# Levels of Plasma Angiotensin-(1-7) in Patients With Hypertension Who Have the Angiotensin–I-Converting Enzyme Deletion/Deletion Genotype

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**In patients with hypertension who have the DD-ACE genotype (higher angiotensin-converting enzyme [ACE] activity), plasma levels of angiotensin-(1-7) are 4 times lower than in patients with the II-ACE genotype (lower ACE levels). Angiotensin II levels are similar in both groups.**

Angiotensin-(1-7), a heptapeptide component of the renin–angiotensin system, has vasodilatory actions opposed to angiotensin II. The vasodilator actions of angiotensin-(1-7) depend on an intracellular signaling mechanism, as yet not fully characterized, that may be caused by secretion of prostacyclin, release of nitric oxide, amplification of the vasodilator effects of bradykinin, or vasopressin liberation alone or in combination.[1–4] Angiotensin-(1-7) is formed from angiotensin I and angiotensin II through the effect of tissue peptidases.[1,2] Angiotensin-(1-7) is rapidly hydrolyzed, mainly by angiotensin–I-converting enzyme (ACE).[1,5] .

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The presence of an ACE gene polymorphism in humans has been postulated from segregation analysis of plasma ACE levels in several families.[6] These variations have been correlated with different plasma and tissue ACE levels, probably by modulating the ACE gene transcription. The insertion/deletion ACE gene polymorphism, characterized by the presence (insertion [I]) or absence (deletion [D]) of a fragment of 287 bp, has been identified in the intron 16 of this gene.[6] Men with the D allele have a higher risk of hypertension.[7–9] This epidemiologic observation has a recent experimental counterpart in male rats with a similar ACE polymorphism in which higher ACE determines higher angiotensin II levels and higher levels of chronic hypertension in the Goldblatt model.[10] The presence of the D allele in humans is associated with higher ACE levels and a shorter plasma half-life of bradykinin.[11,12] However, in normotensive subjects, no differences in the levels of renin, angiotensin II, or aldosterone—or in the conversion from angiotensin I to angiotensin II—have been found.[9,13] Currently, no data are available on the relation of this polymorphism with angiotensin-(1-7) levels in patients who have hypertension. We hypothesized that the I/D ACE gene polymorphism, through different levels of ACE activity, determines circulating angiotensin-(1-7) plasma levels in patients with hypertension.

Our institutional review board and ethics committee approved this study. Participants were consecutive homozygous patients with essential hypertension from our hypertension clinic with these criteria: blood pressure (BP) ≥140/90 mm Hg, not taking antihyperten-

**TABLE 1** Demographic, Clinical, and Laboratory Data According to the Angiotensin–I-converting Enzyme Genotype

| Demographic Data | II-ACE (n = 9) | DD-ACE (n = 9) |
|---|---|---|
| Age (yrs) | 42 ± 3 | 45 ± 4 |
| Men/women | 4/5 | 3/6 |
| Body weight (kg) | 77 ± 5 | 68 ± 6 |
| Height (cm) | 168 ± 3 | 161 ± 5 |
| Systolic BP (mm Hg) | 161 ± 5 | 155 ± 5 |
| Diastolic BP (mm Hg) | 98 ± 3 | 89 ± 4 |
| Creatinine, serum (mg/dl) | 0.94 ± 0.04 | 0.91 ± 0.07 |
| Potassium, plasma (mmol/L) | 4.1 ± 0.18 | 4.3 ± 0.20 |
| Hematocrit (%) | 43 ± 1 | 42 ± 2 |
| White blood cell count (cells/ ml) | 6,438 ± 360 | 7,650 ± 436 |
| Erythrocyte sedimentation rate (mm/1 h) | 11.3 ± 2.6 | 13.7 ± 3.8 |
| Glucose, serum (mg/dl) | 88 ± 3 | 90 ± 4 |
| Total cholesterol, plasma (mg/dl) | 228 ± 16 | 223 ± 15 |
| Serum glutamic oxaloacetic transaminase (U/ml) | 22 ± 3 | 18 ± 3 |
| Plasma renin activity (ng/ml/h) | 2.1 ± 1.7 | 1.4 ± 0.7 |

Valves expressed as mean ± SEM.

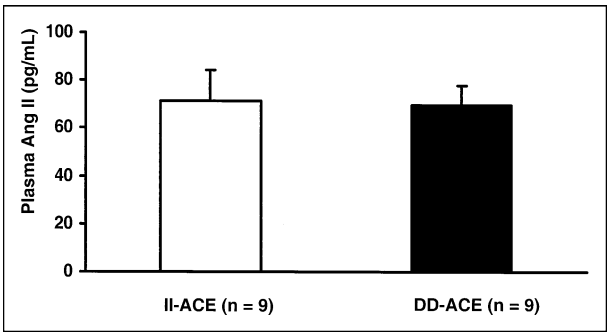


**FIGURE 1. Plasma levels of angiotensin II (Ang II) in patients with hypertension who have the DD-ACE genotype (n = 9) and the II-ACE genotype (n = 9). Values expressed as mean ± SEM.**

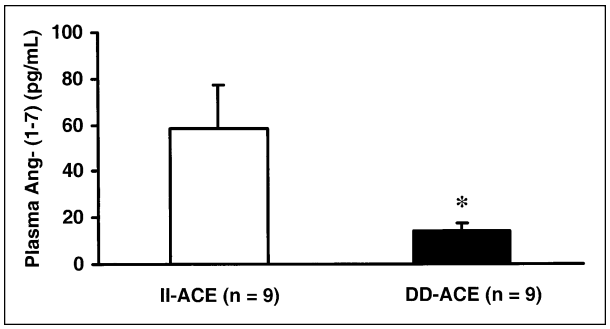


**FIGURE 2. Plasma levels of angiotensin-(1-7) in patients with hypertension who have the DD-ACE genotype (n = 9) and II-ACE genotype (n = 9). *p <0.05. Values expressed as mean ± SEM. Other abbreviation as in Figure 1.**

sive drugs, BP measured twice on 2 different days while sitting, normal renal function, nondiabetic, nonobese, and without secondary hypertension. After the subjects had fasted overnight, signed the informed consent form, and rested in a sitting position for 15 minutes, we obtained fasting blood samples by venipuncture. Serum creatinine, potassium, hematocrit, red and white blood cell counts, erythrocyte sedimentation rate, glucose, cholesterol, serum glutamic oxaloacetic transaminase, and plasma renin levels were measured by standard methods. ACE polymorphism was determined in DNA extracted from leukocytes and amplified by polymerase chain reaction.[14] Another blood sample was obtained in a chilled heparinized tube for plasma ACE activity determination. The sample was centrifuged at 4°C; plasma was stored at −80°C, and ACE polymorphism was determined by a spectrofluorimetric method using Z-phenyl-histidyl-leucine (Bachem Bioscience, King of Prussia, Pennsylvania ) as an ACE substrate.[15] Venous blood was also collected for angiotensin II and angiotensin-(1-7) measurements in chilled tubes containing inhibitors (ethylenediamine tetraacetic acid, phenantroline, and pepstatin A) to prevent angiotensin I generation; conversion of angiotensin I to angiotensin II; and angiotensin I, angiotensin II, and angiotensin-(1-7) degradation.[16,17] The blood was centrifuged at 4°C. Plasma was stored at −80° C, extracted within 2 days, and assayed later. Plasma was directly applied to SepPak cartridges (Merck, Germany). The cartridges were conditioned with methanol and equilibrated with cold distilled water. Adsorbed angiotensins were eluted with methanol. The methanol was evaporated under vacuum rotation at 4°C. Angiotensins were separated by reversed-phase high-performance liquid chromatography using a $\mu$Bondapak C18 column. The concentrated SepPak extracts were dissolved in mobile phase and centrifuged at 10,000g before injection. Elution was performed as follows: 85% mobile phase A and 15% mobile phase B from 0 to 5 minutes, followed by a linear gradient to 40% mobile phase A and 60% mobile phase B for 20 minutes. Eluates were collected in 0.5-ml fractions in polypropylene tubes containing bovine seroalbumin. Fractions containing angiotensin II and angiotensin-(1-7) were neutralized with 1 mol/L sodium hydroxide. Angiotensin II was quantified by radioimmunoassay using an angiotensin II antibody kindly donated by Dr. A.H.J. Danser (Erasmus University, Rotterdam, The Netherlands) with coefficients of variation for inter- and intra-assays of 13% and 6.5%, respectively. The lower limit of detection was 0.4 fmol/fraction for the angiotensin II assay. Angiotensin II-(1-7) levels were determined by radioimmunoassay using a polyclonal antibody developed by us in rabbits using conventional methods. The coefficients of variation for interassay and intra-assay variances were 4.3% and 12% for angiotensin-(1-7), respectively. The range of detection using standards of angiotensin-(1-7) was between 0.92 and 184 pg/ml. Results are shown as mean ± SEM. Statistical comparisons between the 2 groups were performed using Student's *t* test for independent measurements or the Mann-Whitney test. A p value $\leq 0.05$ was considered statistically significant.

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Eighteen homozygous patients with hypertension (9 with the II-ACE genotype and 9 with the DD-ACE

genotype) who did not receive treatment were consecutively evaluated. In all patients, hypertension was detected during the previous 6 months. Both groups were similar in terms of age; gender distribution; weight; renal and hepatic function; and serum cholesterol, glucose, potassium, hematocrit, and plasma renin levels (Table 1). Systolic and diastolic BPs were similar in both groups (Table 1).

As predicted by their genotypes, plasma ACE activity was significantly increased in the patients having the DD genotype compared with patients having the II-ACE phenotype (22.2 ± 2.9 vs 15.5 ± 1.2 U/ml, respectively, $p < 0.05$). Plasma angiotensin II levels were 69.6 ± 8.4 pg/ml in the homozygous patients with hypertension who had the DD-ACE genotype and were similar in the patients with hypertension who had the II-ACE genotype (Figure 1). Plasma levels of angiotensin-(1-7) were 14.5 ± 3.3 pg/ml in the homozygous patients with hypertension having the DD-ACE genotype and were 4 times higher ($p < 0.05$) in the patients with hypertension having the II-ACE genotype (Figure 2).

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The main finding of this investigation is that patients with hypertension who have the II-ACE genotype (lower ACE activity) have much higher circulating levels of angiotensin-(1-7) than do those patients with hypertension who have the DD-ACE genotype (and higher ACE activity). Plasma levels of angiotensin II are similar in both hypertensive groups, as has been reported in normotensive subjects.[9,13] In humans, the presence of the D allele of the ACE polymorphism is associated with higher ACE levels and a shorter plasma half-life of bradykinin.[11,12] In normotensive subjects, however, no difference in levels of renin, angiotensin II, or aldosterone—or in the conversion from angiotensin I to angiotensin II—have been found.[9,13] An additional proposed mechanism that could explain the observed association of the D allele with hypertension[7–9] is the current finding of much lower angiotensin-(1-7) levels in patients with hypertension who have the DD-ACE genotype (and higher plasma ACE activity) compared with the homozygous patients with hypertension who have the II-ACE genotype.

This study has some limitations related to the small number of patients, but the difference in the levels of angiotensin-(1-7) was 4 times between both hypertensive groups. We did not perform studies on angiotensin-(1-7) metabolism in these patients, which may have helped to better explain our findings.


**1.** Ferrario CM. Does angiotensin-(1–7) contribute to cardiac adaptation and preservation of endothelial function in heart failure? *Circulation* 2002;105:1523–1525.

**2.** Santos R, Campagnole-Santos M, Andrade S. Angiotensin-(1-7): an update. *Regul Pept* 2000;91:45–62.

**3.** Heitsch H, Brovkovych S, Malinski T, Wiener G. Angiotensin-(1-7)-stimulated nitric oxide and superoxide release from endothelial cells. *Hypertension* 2001;37:72–76.

**4.** Ferrario CM. Angiotensin I, angiotensin II, and their biologically active peptides. *J Hypertens* 2002;20:805–807.

**5.** Chappell M, Pirro N, Sykes A, Ferrario CM. Metabolism of angiotensin-(1-7) by angiotensin converting enzyme. *Hypertension* 1998;31:362–367.

**6.** Cambien F, Costerousse O, Tiret L, Poirier O, Lecerf L, Gonzales MF, Evans A, Arveiler D, Cambou JP, Luc G. Plasma level and gene polymorphism of angiotensin-converting enzyme in relation to myocardial infarction. *Circulation* 1994;90:669–676.

**7.** O'Donnell CJ, Lindpaintner K, Larson MG, Rao VS, Ordovas JM, Schaefer EJ, Myers RH, Levy D. Evidence for association and genetic linkage of the angiotensin-converting enzyme locus with hypertension and blood pressure in men but not women in the Framingham Heart Study. *Circulation* 1998;97:1766–1772.

**8.** Higaki J, Baba S, Katsuka T. Deletion allele of angiotensin-converting enzyme gene increases risk of essential hypertension in Japanese men: the Suita Study. *Circulation* 2000;101:2060–2065.

**9.** Danser AHJ, Schunkert H. Renin-angiotensin system gene polymorphisms: potential mechanisms for their association with cardiovascular diseases. *Eur J Pharmacol* 2000;410:303–316.

**10.** Ocaranza MP, Piddo AM, Faúndez P, Lavandero S, Jalil JE. Angiotensin I-converting enzyme gene polymorphism influences chronic hypertensive response in the rat Goldblatt model. *J Hypertens* 2002;20:413–420.

**11.** Brown NJ, Blais C Jr, Gandhi SK. ACE insertion/deletion genotype affects bradykinin metabolism. *J Cardiovasc Pharmacol* 1998;32:373–377.

**12.** Murphey LJ, Gainer JV, Vaughan DE, Brown NJ. Angiotensin–converting enzyme insertion/deletion polymorphism modulates the human in vivo metabolism of bradykinin. *Circulation* 2000;102:829–832.

**13.** Lachurié ML, Azizi M, Guyene TT, Alhenc-Geles F, Ménard J. Angiotensin converting enzyme gene polymorphism has no influence on the circulating renin-angiotensin-aldosterone system or on blood pressure in normotensives. *Circulation* 1995;345:2933–2942.

**14.** Jalil JE, Piddo AM, Cordova S, Chamorro G, Braun S, Jalil R, Vega J, Jadue L, Lavandero S, Lastra P. Prevalence of the angiotensin I-converting enzyme insertion/deletion polymorphism, plasma ACE activity and left ventricular mass in normotensive Chilean population. *Am J Hypertens* 1999;12:697–704.

**15.** Jalil JE, Ocaranza MP, Piddo AM, Jalil R. Reproducibility of plasma angiotensin-converting enzyme activity in human subjects determined by fluorimetry with Z-phenylalanine-histidyl-leucine as substrate. *J Lab Clin Med* 1999; 133:501–506.

**16.** Danser AH, van Kats JP, Admiraal PJ, Derkx FH, Lamers JM, Verdouw PD, Saxena PR, Schalekamp MA. Cardiac renin and angiotensins: uptake from plasma versus in situ synthesis. *Hypertension* 1994;24:37–48.

**17.** Admiraal PJ, Derkx FH, Danser AH, Pieterman H, Schalekamp MA. Metabolism and production of angiotensin I in different vascular beds in subjects with hypertension. *Hypertension* 1990;15:44–55.