SQU JOURNAL FOR SCIENTIFIC RESEARCH: MEDICAL SCIENCES
2002, VOL. 4, NO. 1–2, 25–27

Genotypes and allele frequencies of angiotensin converting enzyme (ACE) insertion/deletion polymorphism among Omanis.

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النوع الجيني الوراثي وتكرر الحليل (الاليل) للإنزيم المحول للانجيوتنسين بأشكاله المختلفة في المواطنين العمانيين

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المستخلص: الهدف: معرفة تكرر النوع الوراثي لإنزيم محول الانجيوتنسين في العمانيين ومقارنته بجنسيات اخرى **. الطريقة:** تم استحداث طريقة خاصة من اختبار تفاعل حافز البلمرة التسلسلي والمبنى على تجزئة قطع الحامض النووي ، وذلك من أجل فحص وجود فقد من عدمه في الحامض النووي من عدمه الموجود في مورثات الإنزيم المحول الانجوتنسين . تم فحص ١٢٤ شخصاً من طلاب جامعة السلطان قابوس في مسقط بسلطنة عمان. **النتائج:** كان تكرر حليل (أليل) I هو ٠,٢٩، بينما كان تكرر حليل D هو ٠,٧١ . إن انتشار تكرر المورث لم يحيد عن توازن هاردي و وينبرج **الخلاصة:** إن تكرر أليل D في العمانيين كان مماثلاً لتكرره في العرب الآخرين والأفارقة ولكن يختلف كثيراً عن تكرره في اليابانيين والصينيين.

ABSTRACT. ***Objective:*** To describe the angiotensin converting enzyme (ACE) genotype frequencies among Omani Arabs. ***Method***: A polymerase chain reaction (PCR) test, based on separation of different size DNA fragments, was developed to test the presence or absence (polymorphism) of a small DNA deletion in the ACE gene. The subjects were 124 Omani Arab students of Sultan Qaboos University, Muscat. ***Results***: The frequency of the I allele was 0.29, while that of the D allele was 0.71. The gene frequency distribution did not deviate from Hardy-Weinberg equilibrium. ***Conclusions:*** The frequency of D allele among Omanis is similar to that among other Arabs and Africans, but differs significantly from that among the Japanese and Chinese.

Key Words: Allele, genotype, polymorphism, hypertension.

Essential hypertension (HTN) is a major risk factor for morbidity and mortality from cardiovascular diseases.[1] The causes of HTN have not yet been determined. It is considered as a multifactorial and polygenic disease,[2] and occurs as a consequence of a complex interplay of genetic alterations and environmental factors.[3] The angiotensin converting enzyme (ACE) gene is one of the genes that control blood pressure and has been intensively investigated. The ACE gene, first described by Rigat *et al* in 1990,[4] has an insertion/deletion (I/D) polymorphism in intron 16. Various published reports suggest an association or linkage of the D allele of the ACE gene with myocardial infarction,[5] essential hypertension,[6] left ventricular hypertrophy,[7] renal insufficiency[8] and high fasting blood sugar levels.[9] However, some other investigators have found no association between ACE I/D polymorphism and HTN.[10,11] Inter-ethnic variations in the frequency of allelic forms of certain genes, have been suggested as one of the reasons for such discrepancies.[12] This is particularly true for the ACE gene, since wide inter-ethnic allelic variations have been reported.[13]

The aim of our study was to find out ACE allele and genotype frequencies among Omani Arabs and compare them with those among other ethnic groups. Oman is one of the countries in Arabian Peninsula, and 85% of its population are Arabs of *Bedouin* descent; the remaining 15% originate from Eastern Africa and the Indian subcontinent.[14]

SUBJECTS

One hundred and twenty four (124) unrelated Omani Arabs were randomly selected from among students of Sultan Qaboos University. After getting an informed consent, 5 ml of blood was collected from each into an EDTA-containing tube.

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Table 1. *Frequency of genotypes and alleles of ACE (I/D) polymorphism in 124 Omani Arabs.*

Total Number	Genotypes			Allele fequency	
	II (n) O(E)	ID (n) O(E)	DD (n) O(E)	I	D
124	8(10.1)	55(50.6)	61(63.2)	0.29	0.71

0: Observed, (E): Expected
χ^2: 0.896, for Goodness of Fit Test.
No deviation from the Hardy-Weinberg equilibrium was observed.

DNA Analysis

DNA was isolated from leukocytes by phenol/chloroform extraction. The insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene was detected by the polymerase chain reaction (PCR), following the method described previously by Rigat *et al,*[15] using a set of oligonucleotide primers which differentiate the three ACE genotypes: II, ID and DD. Using 2% agarose gel electrophoresis and ethidium bromide staining, the homozygote deletion (DD) alleles were visualised as 190 base pairs (bp) bands, homozygote insertion (II) alleles as 490bp bands, and heterozygotes (ID) alleles as two bands of 490 bp and 190 bp.

Since the D alleles in heterozygous samples (ID) are preferentially amplified, some samples with ID genotypes are liable to be mistyped as DD.[16] Therefore, each sample found to have the DD genotype was verified in an independent second PCR amplification. A 335 bp band was visualised only in the presence of an I allele and no bands were visualized in samples with DD.

RESULTS

The frequency distribution of genotypes and alleles of the ACE (I/D) polymorphism among Omani Arabs is summarised in Table 1. The frequency of the I allele was 0.29 while that of the D allele was 0.71. Ten heterozygotes were mistyped as DD, but proved to be heterozygotes on independent second PCR using insertion specific primers. The gene frequency distribution did not deviate from the Hardy-Weinberg equilibrium.

DISCUSSION

The frequency of the D allele of the ACE gene among Omanis (0.71) is comparable to that in other Arabs, such as the Emiratis (0.66), Somalis (0.73) and Sudanese (0.64). Compared to other ethnic groups (Table 2) the frequency of the D allele seems to be among the highest reported. The frequency of the D allele seems to follow a clinal distribution, being highest among Africans and Arabs (0.70–0.60), followed by Caucasians (0.46–0.58), then the Japanese (0.33–0.35) and the Chinese (0.29). The Yanomami Indians and Samoans seem to have the lowest frequencies: 0.15 and 0.09, respectively.

CONCLUSION

This study confirms the results obtained among other Arabs and adds to the data indicating the wide variations observed in the frequency of the ACE alleles among the peoples of the world. Therefore, the interpretation of clinical data on the association of the ACE alleles with cardiovascular disease must be approached with the greatest of care.

Table 2. *Prevalence of insertion/deletion polymorphisms of ACE gene among different ethnic groups as per different studies.*

Ethnic group	Allele Frequency		Number of Individuals tested
	I	D	
I. Arabs	0.34	0.66	159
Emirates[17, 18]	0.39	0.61	111
Sudanese[18]	0.36	0.64	121
Somalis[18]	0.27	0.73	53
Omanis (*present study*)	0.29	0.71	124
II. Africans			
Nigerians[13]	0.41	0.59	80
African-Americans[19]	0.30	0.70	40
III. Caucasians[5,20,21]	0.42–0.54	0.46–0.58	1212
IV. Asians			
Japanese[22,23,24]	0.65–0.67	0.33–0.35	354
Chinese[25]	0.71	0.29	189
V. Others:			
Yanomami Indians[13]	0.85	0.15	49
Samoans[13]	0.91	0.09	58

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