Mol Cell Biochem (2012) 362:219–223
DOI 10.1007/s11010-011-1146-1

Genotypes and allele frequencies of angiotensin-converting enzyme (ACE) insertion/deletion polymorphism among Bahraini population with type 2 diabetes mellitus and related diseases

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Received: 11 August 2011 / Accepted: 3 November 2011 / Published online: 13 November 2011

Abstract Insertion/deletion (*I/D*) polymorphism, of a 287-bp *Alu* repetitive sequence in intron 16 of the angiotensin-converting enzyme (ACE) gene has been shown to be associated with different types of diseases and has been widely investigated in different populations with different ethnic origins. Various reports were published suggesting inter-ethnic variations in the frequency of allelic forms of the ACE gene. The goal of this study was to test the distribution of alleles and the different genotypes of ACE (*I/D*) polymorphism in Bahraini subjects and compare the results with those obtained from other population studies. The Bahraini population is an Arabic peninsula population with a high prevalence of T2DM and hypertension. A total of 560 unrelated Bahraini individuals were recruited in this study and the presence (insertion)/absence (deletion) (*I/D*) polymorphism of a 287-bp Alu1 element inside intron 16 of the ACE gene was done by PCR-based assays and the presence or absence of the genotypes were analyzed by the gel electrophoresis. The distribution of *II*, *ID*, and *DD* genotypes showed differences among Bahraini subjects, and the frequency of the *D* allele was significantly ($P < 0.05$) higher in the studied group. The results obtained for the *D* allele are consistent with those obtained from previous studies among Arabs, Africans, and Caucasians, but differs significantly ($P < 0.05$) from those in Japanese and Chinese, thus proving the ethnic variation in the distribution of the ACE alleles in different populations.

Keywords Angiotensin-converting enzyme (ACE) · *I/D* polymorphism · Bahrain · Bahraini population · Type 2 diabetes mellitus

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Introduction

The angiotensin-converting enzyme (ACE) is a key enzyme of the renin–angiotensin system (RAS) that has long been recognized as crucial in the regulation of systemic blood pressure and renal electrolyte homeostasis [1, 2]. This enzyme acts by converting the inactive Angiotensin I to the vasoconstrictor Angiotensin II, and inactivates the vasodilator proinflammatory bradykinin and substance P peptides [3]. The ACE gene (encoding enzyme type kinase II, EC 3.4.15.1) is located on chromosome 17 and the gene polymorphisms have been widely investigated. The insertion/deletion (*I/D*) polymorphism of ACE has been discovered and described by Rigat et al. [4]. The polymorphism is characterized by either the presence/insertion (*I*) or absence/deletion (*D*) of a 287-base pair Alu1 element inside intron 16 of the ACE gene, producing three genotypes (*DD*, *II* homozygote, and *ID* heterozygote) [4, 5].

Several investigations have been shown that the *D* allele of an insertion/deletion (*I/D*) polymorphism of the gene encoding ACE is associated with higher plasma ACE concentrations [4, 5] and suggested an association of the ACE *I/D* polymorphism with incidence, pathogenesis and

progression of several diseases including diabetes mellitus and its long-term macro- and microvascular complications [6–8], including diabetic nephropathy [9–11]. Moreover, other studies have also provide a positive association of the ACE *I/D* polymorphism and cardiomyopathy [12, 13] and hypertension [14]. Since the ACE *I/D* polymorphism is also associated with the overall plasma ACE concentration [15], it was suggested that ACE might be a good candidate gene for the metabolic syndrome, which is clustered together with hypertension, DM and dyslipidemia [16]. However, conflicting results have been reported regarding the association between ACE *I/D* polymorphism and diseases [17–24]. Moreover, various published reports have suggested inter-ethnic variations in the frequency of allelic forms of the ACE genes [25–27].

The aim of our study was to test the distribution of allele and genotype frequencies of ACE (*I/D*) polymorphism among Bahrainis and to compare the results with those of other ethnic groups. The Bahraini population is an Arabic peninsula population with a high prevalence of T2DM occurring in 20–30% of adult population [28] and hypertension [29]. The characteristics of Arabic population make them ideal for the study of complex, polygenic, multifactorial disorders such as diabetes. Arabic populations are characterized by genetic homogeneity due to tribal structure of their society, large family size and extensive consanguinity, as a result of these factors, the statistical power of susceptibility and target gene discovery studies is greatly increased if homogeneous and consanguineous populations like the Arabic populations are used.

Materials and methods

Study population

This epidemiological study comprised a total of randomly selected 560 unrelated adults Bahrainis attending the two major hospitals in Bahrain: Salmaniya Medical Center (SMC) and Bahrain Defense Force (BDF) with or without problems. Each subject received a questionnaire which included an informed consent form to sign agreeing to participate in the study. The project also had the relevant ethical clearance from the Ethics Committee from the Arabian Gulf University to undertake the study.

DNA extraction

Venous blood was collected by venipuncture from each individual in vacutainer tubes with EDTA as anticoagulant. Peripheral blood mononuclear cells were used as a source of genomic DNA. Extraction of DNA was performed according to the protocol of the QIAamp DNA Blood mini-spin column (QiagenGmbH, D-40724 Hilden, Germany).

ACE *I/D* polymorphism genotyping

Genotyping was performed for the insertion/deletion (*I/D*) polymorphisms in intron 16 of ACE gene by PCR-gel electrophoresis on the extracted DNA using a pair of oligonucleotide primers 5′-CTGGAGAC CACTCCCAT CCTTTCT-3′ and 5′-GATGTG GCCATCACATTCG TCAGAT-3′ (Biobasic, Markham, Canada). The reactions were run with 5 pmol of each primer in combination with Taq PCR Master Mix (Qiagen, Valencia, CA). DNA was amplified using cycling conditions described previously [15]. PCR products were separated on 2% agarose gels after staining with Vistra Green TM. Two DNA fragments were detected; 190 bp indicating the deletion allele (*D*) and 490 bp indicating the insertion (*I*) allele. Under some conditions, the ACE *D* allele amplifies more effectively than the longer *I* allele, resulting in mistyping of the *ID* as the *DD* genotype. To eliminate the possibility, each sample that had the *DD* genotype was re-amplified with an insertion-specific primer pair, which recognized an insertion-specific sequence DD F 5′-TGG GAC CAC AGC GCC CGC CAC TAC-3′ DD R 5′-TCG CCA GCC CTC CCA TGC CCA TAA-3′ [30]. The interpretation of PCR results was based on the detection of a PCR product of a 335 bp band of insertion (*I* alleles) to determine whether mistype of the DNA, was present, or whether no PCR product appeared on the gel [31].

Data analysis

Statistical analysis was performed using SPSS version 14.0 statistical package for Windows. Allele and genotype frequencies were determined by allele counting and the χ^2 (Chi-squared) were used to compare observed versus expected outcomes. A value of $P < 0.05$ was taken as significant.

Results

Characteristics of the randomly selected Bahraini population

In this study, the Bahraini subjects ($n = 560$) comprised of 295 (52.6%) males and 265 (47.2%) females with a mean age (±SD) of 49.5 ± 15 years and a mean body mass index (BMI) of 26.34 ± 6.5 kg/m^2 (Table 1). In this population, 65 (11.6%) were diagnosed as T2DM based on 1998 World Health Organization diagnostic and classification criteria [32]. In addition, there were 6.2% with

Table 1 Characteristics of 560 Bahrainis

Characteristics	Bahraini individuals
No of participants	560
Gender	
Male (%)	295 (52.6%)
Female (%)	265 (47.2%)
Age[a] (years)	49.5 ± 15
Male	50 ± 15
Female	48 ± 14
Body mass index[a] (kg/m^2)	26.34 ± 6.5
Male	24.68 ± 6
Female	28 ± 6.5
No of T2DM Patients	65 (11.6%)
No. of study participants with the following diseases (%)	
Nephropathy	6.2
Retinopathy	2.3
Neuropathy	1.8
Hypertension	30.3
Hypercholesterolemia	25.3
Cardiovascular disease	7.3

[a] Mean ± SD

Nephropathy, 2.3% with Retinopathy, 1.8% with Neuropathy, 30.3% with Hypertension, 25.3% with Hypercholesterolemia, and 7.3% with Cardiovascular Diseases (Table 1).

Genotypes and allele frequencies of *I/D* polymorphism of the ACE gene in the randomly selected Bahraini population

Allele distribution of ACE *I/D* polymorphism among the Bahraini subjects was calculated by allele counting using the Hardy–Weinberg (H–W) equation. The results are summarized in Table 2. The frequency of the *I* allele was 0.38 while that of the *D* allele was 0.62. Among the 560 Bahraini individuals, 3 of total 228 of *DD* genotypes were confirmed to be *ID* and the frequencies of the *II*, *ID*, and *DD* genotypes of the ACE gene in the studied subjects were 15.5, 44.3, and 40.2%, respectively (Table 2). The gene frequency distribution did not deviate from the H–W equilibrium ($p^2 = 0.1444$; $2pq = 0.4712$; $q^2 = 0.3844$) (Table 2).

Table 2 Frequency of genotypes and allele distribution of *ACE I/D* polymorphism in Bahraini subjects

Number	Genotypes			Allele frequency	
	II	*ID*	*DD*	*I*	*D*
Observed	87	248	225		
%	15.5	44.3	40.2		
Expected	80.864	263.872	215.264		
560 total				0.38	0.62

$\chi^2 = 1.86$ for goodness of fit test

Discussion

The present study investigated for the first time, the frequency of the ACE gene *I/D* polymorphism among randomly selected unrelated Bahraini subjects and the results obtained were compared with other geographic groups (Table 3). Among the randomly selected unrelated Bahraini subjects, the frequency of the *D* allele of the ACE *I/D* gene polymorphism was found to be (0.62), which is consistent with those obtained from the Gulf Region with similar identity to the Omanis (0.71) [33, 34], the Emiratis (0.66) [34, 35], and similar to other Arabs, including such as the Tunisians (0.76) [36], Algerians (0.73) [36], Somalis (0.73) [33, 34], Egyptians from Ismailia (0.68) and from Sinai (0.66) [37], Jordanians (0.66), Sudanese (0.64) [33, 34], and Syrians (0.60) [37].

This high frequency of the *D* allele among Bahrainis is similar to that observed previously not only among Arabs but also in Africans (0.70–0.60) [25, 38]. This is close to the frequency found in Caucasians (0.46–0.58) [5, 39, 40] but much higher than that found in Japanese (0.33–0.35) [41–43] and Chinese (0.29) [44]. On the other hand, the Yanomami Indians and Samoans have the lowest frequencies, 0.15 and 0.09, respectively [25] (Table 3).

The high level of *D* allele among Bahrainis might have an impact on the high prevalence of diabetes and its complications in addition to hypertension in Bahraini population, which is one of the highest populations in the world regarding rate of diabetes. The positive association of *DD* genotype with the development of hypertension was previously reported [7, 14, 45, 46] and with the development of cardiovascular disease [5, 6, 12, 13, 47].

Conclusion

The results obtained for the *D* allele of the *ACE I/D* gene polymorphism among Bahrainis is consistent with those obtained from previous studies in Arabs, Africans, and Caucasians. This study adds to the data indicating the wide variations in the distribution of the ACE alleles in different populations and highlights that great care needs to be taken when interpreting clinical data on the association of the ACE alleles with different diseases. Further studies need to be done on diabetic and hypertensive patients among

Table 3 Prevalence of *I/D* polymorphisms of the ACE gene among different ethnic group

Ethnic group	Allele frequency		Number of individuals included/studied
	I	*D*	
I. Arabs			
Syrians [37]	0.4	0.6	70
Emiratis [33–35]	0.39	0.61	111
Bahrainis	0.38	0.62	560
Sudanese [33, 34]	0.36	0.64	121
Jordaniens [37]	0.342	0.658	60
Egypt—Sinai [37]	0.337	0.663	52
Egypt—Ismailia [37]	0.321	0.679	112
Omanis [33, 34]	0.29	0.71	124
Somalis [33, 34]	0.27	0.73	53
Algerians [36]	0.27	0.73	48
Tunisians [36]	0.24	0.76	47
II. Africans			
Nigerians [25]	0.41	0.59	80
African-Americans [38]	0.30	0.70	40
III. Caucasians [5, 39, 40]	0.42–0.54	0.46–0.58	1212
IV. Asians			
Chinese [44]	0.71	0.29	189
Japanese [41–43]	0.65–0.67	0.33–0.35	354
V. Others			
Yanomami Indians [25]	0.91	0.09	58
Samoans [25]	0.85	0.15	49

I insertion, *D* deletion

Bahraini population compared with control to study the association of ACE polymorphisms with both diseases.

Acknowledgment The authors gratefully acknowledge Colonel Dr. Abdulla Darweesh, Chairman of Department of Pathology in BDF hospital & Major Khalid Matar, Chief of Medical Technologists for giving the opportunity to collect blood samples, for the current study, from the BDF hospital.

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