Angiotensin-Converting Enzyme Gene Insertion/Deletion Polymorphism and Left Ventricular Hypertrophy in Hemodialysis Patients

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SUMMARY

The relationships between angiotensin-converting enzyme (ACE) gene insertion (I) / deletion (D) polymorphism and left ventricular hypertrophy induced by hypertension or idiopathic hypertrophic cardiomyopathy have been studied. However, little is known about the association between this polymorphism and left ventricular hypertrophy induced by volume overload.

The relationship between left ventricular hypertrophy and the ACE gene I/D polymorphism was examined in 80 maintenance hemodialysis patients (mean age: 60.1 ± 1.4 <SEM> years).

Multivariate regression analysis showed that the left ventricular mass index calculated by M-mode echocardiography was associated with serum creatinine ($p = 0.040$), male gender ($p = 0.027$), antihypertensive drug treatment ($p = 0.026$), weight gain between hemodialysis ($p = 0.018$) and mean blood pressure after hemodialysis ($p = 0.010$), but not with ACE I/D genotype ($p = 0.69$).

These findings suggest that although hemodialysis patients seem to be under volume overload, ACE genotype may not be involved in their left ventricular hypertrophy. Hypertension and other factors related to renal failure are involved in the left ventricular hypertrophy in chronic hemodialysis patients. (Jpn Heart J 1997; 38: 821-830)

Key words: ACE gene polymorphism, Hypertrophy, Left ventricular, Echocardiography, Left ventricular mass, Hemodialysis

EPIDEMIOLOGICAL studies have shown that left ventricular hypertrophy (LVH) is a major predictor of death from cardiovascular diseases.^{1,2} Fur-

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thermore, among maintenance hemodialysis (HD) patients, LVH is common because of volume overload and hypertension resulting from renal disease.^{3,4)} Heart diseases account for approximately 40% of all causes of death in maintenance HD patients.^{3,5)} Therefore, LVH seems to be a very important factor that worsens their prognosis.

The renin-angiotensin system has been shown to be an important system that regulates blood pressure and homeostasis of body fluid, and abnormalities in the system may be involved in the development of cardiovascular diseases. Recently, several gene polymorphisms, such as the ACE, angiotensinogen and angiotensin II type 1 receptor genes, have been reported to be associated with cardiovascular disorders by many investigators, including our group.⁶⁾ In particular, since Cambiens *et al.* reported the association of this ACE gene polymorphism with the development of myocardial infarction,⁷⁾ ACE gene I/D polymorphism has been shown to be associated with not only ischemic heart disease^{8,9)} but also LVH,^{10,11)} cardiomyopathy,¹²⁾ cerebral infarction,¹³⁾ diabetic nephropathy¹⁴⁾ and restenosis after PTCA.¹⁵⁾ However, opposite findings have also been reported.^{16,17)}

Although there have been many studies examining the relationship between ACE gene polymorphism and LVH, the subjects were from general populations free of advanced renal failure. Studies in patients who are under volume overload, such as HD patients, have never been studied. Compared to hypertensive patients with LVH, maintenance HD patients are exposed to prominent volume overload because of renal failure and arterio-venous fistula.¹⁸⁾ The involvement of the ACE gene polymorphism in LVH may be different in these patients from those with LVH induced by pressure overload. In order to study the involvement of ACE gene polymorphism in volume overload induced LVH, we examined it in maintenance HD patients and attempted to identify factors which are involved in LVH in these patients.

SUBJECTS AND METHODS

Patient population: The subjects were randomly selected from patients who had been under maintenance HD. Patients who had a history of myocardial infarction, valvular heart disease or dilated cardiomyopathy were excluded from the study. Eighty patients, aged from 40 to 72 years old, were subsequently enrolled in the study. Informed consent was obtained after thorough explanation of the purpose and protocol of the study. Causative renal diseases included chronic glomerulonephritis (29%), diabetic nephropathy (38%) and nephrosclerosis (13%). History of hypertension was positive in 58 of the 80 patients and 33 were under antihypertensive treatment. The duration of HD was

from 3.7 to 8.0 years (mean 6.2 years).

The patients underwent HD 3 times a week, each performed for approximately 3 hours. Blood pressure was measured with a sphyngomanometer in a supine position. A standard 12-lead electrocardiogram (ECG), chest X-ray film and echocardiogram were taken before HD.

Echocardiography: Echocardiography was performed using a SSH140A system with a 2.5 MHz or 3.75 MHz probe (Toshiba Medical Corp, Tokyo) just before HD.

Under 2-dimensional guidance, LVM was calculated from M-mode echocardiographic measurements of left ventricular diastolic diameter (LVDd), interventricular septum (IVSd) and left ventricular posterior wall thickness (LVPWd) at end-diastole, according to the recommendations of the American Society of Echocardiography. The formula of Devereux and Reichek¹⁹⁾ was used to estimate LVM as follows:

$$\text{LVM(g)} = 1.04[(\text{LVDd} + \text{IVSd} + \text{LVPWd})^3 - (\text{LVDd})^3] - 13.6.$$

Body surface area (BSA) was estimated based on the Fujimoto and Watanabe equation:

$$\text{BSA (m}^2\text{)} = \text{weight}^{0.444} \times \text{height}^{0.663} \times 88.83 - 10^4.$$

A LVM index (LVMI) was obtained by dividing LVM by body surface area. As mentioned above, when valvular stenosis or regurgitation was found by 2-dimensional echocardiography or Doppler echocardiography in the mitral valve or aortic valve, the patient was excluded from the study.

Genotype determination: Approximately 7 ml of blood were collected into a cold test tube containing EDTA from a dialysis circuit during HD. DNA was extracted using a DNA extraction reagent kit, DnaQuick (Dainippon Pharmaceutical Co., Ltd., Tokyo). ACE genotype was determined using the PCR methods according to a protocol reported previously.²⁰⁾ The sense oligonucleotide primer was 5'-GAT GTG GCC ATC ACA TTC GTC AGAT-3' and the antisense primer 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3'. The reaction was performed using 25 pmol primer, 1.5 mmol/l MgCl₂, 50 mmol/l KCl, 10 mmol Tris-HCl (pH 8.3), 0.1 mg/ml gelatin, 0.5 mmol dNTP and 2.5 U Taq polymerase. To prevent mistyping, 5% DMSO was added to this mixture. Amplification was carried out in a DNA Thermal Cycler (PJ2400, Perkin-Elmer Corp., Norwalk, Conn, USA) for 30 cycles of denaturation at 93°C for 1 minute, annealing at 58°C for 1 minute, and extension at 72°C for 1 minute. DNA was visualized after electrophoresing the PCR products on 1.6% agarose gels and staining with ethidium bromide. ACE polymorphism was demonstrated by the presence of a 190-bp fragment, deletion (D) allele, and a 490-bp fragment, inser-

tion (I) allele. Finally, genotypes were determined in 80 of 88 cases from whom high quality echocardiograms had been obtained.

Statistical Analysis: The following factors which may be related to LVMI were examined: gender, age, history of hypertension, the presence or absence of antihypertensive drug treatment, duration of HD, causative renal disease, blood pressure, heart rate, average weight gain between each dialysis, average fluid depletion during dialysis, electrocardiographic findings, cardiothoracic ratio (CTR), laboratory data (serum concentrations of Na, K, Cl, Ca, P, total protein, blood urea nitrogen, creatinine and uric acid, and hematocrit) and ACE genotype.

ANOVA methods were used with StatView 4.11 software for Macintosh to compare LVMI among subgroups according to ACE genotype. Multiple linear regression analysis methods were used with SPSS for Macintosh to analyze LVMI precisely.

RESULTS

Chronic glomerulonephritis and diabetic nephropathy were the two major causative renal diseases, as is consistent with the prevalence reported in Japan.²¹⁾ Thirty-three of 58 patients who had a history of hypertension were taking antihypertensive drugs. No significant differences in the distribution of ACE genotype were found in relation to gender, history of hypertension or causative renal disease.

In the analyses of variance of LVMI among ACE genotype subgroups,

Table I. Left Ventricular Mass Index in ACE Genotypes and Clinical Subgroups

Subjects	ACE genotype					p
	Total	DD	ID	II		
Total subject population	188.5 ± 6.7 (80)	183.4 ± 13.3 (8)	193.5 ± 12.5 (30)	185.9 ± 8.9 (42)		0.84
History hypertension						
without hypertension	169.8 ± 10.5 (22)	186.0 (1)	182.6 ± 27.0 (7)	162.1 ± 9.9 (14)		0.18
with hypertension	195.7 ± 8.2 (58)	183.1 ± 15.3 (7)	196.9 ± 14.4 (23)	197.8 ± 11.8 (28)		0.75
Antihypertensive drugs						
no	173.6 ± 7.5 (47)	195.9 ± 19.5 (4)	179.2 ± 16.1 (15)	167.4 ± 8.8 (28)		0.52
yes	209.9 ± 11.4 (33)	171.0 ± 18.3 (4)	208.0 ± 19.0 (15)	223.0 ± 16.3 (14)		0.38
Gender						
male	197.4 ± 7.9 (57)	197.0 ± 12.9 (5)	211.6 ± 16.7 (19)	193.8 ± 10.1 (33)		0.82
female	166.6 ± 11.6 (23)	148.3 ± 27.7 (2)	176.8 ± 18.4 (12)	157.2 ± 16.2 (9)		0.67
Weight gain						
above or equal to median	203.3 ± 9.7 (44)	198.3 ± 18.0 (4)	215.2 ± 18.2 (15)	196.9 ± 13.0 (25)		0.69
below median	170.5 ± 8.1 (36)	168.5 ± 18.8 (4)	171.8 ± 16.0 (15)	169.8 ± 9.8 (17)		0.99

Values are means ± SEM. The number of patients in each subgroup is shown in parentheses. The median value for weight gain between hemodialysis is 1.7 kg. HD = hemodialysis; DD = deletion/deletion; ID = insertion/deletion; II = insertion/insertion.

Table II. Multivariate Analysis of LVMI

Variable	B	SEB	Beta	T	p
Age	-0.063	0.572	-0.013	-0.109	0.91
ACE genotype	-3.797	9.343	-0.043	-0.406	0.69
Serum creatinine	-4.976	2.381	-0.253	-2.09	0.04*
Hematocrit	-0.411	1.503	-0.027	-0.274	0.79
HD duration	0.275	1.266	0.024	0.217	0.83
Antihypertensive drugs	28.369	12.467	0.235	2.276	0.03*
Gender	32.667	14.437	0.249	2.263	0.03*
Weight gain	22.844	9.419	0.245	2.425	0.02*
Mean BP after HD	1.541	0.582	0.307	2.647	0.01*
(Constant)	42.338	94.746		0.447	0.66

LVMI; regression df = 9, F = 3.803, p = 0.0006. ACE = angiotensin converting enzyme; HD = hemodialysis; BP = blood pressure. *p < 0.05.

there were no significant differences in LVMI among the entire subject population or among the clinical subgroups (Table I). However, when LVMI was analyzed in clinical subgroups ignoring the ACE genotype, there were significant differences in LVMI between males and females ($p = 0.04$), between the presence and absence of antihypertensive drug treatment ($p = 0.007$) and between above or equal to and below median value of weight gain during each hemodialysis interval ($p = 0.01$). When analyzed by multiple linear regression analysis, LVMI exhibited a significant relationship with serum creatinine, male gender, the presence of antihypertensive drug treatment, averaged weight gain between two successive HDs and mean blood pressure after HDs, but not with age, ACE genotype, hematocrit or duration of HD (Table II). Although we also performed other multiple linear regression analyses which took into consideration ACE genotype, history of hypertension, clinical characteristics, laboratory data, CTR and HD data as independent variables, LVMI was found to have no definite relationship with any.

DISCUSSION

LVH is an important predictor for cardiovascular disease and sudden death in the general population.²²⁻²⁴ Various studies, including the Framingham Study,^{25,26} have shown that the development of ischemic heart disease and cerebral infarction²⁷ is more frequent in patients with LVH than those without. Morbidity and mortality after myocardial infarction²⁸ are also greater in patients with LVH. There have been many reports concerning the relationship between ACE polymorphism and LVH. Schunkert *et al.* reported an association between ACE polymorphism and LVH identified by ECG in a population-based study.¹⁰ Iwai *et al.* reported a positive relationship in an out-patient clinic population using

echocardiography.¹¹⁾ However, little is known about the relationship between ACE gene polymorphism and LVH in which volume overload may be involved. Thus, we examined the relationship between ACE gene polymorphism and LVH in 80 HD patients to assess the involvement of this gene in LVH caused, in part, by volume overload. Previous experimental studies have demonstrated that LVH induced by an aortocaval shunt increased cardiac as well as circulating angiotensin II levels in rats. Moreover, this cardiac hypertrophy and increased angiotensin II were prevented by treatment with an ACE inhibitor or the angiotensin II receptor blocker losartan.^{29,31)} These studies suggest the involvement of the renin-angiotensin system in LVH induced by volume overload as well as in that induced by pressure overload.^{32,33)} However, we found no significant relationship between ACE gene polymorphism and LVH in HD patients when the relationship was analyzed using a multivariate analytical method.

Several reasons may explain the lack of a significant relationship between LVH and ACE gene polymorphism in HD patients. Firstly, in contrast to LVH seen in essential hypertension, LVH in HD patients seems to be attributable to multiple determinant factors, i.e., pressure-overload, volume-overload and other factors which are closely related to renal failure. Hypertension which had existed before the development of renal failure and volume-overload which was associated with renal failure must contribute to LVH to various degrees in HD patients. Furthermore, it is known that the renin-angiotensin system is inappropriately activated in hypertensive patients with renal disease^{34,35)} and that sympathetic nerve activity in patients with renal failure assessed by microneurography is extremely enhanced.³⁶⁾ Thus, the complicated involvement of many factors in hypertension and LVH may have made it difficult to assess the contribution of ACE gene polymorphism to LVH.

Although the multivariate analysis did not demonstrate a significant contribution of the ACE gene to LVH, LVH was associated with serum creatinine, male gender, antihypertensive drug treatment, weight gain between two successive HDs and mean blood pressure after HD. The significant contribution of antihypertensive drug treatment and also elevated mean blood pressure after HD to LVH seems to be consistent with the concept that hypertension is an important factor of LVH in HD patients.^{37,38)} As demonstrated in patients with essential hypertension,³⁹⁾ LVH was also significantly associated with male gender in the present study. Although the reason is not clear, this may reflect the greater prevalence of hypertension in males in the general population.^{40,41)} Also, the difference in gonadism may be a cause since testosterone is known to promote protein synthesis in cardiac myocytes.

In this study, we included patients who had antihypertensive drug treatment that might reduce LVMI. However, even these patients had higher LVMI and

were found to be associated with LVMI in multiple regression analysis. This is because these patients may be under larger pressure overload than those who did not need to take antihypertensive drugs. This finding also suggests that the HD patient is not merely a simple volume overload model, but also a multifactorial one.

LVH is very important in predicting prognosis among patients with chronic renal failure. In 1995, the Japanese Society for Dialysis Therapy conducted a statistical survey in Japan and received replies from 2866 facilities (99.82%).²¹⁾ The survey found a gross annual mortality rate of 9.7%, of which 25.4% was from heart failure, 13.5% from cerebrovascular accident and 7.5% from myocardial infarction. In other words, the cause of death in 46.4% of the cases was either a cardiovascular or a cerebrovascular event. Several studies have shown that show LVH is strongly associated with cardiovascular or cerebrovascular events in chronic renal failure patients.⁴³⁻⁴⁵⁾ Therefore, it is very important to investigate the cause of LVH and prevent it. According to the present findings, average weight gain between each dialysis was significantly associated with LVH. Thus, for the prevention of LVH in dialysis patients, not only medical treatment but also a modification of life style is of great importance.

The present study has some limitations. First, the sample volume was relatively small and the D/D genotype accounted for only 10%, which might create some bias in subjects. However, the relatively low frequency of the D/D genotype might be due to ethnic differences.⁴⁶⁾ Secondly, we did not use magnetic resonance imaging or electrocardiography but rather echocardiography to calculate left ventricular mass. Estimating left ventricular mass using electrocardiography is thought to be somewhat unreliable⁴⁷⁾ in comparison with the other two methods. At present, although magnetic resonance imaging is thought to produce the highest quality LVMI assessment, it is complicated and expensive. However, since we excluded myocardial infarction and valvular heart disease in order to minimize the estimation error for left ventricular mass, our estimation of left ventricular mass may be adequate.

In conclusion, the present findings support the concept that the HD patient can be not only a volume-overload but also a pressure-overload model because antihypertensive drug administration and weight gain between each hemodialysis influence left ventricular mass in these patients. However, ACE gene polymorphism has little association with left ventricular hypertrophy in these patients, suggesting that this polymorphism is not involved in left ventricular hypertrophy in HD patients.

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