RESEARCH NOTE

Angiotensin-I converting enzyme gene and I/D polymorphism distribution in the Greek population and a comparison with other European populations

SEKERLI ELENI[1,*], KATSANIDIS DIMITRIOS[1], PAPADOPOULOU VAYA[1], MAKEDOU ARETI[1], VAVATSI NORMA[2] and GATZOLA MAGDALINI[1]

[1]*2nd Department of Pediatrics, Aristotle University of Thessaloniki, AHEPA Hospital, 54636 Thessaloniki, Greece*
[2]*Department of Biochemistry, School of Medicine, Aristotle University of Thessaloniki, 54636 Thessaloniki, Greece*

Introduction

Angiotensin-I converting enzyme (*ACE*) gene is one of the most intensely studied genes because of the key role it plays in the renin–angiotensin system (RAS). *ACE* catalyses the conversion of angiotensin I to angiotensin II, a vasoactive and aldosterone-stimulating peptide, and inactivates bradykinin (Erdos and Skidgel 1987). *ACE* gene is located on chromosome 17q23 and consists of 26 exons and 25 introns. The insertion deletion (I/D) polymorphism in this gene refers to an *Alu* repetitive sequence 287 bp long, in intron 16, resulting in three genotypes, *DD* and *II* homozygotes and *ID* heterozygotes.

The I/D polymorphism is reported to determine circulating and tissue *ACE* levels, such that individuals homozygous for the *D* allele have higher tissue and plasma *ACE* concentrations than heterozygotes and *II* homozygotes (Rigat *et al*. 1990; Costerousse *et al*. 1993). The I/D polymorphism is associated with cardiovascular diseases (Cambien *et al*. 1992; Marian *et al*. 1993; Schunkert *et al*. 1994; Kario *et al*. 1996) as well as chronic renal diseases (Hohenfellner *et al*. 2001; Ohtomo *et al*. 2001).

However, little is known about the distribution of the *ACE* gene I/D polymorphism in the general population and the differences it may present among different ethnicities. In this study, we examined the distribution of the *ACE* I/D polymorphism in a sample of a Greek population. We have also presented a comparision of our data with data for other population in the literature.

*For correspondence. E-mail: elenisekerli@yahoo.gr.

Keywords. *ACE* gene; I/D polymorphism; Greek population.

Materials

Subjects

This study included 352 children, (159 boys and 193 girls). Subjects were recruited from the 2nd Department of Pediatrics, Aristotle University of Thessaloniki, AHEPA Hospital, where they were attended for various reasons. Blood samples were randomly selected between March 2005 and September 2007. Authorization was required from the parents prior to inclusion in the study.

Methods

Genomic DNA was obtained from peripheral leucocytes (300 μl of whole blood), using a DNA extraction kit (Gentra, USA).

The *ACE* I/D polymorphism was determined by PCR. PCR was performed with sense primer 5′ CTG GAG ACC ACT CCC ATC CTT TCT 3′ and antisense primer 5′ GAT GTG GCC ATC ACA TTC GTC AGA 3′ (Invitrogen, USA). The final volume of the mixture was 50 μl and contained 0.76 μg DNA, 50 mM KCl, 10mM Tris-HCl, pH 8.3, 3mM $MgCl_2$, 0.5 mM dNTPs, 40 pM of each primer and 1 U *Taq* polymerase. PCR cycle was 10 min of denaturation at 94°C, 30 cycles of 1 min each at 94°C denaturation, 58°C annealing, 72°C extension and 7 min of final extension at 72°C. The PCR products were separated on 2% agarose gel and visualized by ethidium bromide staining. The *I* allele produced a fragment in the 490-bp area and the *D* allele produced a fragment in the 190-bp area. The *ID* genotype showed two separate bands in the 490 bp and in the 190 bp areas.

To avoid the possibility of mistyping the *ID* heterozygotes as *DD* homozygotes, all *DD* genotypes were reamplified by using a second primer pair specific for the inserted

sequence. As above, PCR was performed in a final 50 μl mixture of 0.76 μg DNA, 50 mM KCl, 10 mM TRIS-Cl, pH 8.3, 3 mM $MgCl_2$, 0.5 mM dNTPs, 40 pM of each primer and 1U *Taq* polymerase. The primers for the inserted sequence were 5′ TGG GAC CAC AGC GCC CGC CAC TAC 3′ and 5′ TCG CCA GCC CTC CCA TGC CCA TAA 3′. PCR cycle was 10 min of denaturation at 94°C, 30 cycles of 30 s each at 94°C denaturation, 67°C annealing temperature, 72°C extension and 7 min of final extension at 72°C. Only the *I* allele produced a 335-bp fragment, while *DD* homozygotes showed no product.

Statistical analysis

Agreement with Hardy–Weinberg equilibrium was evaluated by χ^2 testing of the difference between observed and expected frequencies. Chi-square test was also used to compare our results with the *ACE* I/D genotype frequencies in the literature. A *P* value less than 0.05 was considered to indicate significant difference.

Results

A total of 352 children, (159 boys and 193 girls), aged 40 days to 16 years were recruited. The frequencies of the *II, ID* and *DD* genotypes in the study population were 13% (n = 46), 50.6% (n = 178), 36.4% (n = 128), respectively. The frequencies of the *I* and *D* alleles were 0.384 and 0.616. The genotype frequencies for *ACE* I/D polymorphism were in Hardy–Weinberg equilibrium.

Our results are significantly different from the *ACE* I/D genotype distribution reported for other European population ($P < 0.05$).

Discussion

The *ACE* gene is located on chromosome 17q23 and spans 21 kb on 26 exons. Insertion or absence of the 287-bp *Alu* sequence in intron 16 determines the *II, ID* and *DD* genotypes. The *DD* genotype is known as an independent risk factor in several cardiovascular diseases such as hypertrophic cardiomyopathy (Marian *et al*. 1993), myocardial infarction (Cambien *et al*. 1992; Kario *et al*. 1996) and ventricular hypertrophy (Schunkert *et al*. 1994), as well as chronic renal diseases such as IgA nephropathy (Yoshida *et al*. 1995), diabetic nephropathy (Doria *et al*. 1994), renal scarring (Ozen *et al*. 1999; Ohtomo *et al*. 2001; Erdogan *et al*. 2004) and congenital urological anomalies (Hohenfellner *et al*. 2001).

Alu insertion polymorphisms, like *ACE* I/D polymorphism, are also suitable markers for studying genetic variation in human populations. They can be easily detected by PCR amplification and gel electrophoresis and they are stable markers that represent a unique evolutionary event. The distribution of the *ACE* genotypes differs between races and it is used as a marker in population structure analyses (Barbalic *et al*. 2004).

In this study, we studied at the distribution of the *ACE* I/D polymorphism in 352 Greek children. Our estimated I/D genotype frequencies are significantly different from those reported for Korean and Japanese populations. As shown in table 1, Koreans and Japanese have a relatively low

Table 1. Comparison of *ACE* I/D genotype distributions and allele frequencies.

	n	*DD* (%)	*ID* (%)	*II* (%)	*D*	*I*
Current study group	352	36.4	50.6	13	0.616	0.384
France (Marre *et al*. 1997)	346	34	44	22	0.56	0.44
Spain (Gallego *et al*. 2001)	100	30	49	21	0.545	0.455
Slovenia (Zork *et al*. 2005)	218	27.5	47.7	24.8	0.513	0.487
Turkey (Erdogan *et al*. 2004)	103	26.2	54.4	19.4	0.534	0.466
Germany (Hohenfellner *et al*. 2001)	163	24	50	26	0.49	0.51
Croatia (Barbalic *et al*. 2004)	172	22.1	54.7	23.3	0.493	0.506
Hungary (Haszon *et al*. 2002)	80	22	60	18	0.53	0.47
Poland (Zak *et al*. 2003)	111	22	43	35	0.435	0.565
Italy (Rigoli *et al*. 2004)	92	22	52	26	0.48	0.52
Korea (Choung *et al*. 1999)	739	16	49	35	0.405	0.595
Japan (Yoshida *et al*. 1995)	46	7	52	41	0.33	0.67

percentage of the *DD* genotype. The frequency of the *D* allele is 0.406 in the Koreans (Choung *et al*. 1999) and 0.33 in the Japanese population (Yoshida *et al*. 1995), contrast to Caucasians, where the *D* allele frequency is higher.

The present, Greek I/D frequencies are closer to those reported for a French population (Marre *et al*. 1997). According to the French study, the observed genotype distribution in 346 healthy controls was $II = 22\%$, $ID = 44\%$ and $DD = 34\%$. The *D* allele frequency was 0.56 which is similar to the 0.616 we have estimated for the Greek population.

Significant differences can be noticed between the European populations. As we can see in table 1, the Greek population has the highest *D* allele frequency (0.616), where the lowest (0.435) is reported in the polish population. In the rest of the european countries, the *D* allele frequency varies from 0.49 to 0.53.

Large differences between the reported I/D frequencies for European populations makes it hard to speak of average *AcE* I/D frequencies for the Caucasian populations as a group. We suggest that, in examining the association of the *ACE* I/D polymorphism in cardiovascular and other diseases it is not safe to compare I/D frequencies found in study groups from different countries.

References

Barbalic M., Pericic M., Skaric-Juric T. and Smolej-Narancic N. 2004 ACE alu insertion polymorphism in Croatia and its isolates. *Coll. Antropologica* **28**, 603–610.

Cambien F., Poirier O., Lecerf L., Evans A., Cambou J., Arveiler D. *et al*. 1992 Deletion polymorphism in the gene of angiotensin converting enzyme is a potent risk factor for myocardial infraction. *Nature* **359**, 641–644.

Choung H.-J., Yoon S.-R. and Choi S.-K. 1999 Frequency of the angiotensin-converting enzyme (ACE) gene polymorphism in the general population and the elite endurance students in Korea. *J. Genet. Med.* **3**, 11–14.

Costerousse O., Allegrini J., Lopez M. and Alhenc-Gelas F. 1993 Angiotensin I converting enzyme in human circulating mononuclear cells: genetic polymorphism of expression in T-lymphocytes. *Biochem. J*. **290**, 33–40.

Doria A., Warram J.-H. and Krolewski A.-S. 1994 Genetic predisposition to diabetic nephropathy. Evidence for a role of the angiotensin I-converting enzyme gene. *Diabetes* **43**, 690–695.

Erdogan H., Mir S., Serdaroglu E., Berdeli A. and Aksu N. 2004 Is ACE gene polymorphism a risk factor for renal scarring with low grade reflux? *Pediatr. Nephrol*. **19**, 734–737.

Erdos E. and Skidgel R.-A. 1987 The angiotensin I-converting enzyme. *Lab Invest*. **56**, 345–348.

Gallego N., Estepa R., Telleria D., SanMillan J.-L., Belanger A. and Ortuno J. 2001 Angiotensin I converting enzyme gene polymorphism and reflux nephropathy in children. *Nephron* **89**, 231–232.

Hohenfellner K., Wingen A.-M., Nauroth O., Wuhl E., Mehls O. and Schaefer F. 2001 Impact of ACE I/D polymorphism on congenital renal malformations. *Pediatr. Nephrol*. **16**, 356–361.

Kario K., Kanai N., Saito K., Nago N., Matsuo T. and Shimada K. 1996 Ischemic stroke and the gene for angiotensin converting enzyme in Japanese hypertensives. *Circulation* **93**, 1630–1633.

Marian A., Yu Q., Workman R., Greve G. and Roberts R. 1993 Angiotensin converting enzyme polymorphism in hypertrophic cardiomyopathy and sudden cardiac death. *Lancet* **342**, 1085–1086.

Marre M., Jeunemaitre X., Gallois Y., Rodier M., Chatellier G., Sert C. *et al*. 1997 Contribution of genetic polymorphism in the renin-angiotensin system to the development of renal complications in insulin-dependent diabetes. *J. Clin. Invest*. **99**, 1585–1595.

Ohtomo Y., Nagaoka R., Kaneko K., Fukuda Y., Miyano T. and Yamashiro Y. 2001 Angiotensin converting enzyme gene polymorphism in primary vesicoureteral reflux. *Pediatr. Nephrol*. **16**, 648–652.

Ozen S., Alikasifoglu M., Saatci U., Bakkaloglu A., Besbas N., Kara N. *et al*. 1999 Implications of certain genetic polymorphisms in scarring in vesicoureteric reflux: importance of ACE polymorphism. *Am. J. Kidney Dis*. **34**, 140–145.

Rigat B., Hubert C., Alhenc-Gelas F., Cambien F., Corvol P. and Soubrier F. 1990 An insertion / deletion polymorphism in the angiotensin I converting enzyme gene accounting for half the variance of serum enzyme levels. *J. Clin. Invest*. **86**, 1343–1346.

Rigoli L., Chimenz R., diBella C., Cavallaro E., Caruso R., Briuglia S. *et al*. 2004 Angiotensin converting enzyme and angiotensin type 2 receptor gene genotype distributions in Italian children with congenital uropathies. *Pediatr. Res*. **56**, 988–993.

Schunkert H., Hense H.-W., Holmer S.-R., Stender M., Perz S., Keil U. *et al*. 1994 Association between a deletion polymorphism of the angiotensin converting enzyme gene and left ventricular hypertrophy. *N. Engl. J. Med*. **330**, 1634–1638.

Yoshida H., Mitarai T., Kawamura T., Kitajima T., Miyazaki Y., Nagasawa R. *et al*. 1995 Role of the deletion polymorphism of the angiotensin converting enzyme gene in the progression and therapeutic responsiveness of IgA nephropathy. *J. Clin. Invest*. **96**, 2162–2169.

Zak I., Niemiec P., Sarecka B., Balcerzyk A., Ciemniewski Z., Rudowska E. *et al*. 2003 Carrier-state of D allele in ACE gene insertion/deletion polymorphism is associated with coronary artery disease, in contrast to the C677→T transition in the MTHFR gene. *Acta Biochim. Pol*. **50**, 527–534.

Zorc-Pleskovic R., Teran N., Pleskovic A., Terzic R. and Milutinovic A. 2005 Deletion/deletion genotype of angiotensin I converting enzyme gene is not associated with coronary artery disease in Caucasians with type 2 diabetes. *Coll. Antropol*. **29**, 149–152.

Received 24 October 2007; accepted 31 October 2007
Published on the Web: 12 February 2008