Angiotensin-converting enzyme gene insertion/deletion polymorphism in Saudi patients with rheumatic heart disease

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ABSTRACT

Objectives: To investigate the association between angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism and rheumatic heart disease (RHD) in Saudi patients.

Methods: A case-control study was conducted in Saudi RHD patients. Genomic DNA was isolated from 99 RHD patients attending the Pediatric Cardiology Clinic at the Maternity and Children Hospital, Al-Madinah, Saudi Arabia from March 2013 to June 2014, and from 145 age- and gender-matched controls. Patient clinical records were reviewed to report major and minor modified Jones’ criteria for diagnosis. The diagnosis was confirmed by echocardiography. The ACE I/D polymorphism was identified by polymerase chain reaction.

Results: A significant difference in ACE D allele carriage (DD+ID) distribution between RHD cases and controls was identified (p=0.02, odds ratio = 3.6, 95% confidence interval: 1.2-10.8). The D allele carriage was significantly associated with development of mitral valve lesions alone (p=0.03).

Conclusion: The ACE I/D polymorphism is associated with an increased risk of RHD in the Saudi population. Further studies are needed to confirm our findings and to understand the molecular mechanisms underlying this association.

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Rheumatic fever (RF) is a systemic autoimmune disease that can develop after infection with Group A Streptococcus (GAS). Abnormal immune responses to GAS, most often during childhood, can cause valvular heart damage and lead to rheumatic heart disease (RHD). Rheumatic fever remains one of the most common global causes of acquired heart disease in pediatric populations, especially in developing countries. In Saudi Arabia, Al-Sekait et al assessed the prevalence of RHD among 9,418 school children in the western district of the country. After rigorous clinical and cardiological examination, they found that the prevalence of RHD in this cohort was 2.4 per 1000 children aged 6-15 year old. In another retrospective study in Riyadh covering a period between 1994 and 2003, RF/RHD was still reported as a serious complication despite socioeconomic progress in Saudi Arabia, especially in rural areas with limited medical resources. Carditis is the most serious complication of RF that significantly contributes to morbidity and mortality. Worldwide, 30-50% of patients with RF develop carditis. However, in Saudi Arabian patients, carditis has been reported to occur in 53-65% of patients with RF and, of those, mitral regurgitation occurred in 93.3%. Carditis results from chronic inflammation after several attacks of acute RF, causing fibrosis and valve damage. It has been shown that in human hearts, angiotensin-converting enzyme (ACE) mRNA is significantly increased in patients with aortic valve stenosis, which leads to increases in collagen I and III and fibronectin activity. Plasma ACE levels are genetically controlled by an insertion/deletion (I/D) polymorphisms (287 bp) in intron 16 of the ACE gene that functions as a quantitative trait locus (QTL). This locus (rs4340) controls up to 44% of the variability in plasma ACE levels. Other genetic variations in ACE have been reported to influence ACE protein levels in different populations, but the functional role of these variations has not been confirmed. The I/D polymorphism in intron 16 of ACE therefore remains an important genetic variation associated with ACE protein levels. The DD genotype carriers have the highest plasma levels of ACE protein, while those with the II genotype have the lowest levels. Heterozygous individuals (ID genotype) have intermediate protein plasma levels, indicating co-dominance. The ACE I/D polymorphism has been associated with an increased risk of RHD in different populations. With respect to Arab populations, only one study from Egypt has reported that the ACE DD genotype is associated with an increased risk of RHD. Since there is a lack of data regarding the role of this polymorphism in the Saudi population, we evaluated the ACE I/D polymorphism in Saudi patients with RHD.

Methods. Study population. This case-control study was conducted at Taibah University, Al-Madinah, Kingdom of Saudi Arabia. Ninety-nine unrelated RHD patients attended the Pediatric Cardiology Clinic at the Maternity and Children Hospital, Al-Madinah region, Saudi Arabia between March 2013 and June 2014. Using the terms (rheumatic heart disease AND ACE polymorphism), PubMed/MEDLINE, SCOPUS, and Google Scholar were searched for all related literature. Complete and thorough clinical and laboratory assessments were performed in all patients. Diagnosis was made according to modified Jones’ criteria at initial diagnosis, and the diagnosis was confirmed by echocardiography. Patients were sub-grouped according to echocardiographic findings into “mitral valve lesion alone” (MVL) or multiple valves lesions including the mitral valve, termed the “combined valve lesion” (CVL). Patients with suspected or confirmed rheumatic fever without valve involvement, other heart complications, and/or other inflammatory conditions were excluded from the study. One hundred and forty-five age-, gender-, and ethnically-matched unrelated healthy volunteers without evidence or family history of cardiac illness or history of rheumatic fever or autoimmune disease were studied as the control group. All participants were of Saudi Arabian ethnicity. The Center for Genetics and Inherited Diseases (CGID) research ethical committee and the Maternity and Children Hospital ethical committee approved the study, and the authors followed the norms of the World Medical Association Declaration of Helsinki. All adult patients and donors and the parents/guardians of children participants (<18 years old) signed fully informed and written consent approved by the committees.

Genotyping of the ACE I/D polymorphism. Genomic DNA was prepared from 2 ml of whole peripheral blood using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Extracted DNA was quantified by spectrophotometry (MaestroNano, MaestroGen, Las Vegas, NV, USA). Samples were stored at -20°C until used. The ACE I/D polymorphism was assessed by detection of the presence or absence of the 287 bp sequence in intron 16 of the gene. The I/D region-flanking primers were used as previously described. This method has been shown to give accurate results compared with other reported methods. Reactions were performed with 10 pmol of each primer: forward primer (5’-CTG GAG ACC ACT CCC ATC TT’ TCT-3’) and reverse primer (5’-GAT GTG GCC ATC 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 MgCl₂, 10x PCR buffer, dNTPs, and 10 units of Taq DNA polymerase (Cat # M7132, Promega, Madison, WI, USA). Samples were amplified using the Veriti thermal cycler (Life Technologies, Forester City, CA, USA). The cycling and amplification conditions were as follows: an initial denaturation at 95°C for 2 minutes (mins) followed by 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 polymerase chain reaction (PCR) products were separated on 2% agarose gels after staining with ethidium bromide and visualized with UV light (G:BOX, Syngene, Cambridge, UK). Samples with an insertion allele (I) produced a 490 bp PCR fragment, while the deletion allele (D) was identified by a 190 bp product without the 287 bp Alu insertion. In heterozygous samples, 2 PCR fragments (490 and 190 bp) were detected. Randomly selected samples were repeated to confirm the accuracy of the method. Genotypes were determined by direct counting and labeled 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 analysis of the data was performed using the Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA) version 21. All results were confirmed using an online freely available statistical tool (www.vassarstats.net). The unpaired Student's t test was used to compare the mean age between groups. Genotype and allele frequencies were determined by direct counting in patients and controls. The differences in genotype distributions of the polymorphism between cases and controls were analyzed using chi-squared contingency table analysis or Fisher's exact test as appropriate. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. A p-value of <0.05 was considered statistically significant. All genotyping data were checked for any deviation from Hardy-Weinberg equilibrium (HWE) using the chi-squared test.

Results. Patient characteristics. Two hundred and forty-four Saudi Arabian individuals (145 controls and 99 patients) participated in the study. The baseline characteristics of the RHD patients at disease presentation are shown in Table 1. There was no statistically significant difference between the groups in terms of age and gender (Table 1). The MVL group included 48 patients, and the CVL group included 51 patients. Carditis was found in 79.8% of patients, and arthritis was found in 63.6%.

Disease susceptibility. The distributions of ACE I/D polymorphism genotype and allelic frequencies were confirmed using an online freely available statistical tool (www.vassarstats.net). The unpaired Student's t test was used to compare the mean age between groups. Genotype and allele frequencies were determined by direct counting in patients and controls. The differences in genotype distributions of the polymorphism between cases and controls were analyzed using chi-squared contingency table analysis or Fisher's exact test as appropriate. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. A p-value of <0.05 was considered statistically significant. All genotyping data were checked for any deviation from Hardy-Weinberg equilibrium (HWE) using the chi-squared test.

Table 1 - Demographic characteristics and clinical manifestations of the Saudi RHD patients and controls (controls N=145 and patients N=99).

| Parameter | Values | P-value |
|-----------|--------|---------|
| Average age (mean ± SD years) | | |
| Patients | 20.6±4.5 | 0.06 |
| Controls | 19.4±5.2 | |
| Gender: male/female (%) | | |
| Patients | 54.5±5.5 | |
| Controls | 49.6±5.0 | 0.45 |
| Clinical manifestations of patient group | | |
| Mitral valve lesion | 48 (48.5) | |
| Combined valve lesion | 51 (51.5) | |
| Carditis | 79 (79.8) | |
| Arthritis | 63 (63.6) | |
| Chorea | 8 (8.1) | |
| Skin rash | 3 (3.0) | |
| Subcutaneous nodules | 2 (2.0) | |
| Recurrence | NA | |

RHD - rheumatic heart disease, SD - standard deviation, NA - not available

Table 2 - Distribution of angiotensin-converting enzyme I/D polymorphism genotypes and allele frequencies between Saudi rheumatic heart disease patients and controls.

| Genotype | Control (N=145) Count % Frequency | Patients (N=99) Count % Frequency | X² | P-value | OR (95% CIs) |
|----------|-----------------------------------|-----------------------------------|-----|---------|--------------|
| DD       | 64 (44)                           | 50 (51)                           | 5.7 | 0.06    |              |
| ID       | 62 (43)                           | 45 (45)                           |     |         |              |
| II       | 19 (13)                           | 4 (4)                             |     |         |              |
| D        | 190 (66)                          | 145 (73)                          | 3.2 | 0.07    | 1.4 (0.97 - 2.1) |
| I        | 100 (34)                          | 53 (27)                           |     |         |              |
| DD+ID    | 126 (87)                          | 95 (96)                           | 5.7 | 0.02    | 3.6 (1.2 - 10.8) |
| II       | 19 (13)                           | 4 (4)                             |     |         |              |
| II+ID    | 64 (56)                           | 49 (49)                           | 0.0 | 0.96    | 1 (0.6 - 1.7) |
| DD       | 81 (44)                           | 50 (51)                           |     |         |              |

D - deletion allele, I - insertion allele, OR - odds ratio, CI - confidence interval
in RHD patients and controls are shown in Table 2. Both populations were in Hardy-Weinberg equilibrium for all genotype frequencies. The genotype frequencies between RHD cases and controls were not significantly different, despite a trend towards higher DD genotype frequencies between RHD cases and controls were not significantly different, despite a trend towards higher DD genotype frequencies. The genotype frequencies in RHD patients and controls are shown in Table 2. Both populations were in Hardy-Weinberg equilibrium for all genotype frequencies. The genotype frequencies between RHD cases and controls were not significantly different, despite a trend towards higher DD genotype frequencies between RHD cases and controls were not significantly different, despite a trend towards higher DD genotype frequencies. The genotype frequencies in RHD patients and controls are shown in Table 2. Both populations were in Hardy-Weinberg equilibrium for all genotype frequencies. The genotype frequencies between RHD cases and controls were not significantly different, despite a trend towards higher DD genotype frequencies between RHD cases and controls were not significantly different, despite a trend towards higher DD genotype frequencies. The genotype frequencies in RHD patients and controls are shown in Table 2. Both populations were in Hardy-Weinberg equilibrium for all genotype frequencies. The genotype frequencies between RHD cases and controls were not significantly different, despite a trend towards higher DD genotype frequencies between RHD cases and controls were not significantly different, despite a trend towards higher DD genotype frequencies. The genotype frequencies in RHD patients and controls are shown in Table 2. Both populations were in Hardy-Weinberg equilibrium for all genotype frequencies. The genotype frequencies between RHD cases and controls were not significantly different, despite a trend towards higher DD genotype frequencies between RHD cases and controls were not significantly different, despite a trend towards higher DD genotype frequencies. The genotype frequencies in RHD patients and controls are shown in Table 2. Both populations were in Hardy-Weinberg equilibrium for all genotype frequencies. The genotype frequencies between RHD cases and controls were not significantly different, despite a trend towards higher DD genotype frequencies between RHD cases and controls were not significantly different, despite a trend towards higher DD genotype frequencies.
is itself responsible for plasma ACE activity or whether it merely represents a surrogate marker. Different methods have been used in different populations to determine the QTL of ACE gene activity. In a recent study, Chung et al identified major haplotype blocks in the ACE gene in the Taiwanese population that were significantly associated with ACE plasma protein levels. Interestingly, they found that the region between exon 13 and intron 18 and a region between intron 20 and the 3’ UTR had the most QTL of ACE activity. This indicates that the I/D polymorphism in intron 16 remains an important genetic locus for ACE gene activity. High levels of ACE mRNA and protein have been found in stenotic heart valves. Interestingly, in an animal model, ACE inhibition attenuates aortic valve thickening. Moreover, hypertensive patients carrying the D allele are less responsive to ACE inhibitors, which indicates the strong influence of the D allele in gene expression and disease course. The effect of the ACE I/D polymorphism in patients with RHD requires further study. This may open up the possibility of using ACE inhibitors in the management of RHD and provide an opportunity for pharmacological intervention.

This study has some limitations. It was a case-control observational study with a relatively small sample size that limited the statistical power of the study. Also, we selected only one ACE polymorphism to study. Further work is needed in a larger sample size from different areas of Saudi Arabia that includes screening additional known QTLs in the ACE gene for haplotype analysis. This will be useful for future meta-analyses.

In conclusion, our data suggest that the ACE I/D polymorphism is associated with an increased risk of RHD in the Saudi population. We believe that the ACE I/D polymorphism is linked to the presence of the disease, and may play an important role in the inflammatory phase of RHD. Further studies are needed to understand the molecular mechanisms underlying this association.

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