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Impact of renin-angiotensin-aldosterone system gene polymorphisms on left ventricular dysfunction in coronary artery disease patients

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Abstract. *Background*: Left ventricular dysfunction (LVD), followed by fall in cardiac output is one of the major complications in some coronary artery disease (CAD) patients. The decreased cardiac output over time leads to activation of the renin-angiotensin-aldosterone system which results in vasoconstriction by influencing salt-water homeostasis. Therefore, the purpose of the present study was to explore the association of single nucleotide polymorphisms (SNPs) in angiotensin I converting enzyme; *ACE* (rs4340), angiotensin II type1 receptor; *AT1* (rs5186) and aldosterone synthase; *CYP11B2* (rs1799998) with LVD.
Methods and results: The present study was carried out in two cohorts. The primary cohort included 308 consecutive patients with angiographically confirmed CAD and 234 healthy controls. Among CAD, 94 with compromised left ventricle ejection fraction (LVEF $\leqslant$ 45) were categorized as LVD. The *ACE* I/D, *AT1* A1166C and *CYP11B2* T-344C polymorphisms were determined by PCR. Our results showed that *ACE* I/D was significantly associated with CAD but not with LVD. However, *AT1* 1166C variant was significantly associated with LVD (LVEF $\leqslant$ 45) (p value = 0.013; OR = 3.69), but *CYP11B2* (rs1799998) was not associated with either CAD or LVD. To validate our results, we performed a replication study in additional 200 cases with similar clinical characteristics and results again confirmed consistent findings (p value = 0.020; OR = 5.20).
Conclusion: *AT1* A1166C plays important role in conferring susceptibility of LVD.

Keywords: Coronary artery disease, LVD, LVEF, RAAS genetic variants

1. Introduction

Coronary artery disease (CAD) is a complex, multifactorial disease, influenced by pathophysiologic conditions as well as by genetic and environmental factors [1]. The major complication that some CAD patients face over time is the impairment in left ventricular function, followed by fall in cardiac output. Despite being progressively debilitating, this condition does not affect everybody equally [2] and genetic differences may provide an explanation for the fact that some people, irrespective of lifestyle and common classical cardiovascular risk factors, are more prone to develop Left Ventricle Dysfunction (LVD) than others.

Clinically, LVD is defined as a syndrome that develops in response to an ischemic insult, resulting in decline in the pumping capacity of the heart. This is furthermore characterized by the continuous interactions between the underlying myocardial dysfunction and subsequent compensatory neurohumoral mechanisms that are activated [3]. In view of its physiological role and established benefits of ACE inhibitors therapy,

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attention has been focused on the renin-angiotensin-aldosterone system (RAAS) [4]. This system is initially able to compensate for the depressed myocardial function and preserve the cardiovascular homeostasis. However, their long-term activation has deleterious effects on cardiac structure and performance, leading to cardiac decompensation and progression of heart failure. In patients with cardiovascular disease, activity of the RAAS is often increased and contributes to a poor prognosis [5].

In RAAS cascade, angiotensinogen (AGT) is cleaved by renin to produce angiotensin I, which is further converted in the bioactive octapeptide angiotensin II (AngII) through the action of angiotensin I converting enzyme (ACE), a membrane-bound, zinc metalloendopeptidase involved in the metabolism of many small peptides. Many of the known deleterious effects of Ang II relevant to cardiovascular function and structure are mediated by angiotensin II type1 (AT1) receptor stimulation. These effects include vasoconstriction, sodium and water retention, aldosterone release, augmentation of sympathetic nervous system activity and vascular hypertrophy.

In addition, aldosterone synthase (CYP11B2), sensitive to the effects of ATII, catalyses the final step of aldosterone biosynthesis and release from zona glomerulosa in adrenal cortex. Because of the range of physiologic effects of *ACE, AT1* and *CYP11B2*, genetic variations that affect baseline RAAS activity are, therefore, candidates for increasing risk of, and adverse outcomes in heart disease and could probably affect a wide variety of clinical phenotypes. Therefore, we undertook the present study to assess the association of *ACE* insertion/deletion (*ACE* I/D rs4340), *AT1* (A1166C rs5186) and *CYP11B2* (T-344C rs1799998) polymorphisms with LV dysfunction in CAD patients. To the best of our knowledge, this is the first report investigating the association between *ACE, AT1* and *CYP11B2* gene polymorphism with left ventricular dysfunction in coronary artery disease patients.

2. Materials and methods

2.1. Study population

The present study was carried out in two cohorts, primary and replication cohorts. In the primary cohort, we studied 308 CAD patients recruited from July 2008 to January 2010. In the replication cohort, further 200 cases were enrolled. The diagnostic parameters used in the primary cohort, were also applied to the replication cohort. Both the primary and replication cohorts had significant coronary artery disease, (diagnosis, confirmed by coronary angiography and further all these subjects underwent coronary angioplasty) recruited from the Department of Cardiology of Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), Lucknow, Uttar Pradesh, India. The detailed clinical history of CAD patients was based on hospital investigations including coronary angiography. Angiographically identified stenoses $> 70\%$ in the major coronary vessels at the time of the study were used to classify patients as having single-vessel, double-vessel, or triple-vessel disease. The control (non-CAD) population consisted of 234 subjects (187 males and 47 females) (mean age years 54.18 ± 8.47) with no clinical evidence of CAD or LV dysfunction (by echocardiography) and also without positive family history of CAD or myocardial infarction (MI). Furthermore, the inclusion criteria for controls were absence of prior history of high systolic blood pressure, abnormal lipid profile, hypertension and obesity. Both patients and controls were frequency-matched to age, gender and ethnicity. To test the possibility for population stratification, genomic control method was used as described by Devlin et al. [6]. After obtaining informed consent, all the individuals were personally interviewed for information on food habits, occupation and tobacco usage. The study was approved by local ethical review committees of the institute and the authors followed the norms of World's Association Declaration of Helsinki [7].

2.2. Data collection

The clinical data was obtained by reviewing the patient's medical records. Left ventricle ejection fraction (LVEF) was calculated quantitatively by echocardiography, just before angiography procedure, using the Simpson's method [8]. Echocardiography was repeated in 10% of patients and results were totally concordant. Hypertension was defined as systolic blood pressure > 140 mmHg or a diastolic blood pressure > 90 mmHg or patients using antihypertensive drugs. Smoking was classified as smokers (ex-smoker and current smokers) and non smokers. Similarly, diabetes mellitus was defined as patients with fasting plasma glucose > 6.9 mmol/L or patients using anti-diabetic medication. All laboratory parameters, as stated in the medical record, were determined in overnight-fasting patients. Total cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride levels were mea-

Table 1
Clinical characteristics of CAD patients

Clinical characteristics	Primary stage	Replication stage	Combined
Total subjects	308	200	508
*Age–yr	56.26 ± 9.78	55.69 ± 8.88	56.04 ± 9.42
Male sex	260 (84.4%)	179 (89.5%)	439 (86.4%)
Risk factors			
Hypertension	147 (47.7%)	85 (42.5%)	232 (45.7%)
Diabetes	91 (29.5%)	59 (29.5%)	150 (29.5%)
Smoking	78 (25.3%)	54 (27%)	132 (26%)
BMI	24.39 ± 3.36	24.49 ± 2.97	24.45 ± 3.13
***Lipid levels**			
A) High density lipoprotein (mg/dl)	32.31 ± 7.91	31.88 ± 5.86	32.19 ± 7.45
B) Low density lipoprotein (mg/dl)	100.13 ± 24.33	103.74 ± 22.90	100.97 ± 24.40
C) Triglycerides (mg/dl)	155.64 ± 69.19	125.41 ± 35.54	149.11 ± 62.25
D) Total cholesterol (mg/dl)	170.30 ± 21.98	141.75 ± 39.61	163.74 ± 30.53
Clinical syndrome			
Stable angina	91 (29.5%)	63 (31.5%)	156 (30.7%)
Unstable angina/non st elevation myocardial infarction (NSTEMI)	69 (22.3%)	40 (20.5%)	109 (21.5%)
St elevation myocardial infarction (STEMI)	148 (48.2%)	96 (48.0%)	242 (47.7%)
Anterior wall myocardial infarction (AWMI)	84 (27.3%)	47 (23.5%)	130 (25.6%)
Inferior wall myocardial infarction (IWMI)	63 (20.5%)	49 (24.5%)	110 (21.7%)
Lateral wall myocardial infarction (LWMI)	2 (0.4%)	0 (0.0%)	2 (0.4%)
Angiographic profile			
Single vessel disease (SVD)	200 (64.9%)	141 (70.0%)	341 (67.2%)
Double vessel disease (DVD)	85 (27.6%)	27 (13.5%)	112 (22.0%)
Triple vessel disease (TVD)	23 (7.5%)	32 (16.5%)	55 (10.8%)
Left ventricular function			
*mean left ventricle ejection fraction (LVEF)	50.70 ± 11.65	48.56 ± 10.2	50.0 ± 11.18
> 45	214 (69.5%)	127 (63.5%)	341 (67.1%)
$\leqslant 45$	94 (30.5%)	73 (36.5%)	167 (32.9%)

*Values are mean ± SD.

sured by standard enzymatic methods. LDL cholesterol concentrations were calculated using the Friedewald's formula [9].

2.3. Genotyping

Genomic DNA was isolated from peripheral blood leukocytes according to a standard salting out method [10]. The polymorphisms were genotyped using the PCR or PCR-restriction fragment length polymorphism method. As a negative control, PCR mix without DNA sample was used to ensure contamination free PCR product. Genotyping of *ACE* I/D was based on the PCR amplification of a fragment encompassing the 287 bp insertion polymorphism in intron 16 as described by Joshi et al. [11]. Furthermore, the genotyping of *AT1* A1166C was determined by allele specific primers described by Shu Ye et al. [12] whereas *CYP11B2* T-344C polymorphisms were determined by using the polymerase chain reaction (PCR)-restriction fragment length polymorphism method as described by Kupari et al. [13]. The digested PCR fragments were separated on 2% agarose gel, stained with ethidium bromide and observed with ultraviolet imaging system (Vilber Lourmat, Marne-la-Valle'e, France). Genotyping was performed without knowledge of the case or control status. Ten percent of samples for each genotype were sequenced which showed 100% concordance.

2.4. Statistical analysis

The sample size was calculated using QUANTO 1.1, using minor allele frequency data from HapMap (http://www.hapmap.org/). The sample size of both primary (308) and replication (200) cohorts and 234 controls were adequate to give us power of 80% (probability of not making a type II error).

Descriptive statistics were presented as mean and standard deviation (SD) for continuous measures while absolute value and percentages were used for categorical measures. The chi-square goodness of fit test was used for any deviation from Hardy Weinberg Equilibrium in controls. Differences in genotype and allele frequencies between study groups were estimated by chi-square test. The ORs were adjusted for confounding factors such as age and gender. In addition, the association between RAAS gene polymorphisms and significant risk factors of CAD were analyzed using

Table 2
Distributions for RAAS gene Polymorphism in CAD patients and Healthy controls

Genotypes/Alleles	HC (234) N (%)	CAD[a] (308) N (%)	CAD[b] (200) N (%)	P-value[a] OR (95% CI)	P-value[b] OR (95% CI)
ACE I/D					
II	103 (44)	99 (32.1)	79 (39.5)	1 (reference)	1 (reference)
ID	112 (47.9)	141 (45.8)	80 (40.0)	0.154 1.3 (0.9–1.8)	0.734 0.93 (0.62–1.40)
DD	19 (8.1)	68 (22.1)	41 (20.5)	**< 0.001 3.72 (2.08–6.64)**	**0.001 2.81 (1.52–5.22)**
I	318 (67.9)	339 (55.0)	238 (59.5)	1 (reference)	1 (reference)
D	150 (32.1)	277 (45.0)	162 (40.5)	**< 0.001 1.78 (1.38–2.29)**	**0.041 1.37 (0.99–1.88)**
AT1 A1166C					
AA	168 (71.8)	232 (75.3)	155 (77.5)	1 (reference)	1 (reference)
AC	56 (23.9)	60 (19.5)	35 (17.5)	0.230 0.78 (0.51–1.17)	0.108 0.68 (0.42–1.09)
CC	10 (4.3)	16 (5.2)	10 (5.0)	0.723 1.16 (0.51–2.62)	0.861 1.08 (0.44–2.67)
A	392 (83.8)	524 (85.1)	345 (86.2)	1 (reference)	1 (reference)
C	76 (16.2)	92 (14.9)	55 (17.8)	0.446 0.88 (0.63–1.23)	0.231 0.77 (0.50–1.19)
CYP11B2 T-344C					
TT	77 (32.9)	113 (36.7)	77 (38.5)	1 (reference)	1 (reference)
TC	124 (53)	151 (49.0)	101 (50.5)	0.329 0.83 (0.57–1.20)	0.328 0.82 (0.54–1.23)
CC	33 (14.1)	44 (14.3)	22 (11.0)	0.726 0.91 (0.53–1.55)	0.204 0.67 (0.36–1.25)
T	278 (59.4)	377 (61.2)	255 (63.8)	1 (reference)	1 (reference)
C	190 (40.6)	239 (38.8)	145 (36.2)	0.735 0.88 (0.43–1.81)	0.612 0.92 (0.68–1.26)

CAD-Coronary Artery Disease, HC-healthy control, OR–odds ratio, CI–confidence interval;
D,C,C are variant allele; I,A,T are wild-type allele for ACE,AT1 and CYP111B2 respectively;
[a]CAD patients in primary cohort; [b]CAD patients in replication cohort;
$a =$ represents the p values for the comparison in CAD patients (primary cohort) and HC;
$b =$ represents the p values for the comparison in CAD patients (replication cohort) and HC.
Significant values are shown in bold.

binary logistic regression. Overall we performed meta analysis by Stouffers method to combine the results of two cohorts (primary and replication) to calculate the overall z and p values. A two-tailed p-value of less than 0.05 was considered as statistical significant result. All statistical analyses were performed using SPSS software version 16.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. Patient characteristics

Clinical characteristics of CAD patients-Primary, replication and combined cohorts of study are shown in Table 1. There was no significant difference in the mean age of CAD patients and controls. The male/female ratio was comparable in both CAD cases as well as in controls. Evaluation of the defined risk factors in the cohort showed that 45.7% patients were hypertensive and 29.5% patients were diabetic. Moreover, 26.0% patients were associated with tobacco usage. Patients with stable angina were 30.7% and unstable angina/ Non ST Segment Elevation Myocardial Infarction (NSTEMI) formed 21.5% of the clinical syndrome. ST Segment Elevation Myocardial Infarction (STEMI) patients with anterior wall myocardial infarction (AWMI) and inferior wall myocardial infarction (IWMI) were 25.6% and 21.7% respectively. Only two patients were found to be affected with lateral wall myocardial infarction (LWMI) (0.4%). The angiographic profile categorized patients with single vessel disease (SVD), double vessel disease (DVD), and triple vessel disease (TVD) as 67.2%, 22.0% and 10.8% respectively. The mean ejection fraction was 50.0 ± 11.18. The kurtosis and skewness for left ventricular ejection fraction (LVEF) were 0.12 and -0.98. Thus, the data appeared to be normally distributed. Although in clinical practice, LVEF of <50% is considered as LVD but we selected $\leqslant 45\%$ cut off as significant LVD. Of the total 508 CAD patients, 67.1% showed preserved ($> 45\%$) ejection fraction while 32.9% had compromised ejection fractions ($\leqslant 45\%$). All the values in primary cohort matched to replication cohort and there was no significant difference in the values of three cohorts of the study.

3.2. Allelic distribution of studied polymorphisms in controls

The distribution of *ACE* I/D, *AT1* A1166C and *CYP11B2* T-344C genotypes is shown in Table 2. The observed genotype frequencies of all the studied polymorphisms in healthy controls were in accordance with Hardy-Weinberg equilibrium ($p > 0.05$).

Table 3a
Distributions of RAAS gene polymorphisms in primary cohort of CAD patients with preserved (LVEF $>$ 45) and compromised (LVEF $\leqslant$ 45) left ventricular ejection fraction

LVEF categorical	Genotypes	CAD N (%) 308	P-value	OR (95% CI)
ACE **I/D**				
$>$ 45	II	68 (31.8)		Reference
	ID	98 (45.8)		
	DD	48 (22.4)		
$\leqslant$ 45	II	31 (33.0)	–	–
	ID	43 (45.7)	0.89	0.96 (0.55–1.68)
	DD	20 (21.3)	0.79	0.91 (0.47–1.80)
AT1 **A1166C**				
$>$ 45	AA	172 (80.4)		Reference
	AC	35 (16.3)		
	CC	7 (3.3)		
$\leqslant$ 45	AA	60 (63.8)	–	–
	AC	25 (26.6)	**0.018**	**2.05 (1.13–3.70)**
	CC	9 (9.6)	**0.013**	**3.69 (1.32–10.33)**
CYP11B2 T-344C				
$>$ 45	TT	80 (37.4)		Reference
	TC	102 (47.6)		
	CC	32 (15.0)		
$\leqslant$ 45	TT	33 (35.1)	–	–
	TC	49 (52.1)	0.573	1.16 (0.69–1.98)
	CC	12 (12.8)	0.810	0.91 (0.42–1.97)

Significant values are shown in bold.

Table 3b
Distributions of RAAS gene polymorphisms in replication cohort of CAD patients with preserved (LVEF $>$ 45) and compromised (LVEF $\leqslant$ 45) left ventricular ejection fraction

LVEF categorical	Genotypes	CAD N (%) 200	P-value	OR (95% CI)
ACE I/D				
$>$ 45	II	52 (40.9)		Reference
	ID	52 (40.9)		
	DD	23 (18.1)		
$\leqslant$ 45	II	27 (37.0)	–	–
	ID	28 (38.4)	0.91	1.04 (0.54–2.00)
	DD	18 (24.7)	0.30	1.51 (0.70–3.26)
AT1 **A1166C**				
$>$ 45	AA	107 (84.2)		Reference
	AC	17 (13.4)		
	CC	3 (2.4)		
$\leqslant$ 45	AA	48 (65.7)	–	–
	AC	18 (24.7)	**0.024**	**2.36 (1.12–4.97)**
	*CC	7 (9.6)	**0.020**	**5.20 (1.29–20.98)**
CYP11B2 **T-344C**				
$>$ 45	TT	48 (37.8)		Reference
	TC	64 (50.4)		
	CC	15 (11.8)		
$\leqslant$ 45	TT	29 (39.7)	–	–
	TC	37 (50.7)	0.888	0.96 (0.52–1.77)
	CC	7 (9.6)	0.616	0.77 (0.28–2.12)

*Overall z value for primary and replicative cohort = 3.02; P value $\leqslant$ 0.001.
Significant values are shown in bold.

3.3. *Frequency distribution of RAAS gene polymorphism in CAD patients and controls*

Table 2 shows the risk of CAD in relation to each of the SNPs of RAAS genes. On comparing the genotype frequency distribution in CAD patients (both cohorts) with that of controls, the homozygous variant genotypes (DD) of ACE I/D and D allele showed statistically significant increased risk for developing CAD [($p \leqslant$

Table 4
Distributions for *AT1* A1166C gene Polymorphism in STEMI subjects with preserved (LVEF $>$ 45) and compromised (LVEF $\leqslant$ 45) left ventricular ejection fraction

Genotypes/Alleles	$>45\%$	$\leqslant 45\%$	P-value	OR (95% CI)
AA	102 (84.3)	80 (65.6)	–	Reference
AC	17 (14)	29 (23.8)	**0.023**	**2.18 (1.12–4.27)**
CC	2 (1.7)	13 (10.7)	**0.005**	**8.62 (1.88–39.38)**
A	221 (91.3)	189 (77.5)	–	Reference
C	21 (8.7)	55 (22.5)	$<$ **0.001**	**3.16 (1.82–5.46)**

Significant values are shown in bold.

0.001; OR = 3.72 and $p \leqslant 0.001$; OR = 1.78 respectively in primary cohort) and ($p = 0.001$; OR = 2.81 and $p = 0.041$; OR = 1.37 respectively in secondary cohorts) Table 2] On the contrary, no significant difference was observed in the distribution of AT1 A1166C and CYP11B2 T-344C polymorphisms in any of the groups both at genotype and allele levels.

3.4. *Influence of RAAS gene polymorphisms on patients with reduced and preserved ejection fraction*

We segregated CAD patients on the basis of compromised ($\leqslant 45\%$) and preserved ($> 45\%$) left ventricular ejection fraction (LVEF) and compared with their status of *ACE* I/D, *AT1* A1166C and *CYP11B2* T-344C gene polymorphisms. We found that higher percentage of CAD patients carrying heterogeneous *AT1* AC and CC genotypes had compromised ejection fraction ($\leqslant 45\%$) as compared to the patients with preserved ($> 45\%$) ejection fraction. This frequency difference was statistically significant in both the cohorts (p value = 0.018, OR = 2.05 and p value = 0.013, OR = 3.69 in *AT1* AC and CC genotypes respectively in primary cohort; Table 3a and (p value = 0.024, OR = 2.36 and p value = 0.020, OR = 5.20 in *AT1* AC and CC genotypes respectively in secondary cohorts; Table 3b). Also on calculating the overall z and p values of both primary and replication cohorts, we found statistically significant values in *AT1* CC genotypes ($z = 3.02$; $p \leqslant 0.001$ Table 3b). No significant difference for *CYP11B2* T-344C gene polymorphism was observed between CAD patients with preserved and compromised LVEF.

We looked for the association of *ACE, ATI, CYP11B2* on LVD by changing the cut off values for LVEF to $< 40\%$ and $< 50\%$ also. In case of *AT1* the level of significance decreased when we considered the cut off for LV dysfunction to 50% and at 40% cut off, level of significance increased but we would have missed some patients with LV dysfunction. Therefore, we selected the cut off to 45% to include definite patients with LV dysfunction.

3.5. *Distributions for AT1 A1166C gene Polymorphism in STEMI subjects with preserved (LVEF $>$ 45) and compromised (LVEF $\leqslant$ 45) left ventricular ejection fraction*

In clinical practice it is well known fact that STEMI patients are more prone to develop LVD. We also observed that 73.1% of STEMI patients had LVD. Therefore, we looked for distribution of *AT1* genotypes in STEMI patients with preserved and compromised ejection fraction. Our results showed that the subjects with CC genotypes and C allele of *AT1* polymorphism were more likely to develop LVD as compared to wild type (AA) genotype [Table 4].

Furthermore, we also conducted a separate analysis for stable angina and myocardial infarction (STEMI + NSTEMI) and found that none of the genetic variant of RAAS pathway conferred risk for myocardial infarction.

4. Discussion

In the present study we used the candidate gene approach to determine whether the genetic variants of RAAS genes – *ACE*, *AT1* and *CYP11B2*, being important effectors of the renin-angiotensin-aldosterone system, are involved in LV dysfunction in CAD patients. The main finding of the study indicates close association between *AT1* A1166C polymorphism and significantly higher risk of severe left ventricular dysfunction (LVD) in CAD patients.

Remarkably, left ventricular dysfunction (LVD) constitutes the final common pathway for a host of cardiac disorders. Among them, coronary artery narrowing or ischaemic heart disease is the dominant cause of LVD. The LVD produces many changes in the structure and function of the heart through a variety of mechanisms including the renin-angiotensin system, one of the major neuroendocrine axes involved in the development of heart failure [14].

Till date, several researchers have investigated the role of *AT1* A1166C polymorphism in relation to CAD. Also, there are studies supporting the idea that the *AT1* 1166C allele is a predisposing genetic marker for CAD or MI [4,15,16] but there are also reports contrary to these findings [17,18]. Our findings show the lack of association of this polymorphism with CAD, as we observed no differences in the prevalence of the *AT1* 1166C allele and CC genotype between CAD patients and healthy controls.

After segregation of CAD patients on the basis of left ventricle ejection fraction (LVEF), the significantly higher *AT1* CC genotype and 1166C allele frequencies in reduced LVEF subjects point towards an increased risk of LV dysfunction in CAD subjects with LVEF $\leqslant$ 45%.

In humans the effect of angiotensin II (AngII) seems primarily be mediated by AT1 receptors. Increased levels of angiotensin II have been suggested to be involved in the pathophysiology of cardiovascular disease. Angiotensin II, as an acute vasoconstrictor regulates systemic blood pressure and vascular tone. Among several biallelic polymorphisms present in the *AT1* receptor gene, A1166C transversion, located at 3' UTR, has been associated with cardiovascular phenotypes such as essential hypertension [19,20], myocardial stiffness [21], and left ventricular hypertrophy [22]. Being present in the non-coding region, the polymorphism can affect gene regulation by interfering with posttranscriptional activities. In a previous study [23] it was reported that A1166C polymorphism is located within the target sites of miRNA binding. Using reporter silencing assays, it has been shown that miR155 downregulates the expression only of the 1166A, and not the 1166C allele of *AT1* gene. So in case of 1166C allele, the expression of AT1 increases, which can lead to arteriolar vasoconstriction and increased blood pressure which ultimately results in reduced cardiac output. There are also reports that density of AT1 receptors increases in myocardial infarction [24]. The increased expression of AT1 receptors in carriers of 1166C allele, may lead to higher susceptibility for MI, which may later progress to LVD.

Various comparative studies suggest that angiotensin receptor blockers (ARBs) may lower the risk of HF beyond their ability to reduce hypertension. In the field of cardiovascular disease, ACE I/D and the AT1 A1166C gene polymorphisms are probably the most studied genetic variants because the products of these two genes are directly implicated in the pharmacological modulation of the RAAS, and they evidently provoke great interest. In line to this, our data also demonstrates that there is a strong association between ACE I/D gene polymorphism and CAD patients but not with left ventricular dysfunction. Studies on the association of ACE I/D polymorphism and CAD or MI have shown variable results. A recent meta-analysis of 15 studies into the association between the ACE I/D polymorphism and MI in male dominated populations found a mean OR of 1.26 for MI of the DD vs ID+II genotypes (95% CI 1.15–1.39, $p = 0.001$) [25]. Also few other studies, have suggested a positive association [26,27]. However, a number of other studies have reported negative results [28–30]. Also a previous study demonstrated that the higher level of ACE activity is associated with the development of the moderate left ventricular dysfunction in comparison with ACE activity in patients with preserved left ventricular function. However the severe left ventricular dysfunction (EF $< 40\%$) was associated also with low ACE activity [31]. Apart from conferring susceptibility, the ACE gene has been also proposed to play a role in modifying the effect of various treatments in CAD. We also found no association of CYP11B2 T-344C with either CAD or left ventricular dysfunction. Previous studies on T-344C polymorphism have shown positive [32,33] as well as negative association [34,35] with hypertension and other cardiovascular parameters. There is significant heterogeneity among published reports, with some studies showing association with T allele [32,36] and others with C allele [37,38]. Since LVD is a polygenic disorder influenced by multiple genes, further association studies and screening of other candidate gene polymorphisms is required to elucidate the precise genetic susceptibility of the disease.

4.1. Study strengths and limitations

Being a case-control association study, we have replicated our findings in an independent cohort. Although our sample size is limited, but we have sufficient power ($\beta = 0.8$) to detect a risk of 1.6 or higher. However, it will be worthwhile to replicate the study in different populations before any clinical applications.

In conclusion, our study showed that the *AT1* 1166C allele is closely associated with left ventricle dysfunction in CAD patients. These findings may have important implications in the understanding of pathobiology of heart failure.

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Conflict of Interest

None declared.

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