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Original article

Analyses of *HSP90α* gene polymorphism in arrhythmogenic right ventricular cardiomyopathy/dysplasia

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ABSTRACT

Introduction: Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia (ARVC/D) is a genetic disorder characterized by fibrofatty replacement of right ventricle. Extensive research revealed the involvement of desmosomal, non-desmosomal and modifier genes in the etiology of the ARVC/D. Further, environmental factors with modifier genes influence the severity of the disease. One of the well-known modifier genes is *HSP90α* polymorphism, which is involved in protein folding and regulation as well as apoptosis etc. Therefore, in this study, an investigation was carried out to find the possible association between *HSP90α* polymorphism and ARVC/D.
Material and methods: This study included 240 healthy control samples without any family history of cardiac diseases and 61 ARVC/D patients. Diagnosis of patients was carried out using ECG and 2-D echocardiography, following revised diagnostic criteria: definite, borderline diagnosis and possible diagnosis. Scoring of genotypes was carried out by allelic-specific PCR.
Results: A statistically significant association was observed between *HSP90α* polymorphism and ARVC/D. In codominant and overdominant models, **'C/G'** was found to be risk conferring. Further, in dominant model, **'C/G'** and **'G/G'** genotypes were susceptible for ARVC/D. Moreover, **'G'** allele which was predominant in ARVC/D was found to risk conferring towards ARVC/D.
Conclusion: These findings suggest that possible role of*HSP90α* polymorphism in the etiology of ARVC/D. The **'G'** allele of *HSP90α* polymorphism damages the 3-D native conformations, which is required for the interaction between client proteins such as desmosomes, ryanodine receptor-2 and sodium channel leading to mislocalization of these proteins. Furthermore, loss of inhibitory interaction between HSP90α protein and Apaf-1 trigger apoptosis.

1. Introduction

Marcus et al. (1982) first described ARVD/C as "a rare heritable cardiomyopathy characterized by fibro-fatty replacement of the myocardium, which predisposes patients to life-threatening ventricular arrhythmias".[1] Decades of research on ARVC/D revealed causal mutations in the desmosomal and non desmosomal genes apart from modifier genes, resulting in distorted cellular proteostasis, leading to aggregation of proteins.[2] These insoluble aggregates of misfolded proteins are highly toxic for cellular cardiomyocytes in cardiomyopathy, arrhythmias, and heart failure.[3] Cardiomyocytes may react to these conditions by inducing and /or by enhancing the synthesis of molecular chaperones like HSP70, HSP20, HSP27 and HSP90 etc.[4]

One of the best-known chaperones is HSP90α; which accounts for 1% of total soluble cytosolic proteins in unstressed/normal cells. Recent studies have revealed housekeeping functions of HSP90α, such as protein folding and regulation, providing rationale for its cytoplasmic abundance. In higher eukaryotes, HSP90α is isolated from cytoplasm, nucleus, mitochondria and endoplasmic reticulum. This isolated protein exists in two cytosolic isoforms: an inducible HSP90α1 and α2 encoding *HSP90 A AA1*and *HSP90 A AA12*, respectively; located at **14q32.33**, whereas HSP90β, a constitutive protein is encoded by *HSP90 A AB1* localized to **6p12** region.[5]

Since reports have implicated the role of HSP90α in complex protein folding, apoptosis and vesicular transport movement, the present study was carried out to elucidate the possible association between *HSP90α* polymorphism and ARVC/D in Indian cohort, as a

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marker of cardioprotection, as ARVC/D is a disease of inflammation and involves apoptosis.

2. Material and methods

The present study comprised 61 ARVC/D patients; revised 2010 task force criteria was used for diagnosis[6] and randomly selected 240 healthy control samples, sex and age matched, were collected based on the negative family history of cardiac events. The informed consent was signed by all the patients participated in the present study. Further, for genetic and clinical analysis, institutional ethics committee authorization was obtained from Care Hospitals and Osmania University, Hyderabad.

2.1. Diagnosis of ARVC/D/ inclusion criteria

ARVC/D was diagnosed based on revised Task Force Criteria 2010, which consists of three categories; they are

Definite ARVC/D with 2 major or 1 major and 2 minor criteria or 4 minor criteria from different categories;

Borderline ARVC/D with 1 major and 1 minor or 3 minor criteria from different categories; and

Possible ARVC/D with 1 major or 2 minor criteria from different categories.

In the patient consent forms, following information was obtained.

1) Complete information on presenting symptoms such as dizziness, palpitations, presyncope, syncope, chest pain and a family history.
2) ECG at diagnosis with repolarization and depolarization abnormalities
3) Two-dimensional echocardiography was used to determine the global or regional dysfunction and structural alterations in the right ventricle myocardium

2.2. Exclusion criteria

Patients with right ventricular outflow tract and ventricular tachycardia arising from the aortic root and cardiac sarcoidosis were excluded from the present study.

The genomic DNA was isolated from white blood cells following standard protocol.[7,8] To screen *Gln488His* (***C*** > ***G***) polymorphism specific PCR primers for allele specific PCR were obtained from Zagouri et al. (2012). The common primer was 5' TGGATAACTGTGAGGAGCTAA and allele specific primers were CCTGTGATATATAATAGATATGTTTG and CCTGTGATATAATAGATATGTTTC. The PCR-mix comprised of 60 ng template DNA, 10 mM dNTP-mix, 1 mM of each primer, 10xPCR buffer and 1U Taq-polymerase in a reaction volume of 10 ml. Reactions were carried out in an Eppendorf Master Cycler Gradient (**Germany**). The PCR cycle steps were 95 °C for 5', followed by 35 cycles of 95 °C for 30 s, 55 °C for 30 s, 70 °C for 45 s and final cycle at 72 °C for 10 min.[9]

The X^2 test for *Gln488His* (*C* > *G*) polymorphism in cases and controls was carried out to detect the deviation from Hardy-Weinberg equilibrium. Odds ratio with 95% confidence intervals were calculated to associate genotypic and allelic frequencies. SNPStats software was used for statistical analysis, which is freely available online (www.bioinfo.iconcologia.net/SNPstats). For statistical all test, $p > 0.05$ was set as significant level.

3. Results

The allele specific-PCR was adopted to amplify and genotype *Gln488His* polymorphism as reported by Zagouri et al. (2012).

Table 1
Genotype frequency of *Gln488His* polymorphism of *HSP90α* gene in controls and ARVC/D group.

Genotype	Controls n %		ARVC /D n %		χ^2 (p value)
C/C	162	**68**	29	48	**10.088 (0.006)**
C/G	25	10	14	**23**	
G/G	53	22	18	**30**	

The three genotypes of ***HSP90α*** polymorphism were identified in ARVC/D cases and controls and their frequencies are presented in Table 1. The most common genotype was considered as reference genotype '**CC**' **(68%**) and least common as variant '**GG' (22%**). Analysis of the polymorphism revealed '**CC'** genotype was **two**-fold higher in controls than ARVC/D, indicating protective nature of **'C'** allele. However, the frequency of '**C/G'** and '**G/G'** (**23% and 30%)** were higher in ARVC/D cohort when compared to controls (**10% and 22%**), pointing to the risk conferring nature of **'G'** allele and '**GG'** genotype to ARVC/D.

The proportion of allele frequencies were calculated from genotypic frequencies in both ARVC/D cases and controls, wherein higher frequency of **'C'** allele in controls (**0.73** and **0.59**) and **'G'** allele in ARVC/D (**0.41**) than in controls (**0.27**) was observed.

Chi square test of association revealed a significant deviation from Hardy Weinberg equilibrium with reference to genotypic and allelic frequencies (**χ^2 = 10.088 and p = 0.006; χ2 = 8.673, p = 0.003),** signifying proof for '**GG'** genotype to disease susceptibility and linkage disequilibrium between susceptible ARVC/D loci and marker of *HSP90α* gene.

The relative risk estimates revealed that the heterozygote '**C/G'** to be risk conferring to ARVC/D (in codominant model and overdominant models) (**OR: 3.13, CIs:1.46-6.72, p = 0.008 and OR: 2.56; CIs: 1.24–5.30; p = 0.014**), similarly in the dominant model, '**C/G'** and **'G/G'** genotypes were susceptible for ARVC/D (**OR: 2.29 ; CIs: 1.30–4.05; p = 0.004**). These findings point towards **'GC' and 'GG'** as risk genotypes in ARVC/D. The relative risk estimates computed for alleles also revealed risk conferring nature of **'G'** allele, which is predominant in ARVC/D cohort (**OR: 7.81; CIs: 4.16–13.06; p = 0.0001)** (Tables 2–4).

4. Genotype-phenotype correlations

Gender wise comparison revealed that the heterozygote genotype **(CG)** was found to be predominant in female patients **(54.1%)** than males **(48.6%)**.

The frequency distribution of ***HSP90α*** genotypes with respect to the clinical symptoms is provided in Table 5. The patients with **'CG'** genotypes exhibited enhanced symptoms of dyspnea (**53%**), palpitations (**50%),** syncope **(59%**) and tachyarrhythmias **(56%).** This clearly establishes the genotype-phenotype correlation with respect to the **'G'** allele and '**GG**' genotype and its role in etiopathogenesis and progression of ARVC/D, associated with varied prognosis.

Table 6 shows frequency distribution of ***HSP90α*** genotypes in correlation with ECG and 2-D ECHO characteristics. **'CG'** genotype was associated with sinus rhythm (**54%**), T-wave inversion (**62%**), and bundle branch block (**52%**); while **'GG'** genotype with systolic dysfunction (**42%**) and RV dilation (**51%**), further strengthening the role of **'G'** allele in disease manifestation and ascertaining genotype-phenotype correlations in ARVC/D (Fig. 1).

4.1. In silico analysis: Secondary mRNA structure analysis

To determine the stability of the pre-mRNA molecules *in silico* analysis was performed. Vienna webserver (http://rna.tbi.univie.