

Angiotensin Converting Enzyme Gene Polymorphism and Its Enzyme Activity in Serum in Young Japanese Females

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TSUTAYA, S., KITAYA, H., SAITO, Y., NAKATA, S., TAKAMATSU, H. and YASUJIMA, M. *Angiotensin Converting Enzyme Gene Polymorphism and Its Enzyme Activity in Serum in Young Japanese Females*. Tohoku J. Exp. Med., 1997, **182** (2), 151-155 — To assess the potential association between the angiotensin converting enzyme (ACE) gene polymorphism and the activity of the renin-angiotensin system in a Japanese population, we determined the ACE genotype and its enzyme activity in serum in 108 young Japanese females. Genomic DNA was extracted from blood samples and amplified by polymerase chain reaction (PCR). PCR primers flanked the polymorphic region in intron 16 of the ACE gene. The distribution of the DD, ID and II ACE genotypes was 10, 55 and 35%, respectively. The estimated allele frequencies of the deletion and the insertion were 0.375 and 0.625, respectively. The mean serum ACE activity in DD subjects was about 1.4 times that of II subjects ($p < 0.01$), with ID subjects having intermediate levels ($p < 0.05$), whereas the renin profile were not statistically different among the three groups. These results indicate a significant association between ACE gene polymorphism and serum ACE activity levels, suggesting a mechanism by which genotype might have a bearing on the physiology of the renin-angiotensin system axis, ———— angiotensin converting enzyme gene; genetics; serum angiotensin converting enzyme activity; renin-angiotensin system

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Angiotensin converting enzyme (ACE) plays a key role in the production of angiotensin II and in the catabolism of bradykinin (Erdös and Skidgel 1987), two peptides involved in the modulation of vascular tone and in the proliferation of smooth muscle cells (Ballerman et al. 1991). Rigat et al. (1990) reported an insertion/deletion (I/D) polymorphism in intron 16 of ACE human gene. There is emerging evidence that the deletion variant of the gene confers increased risk of cardiovascular diseases (Cambien et al. 1992; Schunkert et al. 1994; Mattu et

Received March 13, 1997; revision accepted for publication April 30, 1997.

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al. 1995) and also contributes to the development of renal diseases (Marre et al. 1994; Harden et al. 1995). In addition, many reports have shown that the circulating ACE levels are determined partially genetically (Cambien et al. 1988; Costerousse et al. 1993). Thus, these observations are thought to be dependent on the effects of ACE in regulating angiotensin II and kinin levels and on the opposite effects of these peptides on the target tissues or cells.

Therefore, in the present study we evaluated the relationship between ACE genotypes and serum ACE activity in a group of young females considered to be relatively homologous as a subject population.

MATERIALS AND METHODS

Hundred and eight healthy female students of Japanese origin aged 18–22 (19.9 ± 1.1) years were selected for the study in a School of Allied Medical Sciences, Hirosaki University. Inclusion was based both on clinical characteristics and routine laboratory tests performed at our division of laboratory medicine. After an overnight fast and a bedrest for at least 30 minutes, blood samples were taken for DNA studies and to determine serum ACE activity and plasma renin activity with informed consent.

Genomic DNA was extracted from whole blood using a DNA extraction kit, SMI TEST^R (Sumitomo Bio-Medical, Kashima). The genotype of the ACE was determined by polymerase chain reaction according to Rigat et al. (1992). Using extracted DNA, a 287 base pair (bp) insertion or deletion polymorphism in intron 16 of the ACE gene was identified by the polymerase chain reaction method. Polymerase chain samples were separated electrophoretically on 2% agarose gels and DNA was visualized by ethidium bromide staining. ACE gene polymorphism was classified into three genotypes; the 479 bp insertion homozygous (II) genotype, the 192 bp deletion homozygous (DD) genotype and 479 bp insertion 192 bp deletion heterozygous (ID) genotype. To avoid mistyping of the ID genotype, an additional polymerase chain reaction was performed in samples classified as DD, with a set of primers flanking only the insertion allele, as reported by Shanmugam et al. (1993). Serum ACE activity was measured in duplicate by a colorimetric method based on the quininemine dye produced from the substrate *p*-hydroxyhippuryl-L-histidyl-L-leucine (Kasahara and Ashihara 1981). Plasma renin activity was radioimmunologically determined with a commercial kit (Dainabot Pharmaceutical Company, Tokyo).

Data are given as mean and standard deviation (s.d.). The differences among groups were analyzed by Student's *t*-test. $p < 0.05$ was considered statistically significant.

RESULTS

ACE genotypes were determined for all subjects (Table 1). The distribution of the DD, ID and II ACE genotypes was 10, 55 and 35%, respectively. The

TABLE 1. *ACE genotype distribution and allele frequency in 108 young Japanese females*

Number	Genotypes			Alleles	
	II	ID	DD	I	D
108	38	59	11	0.625	0.375
(%)	(35)	(55)	(10)		

estimated allele frequencies of the deletion and the insertion were 0.375 and 0.625, respectively. When mean serum ACE activities were compared among the three groups defined by the ACE genotypes, the present results show a significant relationship between the genotypes and the activities of ACE in the serum, with an additive effect of the alleles (DD 17.2 ± 3.2 IU/liter, ID 15.1 ± 3.4 IU/liter and II 12.1 ± 3.3 IU/liter, respectively; $p < 0.05$ DD vs. ID, $p < 0.01$ DD vs. II and $p < 0.01$ ID vs. II, respectively) (Fig. 1). In contrast, plasma renin activity was not statistically different among the three groups (DD 1.3 ± 1.3 ng/ml/hr, ID 0.9 ± 0.8 ng/ml/hr and II 0.9 ± 0.8 ng/ml/hr, respectively).

DISCUSSION

In the young female population of a northern area in Japan we found allele frequencies of 0.375 and 0.625 for the D and I alleles respectively, with genotype frequencies of 10, 55 and 35% for DD, ID and II, respectively. In addition, in the present study, the D allele is significantly associated with elevated ACE activity. Although in healthy Japanese many studies on ACE gene frequencies have been performed, the reported DD genotype frequencies from Japan are not entirely in agreement. Our present finding on DD genotype frequency of 10% are consistent

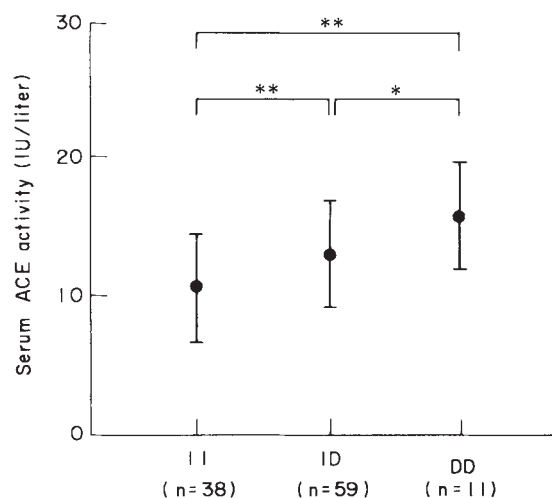


Fig. 1 Serum ACE activities for individuals with the II, ID and DD genotypes shown in left, middle and right panels, respectively. Solid vertical bars indicate mean values and standard deviation for each group. * $p < 0.05$, DD vs. ID; ** $p < 0.01$, DD vs. II; ** $p < 0.01$, ID vs. II

with the study by Morise et al. (1994), whereas those by Ohishi et al. (1994) and Nakai et al. (1994) actually report DD genotype frequencies of 19 and 26% respectively in their control populations. These differences may represent problems in genotyping, recruitment bias or true intra-ethnic differences. Mistyping of the heterozygotes could be caused by preferential amplification of the deletion allele over the insertion allele. To avoid this problem, we used an allele-specific amplification method and have found no examples of mistyping to date. It is also conceivable that the variation in the DD frequency found in Japanese could be caused by the effects of migration and population interbreeding. However, at the moment, we have no evidence to confirm this hypothesis.

Circulating ACE probably originates from the vascular endothelial cells (Erdös and Skidgel 1987), however the biochemical mechanism of ACE secretion is not clear. Whatever the mechanism involved, it is likely that the significant association between the D allele and elevated ACE activity in serum indicates the genetic control of serum ACE level as reported previously (Cambien et al. 1988; Costerousse et al. 1993), presumably exerting at the transcriptional level. In that case, the insertion/deletion itself may not play a direct role in controlling ACE transcription but is more likely to be in linkage disequilibrium with transcriptionally regulatory elements of the ACE gene (Rigat et al. 1990). More recent study by Villard et al. (1996) has identified new polymorphism of ACE gene that might have a functional role in determining serum ACE levels. In several tissues considerable compartmentation of angiotensin II has been noted. These observation of genetic polymorphisms that explain much of the inter-individual variability in serum and tissue ACE activity have clinical implications, particularly for regulating angiotensin II and kinin levels and on the opposite effects of these peptides on vascular tone, myocardial and vascular smooth muscle cell growth and on the production of extracellular matrix. Chronic exposure to higher levels of ACE might therefore result in cardiovascular diseases and the development of renal impairments.

In conclusion, the present results indicate a significant association between ACE gene polymorphism and serum ACE activity levels, suggesting a mechanism by which genotype might have a bearing on the physiology of the renin-angiotensin system axis.

Acknowledgments

This study was partly supported by Grant for Scientific Research No. 09672347 from the Ministry of Education, Science, Sports and Culture, Japan. We wish to acknowledge the secretarial assistance of Miss Akiko Inaba.

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