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−344C/T Polymorphism of CYP11B2 Gene in Italian Patients With Idiopathic Low Renin Hypertension

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Most patients with low renin essential hypertension are not qualitatively different from patients with idiopathic hyperaldosteronism, as in both conditions aldosterone secretion is not appropriately reduced. The aim of the study was to investigate allele and genotype frequencies of the −344C/T polymorphism, located in the promoter region of the aldosterone synthase gene, in 83 patients with idiopathic low renin hypertension characterized by an increased aldosterone to renin ratio, including both patients with low renin essential hypertension ($n = 53$) and subjects with idiopathic hyperaldosteronism ($n = 30$), compared with 78 patients with normal to high renin essential hypertension and 126 normotensive control subjects. The relationship of −344C/T genotypes to basal and post-captopril plasma aldosterone/plasma renin activity ratio was also examined in the entire hypertensive population. An increased frequency of the T allele and a relative excess of TT homozygosity over CC homozygosity were found in patients with idiopathic low renin hypertension in comparison with both normal to high renin hypertensives and normotensive controls. A higher post-captopril aldosterone to renin ratio was found in the hypertensives with TT genotype than in those with CC genotype, and TT+TC genotypes were associated with a smaller decrease in the aldosterone-to-renin ratio elicited by captopril administration. The present study suggests that the −344C/T polymorphism, or a functional variant in linkage disequilibrium with it, may play a role in the abnormal regulation of aldosterone secretion in idiopathic low renin hypertension. Am J Hypertens 2001;14:934–941 © 2001 American Journal of Hypertension, Ltd.

Key Words: CYP11B2 polymorphism, low renin essential hypertension, idiopathic hyperaldosteronism.

Essential hypertension (EH) is considered to be a complex trait, resulting from the interplay between several genes and multiple environment factors.[1] The association of more than 20 genes with EH has been assessed with variable findings.[1] Such inconsistent results may partly reflect the circumstance that EH is not a disease but quite a heterogeneous syndrome. The isolation of intermediate phenotypes that identify more homogeneous subsets within the hypertensive population may mean that the role of candidate genes potentially involved in specific phenotypes may be assessed more reliably.[1]

Low renin essential hypertension (LREH) refers to the subset of EH patients characterized by decreased plasma renin activity (PRA) when compared to normotensive subjects on the same sodium-restricted diet.[2] In most patients with LREH, plasma aldosterone levels are normal in the face of the low PRA. As a result, an abnormally increased plasma aldosterone-to-plasma renin activity (A/R) ratio is observed in a substantial proportion of LREH patients compared with both normotensives and patients with normal renin essential hypertension (NREH).[2–4] On the basis of these findings, a primary increase in aldosterone secretion has been suggested as the mechanism underlying LREH.

The above features are not distinguishable from those observed in idiopathic hyperaldosteronism (IHA). In fact, LREH and IHA have many other similarities. Familial, probably genetic, determinants contribute to both LREH[5] and IHA.[6] An enhanced response of plasma aldosterone to both upright posture[7–12] and angiotensin II infusion,[8,10,13–15] blood pressure (BP) sensitivity to changes in salt intake[2] and increased BP response to spironolactone,[3,16] characterize both conditions. Finally, bilateral adrenal cortical hyperplasia, as assessed by adrenal scin-

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tigraphy, computed tomographic (CT) scan, and histologic examination of the adrenal tissue, has been found in patients with LREH as well as in IHA subjects.[3,16] Even hypokalemia and absolute increase in plasma aldosterone level cannot be considered as distinguishing features for IHA, as normal plasma levels of potassium and aldosterone occur in a substantial number of IHA patients.[17] Diagnostic separation of the two conditions relies on a lower degree of aldosterone suppression after intravenous or oral sodium chloride load in IHA than in LREH.[18] However, even in LREH aldosterone levels do not suppress normally in response to sodium chloride loading.[19]

Taken together, these findings strongly suggest that the separation of LREH (or rather, of the LREH group with increased A/R ratio) and IHA has no firm basis. Indeed, the functional and structural adrenal abnormalities in LREH appear to differ only quantitatively from those in IHA. Therefore, LREH and IHA are more likely to be parts of a same condition of idiopathic low renin hypertension (ILRH) than different conditions, with IHA representing one extreme of the spectrum of ILRH.

Although the mechanisms responsible for ILRH have not yet been elucidated, an enhanced adrenal sensitivity to angiotensin II could theoretically result from an increase in either intrinsic activity or expression of aldosterone synthase (AS), which catalyzes the final steps of aldosterone biosynthesis in adrenal glomerulosa.[20] In humans, the gene for AS (CYP11B2) is located in chromosome 8q24, adjacent to the gene (CYP11B1) for steroid 11β-hydroxylase, which is required for cortisol biosynthesis in adrenal fasciculata.[20–22] CYP11B2 allelic variants in either the coding sequences or in the promoter could hypothetically increase AS activity or expression. Both an enhanced aldosterone production and an overexpression of CYP11B2 mRNA, in the absence of any mutation in the CYP11B2 exons, have recently been found in mononuclear leukocytes of patients with IHA.[23] This finding suggests that some variants in the CYP11B2 promoter may be responsible for increased AS expression in ILRH.

A few polymorphisms in CYP11B2 have recently been identified.[24,25] Among them, −344C/T is a single nucleotide biallelic polymorphism located in the promoter of CYP11B2,[24] 344 nucleotides from the transcription start site, where the residue could be a cytosine (−344C) or thymine (−344T). Position −344 is located in a regulatory sequence that interacts with steroidogenic factor 1, which is involved in the expression of steroid biosynthetic enzymes in the adrenal cortex.[24] Therefore, it is possible to hypothesize that −344C/T polymorphism itself, or an adjacent functional variant in linkage disequilibrium with it, may affect CYP11B2 transcription and aldosterone production.

The aim of the present study was to investigate allelic and genotype frequencies of the −344 C/T polymorphism in white patients with ILRH, including both LREH with elevated A/R ratio and IHA, compared with normotensive controls and NREH subjects. We also examined the relationship of −344C/T genotypes to basal and post-captopril A/R ratios in the entire hypertensive population.

Methods

Study Population

Eighty-three patients with ILRH, 30 of whom were defined as having IHA based on the results of aldosterone suppression tests (see below), 78 patients with NREH and 126 normotensive controls were enrolled in the study. All subjects were Italian, and their families had lived in Emilia Romagna, a region of northern Italy, for at least three generations. Informed consent was obtained for all subjects, and the study protocol was approved by the local ethics committee. The diagnosis of EH was established according to the following criteria: 1) BP levels >140/90 mm Hg on at least three separate occasions in the absence of therapy; and 2) exclusion of renal disease, renal artery stenosis, and pheochromocytoma by appropriate biochemical and instrumental examinations. Primary aldosteronism was excluded as detailed; specifically, patients with ILRH who displayed normal suppression of plasma aldosterone levels during dynamic tests were classified as having LREH. Diabetic patients were also excluded from the study.

Since January 1, 1995, all hypertensive patients referred to our clinic have systematically been submitted to a captopril test. Angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists, diuretics, aldosterone antagonists, β-blockers, calcium antagonists, and clonidine were withdrawn at least 4 weeks (8 weeks for aldosterone antagonists) before the test, and substituted with doxazosin if necessary. Patients were instructed to follow a diet containing 100 mmol NaCl and 80 mmol KCl for 5 days before the test, and compliance was checked by measurement of 24-h urinary excretion of electrolytes on the day before the test. On the day of the test, a blood sample for the measurement of plasma aldosterone (ALDO) and PRA was drawn after 1 h in the seated position (baseline value), and 50 mg of oral captopril was then administered; after 90 min in the seated position, another blood sample was drawn for both ALDO and PRA measurements.

Classification Criteria for the Different Groups

ILRH Low renin hypertensives were selected by screening patients' records according to the following criteria: 1) age <60 years; 2) PRA <0.40 ng Ang I/mL/h; and 3) A/R ratio >20 ng/dL per ng/mL/h at baseline. The PRA value of 0.40 ng Ang I/mL/h represents the upper limit of the lowest quartile of PRA distribution in an unselected hypertensive population screened by the captopril test (n = 815). Normal reference values for PRA in our laboratory are 0.20 to 2.80 ng Ang I/mL/h in the supine position and 1.50 to 5.70 ng Ang I/mL/h in the

Table 1. Characteristics of the study groups

	ILRH (n = 83)	NREH (n = 78)	Controls (n = 126)	P
Age (y)	51.5 ± 7.5	50.5 ± 9.8	49.4 ± 9.4	NS
Female/male	39/44	38/40	66/60	NS
BMI (kg/m^2)	26.1 ± 3.9	25.9 ± 3.9	25.6 ± 2.9	NS
SBP (mm Hg)	166.6 ± 16.4	165.5 ± 13.9	129.5 ± 8.5*†	<.0001
DBP (mm Hg)	104.4 ± 10.4	103.1 ± 6.8	81.4 ± 4.7*†	<.0001
Serum creatinine (mg/dL)	0.91 ± 0.13	0.98 ± 0.16	0.96 ± 0.13	NS
Baseline ALDO (ng/dL)	14.2 ± 8.3	15.2 ± 8.6	—	NS
Baseline PRA (ng/mL/h)	0.21 ± 0.12	2.01 ± 1.39*	—	<.0001‡
Baseline A/R (ng/dL per ng/mL/h)	88.2 ± 49.9	10.9 ± 8.8*	—	<.0001‡
Serum Na (mmol/L)	140.7 ± 2.3	140.4 ± 2.0	140.1 ± 1.8	NS
Serum K (mmol/L)	3.8 ± 0.3	4.1 ± 0.3*	4.2 ± 0.4*	.0003
Urinary Na (mmol/24 h)	94.1 ± 40.5	97.0 ± 42.4		NS

ILRH = low renin essential hypertension; NREH = normal renin essential hypertension; NS = not significant; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; ALDO = plasma aldosterone; PRA = plasma renin activity; A/R = plasma aldosterone-to-plasma renin activity.

* $P < .05$ *v* ILRH; † $P < .05$ *v* NREH; ‡ After log transformation.

upright position. Twenty-four-hour urinary sodium excretion ranged between 70 and 130 mmoles in all patients. In patients with PRA <0.40 ng Ang I/mL/h and A/R ratio >20 at baseline, aldosterone suppression tests by either an intravenous saline load[26] or oral fludrocortisone administration for 4 days[27] were performed. The diagnosis of primary aldosteronism was established according to the following criteria: 1) ALDO >7.5 ng/dL in the supine position after the saline load (500 mL/h NaCl 0.9% over 4 h) or 2) ALDO >5 ng/dL in the upright position after 4 days of oral fludrocortisone. If the diagnosis of primary aldosteronism was confirmed, patients underwent a CT scan of the adrenal glands and a dexamethasone-suppressed scintigraphy with ^{131}I-6-β-iodomethyl-norcholesterol. The diagnosis of IHA was established by exclusion of unilateral adrenal lesions in both CT and scintigraphic scanning. Glucocorticoid-remediable aldosteronism was excluded by a long polymerase chain reaction (PCR)-based method.[28]

NREH Hypertensive patients aged <60 years with PRA values >0.40 ng Ang I/mL/h at baseline were randomly selected from our records. Twenty-four-hour urinary sodium excretion before the captopril test was comparable to that observed in ILRH patients (Table 1).

Normotensive Controls White healthy subjects screened for blood donation, born in the same geographic area as the patients, were randomly selected from records kept at the local Center for Immuno-Hematology and Blood Transfusion. They were also matched with patients for age and gender.

Molecular Analysis of the AS Gene

Subject genotyping was performed according to the method of Kupari et al.[29] Briefly, genomic DNA was obtained by peripheral blood leukocytes, and amplification of the relevant promoter region of the AS gene was carried out with PCR using specific primers (sense, CAGGAGGAGACCCCATGTGAC; antisense, CCTCCACCCTGTTCAGCCC). The PCR conditions were as follows: denaturation at 94°C for 5 min and manual hot start at 80°C for 1 min, followed by 35 cycles of 94°C for 1 min, annealing at 68°C for 1 min and extension at 72°C for 2 min. The amplicon was subsequently digested with *Hae*III at 37°C for 2 h and subjected to electrophoresis in 2.5% agarose gel, yielding two main fragments of 273 bp (−344T alleles) and 202 bp (−344C alleles), respectively.

Glucocorticoid-remediable aldosteronism was excluded by a long PCR-based assay following the method of Stowasser et al.[28]

Analytical Techniques

Blood samples drawn for PRA and ALDO measurements were collected in prechilled tubes containing EDTA and kept on ice until assay. The PRA was determined by radioimmunoassay using a commercial kit (Radim, Pomezia, Italy). Intra-assay and interassay variation coefficients are 7.6% and 9.1%, respectively. The ALDO was also measured by radioimmunoassay with a commercially available kit (Sanofi Diagnostic Pasteur, Marnes-La-Coquette, France). Normal reference values for ALDO in our laboratory are 1.0 to 10.5 ng/dL and 3.5 to 27.5 ng/dL in the supine and upright position, respectively. Intra-assay and interassay coefficients of variation are 5.3% and 8.6%, respectively.

Serum electrolytes and metabolites were measured by standard analytical techniques.

Statistics

Genotype distribution according to Hardy-Weinberg equilibrium was tested by χ^2 with *(k-1)* degrees of freedom, where k represents the number of different genotypes.

Table 2. Allelic and genotype frequencies of −344 C/T polymorphism in the different study groups

	ILRH (n = 83)	NREH (n = 78)	Controls (n = 126)
−344 TT (%)	35 (42.2)	21 (26.9)	33 (26.2)
−344 TC (%)	37 (44.6)	37 (47.4)	58 (46.0)
−344 CC (%)	11 (13.2)	20 (25.7)	35 (27.8)
		χ^2 9.684 P = .046	
% −344 T	64.5	50.6	49.2
% −344 C	35.5	49.4	50.8
		χ^2 10.349 P = .006	

Abbreviations as in Table 1.

Differences in allelic and genotype frequencies among groups were also tested by χ^2 with *(r-1)(k-1)* degrees of freedom, where *r* is the number of the tested groups and *k* represents the number of different alleles or genotypes. The association between homozygosity or heterozygosity for one allele and each study group, as defined by renin profile, was tested by contingency table analysis. Differences in continuous variables among groups were tested by one-way ANOVA followed by post-hoc Scheffé's F test or Student's *t* test for independent samples, as appropriate. For non-normally distributed data, log transformation was applied before analysis.

A two-sided α level of 0.05 was considered significant.

Results

The general characteristics of the studied population are summarized in Table 1. Serum potassium was significantly lower in the patients with ILRH (ie, LREH and IHA considered as one group) as compared with those with NREH and normotensive controls. The PRA was equally suppressed in the patients with LREH (n = 53) and those (n = 30) defined as having IHA (0.21 ± 0.14 *v* 0.20 ± 0.11 ng Ang I/mL/h, P = not significant), although plasma aldosterone levels were higher in the latter group (19.6 ± 11.2 *v* 11.2 ± 6.8 ng/dL, $P < .0001$). In the patients defined as having LREH, A/R ratio was clearly higher compared to that of the patients with NREH (69.4 ± 58.9 *v* 10.9 ± 8.8 ng/dL per ng/mL/h, $P < .0001$ after log transformation); the patients defined as having IHA showed the highest A/R ratio (121.3 ± 78.6 ng/dL per ng/mL/h, $P < .0001$ by ANOVA).

Allelic frequencies were different in the patients with ILRH as compared with those found in the patients with NREH (T: 107/166 *v* 79/156; C: 59/166 *v* 77/156; χ^2 = 6.293, P = .012) and normotensive controls (T: 124/252; C: 128/252; χ^2 = 9.416, P = .002; Table 2). Genotype distribution was in Hardy-Weinberg equilibrium in the controls (TT: 33/126, TC: 58/126, CC: 35/126; χ^2 = 0.476, P = .808) and in the patients with NREH (TT: 21/78, TC: 37/78, CC: 20/78; χ^2 = 0.115, P = .944), but not in the patients with ILRH (TT: 35/83, TC: 37/83, CC: 11/83; χ^2 = 6.894, P = .032). Consequently, genotype distribution was found to be significantly different among the different groups, with homozygosity for the T allele being more frequent in the patients with ILRH (Table 2). Separating the group of patients with ILRH in the two different classification subsets, namely patients with LREH (n = 53) and IHA (n = 30) yielded virtually identical allelic (T: 38/60 *v* 69/106, C: 22/60 *v* 37/106; χ^2 = 0.003, P = .953) and genotype (TT: 12/30 *v* 23/53, TC: 14/30 *v* 23/53, CC: 4/30 *v* 7/53; χ^2 = 0.099, P = .952) frequencies. Thus, the patients with LREH and IHA appeared to be genetically homogeneous for the −344C/T polymorphism and different from the patients with NREH or the normotensive controls. When homozygotes and heterozygotes for the T allele (ie, TT and TC) were combined, a relative excess of this allele appeared to be significantly associated with the condition of ILRH (odds ratio [OR] 2.416, 95% confidence interval [CI] 1.193–4.895, P = .012), but not with the condition of NREH (OR 1.115, 95% CI 0.588–2.117, P = .738).

In the entire hypertensive population (ie, irrespective of the renin profile), no significant differences were noted in the A/R ratio at baseline according to the three separate genotypes (Fig. 1); conversely, after captopril administration, the value of the A/R ratio was significantly higher in the TT genotype compared to the CC genotype (Fig. 1), and a relative excess of the T allele was associated with a lower relative change in the A/R ratio (−55.8% ± 26.4% *v* −70.6% ± 19.5%, P = .004, TT+TC *v* CC, respectively).

Discussion

Our results suggest an association between the −344C/T polymorphism of the CYP11B2 and ILRH, which comprises both LREH with increased A/R ratio and IHA. We have found an increased frequency of the −344T allele and an excess of TT genotype over CC genotype in ILRH patients in comparison with NREH subjects and normotensive controls. Previous studies in European populations have found a higher frequency of the T allele and a relative excess of TT homozygosity over CC homozygosity in patients with EH compared with normotensive controls.[30,31] However, in a Japanese population, the frequency of the TC+CC genotypes combined into a single group was higher in EH patients than in normotensive

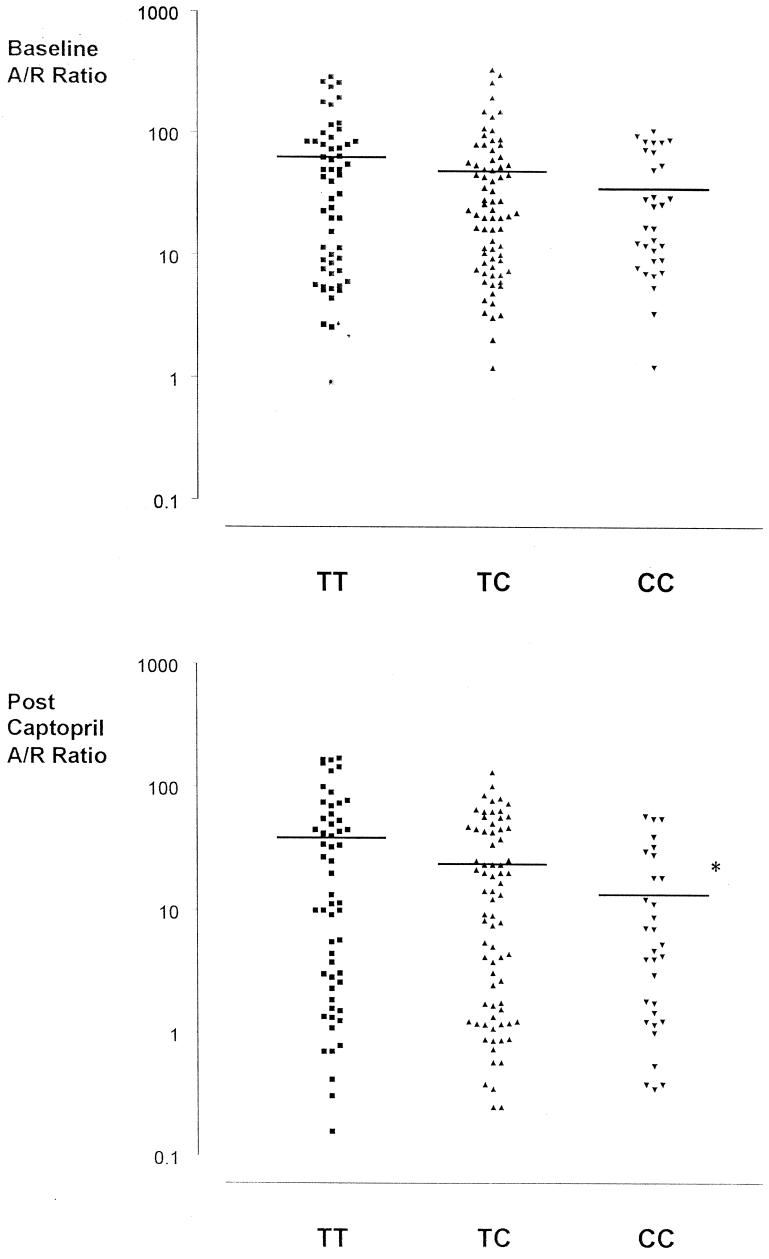

FIG. 1. Individual data on plasma aldosterone (in nanograms per deciliter) to plasma renin activity (in nanograms per milliliter per hour) ratio (A/R ratio) before and after captopril administration in relation to the −344C/T genotypes in the hypertensive patients. *$P < .05$ *v* TT genotype.

controls, which suggests an association of the −344C allele with EH.[32] Finally, no difference in the distribution of the −344C/T polymorphism between EH patients considered as one group and normotensive controls has even been reported.[33] Such an inconsistency may partly depend on the different proportion of subjects with LREH included in the various studies, inasmuch as CYP11B2 might be involved only in the low renin hypertensive subset. In our study we have found a relative excess of the T allele only in the ILRH patients, without any difference in the distribution of the −344C/T polymorphism between NREH and the normotensive control group. Our results are in agreement with those of Komiya et al,[33] who found no difference in the frequencies of the −344C allele between EH patients and normotensive controls, but a lower frequency of the −344C allele in the group of EH subjects with elevated A/R ratio compared with either the remaining EH population or the normotensive controls. Even the analysis of the CYP11B2 Lys173Arg polymorphism, which is in linkage disequilibrium with the −344C/T polymorphism, in a Chilean population[25] has yielded results partly in agreement with our findings. In fact, a relative excess of the Arg173 allele was associated with EH patients compared with normotensive subjects, but a lower frequency of the same allele was found in EH patients with elevated A/R than in those with normal A/R

ratio. Because the Arg173 allele is in linkage disequilibrium with the −344C allele,[32–34] a decreased frequency of the −344C allele may be expected in Chilean EH patients with elevated A/R ratio.

Aplotypes resulting from three biallelic CYP11B2 polymorphisms, including −344C/T and Arg173Lys, have recently been examined in Italian patients with IHA compared to patients with aldosterone-producing adenoma, EH subjects, and normotensive controls.[34] The frequency of an aplotype in which the −344C and Arg173 alleles were combined, was higher in the IHA group than in the other groups. Because of the complete linkage disequilibrium between the −344C/T and Arg173Lys polymorphisms, the frequency of both −344C and Arg173 alleles was higher in IHA than in the other groups. Furthermore, when the EH patients were divided in low renin and normal renin groups, the aplotype and allele frequencies in the low renin group, although not statistically different, were between those of the IHA patients and the other groups. Although the different study design is taken into account, these findings are in sharp contrast with our results. We believe that our results are not due to unsuspected admixture of populations, as we took care to ensure that the study groups were samples of the same ethnic population. Allele frequencies of −344C/T polymorphism in our normotensive group are very similar to those previously reported in normotensive populations in various European countries,[29–31,35–38] but rather different from those found by Mulatero et al,[34] who report in their normotensive control group a frequency of the −344C allele lower than that observed in other European populations, but curiously similar to that observed in Japanese normotensives.[32,33] Therefore, we cannot exclude that genetic (ethnic) differences between the source populations may partly account for the divergence of our results from those of Mulatero et al.[34]

In the entire hypertensive population enrolled in this study we found a higher post-captopril A/R ratio in the TT genotype than in the CC genotype. The remarkable overlap seen in this population both in the baseline and post-captopril A/R ratio (Fig. 1) reflects the admixture of patients with either normal-to-high or low renin profile with respect to daily sodium intake; however, the higher A/R ratio found in the patients carrying the TT genotype irrespective of their renin profile suggests an association of the T allele with an altered functional relationship between angiotensin II and adrenal aldosterone response, and therefore, is in line with the higher prevalence of the T allele and the TT genotype that we have detected in ILRH. In previous studies, the −344T allele has been associated with higher plasma[30,36] or urinary aldosterone levels[31,37] in either normotensive subjects or EH patients. However, these findings are not consistent with the results of other studies, where the opposite association between the −344C allele and plasma aldosterone level[39] or even no association of the −344C/T polymorphism with plasma aldosterone level[40] has been found. The absence of either a standardized salt intake or an adjustment of aldosterone level for PRA values in these studies may partly explain the contrasting results. Moreover, given the complexity of aldosterone regulation, a relationship between −344C/T genotypes and adrenal function could possibly be revealed only in standardized conditions of adrenal suppression or stimulation. We have found a significant association between −344C/T genotypes and A/R ratio values only after captopril administration, which is expected to result in an acute reduction of angiotensin II circulating level.

The diagnostic criteria of LREH used in our study may not have conformed to rigid requirements, as we did not select our low renin hypertensives on the basis of decreased PRA values on a low sodium diet in comparison with those in normotensive controls.[2] However, we have selected the lowest quartile of PRA distribution in a hypertensive population placed on a standardized sodium intake, which corresponds to the prevalence of LREH reported in previous studies.[4] Furthermore, a PRA value of 0.40 ng Ang I/mL/h, which separates our LREH patients from NREH subjects, actually corresponds to the upper limit of the PRA range we have found in our patients with IHA on the same salt intake. This might advantageously have made the entire group with ILRH more homogeneous. Moreover, the added criterion of an A/R ratio >20, which we have used in selecting our low renin hypertensives, may have substantially limited the number of hypertensives erroneously included in the LREH group. Indeed, an A/R ratio <20 has been reported in both normotensives[41] and normal renin hypertensives.[42] Furthermore, an A/R ratio >20 is used as a positive criterion in screening hypertensives for primary aldosteronism.[18,41]

Our inclusion of LREH and IHA into the single group of ILRH may appear rather arbitrary. In fact, a blunted aldosterone response to both upright posture and angiotensin II infusion, similar to that observed in nonmodulating NREH patients, has recently been reported in LREH patients placed on a low salt diet.[43] This finding contrasts with previous observations that showed enhanced adrenal responsiveness to upright posture or angiotensin II infusion in LREH patients even after sodium restriction.[7,10] This discrepancy may possibly reflect the heterogeneity of the LREH itself. In fact, both an elevated A/R ratio and an enhanced adrenal responsiveness to angiotensin II infusion on a low sodium diet may be shown only in a proportion of LREH subjects.[7,10] Even the recent elucidation of some rare mendelian forms of low renin hypertension,[44] such as glucocorticoid-remediable aldosteronism, apparent mineralocorticoid excess, and Liddle's syndrome, suggests that different mechanisms, resulting in either an elevated or normal A/R ratio, may possibly underlie more common forms of idiopathic low renin hypertension. Because both a decreased PRA and an elevated A/R ratio at a standardized sodium intake have been used as selection criteria for ILRH in our study, we believe that a homogeneous clinical phenotype within the population of low renin hypertensives has been isolated.

Finally, a limitation of the present study may reside in the fact that we did not perform adrenal venous sampling in the patients having biochemical evidence of primary aldosteronism, and relied on both adrenal CT scans and ^{131}I-nor-cholesterol scintigraphy under dexamethasone suppression for the identification of IHA. Given the relatively low sensitivity of CT in the identification of small (ie, <1 cm) adrenal adenomas,[45] we may have inadvertently included cases of aldosteronoma in our series. However, as we selected only cases where the results of both CT scan and scintigraphy were concordant with respect to the exclusion of unilateral functioning adrenal lesions, conceivably the number of aldosteronomas erroneously included would be very small.

In conclusion, our study suggests an association of a common variant of the CYP11B2 promoter, the -344T allele, with the subset of ILRH patients characterized by inappropriate plasma levels of aldosterone. The same allelic variant seems to be related to a blunted suppression of aldosterone production after acute reduction of angiotensin II. Taken together, our findings suggest that the -344C/T polymorphism, or a functional variant in linkage disequilibrium with it, may play a role in the abnormal regulation of aldosterone levels in patients with ILRH.

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