Is ACE Gene Polymorphism Associated with Lone Atrial Fibrillation?

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SUMMARY

ACE (angiotensin converting enzyme) gene genotypes have been shown to be a risk factor for development of left ventricular hypertrophy and cardiomyopathy, upon the assumption that the DD genotype is linked to higher cellular ACE activity leading to myocardial fibrosis. To test an analogous hypothesis that the DD genotype favors myocardial fibrosis in the atrium and thus promotes atrial fibrillation without any structural heart diseases, we determined the distribution of the ACE gene genotypes in 77 patients with lone atrial fibrillation and investigated the effects of the ACE genotypes on the clinical characteristics of atrial fibrillation. The distribution of ACE genotypes was not significantly different between the patients and healthy volunteers. Also, the ACE gene genotypes did not affect the types of atrial fibrillation and the age at the onset of atrial fibrillation. Thus, these results refuted the hypothesis of possible relationships between ACE genotypes and lone atrial fibrillation through myocardial fibrosis, and indicated some unknown differences between the atrium and ventricle. (Jpn Heart J 1997; 38: 637–641)

Key words: Atrial fibrillation, ACE gene polymorphism

SYSTEMIC and/or local activities of the renin-angiotensin system have been reported to play an important role in cardiac remodeling and fibrosis, since angiotensin II is one of the potent stimulants of both cardiac myocytes and fibroblasts.1,2) Some of the components of the renin-angiotensin system are also synthesized in the heart, and increased expression of these components is reported to result in progression of cardiac diseases.3)

In human hearts, because ACE (angiotensin converting enzyme) is the predominant pathway for angiotensin II formation, its higher local activity is believed to promote cardiac remodeling, including myocardial fibrosis.1-3) Cloning

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Received for publication April 2, 1997.
Accepted May 14, 1997.
of human ACE cDNA and restriction fragment length polymorphism analysis identified an insertion/deletion (I/D) polymorphism of the ACE gene that consists of the presence (I) and absence (D) of a 287 bp DNA fragment located in intron 16. Although the ACE gene polymorphism is probably only a neutral marker, DD genotype is known to be linked to higher levels of cellular ACE. In this context, the DD genotype has been shown to be a risk factor for the development of cardiomyopathy and cardiac hypertrophy, possibly through myocardial fibrosis in the ventricle. In the present study, to test an analogous hypothesis that the DD genotype might favor myocardial fibrosis also in the atrium leading to atrial degenerative changes, the distribution of the ACE genotype polymorphism was determined in patients with lone atrial fibrillation (Af), in which fibrotic degenerative changes have been pathologically demonstrated.

**METHODS**

**Study population:** Seventy-seven patients with lone Af (41 with paroxysmal and 36 with chronic type, 65 ± 14 years old) and 83 healthy volunteers (64 ± 15 years old) were included in the present study. All subjects were free from hypertension and symptoms suggestive of coronary heart diseases. Echocardiography excluded the existence of left ventricular hypertrophy and valvular heart diseases in the patients with atrial fibrillation. Age at the onset of Af was determined from the history. Thirty-two patients with chronic Af were taking digoxin, verapamil or β blocker, and 39 patients with paroxysmal Af were taking class I antiarrhythmic drugs (disopyramide, flecainide, pilsicainide, aprindine).

**DNA studies:** Genomic DNA was prepared from peripheral blood leukocytes as previously reported. The genotype of the ACE gene was determined by the polymerase chain reaction (PCR) according to the report by Rigat et al. The sense oligonucleotide primer was 5'-CTGGAGACCACTCCCATCCTTTCT-3', and the antisense primer was 5'-GATGTGGCCATCACATTCGTCAGAT-3'. These primers allowed detection of a genomic DNA segment with 490 bp corresponding to the insertion allele (I) as well as a segment with 190 bp corresponding to the deletion allele (D). PCR reactions were performed in a final volume of 50 µl containing 50 pmol of each primer, 2.5 mmol/l MgCl₂, 50 mmol/l KCl, 10 mmol/l Tris-HCl, 0.2 mmol/l of each dNTP, and 0.5 U of Taq DNA polymerase. The amplification condition was initial denaturation at 94°C for 2 min and 35 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec and extension at 72°C for 60 sec. The PCR products were resolved in 2% agarose gels and visualized with ethidium bromide staining.

**Statistical analysis:** Differences in the distribution of the DD, DI and II genotypes were assessed by contingency table/chi-square analysis. Numeric data be-
ACE GENOTYPES AND ATRIAL FIBRILLATION

RESULTS

**ACE gene genotypes:** ACE genotypes could be determined in all of the subjects. ACE genotype distribution and allele frequencies were not different between patients with AF and control subjects (DD/DI/II: 11/37/29 in AF vs 13/40/30 in control; D/I allele frequencies: 0.38/0.62 in AF vs 0.40/0.60 in control, N.S.). Also this distribution was compatible with the previous result from Japan.11) **Effects of ACE gene genotypes on atrial fibrillation:** We compared the clinical characteristics of patients with AF among the ACE gene genotypes (Table). In the present study, the patients with the D allele tended to be younger than those with the I allele, possibly because of early death of individuals with the DD genotype as previously suggested,12) but the difference was not statistically significant. The types of atrial fibrillation (chronic/paroxysmal) were not significantly different among the ACE gene genotypes. Age at the onset of AF tended to be younger in DD than in DI and II groups, but it was not significantly different among the genotype groups, although this tendency might have been resulted from differences in patient ages in the present study.

DISCUSSION

After many investigators have reported specific relations between ACE gene polymorphism and cardiovascular diseases (left ventricular hypertrophy and idiopathic dilated cardiomyopathy),2-4) several mechanisms are under investigation. One of the speculated mechanisms is myocardial fibrosis: higher levels of angiotensin II associated with the DD genotype stimulate cardiac fibroblasts, resulting in an increase of extracellular matrix that would lead to progression of the diseases.5-7) This speculation leads to a hypothesis that the DD genotype favors
myocardial fibrosis. If this view holds true also with the atria, it is conceivable that fibrotic degeneration, which has been pathologically demonstrated in Af, is causative of Af or a progressive factor of Af in a considerable number of patients with DD genotype. This hypothesis predicts that the distribution of the D allele would be increased in patients with Af and without any structural heart diseases. However, this seemed not to be the case from the present result. The distribution of the ACE genotypes was similar between patients with lone Af and healthy volunteers. Also, because the types of Af (paroxysmal/chronic) did not differ among the ACE gene genotypes, the ACE gene genotypes seemed not to be related to progression of Af. Rather, our study questioned the speculated relationship between ACE genotypes and myocardial fibrosis in lone Af, while it might indicate some unknown differences between the atrium and ventricle. And, although the patients with Af were excluded from the study population in many previous reports that investigated the relationships between the ACE genotypes and cardiovascular diseases,\(^5\text{-}^7\) the present result indicates no necessity for the exclusion.

However, we should recognize that Af does not result only from myocardial fibrosis in the atrium. Because Af in itself causes various changes in the atrium, including electrical remodeling,\(^1^3\) the pathological changes such as fibrosis or fatty changes could be the result of atrial fibrillation. In this respect, to determine the relationships between atrial myocardial fibrosis and ACE gene polymorphism, it would be preferable to examine the distribution of ACE gene polymorphism in patients with atrial fibrillation without Af. Atrial standstill may be a candidate disease, but this patient population is small and inappropriate for epidemiological study. Therefore, because the present result is limited to lone atrial fibrillation, the relationship between ACE gene polymorphism and atrial fibrosis requires further study.

REFERENCES

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