RESEARCH NOTE

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

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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 suggested that there is no association of disease etiology with ACE I/D gene polymorphism (Schmidt 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,
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® 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 µl containing 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 0.8% Nonidet P40, 1.5 mM MgCl2, 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 genotype. 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
The 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>
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<th>Ethnic</th>
<th>Allele frequency</th>
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<td></td>
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<tr>
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(2005) on Javanese-Indonesian, Young et al (1995) on Asian Chinese, and Movvaa 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

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REFERENCES


