

## RESEARCH NOTE

# ANGIOTENSIN-1 CONVERTING ENZYME I/D GENE POLYMORPHISM: SCENARIO IN MALAYSIA

JJ Jayapalan<sup>1</sup>, S Muniandy<sup>1</sup> and SP Chan<sup>2</sup>

<sup>1</sup>Department of Molecular Medicine, <sup>2</sup>Department of Medicine, University of Malaya, Kuala Lumpur, Malaysia

**Abstract.** Discrepancies in angiotensin-1 converting enzyme (ACE) allele genetic susceptibility with disease etiology have been attributed to ethnic differences. We investigated ACE gene polymorphism of the multiethnic Malaysian population by utilizing nested polymerase chain reaction. Allelic frequency of 0.65 and 0.35 for I and D allele, respectively in the pooled population was comparable with other Asian populations. A significant association was found between the Malaysian ethnic groups and ACE I/D genotype. The II genotype was found at higher frequency among the Malays but a greater frequency of DD genotype among Indians.

### INTRODUCTION

Polymorphism at intron 16 of the angiotensin-1 converting enzyme (ACE) gene, located at chromosome 17q23, has been implicated in various disease etiologies, including coronary artery disease (Sekin *et al*, 2006), myocardial infarction (Araujo *et al*, 2005), left ventricular hypertrophy (Saeed *et al*, 2005), diabetes (Daimon *et al*, 2003), hypertension (Zee *et al*, 1992), venous thrombosis (Fatini *et al*, 2003), diabetic nephropathy (Movvaa *et al*, 2007), coronary restenosis (Ribichini *et al*, 1999), Alzheimer (Wang *et al*, 2006), and ischemic stroke (Tseng *et al*, 2007), and in a number of such physiological events such as athletic mechanical efficiency and in performance endurance (Williams *et al*, 2000; Amir *et al*, 2007), and in senescence (Schachter *et al*, 1994). However, other studies have sug-

gested that there is no association of disease etiology with ACE I/D gene polymorphism (Schimdt *et al*, 1995, Agerholm-Larsen *et al*, 1997; Clark *et al*, 2000; Moleda *et al*, 2006; Nacmias *et al*, 2007).

As a result, genotype-phenotype interaction between ACE gene polymorphism and diseases have not been fully appreciated nor understood. Wide inter-ethnic variations of the ACE alleles distribution are thought to be responsible for these inconsistent findings (Barley *et al*, 1994). This prompted us to investigate ACE I/D gene polymorphism in the Malaysian population and ethnic groups to observe ethnicity specific ACE genotypic patterns. To our knowledge there has been no report thus far of ACE I/D gene polymorphism in the multiethnic Malaysian population.

### MATERIALS AND METHODS

#### Subject recruitment

Subjects recruited in this study were from the University Malaya Medical Center, Kuala Lumpur, Malaysia and other establishments, such as Kuala Lumpur Police Headquarters,

---

Correspondence: Dr Sekaran Muniandy, Department of Molecular Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.  
Tel: 603 7967 4953; Fax: 603 7967 4957  
E-mail: sekaran@um.edu.my

Petaling Jaya Municipal Council, and community centers such as Sai Baba Centers, and the Hokkien Association. Subjects were briefed on the purpose of the study and risks involved prior to obtaining informed consent. Whole blood (3 ml) was collected from each subject into EDTA-containing tubes.

#### DNA extraction

Genomic DNA from blood sample was isolated using Wizard<sup>®</sup> Genomic DNA purification kit (Promega, Madison). DNA was concentrated and precipitated by isopropanol.

#### Nested polymerase chain reaction (PCR)

Nested PCR was used to amplify target sequence to avoid mistyping errors and was performed essentially as described by Clark *et al* (2000). The following primers were synthesized (Research Biolabs, Singapore) and used to detect the presence or absence of a 287bp *Alu* sequence in intron 16 of the ACE gene: forward 5' CCC ATC CTT TCT CCC ATT TCT C 3'), nested (5' GGT TTC ACC GTT TTA GCC GGG A 3') and reverse (5' CCA TGC CCA TAA CAG GTC TTC A 3') PCR was performed in a final volume of 25  $\mu$ l containing 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 0.8% Nonidet P40, 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 20 pmol of each primer, 1 U of Taq DNA polymerase (Fermentas, Lithuania), and approximately 100 ng of genomic DNA. Protocol optimized for the nested PCR was as follows: initial denaturation step at 94°C for 3 minutes, followed by 30 cycles of 94°C for 30 seconds, 64°C for 30 seconds, and 72°C for 30 seconds, and a final extension step at 72°C for 7 minutes. PCR was performed in duplicate and a negative control was included in every batch. Agarose gel (1.5%)-electrophoresis enabled genotypic determination of ACE I/D allele as II, ID and DD genotype. Amplicons with sizes of 498 bp and 264 bp were designated as II genotype, of 498 bp, 264 bp and 210 bp as ID genotype and of 210 bp as DD genotype.

#### Sequencing

PCR products from nine samples distinctly representing ACE I/D genotype were selected randomly for sequencing. The resulting amplicons were purified using a spin-column (Qiagen, Germany), and sequenced using Applied Biosystems 3730 DNA analyzer at Research Biolabs (Singapore).

#### Statistical analysis

Statistical analysis was performed using SPSS package, version 11.5 (SPSS software, USA). Chi-square test was used to study the genotypic and allelic frequency distribution of ACE I/D gene polymorphisms.

## RESULTS

A total of 637 subjects were recruited, consisting of Malay ( $n = 274$ ), Chinese ( $n = 150$ ), and Indian ( $n = 213$ ) ethnic subgroups.

The resulting PCR products were from intron 16 of ACE gene based on DNA sequencing result. The genotypic and allelic frequency of ACE I/D gene polymorphism in the pooled population was 43.3, 43.0 and 13.7% for II, ID and DD genotypes, respectively; and 0.65 and 0.35% for I and D allele, respectively. The genotypic frequency of II, ID, and DD among Malay subgroup was 51.1, 39.4, and 9.5%, respectively, for Chinese of 40.0, 46.7, and 13.3%, respectively, and for Indian subgroup of 35.7, 45.2, and 19.2%, respectively. The I and D allele frequency was 0.71 and 0.29, and 0.63% and 0.37, 0.58 and 0.42% among the Malay, Chinese and Indian subgroup, respectively. Genotype and allele frequencies were both consistent with the Hardy-Weinberg equilibrium for all 3 ethnic groups.

There is statistically significant association between the ethnic groups and ACE genotypes ( $\chi^2 = 16.946$ ,  $p = 0.02$ ). Indians were found to have lower frequency of II genotype and greater incidence of DD genotype compared to Malays. However, the genotypic

distribution of ACE gene polymorphism among the Chinese are not different from either Malay ( $p = 0.074$ ) or Indian ( $p = 0.338$ ).

## DISCUSSION

The main objective of the study was to evaluate our observations for ACE allele and genotype frequencies among Malaysians, and to compare the data to findings in the literature. Interestingly, we observed a significant difference in ACE I/D prevalence between the ethnic groups found in Malaysia. The distribution of ACE alleles in the pooled population was comparable to other Asian populations

with the exception of the Asian Indian population that demonstrates an increased frequency of D allele. As expected, these findings differed from those with the African (Barley *et al*, 1994), the Arab (Bayoumi *et al*, 2006) and other minor populations worldwide (Barley *et al*, 1994; Lester *et al*, 1999) (Table 1).

In this study the D allele frequency was found to be lower among the Malay (0.29) and Chinese (0.37), while a higher D allele frequency was observed among the Indian (0.42). Compared with other populations, the distribution of ACE gene alleles among the Malaysian Malay, Chinese and Indian were remarkably similar with previous studies of Sasongko *et al*

Table 1  
Distribution of ACE I/D polymorphism in the Malaysian population compared to Asian, Caucasian and others.

Ethnic	Allele frequency		References	
	I	D		
Asian	Malaysian pooled	0.65	0.35	present study
	Malay	0.71	0.29	present study
	Chinese	0.63	0.37	present study
	Indian	0.58	0.42	present study
	Asian Indians	0.55	0.45	Movvaa <i>et al</i> , 2007
	Chinese	0.63	0.37	Young <i>et al</i> , 1995
	Taiwanese	0.64	0.36	Chuang <i>et al</i> , 1997
	Singaporean	0.69	0.31	Lau <i>et al</i> , 2002
	Japanese	0.67	0.33	Kario <i>et al</i> , 1997
	Thai	0.70	0.30	Nitiyanant <i>et al</i> , 1997
	Javanese-Indonesian	0.76	0.24	Sasongko <i>et al</i> , 2005
Caucasian	European	0.42-0.54	0.46-0.58	Barley <i>et al</i> , 1994, Cambien <i>et al</i> , 1992, Tiret <i>et al</i> , 1992, Raynolds <i>et al</i> , 1993
	Australian	0.46	0.54	Lester <i>et al</i> , 1999
	American	0.48	0.52	Foy <i>et al</i> , 1996
Others	Arabs	0.27-0.39	0.61-0.73	Bayoumi <i>et al</i> , 2006, Al-Hinai <i>et al</i> , 2002
	African American	0.41	0.59	Barley <i>et al</i> , 1994
	Amerindian (Teenek and Nahuas)	0.61-0.78	0.22-0.28	Vargas-Alarcon <i>et al</i> , 2003
	Pima Indian	0.71	0.29	Foy <i>et al</i> , 1996
	Yanomami Indian	0.85	0.15	Barley <i>et al</i> , 1994
	Samoan	0.91	0.09	Barley <i>et al</i> , 1994
	Australian Aboriginal	0.97	0.03	Lester <i>et al</i> , 1999

(2005) on Javanese-Indonesian, Young *et al* (1995) on Asian Chinese, and Movva *et al* (2007) on Asian Indian, respectively (Table 1). This is not surprising considering that the Malaysian Malay, Chinese, and Indian are largely descendants of emigrants from southern part of Sumatra, Southern China (Lynn, 1990) and India (Ampalavanar, 1981), respectively. The ACE gene allele frequencies observed among Japanese and Taiwanese (0.67:0.33) are noticeably similar to the Chinese, and Caucasian is most similar to the Indian in general, while Malay is similar to the Thai. ACE gene polymorphism study on the indigenous people or early inhabitants of the Malay Peninsular who migrated from Thailand should prove interesting.

Our findings demonstrated that the allelic and genotypic distributions of ACE gene polymorphism vary but are specific to the ethnic origin. Therefore, the importance of ethnicity must be carefully considered when studying the association of genetic susceptibility of ACE gene to disease etiology.

#### ACKNOWLEDGEMENTS

This work was funded by UM-PPP Grant: P0103/2006B and PS290/2007B. We wish to express our deep gratitude to Prof Robert Fraser, University of Glasgow (UK) for providing the characterized DNA sample.

#### REFERENCES

- Agerholm-Larsen B, Tybjaerg-Hansen A, Frikke-Schmidt R, Gronholdt MLM, Jensen G, Nordestgaard BG. ACE gene polymorphism as a risk factor for ischemic cerebrovascular disease. *Ann Intern Med* 1997; 127: 346-55.
- Al-Hinai AT, Hassan MO, Simsek M, Al-Barwani H, Bayoumi R. Genotypes and allele frequencies of angiotensin converting enzyme (ACE) insertion / deletion polymorphism among Omanis. *SQU J Sci Res Med Sci* 2002; 4: 25-7
- Amir O, Amir R, Yamin C, *et al*. The ACE deletion allele is associated with Israeli elite endurance athletes. *Exp Physiol* 2007; 92: 881-6.
- Ampalavanar R. Indian. The Indian minority and political change in Malaya 1945-1957. London: Oxford University Press, 1981.
- Araujo MA, Goulart LR, Cordeiro ER, *et al*. Genotypic interactions of renin-angiotensin system genes in myocardial infarction. *Int J Cardiol* 2005; 103: 27-32.
- Barley J, Blackwood A, Carter ND, *et al*. Angiotensin converting enzyme insertion/deletion polymorphism: Association with ethnic origin. *J Hypertens* 1994; 12: 955-7.
- Bayoumi RA, Simsek M, Yahya TM, *et al*. Insertion-deletion polymorphism in the angiotensin-converting enzyme (ACE) gene among Sudanese, Somalis, Emiratis, and Omanis. *Hum Biol* 2006; 78: 103-8.
- Cambien F, Poirier O, Lecerf L, *et al*. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 1992; 359: 641-4.
- Chuang LM, Chiu KC, Chiang FT, *et al*. Insertion/deletion polymorphism of the angiotensin I-converting enzyme gene in patients with hypertension, non-insulin-dependent diabetes mellitus, and coronary heart disease in Taiwan. *Metabolism* 1997; 46: 1211-4.
- Clark CJ, Davies E, Anderson NH, *et al*.  $\alpha$ -Adducin and ACE polymorphism in essential hypertension. *Hypertension* 2000; 36: 990-4.
- Daimon M, Oizumi T, Saitoh T, *et al*. The D allele of the angiotensin-converting enzyme insertion / deletion (I/D) polymorphism is a risk factor for type 2 diabetes in a population-based Japanese sample. *Endocrine J* 2003; 50: 393-8.
- Fatini C, Gensini F, Sticchi E, *et al*. ACE DD genotype: an independent predisposition factor to venous thromboembolism. *Eur J Clin Invest* 2003; 33: 642-7.
- Foy CA, McCormack LJ, Knowler WC, *et al*. The angiotensin I-converting enzyme (ACE) gene I/D polymorphism and ACE levels in Pima Indians. *J Med Genet* 1996; 33: 336-7.
- Kario K, Kanai N, Nishiuma S, *et al*. Hypertensive nephropathy and the gene for angiotensin-converting enzyme. *Arterioscler Thromb Vasc Biol* 1997; 17: 252-6.

- Lau YK, Woo KT, Choong HL, *et al.* ACE gene polymorphism and disease progression of IgA nephropathy in Asians in Singapore. *Nephron* 2002; 91: 499-503.
- Lester S, Heatley S, Bardy P, *et al.* The DD genotype of the angiotensin-converting enzyme gene occurs in very low frequency in Australian Aborigines. *Nephrol Dial Transplant* 1999; 14: 887-90.
- Lynn P. Sons of the Yellow Emperor: A history of the Chinese Diaspora. Boston: Little Brown and Company, 1990: 408.
- Moleda P, Majkowska L, Kaliszczak R, Safranow K, Adler G, Goracy I. Insertion/deletion polymorphism of angiotensin I converting enzyme gene and left ventricular hypertrophy in patients with type 2 diabetes mellitus. *Kardiol Pol* 2006; 64: 959-65.
- Movvaa S, Alluric RV, Komandur S, *et al.* Relationship of angiotensin-converting enzyme gene polymorphism with nephropathy associated with Type 2 diabetes mellitus in Asian Indians. *J Diabetes Complications* 2007; 21: 237-41.
- Nacmias B, Bagnoli S, Tedde A, *et al.* Angiotensin converting enzyme insertion/deletion polymorphism in sporadic and familial Alzheimer's disease and longevity. *Arch Gerontol Geriatr* 2007; 45: 201-6.
- Nitiyanant W, Sriussadaporn S, Ploybutr S, Watanakejorn P, Tunlakit M, Bejrachandra S. Angiotensin converting enzyme gene polymorphism in healthy Thais and patients with non-insulin dependent diabetes mellitus. *J Med Assoc Thai* 1997; 80: 747-52.
- Raynolds MV, Bristow MR, Bush EW, *et al.* Angiotensin-converting enzyme DD genotype in patients with ischemic or idiopathic dilated cardiomyopathy. *Lancet* 1993; 342: 1073-5.
- Ribichini F, Steffenino G, Dellavalle A, *et al.* Plasma activity and insertion/deletion polymorphism of angiotensin I-converting enzyme. A major risk factor and a marker of risk for coronary stent restenosis. *Circulation* 1998; 97: 147-54.
- Saeed M, Saleheen D, Siddiqui S, Khan A, Butt ZA, Frossard PM. Association of angiotensin converting enzyme gene polymorphisms with left ventricular hypertrophy. *Hypertens Res* 2005; 28: 345-9.
- Sasongko TH, Sadewa AH, Kusuma PA, *et al.* ACE gene polymorphism in children with nephrotic syndrome in the Indonesian population. *Kobe J Med Sci* 2005; 51: 41-7.
- Schachter F, Faure-Delanef L, Guenot F, *et al.* Genetic associations with human longevity at the APOE and ACE loci. *Nature Genet* 1994; 6: 29-32.
- Schmidt S, Schone N, Ritz E, *et al.* Association of ACE gene polymorphism and diabetic nephropathy? *Kidney Int* 1995; 47: 1176-81.
- Seckin D, Ilhan N, Ilhan N, Ozbay Y. The relationship between ACE insertion/deletion polymorphism and coronary artery disease with or without myocardial infarction. *Clin Biochem* 2006; 39: 50-4.
- Tiret L, Rigat B, Visvikis S, *et al.* Evidence from combined segregation and linkage analysis, that a variant of the Angiotensin I – Converting Enzyme (ACE) gene controls plasma ACE levels. *Am J Hum Genet* 1992; 51: 197-205.
- Tseng CH, Tseng CP, Chong CK, Sheu JJ, Cheng JC. Angiotensin-converting enzyme gene polymorphism and stroke in type 2 diabetic patients in Taiwan. *Eur J Clin Invest* 2007; 37: 483-91.
- Vargas-Alarcon G, Hernandez-Pacheco G, Rodriguez-Perez JM, *et al.* Angiotensin-converting enzyme gene (ACE) insertion /deletion polymorphism in Mexican population. *Hum Biol* 2003; 75: 889-96.
- Wang B, Jin F, Yang Z, *et al.* The insertion polymorphism in angiotensin-converting enzyme gene associated with the APOE epsilon 4 allele increases the risk of late-onset Alzheimer disease. *J Mol Neurosci* 2006; 30: 267-71.
- Williams AG, Rayson MP, Jubbs M, *et al.* The ACE gene and muscle performance. *Nature* 2000; 403: 614.
- Young RP, Sanderson JE, Tomlinson B, *et al.* Angiotensin converting enzyme insertion-deletion polymorphism in Chinese. *J Hypertens* 1995; 13: 1479-83.
- Zee RY, Lou YK, Griffiths LR, Morris BJ. Association of a polymorphism of the angiotensin I-converting enzyme gene with essential hypertension. *Biochem Biophys Res Comm* 1992; 184: 9-15.