Mol Cell Biochem (2017) 427:1–11
DOI 10.1007/s11010-016-2891-y

Renin–angiotensin system gene polymorphisms as potential modifiers of hypertrophic and dilated cardiomyopathy phenotypes

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Received: 3 August 2016 / Accepted: 22 November 2016 / Published online: 24 January 2017

Abstract The renin–angiotensin (RAS) pathway has an important role in the etiology of heart failure and given the importance of RAS as a therapeutic target in various cardiomyopathies, genetic polymorphisms in the RAS genes may modulate the risk and severity of disease in cardiomyopathy patients. In the present study, we examined the association of RAS pathway gene polymorphisms, angiotensin converting enzyme (ACE), angiotensinogen (AGT), and angiotensin receptor type 1 (AGTR1) with risk and disease severity in Asian Indian idiopathic cardiomyopathy patients. The case–control study was conducted in 400 cardiomyopathy patients diagnosed with HCM, DCM, or restrictive cardiomyopathy (RCM) and 235 healthy controls. Genotyping of patients and controls was done by PCR–RFLP assays. Left ventricular wall thickness and left ventricular ejection fraction were measured by means of M-mode echocardiography. We observed significantly higher prevalence of ACE DD and AGTR1 1166CC genotypes in hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) patients. Also, 235TT genotype of AGT (M235T) was significantly associated with enhanced risk of the disease phenotype in HCM, DCM, and RCM.

Keywords Idiopathic cardiomyopathies · Angiotensinogen · Single nucleotide polymorphisms · Hypertrophy · Modifier genes

Introduction

Primary cardiomyopathies are a clinically heterogeneous group of multifactorial disorders and are frequent cause of sudden death. Hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), and arrhythmogenic right ventricular cardiomyopathy (ARVC) are the major types of primary cardiomyopathies [1]. Mutations in sarcomeric and non-sarcomeric genes have been identified as the predominant causative factor in primary cardiomyopathies. However, incomplete and variable penetrance of the disease has been commonly observed in affected individuals and related family members carrying identical gene mutations. A wide range of heterogeneity has also been shown in disease phenotype, for example; the same mutation in two unrelated individuals may cause DCM or HCM or RCM or varied degree of cardiac hypertrophy/dilatation, impairment of systolic/diastolic function, and incidence of sudden cardiac death. These studies suggest that besides pathogenic gene mutations, other factors such as modifier genes and environment can modulate disease phenotype in primary cardiomyopathies. Modifier genes do not cause the disease as such but can influence the phenotypic expression of the primary mutation. Several recent studies have shown that polymorphisms in genes of renin–angiotensin (RAS) pathway such as ACE, AGT, AGTR1 may influence clinical phenotype of HCM/DCM. Perkins et al. reported that HCM patients with ACE DD genotype showed increased LVH and proposed ACE DD

Electronic supplementary material The online version of this article (doi:10.1007/s11010-016-2891-y) contains supplementary material, which is available to authorized users.

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to be a 'pro-LVH' modifier in HCM [2]. ACE D allele was also found to be significantly associated with increased mean septal thickness in HCM and reduced LVEF in DCM in a small cohort of Asian Indian patients [3]. Similarly, polymorphisms in angiotensinogen (AGT) gene have been also shown to be associated with disease phenotype in both HCM and DCM; Goldbergova et al. found AGT M235T polymorphism to be associated with increased risk of heart failure in dilated cardiomyopathy and ischemic heart disease in Czech population [4]. In another study in Canadian-Caucasians involving ten RAS polymorphisms, haplotype consisting of the variant alleles (AGT M174 and AGT T235) was found to be present at significantly higher frequency in heart failure patients as compared to healthy controls [5], confirming association of AGT gene with heart failure. AGT Receptor type 1 (AGTR1) A1166C polymorphism has been also reported to contribute to cardiac hypertrophy in patients with HCM and essential hypertension [6, 7]. However, AGTR1 (A1166C polymorphism) was not found to be associated with increased mortality in acute myocardial infarction (AMI) patients [8], but a long-term follow-up study reported an unfavorable role of AC genotype [AGTR1 (A1166C polymorphism)] after the acute coronary event [9]. A recent genome wide association study to identify common HCM-associated genetic polymorphisms has identified a common non-synonymous FHOD3 genetic variant to be associated with HCM [10] further emphasizing the role of genetic modifiers in determination of phenotype in primary cardiomyopathies.

Till date, there are very few genetic studies on the role of modifier genes in primary cardiomyopathies in different populations and data so far have been inconsistent and inconclusive. In the present study, we examined association of known SNPs of ACE, AGT, and AGTR1 genes of RAS individually and in combination (gene–gene interaction), with HCM and DCM phenotypes in a large cohort of unrelated primary cardiomyopathy Asian Indian patients. In addition, we also examined association of gene polymorphisms in four major sarcomeric genes, i.e., myosin heavy chain (MYH7), myosin binding protein C (MYBPC3), troponin T (TNNT2), and troponin I (TNNI3) with disease severity in cardiomyopathy patients. Our results show significantly high frequency of variant alleles (ACE D, AGT 235T, and AGTR1 1166C) in HCM and DCM patients. Moreover, patients with a mutation in one of the four sarcomeric genes (MYH7, MYBPC, TNNI3, and TNNT2) and carrying a variant allele showed more severe form of disease phenotype (increased septal thickness in HCM/decreased LVEF and increased LVED in DCM) suggesting a role for these variant alleles in modulating disease phenotype.

Methods

Subject selection

From September 2007 to December 2011, 400 consecutive patients, diagnosed with primary cardiomyopathies (HCM, DCM, RCM, and ARVC) attending the cardiology clinic in out patient department of advanced cardiac center at PGIMER, Chandigarh, India, were enrolled in this study. Two hundred and thirty five healthy, ethnicity-matched unrelated subjects, without any family history of heart disease, hypertension, diabetes, or any other chronic ailments served as controls. A written informed consent was obtained from each subject (patients and controls) and study protocols were approved by institutional ethics committee.

Inclusion criteria for all enrolled patients was as follows: HCM patients: left ventricular septal thickness $\geq$13 mm; DCM patients: left ventricular ejection fraction (LVEF) less than 40% in the absence of any other cause were enrolled as DCM patients. Individuals were diagnosed with RCM when echocardiography revealed the Doppler measurements consistent with restrictive left ventricular filling pattern.

Ten millilitre blood was collected in two EDTA-coated vacutainers for molecular genetic studies. The presence of a putative disease-causing variant(s) was determined by mutational analysis of four sarcomeric genes (MYH7, MYBPC3, TNNT2, and TNNI3).

Genotyping

Genomic DNA was extracted from whole blood by phenol–chloroform method. Gene polymorphisms in ACE (ACE I/D (rs1799752), AGT (T174 M) (rs4762) (supplementary Fig. 1) and AGT M235T (rs699) (supplementary Fig. 2), and angiotensin II type I receptor (AGTR1) A1166C (supplementary Fig. 3) were investigated by PCR–RFLP methods (supplementary Figs. 1, 2, 3) [11]. Primer sequences and PCR conditions are given in Table 1. PCR reactions were carried out in 25 ml volumes using 50 ng DNA, 10 pmol of each primer, 200 mM of each dNTP, 2 mM $MgCl_2$, and 1.0 U Taq polymerase (Bioron). Five percent of samples were randomly selected and sequenced to confirm the various genotypes.

Statistical analysis

Statistical analysis was done using SPSS ver17. Inter group comparison of the baseline characteristics was performed using Student's unpaired t test for continuous data and the Chi square test for categorical data.

Table 1 Primers, product size, and annealing temperature for RAS genes

Primer		Amplicon (bps)	Annealing temperature (°C)
ACE I/D			
Forward	CTGGAGACCACTCCCATCCTTTCT	490	59.5
Reverse	GATGTGGCCATCACATTCGTCAGAT		
AGT (T174 M)			
Forward	TACAGGCAATCCTGGGTGTTCCTTG	404	68.8
Reverse	AGCAGAGAGGTTTGCCTTACCTTG		
AGT (M235T)			
Forward	CAGGGTGCTGTCCACACTGGACCCC	165	68.3
Reverse	CCGTTTGTGCAGGGCCTGGCTCTCT		
AGTR1 (A1166C)			
Forward	ATTCCCCCAAAAGCCAA	500	57.5
Reverse	CTGTTGCTCCTCTAACGA		

Differences in allele and genotype frequencies were analyzed using Chi square test or Fisher's exact test and ORs and their 95% CIs were calculated. AGT haplotype analysis was carried out using PHASE version 2.1.1 [12]. The results were considered statistically significant at the 0.05 level.

For evaluation of gene–gene and gene–environment, the multifactor-dimensionality reduction (MDR) method was used [13]. The best MDR model was selected as the one with the maximum testing accuracy. A testing accuracy of 0.5 is expected under the null hypothesis.

Results

The demographic and clinical characteristics of the patients and control subjects are given in Table 2. HCM was more frequent among men (71.2%), while RCM was found to be more common in females (60.6%) in our cohort. 17.4% of the patients had a putative disease-causing myofilament/mitochondrial variant identified (manuscript in preparation).

Genotype and allele frequencies of ACE, AGT, and AGTR1 genes and clinical phenotype

Genotype and allele frequencies of ACE, AGT, and AGTR1 SNPs in patients and control subjects are shown in Tables 3 and 4, respectively ACE I/D and AGT genotypes were in Hardy–Weinberg equilibrium. The prevalence of ACE I/D variant genotypes (ID and DD) was found to be significantly higher in cardiomyopathy patients as compared to controls and was associated with increased risk of the disease (OR 4.66; CI 2.51–8.64, $p < 0.001$ and OR 2.89; CI 1.4–6.0, $p = 0.004$, respectively). Patients with DD genotype were on average 3.4 years younger than other patients at the time of diagnosis/admission. Also hypertension was documented 1.5 years earlier in these patients as compared to those with ID and II genotypes.

HCM patients with DD genotype had significantly increased left ventricular wall thickness as compared to ID ($p = 0.09$) and II ($p < 0.001$) genotypes. DCM patients with ACE ID and DD genotypes showed significantly decreased LVEF (Table 5) as compared to II and ACE DD genotype in DCM patients was observed to be significantly associated with increased LVED as compared to II genotype ($p \ll 0.01$).

AGT (T174 M)

No significant difference in genotype frequencies of AGT (T174 M) (C3889T) polymorphism was observed between patients and controls (OR 1.48; CI 0.89–2.45; $p = 0.15$ and OR 0.94; CI 0.15–5.69; $p = 1$). Although no significant association between AGT (T174 M) genotypes and age of onset of hypertension was observed, the only hypertensive patient with homozygous variant genotype (AGT (T174 M)) was 10 years younger than hypertensive patients with wild-type genotype (37 years vs 48.86 ± 13.14 years; $p = 0.54$). There were no significant genotype-based differences with regard to LVWT in HCM patients (TT = 23.58 ± 4.66 mm, TM = 23.34 ± 6.82 mm; $p = 0.81$, MM = 27.00; $p = 0.47$) and LVED (TT = 58.11 ± 10.71, TM = 58.80 ± 9.56; $p = 0.73$, MM = 54.71 ± 12.91; $p = 0.41$) in DCM patients; although a trend for increased LVEF was observed in DCM patients carrying TM and MM genotypes (TT = 28.19 ± 9.53, TM = 29.05 ± 9.91, M = 31.29 ± 10.42; $p = 0.41$), it was not statistically significant ($p = 0.62$) (Table 5).

Table 2 Demographic profile and clinical characteristics of study population

Variables	Patients			Controls ($n = 201$)
	HCM ($n = 157$)	DCM ($n = 206$)	RCM[a] ($n = 37$)	
Age (years)	45.46 ± 14.9	41.94 ± 14.37	35.23 ± 15.85	42.86 ± 14.16
LVWT (mm)	22.98 ± 5.80	–	–	–
LVEF (%)	–	28.31 ± 10.26	58.61 ± 15.00	–
LVEDD (mm)	–	58.64 ± 11.12	–	–
Risk factors				
Smoking (%)				
Smokers	21.5	28.1	10.9	55.7
Non-smokers	78.5	71.9	89.1	44.3
Alcohol (%)				
Alcoholic	34	22.7	10.9	43.4
Non-alcoholic	66	77.3	89.1	56.6
Diabetes (%)				
Diabetic	10.1	10.1	0	0
Non-diabetic	89.9	89.9	100	100
Hypertension (%)				
Hypertensives	31.7	25.6	13	0
Non-hypertensives	68.3	74.4	87	100

n number of subjects; data expressed as mean ± SD and percentage

[a] Eight ARVC and three IVCD patients are grouped under RCM for this study due to very small sample size

AGT (M235T)

Consistent with the Asian population (Indian, Chinese, Japanese), in which a high frequency of T allele of AGT M235T polymorphism has been reported to increase the risk of HCM in Asians [14], we too observed similar results in our cohort (Table 4). The frequency of variant allele (235T) and variant genotypes (235TT and 235MT) was significantly higher in all three groups of patients (HCM, DCM, and RCM) as compared to controls ($p = 0.004$, $p \ll 0.001$, and $p = 0.008$). The age of onset of hypertension in homozygous variant carriers was approx 3.4 years less than the patients with wild-type allele ($p = 0.54$). In HCM cohort, patients with TT genotype had significantly increased septal thickness as compared to those with wild genotype (24.97 ± 4.64 vs. 21.63 ± 4.34 mm, $p = 0.05$) and MT genotypes (24.97 ± 4.64 vs. 21.53 ± 5.75 mm; $p = 0.95$). The frequency of the variant allele 235T was significantly higher in DCM patients compared to controls but it was not significantly associated with either decrease in LVEF ($p = 0.48$) or increase in LVED ($p = 0.24$).

Haplotype analysis of AGT polymorphisms

Haplotypes for AGT gene polymorphisms (M235T and T174 M) were inferred using Phase software version 2.1.1 and Haploview. A total of four haplotypes were generated for AGT gene. Haplotype T–T containing wild-type allele for T174 M and mutant allele for M235T was seen to be predominant in our study population (Table 6). The frequencies of T–T (OR 1.73; 95% CI 1.30–2.30, $p = 0.0001$) and M–T (OR 1.75; 95% CI 0.12–2.73, $p = 0.01$) haplotypes were significantly higher in cardiomyopathy patients as compared to controls and were associated with increased risk of disease.

AGTR1 (A1166C)

The study population was not in HWE for A1166C polymorphism as the variant CC genotype was not observed in controls. The frequency of AGTR1 1166CC genotype was significantly higher in patients as compared to controls (Table 3). There was only one hypertensive patient with CC genotype and was only 19 years ($p = 0.14$) old as compared to average age of 45.95 ± 13.39 years of hypertensive patients with AA genotype. The CC genotype was more common in females (66.7%) as compared to males and had significant association with the risk of disease (1166 AA vs. 1166AC; OR 0.29, CI 0.17–0.52, $p = 0.00002$). HCM patients with CC or AC genotype showed increased septal thickness (25.00 ± 4.24 mm; $p = 0.68$; 23.82 ± 6.07 mm; $p = 0.77$) as compared to those with AA genotype (23.46 ± 5.15 mm).

Table 3 Genotype frequencies of ACE I/D, AGT (T174 M), AGT (M235T), and AGTR1 (A1166C) gene polymorphisms in idiopathic cardiomyopathy patients and controls

Polymorphisms	Patients, *n* (%)	Controls, *n* (%)	OR (95% CI)	*p* value
ACE I/D				
HCM				
II	16 (12.12)	72 (36)	1.0	
ID	89 (67.42)	86 (43)	4.66 (2.51–8.64)	**<0.001**
DD	27 (20.46)	42 (21)	2.89 (1.4–6.0)	**0.004**
DCM				
II	15 (8.47)	72 (36)	1.0	
ID	120 (67.80)	86 (43)	6.70 (3.60–12.47)	**<0.001**
DD	42 (23.73)	42 (21)	4.8 (2.38–9.68)	**<0.001**
RCM				
II	9 (27.27)	72 (36)	1.0	
ID	16 (48.48)	86 (43)	1.49 (0.62–3.57)	0.395
DD	8 (24.24)	42 (21)	1.52 (0.55–4.25)	0.43
AGT (T174 M)				
HCM				
CC	114 (73.08)	160 (79.60)	1.0	
CC	40 (25.64)	38 (18.90)	1.48 (0.89–2.45)	0.15
TC	2 (1.28)	3 (1.50)	0.94 (0.15–5.69)	1
DCM				
CC	154 (77)	160 (79.60)	1.0	
TC	39 (19.5)	38 (18.90)	1.07 (0.64–1.76)	0.89
TT	7 (3.5)	3 (1.50)	2.42 (0.62–9.55)	0.21
RCM				
CC	37 (82.22)	160 (79.60)	1.0	
TC	7 (15.56)	38 (18.90)	0.78 (0.33–1.92)	0.68
TT	1 (2.22)	3 (1.50)	1.44 (0.15–14.25)	1
AGT (M235T)				
HCM				
CC	16 (12.21)	26 (15.12)	1.0	
CT	72 (54.96)	126 (73.25)	0.93 (0.47–1.85)	0.86
TT	43 (32.83)	20 (11.63)	3.49 (1.54–7.92)	**0.002**
DCM				
CC	11 (6.75)	26 (15.12)	1.0	
CT	97 (59.51)	126 (73.25)	1.82 (0.86–3.86)	0.14
TT	55 (33.74)	20(11.63)	6.5 (2.72–15.53)	**<0.0001**
RCM				
CC	3 (7.69)	26 (15.12)	1.0	
CT	21 (53.85)	126 (73.25)	1.45 (0.40–5.20)	0.76
TT	15 (38.46)	20 (11.63)	6.5 (1.65–25.58)	**0.005**
AGTR1 (A1166C)				
HCM				
CC	129 (83.76)	130 (65.0)	1.0	
TC	23 (14.93)	70 (35.0)	0.33 (0.19–0.56)	**<0.001**
TT	2 (1.29)	0	–	0.498
DCM				
CC	156 (79.19)	130 (65.0)	1.0	
TC	41 (20.81)	70 (35.0)	0.49 (0.31–0.77)	**0.001**
TT	0	0	–	1
RCM				
CC	38 (82.60)	130 (65.0)	1.0	
TC	7 (15.21)	70 (35.0)	0.34 (0.14–0.80)	**0.01**
TT	1 (2.17)	0	–	0.23

Table 4 Allele frequencies of ACE I/D, AGT (T174 M), AGT (M235T), and AGTR1 (A1166C) gene polymorphisms in idiopathic cardiomyopathy patients and controls

Polymorphisms	Patients, *n* (%)	Controls, *n* (%)	OR (95% CI)	*p* value
ACE I/D				
HCM				
D	0.54	0.425	1.0	
I	0.46	0.575	1.6 (1.17–2.19)	**0.03**
DCM				
D	0.58	0.425	1.0	
I	0.42	0.57	1.84 (1.38–2.46)	**<0.001**
RCM				
D	0.48	0.425	1.0	
I	0.52	0.57	1.27 (0.76–2.15)	0.42
AGT (T174 M)				
HCM				
C	0.86	0.89	1.0	
T	0.14	0.11	1.34 (0.85–2.09)	0.21
DCM				
C	0.87	0.89	1.0	
T	0.13	0.11	1.24 (0.81–1.90)	0.33
RCM				
C	0.90	0.89	1.0	
T	0.10	0.11	0.90 (0.42–1.93)	0.85
AGT (M235T)				
HCM				
M	0.4	0.52	1.0	
T	0.6	0.48	0.63 (1.18–2.26)	**0.004**
DCM				
M	0.37	0.52	1.0	
T	0.63	0.48	1.87 (1.37–2.54)	**<0.0001**
RCM				
M	0.35	0.52	1.0	
T	0.65	0.48	2.03 (1.21–3.38)	**0.008**
AGTR1 (A1166C)				
HCM				
A	0.91	0.82	1.0	
C	0.09	0.18	0.45 (0.28–0.72)	**<0.001**
DCM				
A	0.90	0.82	1.0	
C	0.10	0.18	0.55 (0.36–0.83)	**0.004**
RCM				
A	0.90	0.82	1.0	
C	0.10	0.18	0.51 (0.24–1.06)	0.08

The frequency of variant (AGTR1-1166AC) genotype was significantly higher in DCM patients as compared to controls, but CC genotype was not observed in DCM patients (Table 3). No significant difference either in LVED (58.13 ± 10.64 vs. 58.17 ± 10.18; $p = 0.83$) or in LVEF (28.45 ± 9.06 vs. 28.82 ± 11.41; $p = 0.98$) was seen between AA genotype and AC genotype, respectively, in DCM patients.

Gene–gene interactions

There is increasing evidence that epistatic interactions may modulate disease risk and phenotype of complex-trait diseases [15]. Potential gene–gene interactions were examined using MDR analysis incorporating all the genetic variables (Table 7). ACE ID polymorphism was identified as the best one-factor model with the

Table 5 Comparison of clinical characters in patients carrying wild-type and variant genotypes

Patients	Clinical character	Wild-type	Heterozygous	Homozygous
ACE I/D				
HCM	LVWT	20.60 ± 3.39	23.16 ± 5.62	26.96 ± 3.52
			$p = 0.09$	$\mathbf{p < 0.001}$
DCM	LVEF	38.50 ± 6.17	28.50 ± 9.73	25.7 ± 7.54
			$\mathbf{p < 0.001}$	$\mathbf{p < 0.001}$
	LVEDD	53.07 ± 5.64	56.79 ± 11.50	63.08 ± 7.25
			$p = 0.23$	$\mathbf{p < 0.001}$
AGT (T174 M)				
HCM	LVWT	23.58 ± 4.66	23.34 ± 6.82	27.00
			$p = 0.81$	$p = 0.47$
DCM	LVEF	28.19 ± 9.53	29.05 ± 9.91	31.29 ± 10.42
			$p = 0.62$	$p = 0.40$
	LVEDD	58.11 ± 10.71	58.80 ± 9.56	54.71 ± 12.91
			$p = 0.73$	$p = 0.41$
AGT (M235T)				
HCM	LVWT	21.63 ± 4.34	21.53 ± 5.75	24.97 ± 4.64
			$p = 0.95$	$p = 0.05$
DCM	LVEF	29.05 ± 9.51	28.81 ± 9.77	28.15 ± 5.68
			$p = 0.56$	$p = 0.48$
	LVEDD	54.27 ± 15.88	58.42 ± 11.03	58.68 ± 9.42
			$p = 0.27$	$p = 0.24$
AGTR1 (A1166C)				
HCM	LVWT	23.46 ± 5.15	23.82 ± 6.07	25.00 ± 4.24
			$p = 0.77$	$p = 0.68$
DCM	LVEF	28.45 ± 9.06	28.82 ± 11.41	–
			$p = 0.83$	
	LVEDD	58.13 ± 10.64	58.17 ± 10.18	–
			$p = 0.98$	

Table 6 Haplotype frequency of AGT gene in patients and controls

Haplotype[a]	Patients, *n* (frequency)	Controls, *n* (frequency)	OR (95% CI)	*p* value
T–M	250 (0.36)	164 (0.47)		
T–T	325 (0.50)	123 (0.41)	1.73 (1.30–2.30)	**0.00016**
M–M	3 (0.02)	4 (0.46)	0.49 (0.10–2.23)	0.44
M–T	88 (0.12)	33 (0.07)	1.75 (0.12–2.73)	**0.01**

highest cross-validation consistency (10/10) with 35.52% prediction error among all the factors. The prediction error was statistically significant ($p = 0.04$). We found that four-factor model consisting of all four variant alleles had the lowest prediction error 28.73% and highest cross-validation consistency of 10/10 ($p < 0.01$) (Fig. 1), was the best-fit model for predicting risk of cardiomyopathy, and showed more than sixfold increased risk of cardiomyopathy (OR 6.36; 95% CI 4.44–9.1, $p < 0.001$).

Sarcomeric mutants versus non-mutants and modifier genes

Cardiomyopathy patients with variant allele for the RAS polymorphisms were stratified into two groups on the basis of mutations in sarcomeric genes, i.e., patients with and without sarcomeric gene mutations and clinical parameters were compared among these groups.

An early onset of the clinical symptoms was observed in cardiomyopathy patients carrying a variant allele (ACE

Table 7 Epistatic interactions and risk prediction in RAS pathway candidate genes by MDR method

No. of factors	Best model	Prediction error (%)	CVC	OR (95% CI)	Permutation test: p value
1	ACE ID	35.52	10/10	4.89 (3.29–7.26)	<0.01
2	ACE ID A1166C	32.65	6/10	4.22 (2.99–5.95)	<0.01
3	ACE ID M235T A1166C	30.4	10/10	5.22 (3.67–7.43)	<0.01
4	ACE ID T174 M M235T A1166C	28.73	10/10	6.36 (4.44–9.1)	<0.01

CVC cross-validation consistency

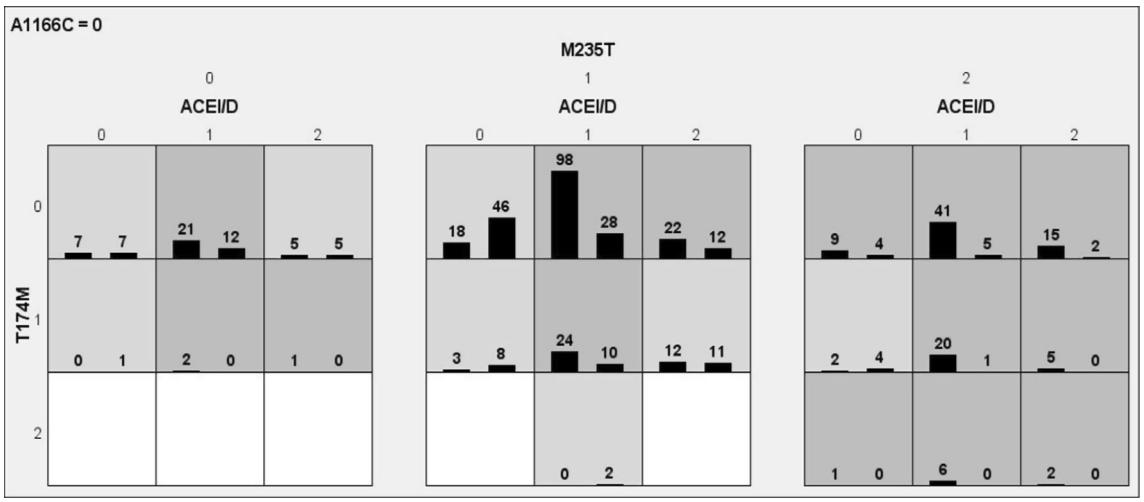

Fig. 1 Summary of the four-locus genotype combinations for RAS pathway genes associated with high risk and low risk for severe phenotypes, along with corresponding distribution of patients (in *left bars* in *boxes*) and of controls (in *right bars* in *boxes*), for each multilocus genotype combination. High-risk combinations appear as *dark gray* and low-risk combinations as *light gray*

D/AGT 174M/AGT 235T or AGTR1 1166C) and a mutation in any of the four sarcomeric genes, but the results were not significant ($p = 0.51$) (Table 8). In HCM patients with variant D allele (ACE I/D) and a mutation in sarcomeric gene, the LVWT was higher than those not carrying a mutation although the results were not significant ($p = 0.34$); however, a significant decrease in LVWT and an increase in LVED were observed in DCM patients carrying sarcomeric mutation ($p < 0.01$ and $p = 0.05$). AGT 235T allele showed significant association with an increase in septal thickness in HCM patients with sarcomeric mutation; however, neither LVED or nor LVEF was found to be altered in mutation carrying DCM patients with AGT 235T allele. DCM patients carrying mutation in sarcomeric gene and AGT 174T allele also showed a decrease in LVEF and an increase in LVED but the results were not statistically significant. No significant difference in LVWT was seen in HCM patients carrying 174T allele and sarcomeric gene mutation as compared to those without mutation (23.53 ± 5.80 vs. 23.67 ± 7.66; $p = 0.9$). An increase in LVED ($p = 0.15$) and a significant decrease in LVEF ($p = 0.03$) were observed in DCM patients carrying sarcomeric gene mutation with AGTR1 1166C allele, but the LVWT was unaffected with respect to the AGTR1 A1166C polymorphic status in HCM patients with sarcomeric gene mutation.

Discussion

This is the first study from N. India on the role of modifier genes in idiopathic cardiomyopathy patients from a single center. We examined the role of ACE, AGT, and AGTR1 genes as disease modifiers in HCM and DCM. Our study provides evidence that SNPs of ACE, AGT, and AGTR1 genes modulate the DCM, HCM, and RCM disease risk

Table 8 RAS gene polymorphisms and clinical characteristics in idiopathic cardiomyopathy patients with and without sarcomeric gene mutations

RAS genotypes	Sarcomeric non-mutants		Sarcomeric mutants	
	Wild-type	Variant	Wild-type	Variant
ACE I/D				
LVWT	19.90 ± 3.28	22.84 ± 5.89 ($p = 0.12$)	21.60 ± 6.15	24.39 ± 5.80 ($p = 0.34$)
LVEF	26.36 ± 9.77	28.22 ± 10.3 ($p = 0.56$)	40.50 ± 0.70	29.00 ± 10.16 **($p > 0.05$)**
LVEDD	59.05 ± 10.76	60.22 ± 8.79 ($p = 0.75$)	57.78 ± 8.64	70.50 ± 6.36 **($p = 0.05$)**
Age of onset	42.42 ± 15.92	41.72 ± 11.28 ($p = 0.81$)	41.25 ± 11.75	38.14 ± 14.21 ($p = 0.51$)
AGT (T174 M)				
LVWT	22.39 ± 5.70	23.81 ± 5.59 ($p = 0.23$)	23.53 ± 5.80	23.67 ± 7.66 ($p = 0.96$)
LVEF	28.01 ± 9.79	28.00 ± 11.52 ($p = 0.99$)	35.34 ± 8.02	27.86 ± 10.89 ($p = 0.13$)
LVEDD	58.15 ± 11.29	60.00 ± 12.31 ($p = 0.41$)	57.95 ± 9.14	60.00 ± 8.74 ($p = 0.63$)
Age of onset	42.21 ± 15.21	42.20 ± 14.25 ($p = 0.99$)	40.76 ± 9.13	39.47 ± 13.69 ($p = 0.69$)
AGT (M235T)				
LVWT	21.62 ± 4.63	22.81 ± 5.88 ($p = 0.48$)	21.19 ± 5.21	26.50 **($p = 0.001$)**
LVEF	28.42 ± 10.55	26.55 ± 7.18 ($p = 0.58$)	32.00	30.29 ± 11.00 ($p = 0.88$)
LVEDD	63.56 ± 13.96	58.11 ± 11.83 ($p = 0.19$)	59.00	59.26 ± 9.09 ($p = 0.97$)
Age of onset	42.49 ± 14.25	41.76 ± 16.18 ($p = 0.81$)	40.13 ± 12.36	40.00 ± 17.45 ($p = 0.98$)
AGTR1 (A1166C)				
LVWT	22.16 ± 5.83	23.02 ± 5.73 ($p = 0.55$)	23.81 ± 5.88	23.34 ± 7.39 ($p = 0.86$)
LVEF	29.85 ± 9.18	27.41 ± 13.46 ($p = 0.22$)	32.09 ± 9.90	21.80 ± 6.87 **($p = 0.03$)**
LVEDD	58.77 ± 11.98	58.00 ± 9.94 ($p = 0.74$)	53.60 ± 6.02	60.00 ± 9.14 ($p = 0.15$)
Age of onset	42.62 ± 14.81	39.61 ± 15.44 ($p = 0.15$)	45.90 ± 13.72	39.04 ± 12.39 ($p = 0.11$)

and severity and thus act as modifier genes in North Indian Asians. These SNPs have been shown earlier to be associated with disease severity in HCM and DCM in other ethnic populations [16, 17].

ACE D allele has been hypothesized to be a gene-modifying factor in HCM [18]. We too observed that ACE D allele was a disease phenotype modifier as it was found to be associated with an early onset of HCM. Further, D allele carrying HCM patients had an early onset of hypertension and increased LVH and DCM patients with D allele showed decreased LVEF, indicating its putative influence on severity of clinical phenotype in both HCM and DCM. The increased disease severity in D allele carriers may be due to increased ACE and Ang II levels, which are known to play an important role in cardiac remodeling [19]. Thus, our results confirm the role of these SNPs in modulating disease phenotype in HCM and DCM. We also observed that the co-occurrence of ACE D allele in conjunction with sarcomeric mutations appeared to increase severity of disease phenotype as shown by non-significant increased LVWT in patients who carried variant allele and a sarcomeric mutation. This is the first report showing a synergism between D allele and sarcomeric mutations, leading to altered disease phenotype. The lack of statistical significance observed in these clinical parameters in these patients appears to be due to relatively less number of patients carrying mutation and D allele.

We observed that besides ACE I/D, AGT M235T polymorphism also modulated the disease severity in HCM/DCM patients. HCM patients carrying TT genotype showed significantly increased LVWT as compared to those carrying MM or MT genotypes. The co-occurrence of 235T allele and a sarcomeric gene mutation in HCM patients increased LVWT as compared to non-mutation carriers, suggesting that AGT 235T allele increased the disease severity in HCM patients. Earlier also AGT 235T has been reported to be significantly associated with HCM in Japanese [20] and a South Indian cohorts [21]. As explained earlier, the increased disease severity in AGT 235T allele carriers may be due to increased AGT levels in these patients.

AGT (T174 M) polymorphism was not found to have an effect on the disease outcome in our cohort of idiopathic cardiomyopathy patients. However, we observed that a haplotype consisting of 174M and 235T alleles conferred higher risk of developing more severe phenotype in these patients. Our results confirm an earlier study

by Marcin et al. who reported higher risk of heart failure in these haplotype carriers in their French Canadian cohort [22].

The adverse effects of AngII in heart are primarily mediated through its receptors. A1166C polymorphism in AGTR1 has been reported to be disease predisposing and a modifying factor in HCM [23, 24]. In our study, AGTR1 1166C allele was associated with increased risk of HCM and DCM; however, it did not appear to modulate disease severity (HCM, DCM) as no genotype-based differences were observed in clinical parameters, i.e., LVWT, LVEF, and LVED in our cohort.

There have been contradictory reports regarding the role of A1166C polymorphism in heart failure. Andrikopoulos et al. [25] found no significant association of A1166C polymorphism with increased mortality in acute myocardial infarction (AMI), whereas Franco et al. [26] reported a significant association of 1166C allele together with ACE D allele with increased cardiovascular risk.

We observed that AGTR1 1166C in the presence of a sarcomeric gene mutation significantly increased LVED and decreased LVEF in DCM patients, indicating that AGTR1 T1166 could modulate disease phenotype in a mutation carrier. This is the first report showing synergistic effect of A1166C polymorphism on DCM phenotype in mutation carriers. This polymorphism occurs in the 3′-untranslated region of the human AGTR1 gene and its biological importance is not well understood. Martin et al. reported that microRNA (miRNA) miR-155 translationally represses the expression of AGTR1 in vivo through A1166C polymorphism which occurs in *cis*-regulatory site recognized by miR-155. These authors showed that the presence of 1166C allele interrupts base-pair complementarity, thereby decreasing the miR-155 interaction with the *cis*-regulatory region resulting in attenuated translation of this gene [27]. These results suggest a role for miR-155 in modulating the effect of AGTR A1166C polymorphism on AGTR expression; however, functional validation of this observation is yet to be confirmed.

Thus, taken together, our findings provide evidence that polymorphisms in RAS genes not only potentially modify the disease outcome or severity of the disease but also account for genotype–phenotype heterogeneity among primary cardiomyopathy patients carrying genetic variants in sarcomeric protein-coding genes.

Acknowledgements Bindu Rani received Junior Research Fellowship and Senior Research Fellowship from the Indian Council of Medical Research (ICMR), New Delhi, India.

Statement of authorship The authors take responsibility for all aspects of the reliability and freedom from any bias of the data presented and their discussed interpretation.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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