Marcoleta 391, Santiago, Chile; and Endocrine Section (C.G.-S.), Harry S. Truman Memorial Hospital, Department of Internal Medicine, Columbia, Missouri 65201-5297

ABSTRACT

There is evidence that primary aldosteronism (PA) may be common in patients with essential hypertension (EH) when determinations of serum aldosterone (SA), plasma renin activity (PRA), and the SA/PRA ratio are used as screening. An inherited form of primary hyperaldosteronism is the glucocorticoid-remediable aldosteronism (GRA) caused by an unequal crossing over between the CYP11B1 and CYP11B2 genes that results in a chimeric gene, which has aldosterone synthase activity regulated by ACTH. The aim of this study was to evaluate the prevalence of PA and the GRA in 305 EH patients and 205 normotensive controls. We measured SA (1–16 ng/dL) and PRA (1–2.5 ng/mL·h) and calculated the SA/PRA ratio in all patients. A SA/PRA ratio level greater than 25 was defined as being elevated. PA was diagnosed in the presence of high SA levels (>16 ng/dL), low PRA levels (<0.5 ng/mL·h), and very high SA/PRA ratio (>50). Probable PA was diagnosed when the SA/PRA ratio was more than 25 but the other criteria were not present. A Fludrocortisone test was done to confirm the diagnosis. GRA was differentiated from other forms of PA by: the aldosterone suppression test with dexamethasone, the high levels of 18-hydroxycortisol, and the genetic detection of the chimeric gene. In EH patients, 29 of 305 (9.5%) had PA, 13 of 29 met all the criteria for PA, and 16 of 29 were initially diagnosed as having a probable PA and confirmed by the fludrocortisone test. Plasma potassium was normal in all patients. The dexamethasone suppression test was positive for GRA in 10 of 29 and 18-hydroxycortisol levels were high in 2 of 29 patients who had also a chimeric gene. In normotensive subjects, 3 of 205 (1.46%) had PA, and 1 of 205 had a GRA. In summary, we found a high frequency of normokalemic PA in EH patients. A high proportion of PA suppressed SA with dexamethasone, but only a few had a chimeric gene or high levels of 18-hydroxycortisol. These results emphasize the need to further investigate EH patients. (*J Clin Endocrinol Metab* **85:** 1863–1867, 2000)

HYPERTENSION is a common disease affecting about 20% of the adult population (1–3). Although a great deal is known about the regulation of blood pressure, a specific causal abnormality can be found in only a small percentage of patients. Thus, about 95% of patients with hypertension are said to have essential or idiopathic hypertension. A key mechanism in the regulation of blood pressure is the renin-angiotensin-system. Angiotensin II is the most potent stimulus to aldosterone synthesis, which, in turn, controls sodium and water excretion (4).

Low levels of plasma renin activity (PRA) are found frequently in essential hypertension (EH), but the significance of low-renin hypertension and the mechanisms involved remain controversial. A survey of 436 Japanese patients with EH showed that 12.4% had low PRA values with normal or high serum aldosterone (SA) levels, suggesting a persistent mineralocorticoid synthesis in the presence of minimal stimulation from the renin-angiotensin system (5). However, the quoted prevalence of primary aldosteronism (PA) is usually cited as being less than 1% for an unselected hypertensive population (6–9). Recently, Gordon *et al.* (10) presented evidence that PA may not be uncommon and demonstrated an incidence up to 12% of PA when determinations of SA, PRA, and the SA/PRA ratio are used in the diagnosis. They also found that hypokalemia is present only in the more severe form of the disease and that most patients with PA are normokalemic.

The biosynthesis of aldosterone is controlled by P450c11AS (aldosterone synthase), which has all three activities needed to convert 11-deoxycorticosterone to aldosterone, *i.e.* 11β-hydroxylase, 18-hydroxylase, and 18-oxidase activities (11–14). This enzyme is encoded by the CYP11B2 gene and is regulated by angiotensin II and potassium via protein kinase C. This enzyme is different from P450c11β (11β-hydroxylase), the product of the CYP11B1 gene, which is expressed in the zona fasciculata and reticularis, and converts 11-deoxycortisol to cortisol. P450c11β is regulated by ACTH via cAMP and protein kinase A. Human CYP11B1 and CYP11B2 have 90% nucleotide sequence identity in the introns and 95% in the exons and lie on chromosome 8q (12, 15). Both CYP11B1 and CYP11B2 have been implicated in the genesis of arterial hypertension through an increase in the aldosterone synthesis, as occurs in the glucocorticoid-remediable aldosteronism (GRA) (16, 17). Moreover, *in vitro* studies have

Received August 31, 1999. Revision received December 12, 1999. Accepted February 7, 2000.

Address correspondence and requests for reprints to: Carlos E. Fardella, Department of Endocrinology, Faculty of Medicine, P. Universidad Católica de Chile, Marcoleta 391, Santiago, Chile. E-mail: cfardella.med.puc.cl.

* This work was supported by grants from the Chilean Fondecyt (1980999) and the Chilean Society of Endocrinology (97.2.05).

demonstrated that specific mutations in CYP11B2 or CYP11B1 should also explain some forms of hyperaldosteronism (18–20).

GRA is an autosomal dominant disorder characterized by hyperaldosteronism (21, 22) and high levels of the abnormal adrenal steroids 18-oxocortisol and 18-hydroxycortisol, which are all under control of ACTH and suppressible by glucocorticoid (23–26). The GRA is caused by an unequal crossing-over between the CYP11B1 and CYP11B2 genes that results in a chimeric gene that has aldosterone synthase activity but is regulated by ACTH rather than angiotensin II (16, 17). *In vitro* studies have shown that breakpoints through exon 4 (codon 247) result in a hybrid enzyme (11β-hydroxylase/aldosterone synthase) that could be expected to lead to full GRA (17). Moreover, Pascoe *et al.* (20) have demonstrated that the product of CYP11B1 may acquire the aldosterone synthase activity if serine-288 and valine-320 are replaced by the corresponding CYP11B2 residues, glycine and alanine. Thus, the amino acid substitution serine-288-glicine (S288G) in CYP11B1 confers on it the 18-hydroxylase activity, and the substitution valine-320-alanine (V320A) confers the 18-oxidase activity required for aldosterone synthesis.

The aim of this study was to establish the prevalence of PA and GRA in a Chilean population of 305 EH patients and 205 normotensive controls, through the routine determinations of SA, PRA, and the SA/PRA ratio. The fludrocortisone suppression test was used to confirm the diagnosis of PA. The prevalence of GRA was studied using an aldosterone suppression test with dexamethasone, determinations of 18-hydroxycortisol, and genetic studies. Genetic studies were done to demonstrate the presence of the chimeric gene and the amino acid substitutions S288G and V320A in the CYP11B1 gene.

Subjects and Methods

Patients were recruited from the Primary Outpatient Clinic Hypertension Program associated with the Universidad Católica de Chile in Santiago. They were considered hypertensives if their diastolic blood pressure was more than 90 mm Hg and the systolic blood pressure more than 140 mm Hg on at least 3 occasions on different days and without taking any antihypertensive medication or estrogen replacement (3). All individuals with clinical evidence of secondary hypertension, diabetes, or renal disease were excluded. Using these criteria, we selected 305 EH patients. A group of 204 normotensive subjects of similar age and gender was included as control. The normotensive subjects were selected from the same primary clinics, according to the criteria that they had a diastolic blood pressure less than 85 mm Hg and a systolic blood pressure less than 140 mm Hg, determined at least on 2 occasions. The normotensives had no family history of hypertension, at least in first-degree relatives. Blood pressure was measured with a mercury sphygmomanometer, by a health care professional, after the patients were seated for 10 min. The first and fifth Korotkoff sounds were used to designate systolic and diastolic blood pressure. Informed consent was obtained from all participants, according to the guidelines of the Declaration of Helsinki, and the protocol was approved by the Research Commission of the School of Medicine at the Catholic University of Chile.

Patients were admitted to our Metabolic Ward between 0800 and 0900 h, after a 12-h fast. All subjects consumed a normal diet, with no attempt to control sodium intake. Weight and height were measured at the time of admission. Upon admission, a catheter was placed in an antecubital vein; and after sitting for 15 min, free-flowing blood was withdrawn to measure sodium, potassium, calcium, albumin, blood urea nitrogen, creatinine, aldosterone, and PRA. Twenty-four-hour urinary sodium was measured in all subjects.

SA was measured by RIA using a commercial kit from Diagnostic Products (Los Angeles, CA). The intra- and interassay coefficients of variation for SA were 5.1% and 7.1%, respectively, and the normal range was 1–16 ng/dL. The PRA was determined as previously described by Menard (27). The intra- and interassay coefficients of variation for PRA were 6.1% and 8.2%, respectively, and the normal range was 1–2.5 ng/mL/h (28). The lower limit of PRA determination was 0.1 ng/mL·h (29). PRA values less than 0.5 ng/mL/h were considered as low renin. A SA/PRA ratio more than 25 was considered as high; and a ratio more than 50, as very high (30–32). The cut point for SA/PRA ratio in 25 was validated in our population of normotensive controls (see *Results*). Serum 18-hydroxycortisol was measured using a biotine-avidine enzyme-linked immunoassay, as described by Gómez-Sanchez *et al.* (33, 34). The normal 18-hydroxycortisol value in our population was 2.67 ± 1.44 nmol/L (unpublished data). A citrated blood sample was also obtained to analyze the genome.

The diagnosis was PA if the patients met the following criteria: high SA levels (>16 ng/dL), low levels of PRA (<0.5 ng/mL·h), and a very high SA/PRA ratio (>50) in at least two determinations. The diagnosis was probable PA if the SA levels were normal but the SA/PRA ratio was more than 25 or when the SA levels were high but the SA/PRA ratio was less than 50. The cut points to define probable PA were arbitrarily established considering patients that did not meet all the classical criteria for PA but had a high SA/PRA ratio in two different determinations. A fludrocortisone test was done to confirm the diagnosis of PA in all patients. In patients with PA, the administration of a sodium-retaining steroid should not suppress SA, indicating the autonomous overproduction of aldosterone. For the fludrocortisone test, supine SA levels were measured under baseline conditions and after 4 days of fludrocortisone (0.4 mg/day, orally; 0.1 mg every 6 h), during a dietary supplementation with 110 mmol of sodium per day (35, 36). Blood samples were taken on the fifth day at 0800 h. A fludrocortisone test was considered positive when SA levels failed to suppress under 5ng/dL (36–38). In patients with confirmed PA, a dexamethasone suppression test was done. For this test, supine SA and cortisol were measured under baseline conditions and after 2 days of dexamethasone (2 mg/day, orally; 0.5 mg every 6 h). Blood samples were taken on the third day at 0800 h. Suppression of aldosterone by dexamethasone was considered positive if the SA levels were below 4 ng/dL (39–40). Plasma cortisol suppression (under 2.5 ug/dL) was assumed as an index of the dexamethasone effect. Serum 18-hydroxycortisol measurements and a computed tomography (CT) scan of the adrenal were performed in patients with PA. Although there are no strict measurements of normal adrenal size, the CT scan was considered abnormal when any area thicker than 10 mm was detected (41).

Analysis of genomic DNA

All patients with confirmed PA were studied for chimeric 11β-hydroxylase/aldosterone synthase genes and the CYP11B1 gene conversions S-288-G and V320A. Genomic DNA was prepared from the citrate-treated blood of 305 EH patients and 205 normotensive controls, as previously described (42). The presence of the chimeric 11β-hydroxylase/aldosterone synthase gene was studied using the long PCR technique described by Jonsson *et al.* (43). This technique uses two amplification reactions in which two sense primers and one antisense primer performed concurrently. In the first reaction, the sense primer was specific for the 5′ untranslated region of the aldosterone synthase gene; whereas, in the second, the sense primer was specific for the untranslated region of the 11β-hydroxylase gene. For both reactions, the antisense primer was specific for the intron-E region of the aldosterone synthase gene. The amplification reactions were carried out using the XL PCR Kit from Perkin-Elmer Corp. (Branchburg, NJ).

The detection of the gene conversion S288G and V320A was performed using two PCR reactions (nested PCR). The first reaction amplified the CYP11B1 gene using primers to amplify exons 3–5 and 6–8, as previously described (44). The second reaction used the product of the first PCR as template. The mutant S288 allele was recognized using a modified sense primer that creates a restriction site for BsaOI (5′-AAC TGG CCT TCA GCC GCC CTC AAC AGT CGA CC-3′) in the presence of the same antisense primer. The mutant V320 allele was recognized using the same sense primer and a new antisense primers (5′-GGG CCG CAC GCA GCA A-3′), and the product was digested with a *Bgl*I enzyme. Positive controls for 288G and 320A mutant were artificially constructed

and used to verify the specificity of the restriction enzyme digestion. The restriction fragments were visualized on nondenaturing polyacrylamide gels at 7.5%, stained with ethidium bromide.

Results

We studied 305 EH patients and 205 normotensive controls of similar age and gender. The hypertensive patients showed higher levels of SA and SA/PRA ratio than the normotensives. The levels of urinary sodium excretion were similar in hypertensives and normotensives (Table 1). In normotensive subjects, the mean SA/PRA ratio plus 2 SD was 24.0, and we considered it a high ratio when it was more than 25.

In the EH patients, the diagnoses of PA was made in 29 of 305 subjects (9.5%). Thirteen of 29 met all criteria for PA, because they had high aldosterone levels (22.3 ± 6.8; range, 18–41), low PRA values (0.29 ± 0.10; range, 0.1–0.5), and very high SA/PRA ratio (95.0 ± 62.2; range, 50–260). A second determination of SA, PRA, and SA/PRA ratio did not show a statistically significant difference, compared with the first one. The fludrocortisone test confirmed the diagnosis of PHA in all 13 patients, because the aldosterone levels were not suppressed at the end of the test (8.5 ± 1.3; range, 7.2–10.3). The other 16 of 29 were initially diagnosed as having a probable PA.

Diagnosis of probable PA was made in 31 EH patients; 26 of 31 cases (83.9%) had normal aldosterone levels (<16 ng/dL), but SA/PRA ratios more than 25. In the other 5 (16.1%) patients, the diagnosis of probable PA was made because they had high SA levels but an SA/PRA ratio less than 50. The fludrocortisone test confirmed the diagnosis of PA in 16 of 31 patients (51.6%); 13 had normal- and 3 had high aldosterone levels. The fludrocortisone test was always negative in patients with aldosterone levels less than 9 ng/dL, independent of the magnitude of the SA/PRA ratio. The SA/PRA ratios were not significantly different between patients with positive and negative fludrocortisone test results (Table 2). Of those patients with very high ratios (>50) but SA levels less than 16 ng/dL, 4 of 9 were confirmed as having PA. The biochemical characteristics of patients with positive and negative fludrocortisone tests are shown in Table 2.

In the 29 patients with PA, the plasma potassium levels were always within the normal range (K = 3.9 ± 0.2; range, 3.5–4.9). CT scans of the adrenal glands showed a bilateral enlargement in 5 of 29 cases and an adrenal nodule in 1 of 29 cases. The CT scan was reported to be normal in the other 23 of 29 cases. The CT abnormalities were found only in patients with high SA levels and very high SA/PRA ratio.

TABLE 1. Clinical and laboratory finding in essential hypertensive patients and normotensive subjects

Parameter	Hypertensives (n = 305)	Normotensives (n = 205)	*P* value
Age (yr)	54.1 ± 11.2	52.5 ± 7.3	NS
Sex (male/female)	65/240	54/151	NS
Blood pressure (mm Hg)			
Systolic	161.3 ± 17.1	117.6 ± 11.3	<0.01
Diastolic	97.2 ± 10.5	71.2 ± 8.3	<0.01
SA (ng/dL)	11.91 ± 7.8	8.64 ± 4.61	<0.01
PRA (ng/mL·h)	2.58 ± 5.73	1.36 ± 0.87	<0.01
SA/PRA ratio	16.9 ± 33.7	9.06 ± 7.48	<0.01
UNa (mEq/24 h)	165 ± 40	170 ± 48	NS

Values are expressed as mean ± SD. UNa, Urinary sodium; NS, not significant; mEq, milliequivalent.

All 29 patients with PA responded to the dexamethasone suppression test with a decrease in SA from 17.4 ± 7.4 ng/dL to 6.5 ± 5.0 ng/dL, $P < 0.05$ (Fig. 1); and the serum cortisol, from 13.6 ± 4.1 ug/dL to 0.75 ± 0.35 ug/dL. The dexamethasone test was positive, as defined by the suppression of aldosterone to less than 4 ng/dL, in 10 of 29 (34.5%) patients. Serum 18-hydroxycortisol level measured in these 29 patients was 4.64 ± 5.67 nmol/L, and 2 of 29 showed significantly elevated levels (19.4 and 26.7 nmol/L).

Long-range PCR was done in the 29 patients with PA. A chimeric 11β-hydroxylase/aldosterone synthase gene was demonstrated in 2 of them. These 2 patients had a positive dexamethasone suppression test and high 18-hydroxycortisol levels. In one of them, the SA levels were always in the normal range, and the diagnosis of PA was confirmed by the fludrocortisone test. We did not find the amino acid substitutions S288G and V320A in the CYP11B1 gene in the other 27 patients with PA.

In normotensive controls, a PA was diagnosed in 3 of 205 (1.46%) cases. Only 1 of them met all the criteria for PA. The other 2 subjects were initially diagnosed as having a probable PA and confirmed by a fludrocortisone test. GRA was diagnosed in 1 of 3 normotensives with PA.

Discussion

The results of this study show a high prevalence of normokalemic PA in an unselected population of EH Chilean patients. We also found a high proportion of patients with PA that suppress the SA levels with dexamethasone. However, the presence of a chimeric 11β-hydroxylase/aldosterone synthase gene, explaining this suppression, is found only in few cases.

The frequency of PA found in our study is clearly higher than that classically described (6–9), and it confirms reports of a significant high incidence of PA in patients with EH when determinations of SA, PRA, and SA/PRA ratio are used as screening (5, 10, 32, 45–48). Screening patients with hypertension for PA has usually been limited to those who presented with unprovoked or easily induced hypokalemia (3, 8); our patients with PA were normokalemic and could not have been diagnosed if the screening was done using these criteria. These results support the hypothesis that normokalemic PA constitutes the most common presentation of the disease, and the hypokalemic variant probably represents only the most severe cases (31, 49). It is also interesting to note that CT scanning revealed only one nodule in 29 patients, suggesting that the hyperplasia variant is the most common.

The SA/PRA ratio has been considered a useful tool in the screening for PA (30–32). In our study, the calculation of SA/PRA ratio was useful in identifying patients with PA who had normal SA levels, but the predictive value was low. The fludrocortisone test confirmed the diagnoses of PA only in 50% of patients with probable PA, and the test was always negative in patients with SA levels less than 9 ng/dL. This fact could be explained because normal or low-normal SA levels were highly amplified by very low PRA levels, given

TABLE 2. Biochemical characteristics of patients with positive and negative Fludrocortisone (Flc) suppression test

Parameter	Positives patients (n = 16)	Negatives patients (n = 15)	*P* value
SA (ng/dL)			
Basal	13.6 ± 5.4 (9.0–28.9)	10.8 ± 3.6 (6.5–16.4)	NS
Post Flc	7.2 ± 1.6 (5.0–10.0)	3.3 ± 0.7 (2.0–4.5)	<0.05
PRA (ng/mL·h)	0.35 ± 0.2 (0.18–0.95)	0.26 ± 0.14 (0.11–0.58)	NS
SA/PRA ratio	43.2 ± 16.8 (25.7–80.0)	48.2 ± 22.0 (27.2–101)	NS

Values are expressed as mean ± SD.

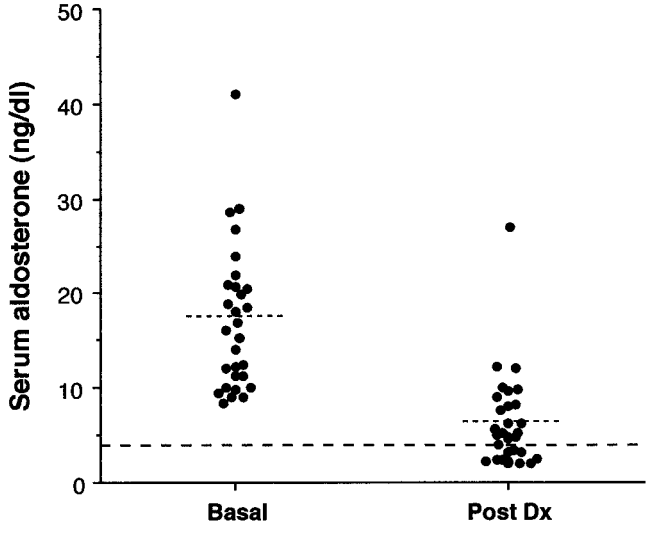

FIG. 1. SA levels before and after dexamethasone (Dx) suppression test. The *horizontal bars* correspond to means. The *dotted line* indicates the cut-off for aldosterone suppression of 4 ng/dL.

a high SA/PA ratio and then a wrong suspicion of PA. In this work, the SA/PRA ratio predictive value improved from 50% to 65% (without losing any positive patients with PA) when the cut-off of the lower limit of PRA was moved from 0.1 to 0.35 ng/mL·h. For those reasons, we propose that the lower limit for PRA should be not less than 0.3 ng/mL·h, and confirmatory testing must be done when SA levels are not clearly elevated. In patients with high SA levels and a SA/PRA ratio more than 50, the PA was always confirmed, suggesting that SA/PRA ratios more than 50 are highly specific in patients with high PA levels, independent of the cut-off used for the lower limit of PRA.

The dexamethasone suppression test was positive in 10 of 29 patients with PA, but only 2 of these patients had a chimeric 11β-hydroxylase/aldosterone synthase gene and elevated levels of 18-hydroxycortisol. Similar results were reported recently by Mulatero *et al.* (49) in 6 patients that showed suppression of SA to less than 2 ng/dL, but none of their patients were positive for the chimeric gene, suggesting than the dexamethasone suppression test can be misleading in identifying GRA. The measurement of 18-hydroxycortisol constituted the most reliable biochemical determination in the diagnosis of GRA in this study, as well as others (50). The existence of other genetic defects responsible for the suppression with dexamethasone is unknown. We were unable to demonstrate the conversions S288G and V320A in the CYP11B1 gene that should also explain a GRA (12).

It is interesting to note that one of the two patients having a chimeric gene had normal SA levels, exemplifying the wide spectrum of SA levels that can be found in patients with GRA. Moreover, we detected one normotensive subject having a chimeric gene, demonstrating that the GRA is also present in a wide spectrum of blood pressure. The presence of GRA in normotensives had been previously demonstrated and probably reflect a prehypertensive state (39).

In summary, we found a high frequency of normokalemic PA in a group of Chilean EH patients. The measurement of the SA/PRA ratio constitutes only a screening test that is especially useful in patients with normal SA levels. Dexamethasone suppresses SA in many patients with PA and, thus, is very unspecific in the identification of patients with chimeric gene. We suggest the routine determination of SA and PRA in all EH patients and serial determinations of 18-hydroxycortisol levels in the positive patients.

References

1. **Dannenberg AL, Garrison RJ, Kannel WB.** 1988 Incidence of hypertension in the Framingham study. Am J Public Health. 78:676–679.
2. **Berríos X, Jadue L, Zenteno J, Ross MI, Rodriguez H.** 1990 Enfermedades crónicas del adulto. Estudio de algunos factores de riesgo en población general urbana de la Región Metropolitana, 1986–1987. Rev Med Chil. 118:1042–1043.
3. **Joint National Committee.** 1997 The sixth report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Arch Intern Med. 157:2413–2446.
4. **Fardella C, Miller W.** 1996 Molecular biology of mineralocorticoid metabolism. Annu Rev Nutr. 16:443–470.
5. **Komiya I, Yamada T, Aizawa T, et al.** 1991 Inappropriate elevation of the aldosterone/plasma renin activity ratio in Hypertensive patients whit increases of 11-deoxycorticosterone and 18-hydroxy-11-deoxycorticosterone: a subtype of EH?. Cardiology. 78:99–110.
6. **Conn JW, Cohen EL, Rovner DR, Nesbit RM.** 1965 Normokalemic primary aldosteronism. A detectable cause of curable "essential" hypertension. JAMA. 193:200–206.
7. **Tucker RM, Labarthe DR.** 1977 Frequency of surgical treatment for hypertension in adults at the Mayo Clinic from 1973 to 1975. Mayo Clin Proc. 52:549–555.
8. **Ganguly A.** 1998 Primary aldosteronism. Review article. N Engl J Med. 339:1828–1834.
9. **Blumenfeld JD, Sealey JE, Schlussel Y, et al.** 1994 Diagnosis and treatment of primary hyperaldosteronism. Ann Intern Med. 121:877–885.
10. **Gordon RD, Zlesak MD, Tunny J, Stowasser M, Klemm SA.** 1993 Evidence that primary aldosteronism may not be uncommon: 12% incidence among antihypertensive drug trial volunteers. Clin Exp Pharmacol Physiol. 20:296–298.
11. **Curnow KM, Tusie-Luna M, Pascoe L, et al.** 1991 The product of the CYP11B2 gene is required for aldosterone biosynthesis in the human adrenal cortex. Mol Endocrinol. 5:1513–1522.
12. **Kawamoto T, Miysuuchi Y, Ohnishi T, et al.** 1990 Cloning and expression of a cDNA for human cytochrome P450aldo as related to primary aldosteronism. Biochem Biophys Res Commun. 173:309–316.
13. **Ogishima T, Shibata H, Shimada H, et al.** 1991 Aldosterone synthase cytochrome P450 expressed in the adrenals of patients with primary aldosteronism. J Biol Chem. 266:10731–10734.
14. **Kawamoto T, Mitsuuchi Y, Toda K, et al.** 1992 Role of steroid 11β-hydroxylase and 18-hydroxylase in the biosynthesis of glucocorticoids and mineralocorticoids in humans. Proc Natl Acad Sci USA. 89:1458–1462.
15. **Mornet E, Dupont J, Vitek A, White PC.** 1989 Characterization of two genes encoding human steroid 11β-hydroxylase (P45011β). J Biol Chem. 264:20961–20967.

16. **Lifton R, Dluhy RG, Powers M, et al.** 1992 A chimaeric 11β-hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. Nature. 335:262–265.
17. **Pascoe L, Curnow K, Slutsker L, et al.** 1992 Glucocorticoid-suppressible hyperaldosteronism results from hybrid genes created by unequal crossover between CYP11B1 and CYP11B2. Proc Natl Acad Sci USA. 89:8372–8331.
18. **Fardella CE, Rodriguez H, Hum DW, Mellon SH, Miller WL.** 1995 Artificial mutations in P450c11AS (aldosterone synthase) can increase enzymatic activity: a model for low-renin hypertension? J Clin Endocrinol Metab. 80:1040–1043.
19. **Fardella CE, Rodriguez H, Montero J, et al.** 1996 Genetic variation in P450c11AS in Chilean patients with low renin hypertension. J Clin Endocrinol Metab. 81:4347–4351.
20. **Curnow K, Mulatero P, Emeric-Blanchouin N, Aupetit-Faisant B, Corvol P, Pascoe L.** 1997 The aminoacid substitutions Ser288Gly and Val 320Ala convert the cortisol producing enzyme, CYP11B1, into an aldosterone producing enzyme. Nat Struct Biol. 4:32–35.
21. **Sutherland DJ, Ruse JL, Laidlaw JC.** 1966 Hypertension, increased aldosterone secretion, and low plasma renin activity relieved by dexamethasone. Can Med Assoc J. 95:1109–1119.
22. **Fallo F, Mantero F.** 1990 dexamethasone-suppressible hyperaldosteronism. In: Biglieri EG, Melby JC, eds. Endocrine hypertension. Comprehensive endocrinology, revised series. New York: Raven Press; 87–92.
23. **Chu MD, Ulick SJ.** 1982 Isolation and identification of 18-hydroxycortisol from the urine of patients with primary aldosteronism. J Biol Chem. 257:2218–2224.
24. **Ulick SJ, Chu MD, Land MJ.** 1983 Biosynthesis of 18-oxocortisol by aldosterone-producing adrenal tissue. J Biol Chem. 258:5498–5502.
25. **Gomez-Sanchez CE, Montgomery M, Ganguly A, et al.** 1984 Elevated urinary excretion of 18-oxocortisol in glucocorticoid-remediable aldosteronism. J Clin Endocrinol Metab. 59:1022–1024.
26. **Ulick SJ, Chan CK, Gill GR, et al.** 1990 Defective fasciculata zone function as the mechanism of glucocorticoid-remediable aldosteronism. J Clin Endocrinol Metab. 71:1151–1157.
27. **Kreft C, Menard J, Corvol P.** 1979 Value of renin measurement, saralasin test, and acelretolol treatment in hypertension. Kidney Int. 15:176–183.
28. **Sealey JE, James GD, Laragh JH.** 1995 Interpretation and guidelines for the use of plasma and urine aldosterone and plasma angiotensin II, angiotensinogen, prorenin, peripheral, and renal vein renin tests. In: Laragh JH, Brenner BM, eds. Hypertension: pathophysiology, diagnosis, and management. New York: Raven Press; 1953–1967.
29. **Montero J, Soto J, Fardella C, Foradori A, Valdés G.** 1998 Medición de la actividad de renina plasmática a niveles bajos. Optimización del método. Rev Med Chil. 126:151–154.
30. **McKenna TJ, Sequeira SJ, Heffernan A, Chambers J, Cunningham S.** 1991 Diagnosis under random conditions of all disorders of the renin-angiotensin-aldosterone axis, including primary hyperaldosteronism. J Clin Endocrinol Metab. 73:952–957.
31. **Gordon R.** 1994 Mineralocorticoid hypertension. Lancet. 344:240–243.
32. **Lins PE, Adamson U.** 1986 Plasma aldosterone-plasma renin activity ratio: a simple test for primary aldosteronism. Acta Endocrinol (Copenh). 113:564–569.
33. **Gómez-Sánchez C, León L, Gómez-Sánchez E.** 1992 Biotin-hydrazide derivatives for the development of steroid enzyme-linked inmunoassays. J Steroid Biochem Mol Biol. 43:523–527.
34. **Yamakita N, Gomez-Sanchez C, Mune T, et al.** 1994 Simultaneous measurement of plasma 18-oxocortisol and 18-hydroxycortisol levels in normal man. Eur J Endocrinol. 131:74–79.
35. **Streeteen A.** 1979 Fludrocortisone suppression test. Am J Med. 67:403.
36. **Vallotton MB.** 1996 Primary aldosteronism. Part I. Diagnosis of primary hyperaldosteronism. Clin Endocrinol (Oxf). 45:47–52.
37. **Gill J.** 1995 Hyperaldosteronism. In: Becker K, ed. Principles and practice of endocrinology and metabolism. 2nd ed. Philadelphia: JB Lippincott Company; 716–729.
38. **Litchfield WR, Dluhy RG.** 1995 Primary aldosteronism. Endocrinol Metab Clin North Am. 24:593–612.
39. **Dluhy R, Lifton R.** 1995 Glucocorticoid-remediable aldosteronism (GRA): diagnosis, variability of phenotype and regulation of potassium homeostasis. Steroids. 60:48–51.
40. **Lichfield W, New M.** 1997 Evaluation of the dexamethasone suppression test for the diagnosis of glucocorticoid-remediable aldosteronism. J Clin Endocrinol Metab. 82:3570–3573.
41. **Kenney PJ, Lee JKT.** 1998 The adrenals. In: Lee JKT, Sager SS, Stanley RJ, Heiken JP, eds. Computed body tomography with MRI correlation. Philadelphia: Lippincott-Raven Press; 1171–1208.
42. **Lin P, Harikrishna JA, Moore CCD, Jones KL, Miller WL.** 1991 Missence mutation Sr106 $\rightarrow$ Pro causes 17 α-hydroxylase deficiency. J Biol Chem. 266:15992–15998.
43. **Jonsson J, Klemm S, Tunny T, Stowasser M, Gordon R.** 1995 A new genetic test for familial hyperaldosteronism type I aids in the detection of curable hypertension. Biochem Biophys Res Commun. 207:565–571.
44. **White PC, Dupont J, New MI, Lieberman E, Hochberg Z, Rósler A.** 1991 A mutation in CYP11B1 (Arg 448 $\rightarrow$ His) associated with steroid 11β-hydroxylase deficiency in Jews of Moroccan origin. J Clin Invest. 87:1664–1667.
45. **Brown MA, Cramp HA, Zammit VC, Whitworth JA.** 1996 Primary hyperaldosteronism: a missed diagnosis in "essential hypertensives"?. Aust N Z J Med. 26:533–538.
46. **Hiramatsu K, Yamada T, Yukimura Y, et al.** 1981 A screening test to identify aldosterone-producing adenoma by measuring plasma renin activity. Results in hypertensive patients. Arch Intern Med. 141:1589–1593.
47. **Bravo EL.** 1994 Primary aldosteronism: issues in diagnosis and management. Endocrinol Metab Clin North Am. 23:271–283.
48. **Dluhy R, Gordon W.** 1998 Endocrine hypertension. In: Wilson J, Foster D, Kronenberg H, Larsen P, eds. Williams textbook of endocrinology. 9th ed. Philadelphia: Southern Company; 729–749.
49. **Mulatero P, Veglio F, Pilon C, et al.** 1998 Diagnosis of glucocorticoid -remediable aldosteronism in primary aldosteronism: aldosterone response to dexamethasone and long polymerase chain reaction for chimeric gene. J Clin Endocrinol Metab. 83:2573–2575.
50. **Gomez-Sanchez C, Gill JR, Ganguly A, Gordon RD.** 1993 Glucocorticoid-suppressible aldosteronism: a disorder of the adrenal transitional zone. J Clin Endocrinol Metab. 76:873–878.