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Angiotensin-converting enzyme gene insertion deletion (ACE I/D) polymorphism in Saudi children with congenital heart disease

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Abstract. – OBJECTIVE: Congenital heart diseases (CHDs) are the leading cause of infant deaths worldwide. Angiotensin-converting enzyme (ACE) gene I/D polymorphism is associated with many cardiovascular diseases. The precise relationship between this polymorphism and CHDs is not clear. The aim of this work is to determine the normal distribution of I/D polymorphism in Saudi citizens and to test for any association between this polymorphism and CHDs in Saudi children.

PATIENTS AND METHODS: Ninety-six CHD cases and 145 controls were included in this study. DNA was isolated from their peripheral blood, and then ACE I/D gene polymorphism was assayed by polymerase chain reaction (PCR).

RESULTS: There was no significant difference among the frequencies of the DD, DI and II genotypes in patients and controls [39 (41%), 64 (44%), 48 (51%) and 62 (43%), 7 (7%), 19 (13%)] respectively (p-value = 0.3 and OR (95% CI) = 0.3). There was no significant difference between D allele (DD+DI) and II genotype distribution among patients and controls [p-value = 0.2 & OR (95% CI) = 1.9 (0.8-4.7)]. Moreover, there was no difference between I allele (II+DI) and DD frequency [*p*-value = 0.8 & OR = 0.9, CI = 0.5-1.5].

CONCLUSIONS: ACE I/D gene polymorphism is not associated with CHDs in Saudi children. Further large-scale studies are necessary to establish our findings.

Key Words:
Congenital heart disease, Saudi Arabia, ACE gene polymorphism.

Introduction

Congenital heart disease (CHD) involves defects in the structure of the great vessels and/or the heart that disrupt the normal flow of blood through the heart. They are present at birth. CHDs are counted among the most common congenital disorders in newborns and the leading cause of infant death and heart failure worldwide[1,2]. The incidence of CHDs is reported to be approximately 1% with a mortality rate of 24.1%[3]. Severe CHD is a major health problem in the Kingdom of Saudi Arabia and worldwide. In Al-Qassim region, the incidence of infant CHDs that are likely to require urgent surgical and medical treatment is 5.4 per 1,000 live births[4]. Moreover, pediatric heart disease constitutes a major health problem in Al-Madinah region, where the majority of pediatric patients with heart disease have CHDs[5]. There are no definitive etiological factors for CHDs. In most CHD patients, the disease is multi-factorial (i.e. influenced by either genetic or environmental factors), but its causative agents are still not fully understood[6]. High CHD incidence has been observed with the consumption of certain medications during pregnancy, drug abuse or smoking during pregnancy, maternal diabetes mellitus and viral infection (such as rubella infection) in the first trimester[7].

Advances in molecular techniques have uncovered evidence that genetic factors or genetic abnormalities have the upper hand as causative agents, but no definite gene has been identified yet. It has been reported that a high incidence of CHDs was characterized by the presence of an abnormal number of chromosomes (aneuploidy). About 50% of children with trisomy 21 have cardiac defects such as atrial and ventricular septal defects and atrioventricular canal lesions[8]. Muta-

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tions in single genes, including TBX5, JAG1, NKX2.5 and GATA4, have been reported to be associated with the development of CHDs. Advances in CHD diagnosis and cardiac surgery over the past decades have led to a rise in the survival rate of neonates with CHDs[9].

The angiotensin-converting enzyme (ACE) is responsible for the conversion of angiotensin I into angiotensin II, the main hormone of the renin-angiotensin system. This results in alterations in membrane ion channel permeability, intracellular calcium cycling and increased oxidative stress[10]. Angiotensin II has direct and indirect effects on the heart: It stimulates myocardial growth by increasing the fractional rate of protein synthesis and increasing the total peripheral vascular resistance[11].

The single ACE gene polymorphism is defined as insertion at the larger allele (I) and deletion at the shorter one (D), beside a heterozygous form (ID). The gene encoding ACE is located on the long arm of chromosome 17 (17q23) and includes 26 exons across 21 kilobases. The DD form was associated with an elevation of ACE action and a high level of plasma angiotensin II. The deletion allele of the ACE I/D polymorphism is associated with gene-dose related increases in levels of both ACE and angiotensin II. Many studies have examined angiotensin-converting enzyme (ACE) and other renin-angiotensin system-related genes in association with premature coronary heart disease; their results indicate the interactive contribution of ACE DD and AGT MM polymorphisms to the risk of the occurrence of premature coronary heart disease[12-14].

Other researchers investigated the association between ACE I/D polymorphism and rheumatic heart disease (RHD). They found a significant association value between the D allele of ACE polymorphism and the risk of RHD development[15-17]. Yet other studies revealed an association between the II genotype and RHD[17,18].

Recently, a few studies described the association of ACE I/D polymorphism with susceptibility to arrhythmia in CHD. They reported that tachyarrhythmia was associated with high angiotensin II activity[19,20]. ACE gene polymorphism is entangled with the mechanisms of the development of different cardiovascular disorders, including left ventricular hypertrophy, cardiomyopathy, and the increased risk of sudden death in hypertrophic cardiomyopathy[21]. The presence of at least 1 copy of the ACE I/D deletion (I/D or D/D) is associated with a significant increase in the occurrence of tachyarrhythmias after congenital heart surgery with cardiopulmonary bypass, and the preoperative modulation of the renin-angiotensin-aldosterone system with ACE inhibition is associated with a significant reduction in the appearance of tachyarrhythmias[22].

The current study is a case-control study that investigates the existence of any association between ACE I/D polymorphism and CHD. It will be the first study to investigate the existence of an association between this polymorphism and CHD.

Patients and Methods

Patient Selection

This study was approved by the Medical Ethics Committee of Taibah University, and the patients recruited gave their informed consent for genetic analysis. The subjects comprised 96 patients. All of the subjects were enrolled in the Pediatric Cardiology Clinic at the Maternity and Children Hospital of Al-Madinah region, Saudi Arabia, between February 2014 and January 2015. They underwent thorough history taking, full clinical examinations and laboratory assessments. Echocardiography was performed to confirm the diagnoses and facilitate proper classifications. A control group of 145 healthy individuals (that is, with no evidence of heart disease or vascular abnormality) was included in the study.

The genotyping of the ACE I/D polymorphism followed. Genomic DNA was extracted from whole peripheral blood (PB) using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Spectrophotometry was used to quantify the extracted DNA (MaestroNano, MaestroGen, Las Vegas, NV, USA). Samples were stored at –20°C until their use.

The ACE I/D polymorphism was estimated by the presence or absence of the 287 bp sequence in intron 16 of the gene. The method used in the I/D region-flanking primers was described, in [23], to get better results. Reactions were performed with 10 pmol each of the forward primer (5`-CTG GAG ACC ACT CCC ATC CTTTCT-3`) and the reverse primer (5`-GAT GTG GCCATC ACA TTC GTC AGA T-3`). The amplification was carried out in a total of 12.5 μL reaction volume containing 30 ng of genomic DNA added to 2x colorless Go-Taq master mix that included $MgCl_2$, 10x PCR buffer, dNTPs and 10 units of Taq DNA polymerase (Cat # M7132, Promega, Madison, WI, USA). The samples were amplified

using the Veriti thermal cycler (Life Technologies, Foster City, CA, USA). The steps applied follow: initial denaturation at 95°C for 2 minutes then 35 cycles with denaturation at 95°C for 30 seconds (s), annealing at 58°C for 15 s and extension at 72°C for 30 s. The PCR products were separated on 2% agarose gels after staining with ethidium bromide and visualized with UV light (G:BOX, Syngene, Cambridge, UK). Insertion allele (I) samples produced a 490 bp PCR fragment, whereas deletion allele (D) samples produced a 190 bp product (The 287 bp Alu insertion was not detected).

Two fragments (490 bp and 190 bp) were evident in heterozygous samples. Genotypes were determined by direct counting as follows: DD homozygous when only the 190 bp fragment was present, II homozygous when only the 490 bp fragment was present and ID heterozygous when both fragments were present.

Statistical Analysis

Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA) version 21 was used for the statistical analysis of the samples. The unpaired Student's *t*-test was used to compare the mean ages of the groups. Genotypes and allele frequencies in patients and controls were determined by direct counting. The differences in the genotype distributions of the polymorphism between cases and controls were analyzed using chi-squared contingency table analysis or Fisher's exact test as appropriate. Moreover, odds ratios (OR) and 95% confidence intervals (CIs) were calculated. A p-value of <0.05 was considered statistically significant.

Results

A total of 241 subjects were recruited in this study. These subjects comprised 96 CHD group patients and 145 normal volunteers (as the control group). A statistical analysis regarding the age and sex of the population under study is described in Table I. There was no significant difference between control and CHD subjects regarding sex (p-value = 0.9). However, there was a significant difference in age (p-value = 0.0001). The latter was not considered a problem as genetic specifications do not change with age. The clinical diagnosis of the patient group is described in Table II.

ACE I/D polymorphism genotypes and allele frequencies are presented in Table III. The homozygous distribution of DD and II between patients and controls was [39 (41%), 64 (44%), 7 (7%), 19 (13%)] respectively with no statistical significance (p-value = 0.3). The heterozygous distribution of DI between patients and controls was [48 (51%), 62 (43%)] and also showed no statistical significance (p-value = 0.3). D allele distribution (DD+DI) between the two groups also showed no statistically significant difference (p-value = 0.2). Furthermore, I allele distribution (II+DI) showed no statistically significant difference (p-value = 0.8).

Discussion

This study is considered to be the first one that has sought an association between ACE polymorphism and CHDs. We studied children with CHDs and compared them with a pool of controls at our genetic center. A comparison of the two groups revealed no difference regarding sex, but there was an age difference. The age difference proved statistically insignificant as genetic material does not change with age.

In our work, we found no difference between various ACE alleles in patients and controls. We were unable to find any association between this polymorphism and the development of CHDs. In

Table I. Age and gender of the population under study.

Group	Age			Sex		
	Mean ±SD	Median (range)	*p*-value	Male no. %	Female no. %	*p*-value
Cases (96)	6.8 ± 6.3	4 (1-35)	0.0001	53 (55.2 %)	43 (44.8 %)	0.9
Controls (145)	20.6 ± 4.5	21 (11-33)		80 (55.1 %)	65 (44.9 %)	

Age is presented as mean ± SD. Other data are presented as number (%).

Table II. Clinical diagnosis of patient group.

Clinical diagnosis of patients	No.	%
Atrial septal defect	18	(18.8%)
Ventricular septal defect	25	(26.0%)
Patent ductus arteriosus	8	(8.3%)
Atrioventricular septal defect	4	(4.2%)
Tetralogy of Fallot	4	(4.2%)
Transposition of great arteries	2	(2.1%)
Double outlet right ventricle	2	(2.1%)
Pulmonary atresia	2	(2.1%)
Tricuspid atresia	2	(2.1%)
Hypoplastic left heart syndrome	1	(1.0%)
Single ventricle	2	(2.1%)
Truncus arteriosus	2	(2.1%)
Mixed cyanotic heart disease	8	(8.3%)
Anomalous pulmonary venous return	2	(2.1%)
Coarctation of aorta	3	(3.1%)
Interrupted aortic arch	2	(2.1%)
Pulmonary stenosis	6	(6.3%)
Aortic stenosis	3	(3%)

the literature, there were only two studies concerning the polymorphism and CHDs, and the studies did not look for an association between normal and CHDs patients as our repor but they focused solely on CHD patients, investigating their risk for post-operative arrhythmia. One study[22] examined the role of ACE I/D gene polymorphism in postoperative tachyarrhythmias in patients undergoing congenital heart surgery. They found that the risk of tachyarrhythmias after surgery was independently affected by ACE I/D polymorphism. Furthermore, they concluded that genotype variation may be an important factor in perioperative preparation for congenital heart surgery and that preoperative ACE inhibition was associated with a lower risk of postoperative tachyarrhythmias. The other study[24] reported that ACE I/D gene polymorphism was associated with a greater than twofold increase in the odds of developing postoperative junctional ectopic tachycardia (JET) in CHD. Furthermore, the authors concluded that ACE I/D polymorphism was not associated with the development of CHD but may carry a prognostic value regarding the post-operative development of arrhythmia.

Conclusions

We would like to underline that a cohort with a larger number of patients may be necessary. Combining our results with those from the two studies described in the preceding paragraph, we concluded that there was no association between ACE I/D polymorphism and CHDs. However, a study with a larger number of patients may be necessary to establish our findings.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

Table III. Distribution of angiotensin-converting enzyme I/D polymorphism genotypes and allele frequencies between Saudi congenital heart disease patients and controls.

	Control (N=145)		Patients (N=94)			
Genotype	Count	Frequency (%)	Count	Frequency (%)	*p*-value	OR (95% CI)
DD	64	44	39	41	0.3	0.3
DI	62	43	48	51		
II	19	13	7	07		
D	190	66	126	67	0.8	1.1 (0.7-1.6)
I	100	34	62	33		
DD+DI	126	87	87	93	0.2	1.9 (0.8-4.7)
II	19	13	7	07		
II+DI	81	56	55	59	0.8	0.9 (0.5-1.5)
DD	64	44	39	41		

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