



## Original Article

# Insertion/deletion polymorphism of ACE gene in females with peripartum cardiomyopathy: A case-control study



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## ABSTRACT

**Background:** The role of polymorphism of Angiotensin converting enzyme (ACE) gene and ACE activity in etiopathogenesis, prognosis, and many other clinical parameters in the various form of the cardiovascular disease has been established to some degree of certainty. The pathophysiology of Peripartum cardiomyopathy (PPCM) remains an area of active research. The main aim of our study was to see pattern of ACE- Insertion/Deletion (I/D) allele in PPCM and its implications on left ventricular performance indices.

**Methods:** This single-center case-control study included 45 cases and 70 controls. The diagnosis of PPCM was established clinically and echocardiographically. ACE genotyping was done by polymerase chain reaction (PCR) method in all subjects.

**Results:** The II, ID, and DD genotype was present in 16, 18 and 11 of subjects with PPCM and 48, 19 and 3 of controls respectively. The odds ratio for ACE-II genotype in cases vs. controls was 0.253 (95% CI = 0.114–0.558;  $p = 0.007$ ), for that of II genotype was 1.93 (95% CI = 0.86–4.3;  $p = 0.107$ ) and for DD genotype was 7.225 (95% CI; 1.88–27.6;  $p = 0.0039$ ). Overall frequency of D allele in cases was significantly higher than controls (odds = 4.25; 95% CI = 2.01–6.7;  $p = 0.0001$ ). Moreover, ejection fraction, left ventricular volume and linear dimensions were worse in patients with DD genotype.

**Conclusion:** ACE DD genotype and overall frequency of D allele is significantly higher in patients with PPCM. Also, the presence of DD genotype is associated with worse systolic performance indices measured echocardiographically.

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## 1. Introduction

Peripartum cardiomyopathy (PPCM) has been traditionally defined as rare, acquired, idiopathic, non-genetic, non-familial and dilated cardiomyopathy that presents with heart failure secondary to left ventricular systolic dysfunction in last month of pregnancy or first five months after delivery, in the absence of any other cause of heart failure.<sup>1–5</sup> Angiotensin-converting enzyme (ACE) is a zinc metallopeptidase that converts Angiotensin I to Angiotensin II which is a potent vasoconstrictor, stimulates adrenal cortex to release aldosterone which increases plasma volume, mediates cell growth and proliferation by stimulating various cytokines, growth factors and induces endothelial dysfunction by reducing nitric

oxide bioavailability. These mechanisms emphasize the importance of angiotensin II in cardiovascular pathophysiology and motivate the exploration of the role of the renin-angiotensin-aldosterone system (RAAS) in cardiovascular pathology.<sup>6,7</sup>

The role of insertion/deletion (I/D) polymorphism of ACE gene and ACE activity in etiology, pathogenesis, prognosis, and other clinical implications in the various form of the cardiovascular disease has been established to some degree of certainty. ACE gene polymorphism in various specific cardiomyopathies like that of ischemic, idiopathic dilated, hypertrophic and alcoholic cardiomyopathy have been studied depicting the positive correlation between the frequency of D allele and development of specific cardiovascular pathophysiology. The fact that idiopathic dilated cardiomyopathy (IDCM), alcoholic cardiomyopathy and other forms of low ejection fraction state bear a phenotypic resemblance to that of PPCM, has prompted us to take on the study of the association of ACE gene I/D polymorphism in patients with PPCM.<sup>8</sup> We for the first time tested the hypothesis that PPCM may be

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associated with higher incidence of ACE-DD genotype and overall frequency of D allele and that the DD genotype in cases may be associated with the lower systolic performance of left ventricle.

## 2. Methods

### 2.1. Study design

This was a single centre hospital-based, Case-control study done in Kashmir (A state of north India) and carried out from August 2012 to March 2015

### 2.2. Study population

**CASES:** The study population included 45 consecutive Kashmiri patients admitted to our hospital with PPCM diagnosed by clinical (Presentation of heart failure in last month of pregnancy or first 5 months postpartum) and echocardiographic (left ventricular ejection fraction  $<0.45$  or M-mode fractional shortening  $<30\%$  (or both) and end-diastolic dimension  $>2.7$  cm/m<sup>2</sup>) criteria. Patients with a history of the pre-existing cardiac disease, hypertension (essential or pregnancy induced) or any structural heart disease inconsistent with PPCM on echocardiography and those with multiple gestations were excluded from the study. **CONTROLS:** A total number of controls were 70. Controls were healthy married normotensive, Kashmiri females, within first five months of puerperium with no history suggestive of cardiac disease, structurally and functionally normal heart (measured echocardiographically) and no history of pregnancy induced hypertension.

### 2.3. Consent and ethical issues.

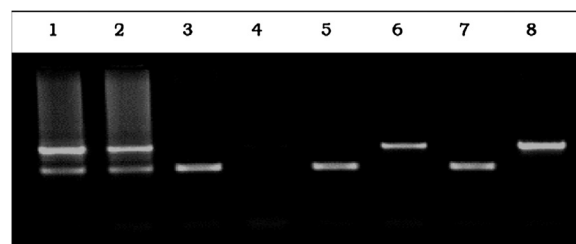
An informed consent was obtained from each subject after explaining the study in detail. The study was cleared by the Institutional Ethics Committee.

### 2.4. Initial evaluation

Patient's New York Heart Association (NYHA) functional class was assessed at first contact by thorough interview.<sup>13</sup> This was followed by complete general physical and specific cardiovascular examination. Standard 12 lead ECG was recorded in all patients and Chest radiography was done in only 31 patients who had presented in the postpartum period in order to avoid radiation exposure to the fetus.

### 2.5. Echocardiography

A comprehensive echocardiographic examination of patients and controls was carried out using a commercially available cardiac ultrasound scanner equipped with 2.5 and 3.5 MHz transducers (Aloka, Prosound, SSD  $\alpha$ -110, South Korea), by an experienced cardiologist. Each patient was examined in the left lateral decubitus and supine position by precordial transthoracic two-dimensional targeted M-mode echocardiography with Doppler color flow mapping. Echocardiographic parameters that were recorded were systolic and diastolic left ventricular dimensions, fractional shortening, diastolic functions by transmitral flow velocities, left ventricular end diastolic volume, end systolic volume and ejection fraction by Simpson's method and left atrial diameter. These measurements taken were strictly according to the joint guidelines by American Society of Echocardiography and European Association of Echocardiography.<sup>14</sup> Measurements of left ventricular dimensions and function were determined by an average of  $\geq 3$  beats. Subjects were categorized as follows on the



**Fig. 1.** Direct visualization of ACE I/D PCR products, electrophoresed on 2% agarose gel, by ethidium bromide staining. A 490 base pair ACE I allele and 190 base pair ACE D allele is seen. Results from 8 patients are shown.

II homozygote genotypes: Lane 6 and 8.

DD homozygote: Lanes 3, 5 and 7

ID heterozygotes: Lane: 1 and 2.

Control (D. H<sub>2</sub>O): 4

basis of echocardiographic parameters: Mild LV dysfunction, EF = 41–45, moderate LV dysfunction, EF = 31–40 and severe LV dysfunction, EF  $\leq 30$ . Furthermore, subjects were classified on the basis of severity of LV dilatation as follows: Normal LV end diastolic volume = 56–104 ml; Mild LV dilatation LVEDV, 105–117 ml; Moderate LV dilatation LVEDV, 118–130 ml; and Severe LV dilatation; LVEDV  $> 130$ .<sup>14</sup>

### 2.6. Extraction and amplification of genomic DNA

Blood samples were taken from both the groups after obtaining written informed consent. Five milliliters of peripheral blood was obtained from each subject in ethylenediaminetetraacetic acid (EDTA) containing vials (200  $\mu$ l of 0.5 M, pH = 8.0) and stored at  $-20^{\circ}\text{C}$  until use. Deoxyribonucleic acid (DNA) extraction from blood sample was performed according to the manufacturer's protocol for Qiagen DNA extraction kits (Qiagen, Hilden, NRW, Germany). DNA content was quantified by spectrophotometric absorption (Nanodrop Spectrophotometer, BioLab, Scoresby, VIC, Australia). Polymerase chain reaction (PCR) was performed using an iCycler Thermal Cycler (Bio-Rad, Hercules, CA, USA). Molecular detection of the ACE I/D polymorphism was performed by PCR amplification of 287-bp sequence of DNA of intron 16. The template DNA (0.5–1.0  $\mu$ g) was used in a PCR under stringent conditions to avoid the possibility of false positives for ACE genotyping. The reactions were carried out with 10 pmol of each primer: Forward primer, 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3', and reverse primer, 5'-GAT GTG GCC ATC TTC GTC AGA T-3', in a final volume of 25  $\mu$ l containing 1.5 mM MgCl<sub>2</sub>, 25 mM KCl, 5 mM Tris-HCl (pH 8.4), 0.25 mM each of deoxyribonucleotide triphosphate (dNTP, Biotools, Spain) and 1 unit Taq polymerase (Biotools, Spain). Amplification was carried out in a DNA thermal cycler (Pamcyler thermocycler) for 30 cycles with denaturation extension at 72  $^{\circ}\text{C}$  for 2 min. The PCR products were separated on a 2% agarose gel and DNA were visualized by ethidium bromide staining. The PCR product is a 490-bp fragment in the presence of the insertion (I) allele. Thus, each DNA sample revealed one of three possible patterns after electrophoresis: A 490-bp band (genotype I/I), a 190-bp band (genotype D/D), or both 490-bp and 190-bp bands (genotype I/D). Restriction fragments were visualized after ethidium bromide staining of the agarose gel with the use of an ultraviolet transilluminator (Fig. 1).

### 2.7. Collection and recording of data

All the patient data including demographic, clinical and diagnostic data as well as hospital outcome were recorded on a specially pre-designed patient record form.

## 2.8. Statistical analysis

All continuous variables of study have been shown in terms of Mean  $\pm$  SD and the categorical variables in terms of frequency and percentage. Continuous variables have been compared using T-test and Chi-square test has been used to analyze categorical variables. All the results obtained have been discussed on 5% level of significance with p value  $< 0.05$  considered as significant. Also suitable statistical charts have been used to represent the data, SPSS version 20 was used for analysis.

## 3. Results

### 3.1. Patient characteristics

After excluding four patients (with two having associated pregnancy induced hypertension one patient had twin pregnancy and one patient had poor echocardiographic window that precluded the proper assessment), total of 45 patients who met the inclusion criteria, were enrolled in the study. Demographic characteristics of cases and controls are depicted in Table 1. There wasn't significant difference in terms of age and parity between the two groups.

### 3.2. Clinical characteristics

NYHA class III was the most common presentation followed by class II and class IV respectively. Breathlessness was present in 91%, nocturnal cough in 51% and palpitations in 26% patients. Most patients had normal blood pressure at presentation (n: 33; 73%), 9 patients (20%) were in hypotension and 3 patients were in clinical shock. There was a significant difference in the mean heart rate between two groups. Chest radiograph was done only in patients presenting postpartum (n = 31) to avoid the radiation exposure to the fetus, out of whom 21 (65.6%) had CTR greater than 5.5.

### 3.3. Echocardiographic parameters

Baseline echocardiographic parameters of the cases and controls are depicted in Table 2. There was a significant difference in mean ejection fraction (EF), left ventricular systolic and diastolic indices between cases and controls. Total of 11 patients (24.4%)

had normal left ventricular volume. Eleven cases (24%) had moderate to severe secondary mitral regurgitation (Fig. 2).

### 3.4. ACE genotype

The ACE genotype was determined in 45 patients with PPCM and compared with 70 controls. The results are shown in Table 3. When mean EF, mean of left ventricular end diastolic and end systolic diameters and volume of cases with II + ID genotype was compared with that of DD genotype, later had worse parameters but same was not statistically significant (Table 4). Mean left atrial size didn't differ much between the two groups.

## 4. Discussion

The main findings of our study were:

1. ACE-DD genotype was statistically significant in patients with PPCM
2. Overall frequency of D allele was significantly higher in cases
3. DD genotype was associated with worse systolic performance indices in terms of ejection fraction, LV end-systolic and end-diastolic dimensions and volumes.

The renin-angiotensin system (RAAS) plays a central role in cardiovascular homeostasis. Angiotensin is the key peptide of the RAAS and exerts its influence on the heart and blood vessels both through its hemodynamic effects (via its influence on after-load and pre-load and determining coronary vasoconstriction) and through its direct cellular effects (via its actions on cell proliferation).<sup>7</sup> The discovery that ACE levels are under genetic control ushered in a new era of investigation; most studies focused on an insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene as a marker for a functional polymorphism. In 1990, Rigat and coworkers published an important report that provided the impetus to further study polymorphisms in this gene.<sup>9</sup> They found a polymorphism involving the presence (insertion, I) or absence (deletion, D) of a 287-bp sequence of DNA in intron 16 of the gene. Mean ACE activity levels in DD carriers were approximately twice that found in II genotype individuals.<sup>10</sup> Subjects with the ID genotype had intermediate levels indicating codominance. The I/D polymorphism accounted for approximately half (47%) of the observed variance in ACE levels in this study group. Later studies

**Table 1**  
Demographic and clinical characteristics.

	Cases	Controls	
Age	Mean (years) 31.8 $\pm$ 5.19 Range (years) 22–42	Mean (years) 31.48 $\pm$ 4.42 Range (years) 24–40	P = NS
Parity	Mean 2.56 $\pm$ 1.27 Range 1–6	Mean 2.14 $\pm$ 0.92 Range 1–5	P = NS
Clinical characteristics of Cases			
NYHA Class	II III IV	n = 13 (29%) n = 25 (55.5%) n = 7 (15.5%)	
Clinical Signs	Systolic BP (mmHg) >90 <90 <90 with clinical signs of shock Mean Heart rate Peripheral edema Raised JVP Murmur Clinical S3	n = 33 73.4% n = 9 20% n = 3 6.6% Controls = 83.8 $\pm$ 10.4 Cases = 104.4 $\pm$ 16.5 n = 29 64.4% n = 26 57.7% n = 8 17.8% n = 19 42.3%	P < 0.05*

NYHA = New York heart association; JVP = jugular venous; BP = Blood Pressure; ECG = Electrocardiogram; CTR = Cardiothoracic ratio.

\* P value significant.

**Table 2**  
Laboratory characteristics of cases.

	Cases	Controls	Controls	
ECG	Normal	n = 9; 20%	n = 63; 90%	
	Sinus tachycardia	n = 27; 60%	n = 7; 10%	
	LBBB	n = 5; 11%	-	
	Atrial fibrillation	n = 4; 8.8%	-	
Chest Xray (done in 31 Patients)	Normal CTR	n = 21; 34.4	-	
	Increased CTR	n = 11; 65.6	-	
Ejection Fraction	Mean	34.24 (±5.84)	66.7(±7.0)	P < 0.05*
	EF = 41–45	n = 7 15.5%	-	
	EF = 31–40	n = 28 62.2%	-	
	EF ≤ 30	n = 10 22.3%	-	
LV dimensions	LVEDD (mm)	Mean = 59.2 (±7.04)	47.4 (±4.6)	P < 0.05*
	LVESD (mm)	Mean = 47.6 (±7.43)	29.6 (±3.5)	P < 0.05*
LV volumes	LVESV (ml)	Mean = 78.8 (±22.6)	34.5(±9.8)	P < 0.05*
	LVEDV (ml)	Mean = 120 (±26.24)	105.7 (±23.8)	P < 0.05*
Severity of LV dilatation (Cases)	Normal	n = 11 24.4%	-	
	Mild dilatation	n = 13 28.9%	-	
	Moderate dilatation	n = 11 24.4%	-	
	Severe dilatation	n = 10 22.3%	-	

EF = ejection fraction; LVEDD = left ventricular end diastolic diameter; LVESD = left ventricular end systolic diameter; LV = left ventricle; Recommendations of report from American Society of Echocardiography's in conjunction with European Association of Echocardiography, a branch of ESC. [\* Normal LV end diastolic volume; 56–104 ml, Mild LV dilatation LVEDV; 105–117 ml, Moderate LV dilatation LVEDV; 118–130 ml, Severe LV dilatation; LVEDV > 130.14

EF was calculated by Simpson's method.

\* Significant P value.

showed that the involvement of the I/D polymorphism is not limited to ACE levels in plasma, and is also detected in tissue ACE levels. 10,11

Although there is no reported study regarding ACE-gene I/D polymorphism in PPCM, various cardiovascular abnormalities bearing phenotypic resemblance to that of PPCM have shown a positive correlation with D allele and specific cardiomyopathy. In our study, we could establish a positive association between subjects with PPCM with DD genotype and D allele of ACE gene, which is in consistency with many other studies primarily involving cardiac muscle. Raynolds et al. determined the genotypes of individuals with end-stage heart failure due to either ischaemic dilated cardiomyopathy or idiopathic dilated cardiomyopathy and compared these to organ donors with normally functioning hearts. Compared with the DD frequency in the control population, the frequency of the ACE DD genotype was 48% higher in individuals with idiopathic dilated cardiomyopathy (p = 0.008) and 63% higher in subjects with ischaemic cardiomyopathy (p = 0.008), suggesting that an ACE gene variant may contribute to the pathogenesis of both types of cardiomyopathy.12 Mahjoub et al. found a similar result in Tunisian population with dilated cardiomyopathy. They concluded that frequencies of the DD genotype and D allele were significantly higher in patients with dilated cardiomyopathy as compared with controls.13 Another form of myocardial disease like hypertrophic cardiomyopathy and alcoholic cardiomyopathy has also been shown to have an association with DD genotype and with a higher frequency of D allele.14–16 In our study, we could establish a positive association between DD genotype and D allele of ACE gene in subjects with PPCM which is in consistency with many other studies involving primarily cardiac muscle.

We also found that echocardiographically determined left ventricular systolic performance indices were affected by ACE I/D

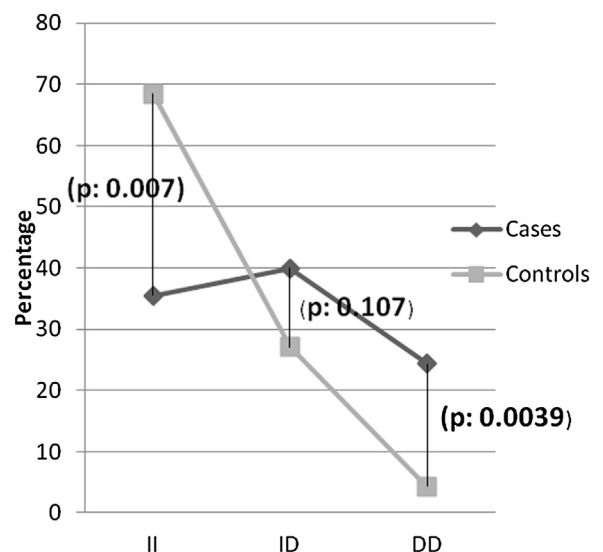
**Table 3**  
Comparison of ACE I/D genotype in cases vs. controls.

	Cases	Controls	Odds ratio	95% CI	P value
II	16 (35.5%)	48 (68.5%)	0.253	0.114–0.558	<b>0.007</b>
ID	18 (40%)	19 (27.1%)	1.93	0.86–4.3	0.107
DD	11 (24.4%)	3 (4.3%)	7.225	1.88–27.6	<b>0.0039</b>
I	50(55.5%)	115 (82.1%)	0.27	0.14–0.49	<b>0.0001</b>
D	40(44.4%)	25 (17.9%)	4.25	2.01–6.7	<b>0.0001</b>

Bold values represent significant p value.

polymorphism. Mean LV-ejection fraction was lower and mean of LV linear dimension and volume was higher in cases with DD genotype when compared with combined mean of cases with ID and II genotype. Previous studies have almost consistently shown worse echocardiographic systolic performance indices (EF, LV dimensions, and volumes) as well as overall prognosis with DD genotype in other phenotypically similar cardiomyopathies.17–19 Moreover, patients with DD genotype have shown to get maximum benefit from pharmacological ACE antagonism.20 This observation may have implication on patients of PPCM as they are usually denied the beneficial effects of ACE inhibitors late in pregnancy owing to deleterious effects on the fetus.

Although exact etiology and pathogenesis of PPCM are an active area of research, our study may open the new dimensions in this area. Moreover, studies have conclusively shown that long-term inhibition of Renin-Angiotensin by various drugs started early in the course of the disease in other phenotypically similar forms of cardiomyopathy alters the natural course and promotes reverse remodeling of left ventricle, same benefit may, however, be denied



**Fig. 2.** Line diagram showing the frequency of I/D polymorphism in cases vs. controls.

**Table 4**

LV systolic functions, LV size, LV volume and LA size in DD genotypes vs II+ID genotypes combined.

	Females with DD genotype	Females with II+ID genotype	P*
Mean EF	32.3	35.3	0.194
Mean LVEDD in mm	61.1	58.5	0.306
Mean LVESD in mm	49.7	47	0.346
Mean LVEDV in ml	126.8	119.26	0.594
Mean LVESV in ml	87	77.39	0.409
Mean LA size in mm	40.6	38.2	0.279

\*P &lt; 0.05 is statistically significant.

in this specific form of cardiomyopathy as inhibitors of Renin-Angiotensin system in late pregnancy (when pathophysiology of this disease entity usually starts) are absolutely contraindicated. The development of so-called “Pregnancy safe ACE inhibitor or ARB” may have a protective role, halt or reverse the disease progression in such cases.

Owing to the rarity of the disease, the number of patients in our study was relatively small, therefore results might be open to interpretation, a clear interrelation between genotypes and PPCM could nonetheless be seen. Further population-based, multicenter studies over longer periods involving multiple geographic regions and different ethnic groups should be done. Also, longitudinal follow-up studies where long-term prognosis and recovery of left ventricular functions in different genetic groups of ACE-gene need to be carried out.

## 5. Conclusion

This case-control study has demonstrated that the ACE DD genotype and overall frequency of D allele is significantly higher in patients with PPCM. Thus, the presence of D allele and DD genotype may predispose and may be an independent risk factor for the development of PPCM. Also presence of ACE-gene DD genotype in our study is associated with worse left ventricular performance. Further studies, however, need to be undertaken with a larger number of patients taking region and ethnicity into account.

## 6. Study limitations

- The major limitation of this study is the small sample size owing to the low prevalence of PPCM in our community. This has resulted in wide confidence intervals and lower statistical power of the study.
- Moreover, our study comprised of the single ethnic population, the polymorphism of ACE genotype may vary in different regions of the world.
- We did not study the long term follow-up of these patients. Polymorphism of ACE gene may have implications on overall prognosis in PPCM. Longitudinal studies studying the death, hospitalisations for heart failure and recovery of LV functions in correlation with I/D polymorphism of ACE gene need to be put into perspective.

## Declaration of interest

The authors have none to declare.

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