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Original Article

# Role of angiotensin II type I (AT1 A1166C) receptor polymorphism in susceptibility of left ventricular dysfunction

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ABSTRACT

*Background:* Left ventricular dysfunction (LVD) with subsequent congestive heart failure (CHF) constitutes the final common pathway for a host of cardiac disorders. The impaired LV function develops in response to an ischemic insult followed by a fall in cardiac output that leads to activation of renin-angiotensin-system (RAS). Angiotensin II type I receptor (AT1), which mediate the vasoconstrictive and salt-conserving actions of the RAS, represent interesting candidate genes for cardiovascular diseases. Therefore, we conducted an association study between single nucleotide polymorphism (SNP) in *AT1* gene and LVD in CAD patients.

*Methods and results:* The present study recruited a total of 950 subjects including 720 angiography confirmed CAD patients and 230 healthy controls. Among 720 CAD patients, 229 with reduced left ventricle ejection fraction (LVEF $\leq$ 45%) were categorized as LVD. The *AT1* (A1166C, rs5186) polymorphism was determined by ARMS-PCR. Our results showed that the frequency of *AT1* 1166AC and CC genotypes were significantly higher in LVD patients in comparison to non-LVD (LVEF >45%) patients ($p$ value = 0.003; OR = 1.81 and $p$ value <0.001; OR = 4.33). Further analysis showed that *AT1* A1166C polymorphism was significantly associated with LV end diastole ($p$-value = 0.031), end systole ($p$-value = 0.038) dimensions, and mean LVEF ($p$-value = 0.035). Moreover, on comparing the *AT1* A1166C polymorphism in CAD patients with healthy controls, we did not find any association both at genotypic and allelic level ($p$ value = 0.927; OR = 1.04 and $p$ value = 0.219; OR = 0.83) respectively.

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*Conclusions:* Our study suggests that *AT1* A1166C polymorphism may play significant role in conferring genetic susceptibility of LVD.



## 1. Introduction

Left ventricular dysfunction (LVD) with subsequent congestive heart failure (CHF) constitutes the final common pathway for a host of cardiac disorders. Coronary artery narrowing or ischemic heart disease is the dominant cause of LVD and is often associated with acute or prior myocardial infarction.[1] Left ventricular remodeling is a key process, determining disease progression and affecting outcome in this condition. This is further characterized by the continuous interactions between the underlying myocardial dysfunction and activated compensatory neurohumoral mechanisms.[2] Being a progressively debilitating condition and despite broad array of treatment, a significant heterogeneity exists in the benefits to individual subject 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 LVD.

New molecular biology techniques applied to genetic diagnosis make it possible to study the mechanisms underlying individual and familial predisposition to suffering certain diseases. Specifically, in relation to coronary artery disease, the genetic markers linked to the renin-angiotensin-system (RAS) have received special attention, not only because of their well-known effects on vascular homeostasis[3] but also the promise of the use of angiotensin-converting enzyme inhibitor (ACEI) and angiotensin receptor blockers (ARBs) to reduce morbidity and mortality in ischemic heart disease.[4]

Angiotensin II (Ang II) is an active component of the RAS and the majority of the known action of Ang II relevant to cardiovascular function and structure are mediated by angiotensin II type1 (AT1) receptor. AT1 receptor, located in vascular smooth muscle cells and myocardium, mediates the vasoconstrictive and salt-conserving actions of the RAS, and therefore represents interesting candidate gene for cardiovascular diseases. A single nucleotide polymorphism (SNP), A1166C, located in the 3′ untranslated region (UTR) of *AT1* gene, has been characterized and investigated in relation to arterial hypertension,[5] hypertension-induced hypertrophy,[6] aortic stiffness,[7] myocardial infarction,[8] and carotid intimal-medial thickening.[9] AT1 A1166C polymorphism has been associated with essential hypertension,[10,11] aortic stiffness,[7] collagen type I synthesis, and myocardial stiffness in patients with hypertensive heart disease[12] and cardiac hypertrophy.[13]

In our previous study we showed that in RAS, *AT1* A1166C polymorphism was associated with LVD in a small subset of CAD patients.[14] However, the aim of the present study was to assess whether *AT1* A1166C polymorphism associated with LV ejection fraction (LVEF) and other echocardiography parameters such as LV end diastole dimension (LVEDD), LV end systolic dimension (LVESD), and LV mass in a larger sample size. In addition, we have done an extensive statistical analysis with different variables to explore a more clear picture of *AT1* A1166C (rs5186) polymorphism in the development of LVD.

## 2. Materials and methods

### 2.1. Study population

The present study recruited a total of 950 subjects including 720 CAD patients and 230 healthy controls. All the patients had significant coronary artery disease (diagnosis, confirmed by coronary angiography and further all these subjects underwent either coronary angioplasty or Coronary Artery Bypass Graft (CABG) surgery), recruited from the Department of Cardiology and Department of Cardiovascular and Thoracic Surgery (CVTS) 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 stenosis >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 population consisted of 230 subjects (191 males and 39 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.[15] 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 (IEC Code No: A-01:PGI/SRF/IEC/54/29.04.2011)and the authors followed the norms of World's Association Declaration of Helsinki.[16]

### 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.[17] LV mass was calculated by using the following formula: 0.8 [1.04 {(LV diastolic internal dimension + inter-ventricular septum + posterior wall)3−(LV diastolic internal dimension)

3}]+0.6.[18] 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.

### 2.3. Genotyping

Genomic DNA was isolated from peripheral blood leukocytes according to a standard salting out method.[19] AT1 A1166C polymorphism was genotyped using ARMS-PCR method. As a negative control, PCR mix without DNA sample was used to ensure contamination free PCR product. Genomic DNA was amplified in a DNA thermal cycler (Eppendorf Germany) using a set of outer (Forward outer (GCCAAATCCCACTCAAACCTTTCAACAA)/Reverse outer AAGCAGGCTAGGGAGATTGCATTTCTGT) and a set of inner [Forward inner (A allele) TCTGCAGCACTTCACTACCAAATGAACA/Reverse inner (C allele) TCTCCTTCAATTCTGAAAAGTAGCTGAG] primers described by Shu Ye et al.[20] PCR was conducted in a total volume of 25 μl with 2 pmol of outer primers and 16 pmol of inner primers, genomic DNA (100–150 ng), 10 mM dNTPs, PCR buffer containing final concentrations of 50 mM KCl; 10 mM Tris–HCl (pH 8.3); 1.5 mM $MgCl_2$ and 1.5 units of Taq DNA polymerase (Bangalore Genei, India).

#### 2.3.1. PCR conditions

Initial denaturation: 95 °C for 2 min; Denaturation, 95 °C for 1 min; Annealing, 58 °C for 1 min; Extension, 72 °C for 1 min and Final extension at 72 °C for 2 min. The PCR was carried out for 35 cycles.

The PCR fragments were separated on 2% agarose gel, stained with ethidium bromide and observed with ultraviolet imaging system (Bio-Rad Gel DocTM EZ Imager, USA). Representative gel picture is given in Fig. 1. Genotyping was performed without knowledge of the case or control status. Ten percent of samples for each genotype were sequenced which showed 100% concordance.

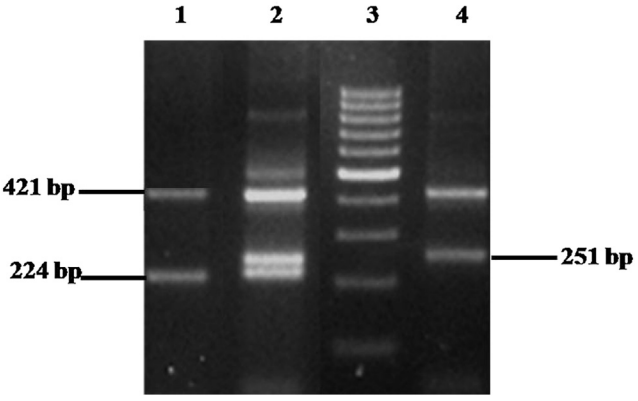


**Fig. 1 – Representative gel picture of *AT1* A1166C polymorphism: Lane 1, CC genotype; Lane 2, AC genotype; Lane 3, 100 bp DNA ladder; Lane 4, AA genotype.**

### 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 720 patients and 230 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 *AT1* A1166C gene polymorphism and significant risk factors of CAD was analyzed by using binary logistic regression. 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

Of the total 720 CAD patients, 68.2% showed preserved (>45%) ejection fraction (Non-LVD) while 31.8% had reduced (≤45%) ejection fractions (LVD), according to criteria used previously.[14,21] A comparison of demographic profile and clinical characteristics between LVD and Non-LVD patients is shown in Table 1. The mean age (56.20 ± 8.47 v/s 55.54 ± 9.54) and male/female ratio (89.1% v/s 86.4%) were not significantly different between the LVD and Non-LVD patients. Hypertension and diabetes are common in CAD patients, but their incidence was not significantly different among LVD and Non-LVD patients. Lipid levels and body mass index (BMI) were also comparable between the two groups. However, a higher percentage of LVD patients were smokers as compared to Non-LVD patients (31.4% v/s 21.6%, $p = 0.005$). The frequency of STEMI (ST-elevation myocardial infarction), a well known predictor of LVD, was also significantly different between the LVD and Non-LVD patients (69.9% v/s 37.5%, $p < 0.001$). The angiographic profile categorized patients with single vessel disease (SVD), double vessel disease (DVD), and triple vessel disease (TVD) as 60.1%, 17.4% and 22.5% respectively in CAD patients. Among all CAD patients, complete revascularization was done in 82.8% patients. There was no significant difference in the frequency of SVD, DVD, and TVD between the two groups. The percentage of complete revascularization was also comparable between these two groups. Among echocardiography traits, LV ejection fraction ($p < 0.001$), LV end diastole dimension ($p < 0.001$), LV end systolic dimension ($p < 0.001$), Posterior wall end diastole dimension ($p = 0.027$) and LV mass ($p = 0.014$) were significantly different between LVD and Non-LVD groups which clearly demarked these two different populations (Table 1).

**Table 1 – Demographic profile and clinical characteristics of CAD patients with LVD v/s Non-LVD.**

| Clinical characteristics | CAD | LVD (LVEF ≤ 45%) | Non-LVD (LVEF > 45%) | *p*-values |
|---|---|---|---|---|
| Total subjects | 720 | 229 | 491 | – |
| *Age – yr | 55.79 ± 9.22 | 56.20 ± 8.47 | 55.54 ± 9.54 | 0.372 |
| Male sex | 628 (87.2%) | 204 (89.1%) | 424 (86.4%) | 0.339 |
| **Risk factors** | | | | |
| Hypertensive | 317 (44.0%) | 96 (41.9%) | 221 (45.0%) | 0.468 |
| Diabetic | 230 (31.9%) | 82 (35.8%) | 148 (30.1%) | 0.145 |
| Smokers | 178 (24.7%) | 72 (31.4%) | 106 (21.6%) | **0.005** |
| *BMI | 24.27 ± 3.06 | 24.51 ± 3.23 | 23.84 ± 2.68 | 0.120 |
| ***Lipid profiles (mg/dl)** | | | | |
| High density lipoprotein, HDL | 32.19 ± 7.92 | 31.05 ± 6.75 | 32.66 ± 8.34 | 0.385 |
| Low density lipoprotein, LDL | 74.31 ± 26.24 | 73.98 ± 24.82 | 74.46 ± 26.95 | 0.909 |
| Triglycerides (TG) | 146.30 ± 64.01 | 146.34 ± 63.68 | 146.27 ± 64.41 | 0.994 |
| Total cholesterol (TC) | 138.21 ± 38.66 | 138.40 ± 37.53 | 138.12 ± 39.30 | 0.963 |
| **Clinical syndrome** | | | | |
| Stable angina | 237 (32.9%) | 36 (15.7%) | 201 (40.9%) | – |
| Unstable angina/NSTEMI | 139 (19.3%) | 33 (14.4%) | 106 (21.6%) | – |
| STEMI | 344 (47.8%) | 160 (69.9%) | 184 (37.5%) | **<0.001** |
| AWMI | 187 (26.0%) | 109 (47.6%) | 78 (15.9%) | – |
| IWMI | 157 (21.8%) | 51 (22.3%) | 106 (21.6%) | – |
| **Angiographic profiles** | | | | |
| Single vessel disease (SVD) | 433 (60.1%) | 134 (58.5%) | 299 (60.9%) | – |
| Double vessel disease (DVD) | 125 (17.4%) | 39 (17.0%) | 86 (17.5%) | – |
| Triple vessel disease (TVD) | 162 (22.5%) | 56 (24.5%) | 106 (21.6%) | 0.691 |
| Complete Revascularization | 596 (82.8%) | 185 (80.8%) | 411 (83.7%) | 0.342 |
| ***Echocardiographic traits** | | | | |
| LVEF, % | 49.97 ± 11.18 | 35.94 ± 7.05 | 56.51 ± 5.05 | **<0.001** |
| LVEDD, mm | 47.21 ± 8.11 | 49.07 ± 6.99 | 44.48 ± 4.58 | **<0.001** |
| LVESD, mm | 31.05 ± 9.31 | 34.42 ± 7.69 | 28.93 ± 4.87 | **<0.001** |
| Posterior wall end diastole dimension, mm | 9.98 ± 1.87 | 9.59 ± 1.42 | 10.07 ± 1.49 | **0.027** |
| Inter-ventricular septum end diastole dimension, mm | 10.01 ± 1.98 | 9.91 ± 1.45 | 10.38 ± 1.76 | 0.051 |
| LV mass (LV mass), gm | 161.31 ± 55.56 | 175.39 ± 53.04 | 158.38 ± 42.76 | **0.014** |

*Values are mean ± SD.
*p*-values = Between LVD and Non-LVD groups, Significant values are shown in bold.
NSTEMI = Non-ST elevation myocardial infarction, STEMI = ST elevation myocardial infarction, AWMI = anterior wall myocardial infarction, IWMI = Inferior wall myocardial infarction, LVEF = LV ejection fraction, LVEDD = LV end diastole dimension, LVESD = LV end systolic dimension.

### 3.2. *Analysis of AT1 A1166C gene polymorphisms in healthy controls, CAD and LVD patients*

The distribution of *AT1* A1166C genotypes is shown in Table 2. The observed genotype frequencies of the studied polymorphism in healthy controls were in accordance with Hardy–Weinberg equilibrium ($p > 0.05$). Table 2 shows the risk of CAD in relation to *AT1* A1166C polymorphism. On comparing the genotype frequency distribution in CAD patients with that of healthy controls, no significant difference was observed in the distribution of *AT1* A1166C polymorphisms (AA *vs* AC; *p*-value = 0.059, OR = 0.71, AA *vs* CC; *p*-value = 0.927, OR = 1.04, and AA *vs* AC + CC; *p*-value = 0.219, OR = 0.83; Table 2).

Further, we segregated CAD patients on the basis of reduced (≤45%) and preserved (>45%) left ventricular ejection fraction (LVEF) and compared with their status of *AT1* A1166C polymorphism. We found that higher percentage of CAD patients carrying *AT1* AC and CC genotypes had reduced ejection fraction (≤45%) as compared to the patients with preserved (>45%) ejection fraction. This frequency difference was statistically significant (AA *vs* AC; *p*-value = 0.003, OR = 1.81, AA

**Table 2 – Analysis of *AT1* A1166C gene polymorphisms in healthy controls, CAD and LVD patients.**

| Genotypes | Controls (230) | CAD (720) | Non-LVD (491) | LVD (229) | OR (95% CI) *p*-value[a] | OR (95% CI) *p*-value[b] |
|---|---|---|---|---|---|---|
| AA | 165 (71.7) | 553 (76.8) | 400 (81.5) | 153 (66.8) | – | – |
| AC | 56 (24.3) | 135 (18.8) | 79 (16.1) | 56 (24.5) | 0.71 (0.49–1.01) 0.059 | **1.81 (1.22–2.70) 0.003** |
| CC | 9 (3.9) | 32 (4.4) | 12 (2.4) | 20 (8.7) | 1.04 (0.48–2.23) 0.927 | **4.33 (2.06–9.09) <0.001** |
| AC + CC | 65 (28.2) | 167 (23.2) | 91 (18.5) | 76 (33.2) | 0.83 (0.62–1.17) 0.219 | **2.19 (1.53–3.14) <0.001** |

Significant values are shown in bold.
[a] *p*-value between healthy controls and CAD.
[b] *p*-value between Non-LVD and LVD.

*vs* CC; *p*-value <0.001, OR = 4.33, and AA *vs* AC + CC; *p*-value <0.001, OR = 2.19; Table 2).

We also looked for the association of *AT1* A1166C polymorphism with LVD by changing the cut-off values for LVEF. When CAD patients were categorized on the basis of different subgroups of LVEF (below 31% to above 55%), the results showed that the patients in lower LVEF subgroups had significantly higher frequency of *AT1* A1166C polymorphism (*p*-value <0.001; Table 3).

### 3.3. *AT1 A1166C polymorphism in CAD patients with clinical characteristics*

After evaluating association of *AT1* A1166C polymorphism with reduced LVEF, further analysis was extended to look for the association of *AT1* A1166C polymorphism with clinical characteristics of CAD. The results showed that *AT1* A1166C polymorphism is significantly associated with reduced LVEF (*p*-value <0.001), LVEF means (*p*-value = 0.035) and other parameters of LV remodeling i.e. LV dimensions (LVEDD; *p*-value = 0.031, and LVESD; *p*-value = 0.038; Table 4).

However when CAD patients were stratified on the basis of risk factors like diabetes mellitus, hypertension and smoking status, *AT1* A1166C polymorphism did not modulate the risk of CAD due to these factors.

### 3.4. *Distributions for AT1 A1166C gene polymorphism in STEMI subjects with preserved (LVEF >45%) and reduced (LVEF ≤45%) left ventricular ejection fraction*

In clinical practice it is well known fact that STEMI patients are more prone to develop LVD. We observed that 70.2% of LVD patients had previous STEMI. Therefore, we looked for distribution of *AT1* A1166C genotypes in STEMI patients with preserved and reduced ejection fraction. Our results showed that the subjects with AC and CC genotypes were more likely to develop LVD as compared to wild type AA genotype (AA *vs* AC; *p*-value = 0.044, OR = 1.79, AA *vs* CC; *p*-value = 0.002, OR = 10.24; Table 5).

### 3.5. *Multivariate analysis of AT1 A1166C polymorphism in CAD patients with risk factors*

Further, multivariate analysis was done to rule out the possibilities of development of LVD in CAD patients due to confounding factors such as smoking, diabetes, hypertension, and STEMI (ST-elevation myocardial infarction). In multivariate analysis we step-wise removed patients of one risk factor at a time and did analysis of CAD patients with *AT1* A1166C polymorphism by excluding patients with known risk factors of LVD such as smoking, diabetes, hypertension, and STEMI (ST-elevation myocardial infarction). In this analysis we found that non-smokers ($p < 0.001$), non-diabetic ($p = 0.003$), non-hypertensive ($p = 0.008$), and their combination ($p = 0.013$) were at higher risk of developing LVD in CAD patients due to *AT1* A1166C polymorphism, while we did not find any significant association in case of excluding CAD patients with STEMI (Table 6).

**Table 4 – Association of clinical characteristics of CAD patients with *AT1* A1166C polymorphism.**

| Characteristics | AA *n* (%) | AC + CC *n* (%) | *p*-value |
|---|---|---|---|
| Patients | 553 | 167 | – |
| *Age at CAD diagnosis, years | 55.63 ± 9.09 | 56.16 ± 9.63 | 0.518 |
| Male | 485 (87.2) | 143 (85.6) | 0.280 |
| **Risk factors** | | | |
| Hypertension | 240 (43.4) | 77 (46.1) | 0.298 |
| Diabetes | 177 (32.0) | 53 (31.7) | 0.514 |
| Smoking | 133 (24.1) | 45 (26.9) | 0.474 |
| *BMI, kg/m$^2$ | 24.33 ± 3.20 | 24.05 ± 2.43 | 0.586 |
| Myocardial Infarction (MI) | 320 (68.8) | 109 (73.2) | 0.356 |
| **Angiographic profiles** | | | |
| Single vessel disease (SVD) | 331 (59.9) | 102 (61.1) | – |
| Double vessel disease (DVD) | 93 (16.8) | 32 (19.2) | – |
| Triple vessel disease (TVD) | 129 (13.3) | 33 (19.8) | 0.559 |
| ***Echocardiography traits** | | | |
| LV end diastole dimension, mm | 45.30 ± 5.51 | 46.97 ± 6.33 | **0.031** |
| LV end systolic dimension, mm | 30.85 ± 5.77 | 32.53 ± 6.52 | **0.038** |
| LV posterior wall thickness, mm | 9.47 ± 1.23 | 9.50 ± 1.22 | 0.914 |
| LV inter-ventricular septum, mm | 9.68 ± 1.35 | 9.63 ± 1.36 | 0.895 |
| LV mass, gm | 146.86 ± 46.21 | 149.77 ± 43.69 | 0.678 |
| LV ejection fraction | 50.45 ± 10.82 | 48.37 ± 12.20 | **0.035** |
| Reduced LVEF (≤45%) | 153 (27.7) | 76 (45.5) | **<0.001** |

Significant values are shown in bold.
* Values are mean ± SD.

## 4. Discussion

In the present study we explored the role of *AT1* A1166C genetic polymorphisms on left ventricular dysfunction in a population of 720 angiographically confirmed CAD patients who had already been on optimal treatment for this condition. The main finding of the present study indicates that the

**Table 3 – Association of *AT1* A1166C gene polymorphism with different subgroups based on left ventricular ejection fraction (LVEF).**

| Genotypes | >55% *n* (%) | 51–55 % *n* (%) | 46–50 % *n* (%) | 41–45 % *n* (%) | 31–40 % *n* (%) | <31% *n* (%) | *p*-value |
|---|---|---|---|---|---|---|---|
| AA | 180 (32.5) | 112 (20.3) | 105 (19.0) | 34 (6.1) | 72 (13.0) | 50 (9.0) | – |
| AC + CC | 56 (33.5) | 22 (13.2) | 12 (7.2) | 23 (13.8) | 33 (19.8) | 21 (12.6) | **<0.001** |

Significant value is shown in bold.

**Table 5 – Distributions for AT1 A1166C gene polymorphism in STEMI subjects with preserved (>45%) and reduced (≤45%) left ventricular ejection fraction.**

| Genotypes | >45% | ≤45% | *p*-value | OR (95% CI) |
|---|---|---|---|---|
| AA | 134 (83.2) | 101 (67.3) | – | Reference |
| AC | 25 (15.5) | 34 (22.7) | **0.044** | **1.79 (1.00–3.19)** |
| CC | 2 (1.2) | 15 (10.0) | **0.002** | **10.24 (2.28–45.99)** |

Significant values are shown in bold.

individuals with AT1 1166 AC and CC genotypes (AA *vs* AC; *p*-value = 0.003, OR = 1.81, AA *vs* CC; *p*-value <0.001, OR = 4.33, and AA *vs* AC + CC; *p*-value <0.001, OR = 2.19) are genetically predisposed to LVD as compared to AA homozygote subjects.

In patients with cardiovascular disease, activity of the RAS is often increased and contributes to a poor prognosis.[22] Angiotensin II (Ang II), as active component of RAS, is an acute vasoconstrictor that regulates systemic blood pressure and vascular tone. Increased levels of Ang II have been suggested to be involved in the pathophysiology of cardiovascular disease.[23] Physiologically AT1 receptors are the primary mediator of Ang II.

Among several biallelic polymorphisms present in the *AT1* receptor gene, A1166C transversion is particularly important, located at 3′ UTR. Although A1166C polymorphism does not appear to be functional but tends to be a key genetic marker or in linkage disequilibrium with unidentified functional loci which would affect the regulation of the gene. Earlier, a report on mapping of 3′ UTR SNPs onto a collection of experimentally supported human miRNA targets, have confirmed that A1166C polymorphism is located within the miRNA binding sites and miR155 down-regulates the expression only of the 1166A, and not the 1166C allele of *AT1* gene.[24] In presence of 1166A allele, miRNA binds with target site and reduces the expression of *AT1* receptor gene while 1166C allele abolishes the target site and impairs the ability of miR-155 binding, thereby elevating the level of AT1 receptors. Also, it has been suggested that after severe myocardial infarction (MI), the level of AT1 receptors increases.[25] Thus, increased AT1 receptor levels in 1166C allele carriers may lead higher probability of arteriolar vasoconstriction and increased blood pressure followed by reduced cardiac output which may give rise to LVD (Fig. 2).

The previous findings that co-relate *AT1* receptor gene to CAD and other cardiovascular diseases are contradicting. Some studies support that the *AT1* 1166C allele is a predisposing genetic marker for CAD or MI[8,26,27] but there other reports contrary to these findings.[28,29] In addition to CAD, *AT1* A1166C polymorphism has been also associated with the severe form of essential hypertension,[10,11] aortic stiffness,[7] and collagen type I synthesis, and myocardial stiffness in patients with hypertensive heart disease[12] and cardiac hypertrophy in hypertrophic cardiomyopathy patients.[13] Previously, *AT1* 1166C allele has also been associated with lower ejection fraction[30] and increased left ventricular mass.[31] We had earlier reported an association of *AT1* A1166C polymorphism with LVD in a small cohort of CAD patients.[14]

Analysis of association of *AT1* A1166C polymorphism with different subgroups based on left ventricular ejection fraction (LVEF) shows a significant association of this polymorphism with severe LVD as the patient in groups LVEF = 31–40% and below 31% were having significantly higher percentage of AC and CC genotypes. Further, analysis of with clinical characteristics shows that this polymorphism not only associated with LVEF but also with other echocardiography parameters such as LV end diastole dimension (LVEDD), and LV end systolic dimension (LVESD). These results strongly support our hypothesis that *AT1* 1166C allele associated with LV dysfunction. Further multivariate analysis results rule out the

**Table 6 – Multivariate analysis between LVD and Non-LVD patients.**

| Genotypes | Non-LVD | LVD | OR (95%CI) | *p*-value | FDR $p_{corr}$ |
|---|---|---|---|---|---|
| **CAD patients without smoking** | | | | | |
| AA | 320 (83.1) | 100 (63.7) | Reference | – | – |
| AC + CC | 65 (16.9) | 57 (36.3) | **2.86 (1.87–4.37)** | **<0.001** | **0.044** |
| **CAD patients without diabetes** | | | | | |
| AA | 276 (80.5) | 100 (68.0) | Reference | – | – |
| AC + CC | 67 (19.5) | 47 (32.0) | **1.96 (1.26–3.04)** | **0.003** | **0.040** |
| **CAD patients without hypertension** | | | | | |
| AA | 220 (81.5) | 93 (69.9) | Reference | – | – |
| AC + CC | 50 (18.5) | 40 (30.1) | **1.93 (1.19–3.15)** | **0.008** | **0.040** |
| **CAD patients without STEMI** | | | | | |
| AA | 245 (79.8) | 48 (70.6) | Reference | – | – |
| AC + CC | 62 (20.2) | 20 (29.4) | 1.62 (0.89–2.95) | 0.111 | – |
| **CAD patients without smoking, diabetes, and hypertension** | | | | | |
| AA | 130 (82.3) | 43 (68.3) | Reference | – | – |
| AC + CC | 28 (17.7) | 20 (31.7) | 2.42 (1.21–4.84) | **0.013** | **0.040** |

Significant values are shown in bold.

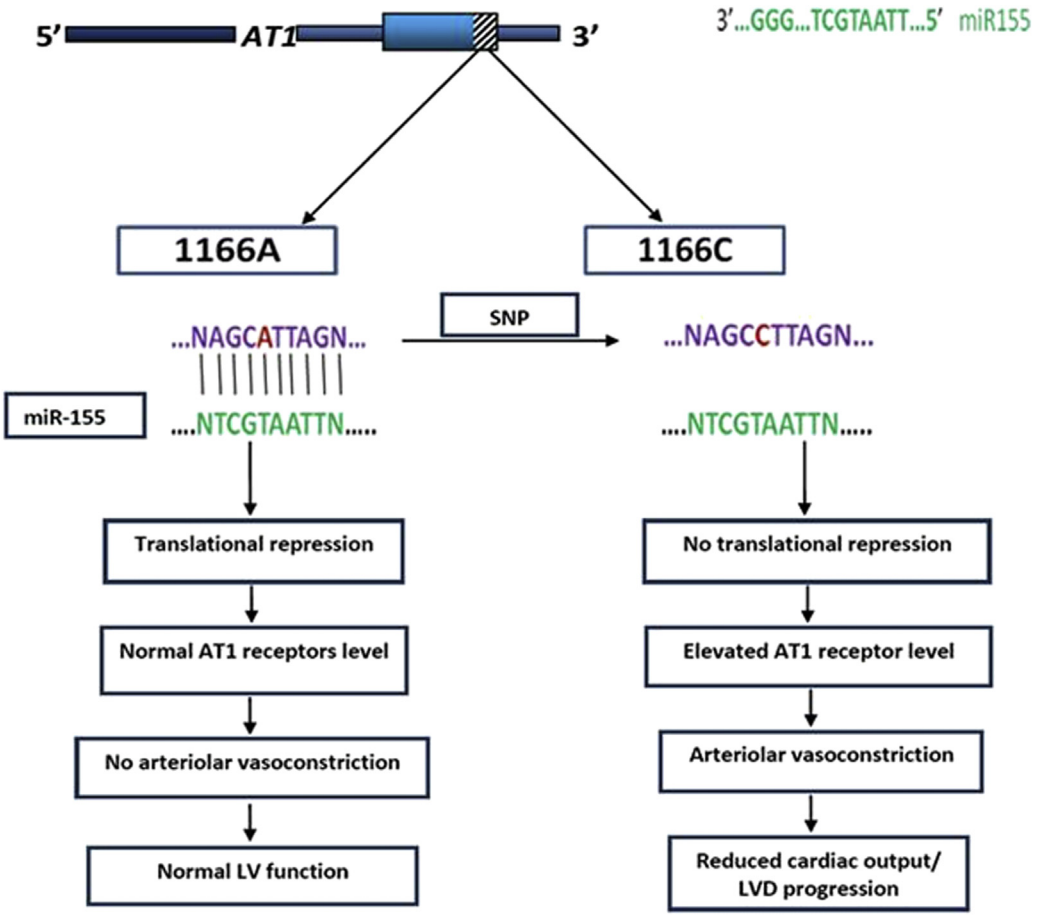


**Fig. 2 – Proposed model for molecular mechanism of association of *AT1* 1166C allele with LV dysfunction: *AT1* 1166C allele in the 3′ UTR of *AT1* gene abolishes miR-155 binding, which induces elevated levels of AT1 receptors that may lead to LV dysfunction.**

possibilities of development of LVD in CAD patients due to confounding factors such as smoking, diabetes, and hypertension. The results confirm that *AT1* A1166C polymorphism may be responsible for development of LVD in CAD patients. On analyzing other parameters of LV remodeling, the patients with *AT1* A1166C polymorphism had significantly higher LV end systolic and diastolic dimensions, which indicates that the patients with AC and CC genotypes were at higher risk of developing LV remodeling.

As, in clinical practice, STEMI patients are more prone to develop LVD. In line to this our data also showed that 70.2% LVD patients were having STEMI. So we looked for *AT1* A1166C genotypes status in STEMI subjects. Also in STEMI subjects, a significantly higher percentage of AC and CC genotypes were found in LVD patients as compared to patients with preserved LVD. These observations also indicate a close association of *AT1* 1166C allele with the susceptibility of LVD.

LVD is a polygenic condition which may be influenced by multiple genes other than *AT1*. We have previously shown the association of *NFKB1*,[32] *MMP9*,[33] and *MYBPC3*[21] in genetic predisposition of LVD. It may be worthwhile that further large, well-designed association studies and screening of other candidate gene polymorphisms is required to elucidate the precise genetic susceptibility of the disease. Moreover, it will be worthwhile to replicate the study in different populations before any clinical implications.

## Conflicts of interest

All authors have none to declare.

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## REFERENCES

1. Armstrong PW. Left ventricular dysfunction: causes, natural history, and hopes for reversal. *Heart*. 2000;84:i15–i17. discussion i50.
2. Triposkiadis F, Karayannis G, Giamouzis G, Skoularigis J, Louridas G, Butler J. The sympathetic nervous system in heart

failure physiology, pathophysiology, and clinical implications. *J Am Coll Cardiol*. 2009;54:1747–1762.
3. Busjahn A, Knoblauch H, Knoblauch M, et al. Angiotensin-converting enzyme and angiotensinogen gene polymorphisms, plasma levels, cardiac dimensions. A twin study. *Hypertension*. 1997;29:165–170.
4. Lonn EM, Yusuf S, Jha P, et al. Emerging role of angiotensin-converting enzyme inhibitors in cardiac and vascular protection. *Circulation*. 1994;90:2056–2069.
5. Ono K, Mannami T, Baba S, Yasui N, Ogihara T, Iwai N. Lack of association between angiotensin ii type 1 receptor gene polymorphism and hypertension in japanese. *Hypertens Res*. 2003;26:131–134.
6. Takami S, Katsuya T, Rakugi H, et al. Angiotensin ii type 1 receptor gene polymorphism is associated with increase of left ventricular mass but not with hypertension. *Am J Hypertens*. 1998;11:316–321.
7. Benetos A, Gautier S, Ricard S, et al. Influence of angiotensin-converting enzyme and angiotensin ii type 1 receptor gene polymorphisms on aortic stiffness in normotensive and hypertensive patients. *Circulation*. 1996;94:698–703.
8. Tiret L, Bonnardeaux A, Poirier O, et al. Synergistic effects of angiotensin-converting enzyme and angiotensin-ii type 1 receptor gene polymorphisms on risk of myocardial infarction. *Lancet*. 1994;344:910–913.
9. Chapman CM, Palmer LJ, McQuillan BM, et al. Polymorphisms in the angiotensinogen gene are associated with carotid intimal-medial thickening in females from a community-based population. *Atherosclerosis*. 2001;159:209–217.
10. Bonnardeaux A, Davies E, Jeunemaitre X, et al. Angiotensin ii type 1 receptor gene polymorphisms in human essential hypertension. *Hypertension*. 1994;24:63–69.
11. Kainulainen K, Perola M, Terwilliger J, et al. Evidence for involvement of the type 1 angiotensin ii receptor locus in essential hypertension. *Hypertension*. 1999;33:844–849.
12. Diez J, Laviades C, Orbe J, et al. The a1166c polymorphism of the at1 receptor gene is associated with collagen type i synthesis and myocardial stiffness in hypertensives. *J Hypertens*. 2003;21:2085–2092.
13. Osterop AP, Kofflard MJ, Sandkuijl LA, et al. At1 receptor a/c1166 polymorphism contributes to cardiac hypertrophy in subjects with hypertrophic cardiomyopathy. *Hypertension*. 1998;32:825–830.
14. Mishra A, Srivastava A, Mittal T, Garg N, Mittal B. Impact of renin-angiotensin-aldosterone system gene polymorphisms on left ventricular dysfunction in coronary artery disease patients. *Dis Markers*. 2012;32:33–41.
15. Devlin B, Roeder K. Genomic control for association studies. *Biometrics*. 1999;55:997–1004.
16. Rickham PP. Human experimentation. Code of ethics of the world medical association. Declaration of helsinki. *Br Med J*. 1964;2:177.
17. Schiller NB, Shah PM, Crawford M, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American society of echocardiography committee on standards, subcommittee on quantitation of two-dimensional echocardiograms. *J Am Soc Echocardiogr*. 1989;2:358–367.
18. Devereux RB, Alonso DR, Lutas EM, et al. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol*. 1986;57:450–458.
19. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988;16:1215.
20. Ye S, Dhillon S, Ke X, Collins AR, Day IN. An efficient procedure for genotyping single nucleotide polymorphisms. *Nucleic Acids Res*. 2001;29:E88. E88.
21. Srivastava A, Garg N, Mittal T, et al. Association of 25 bp deletion in mybpc3 gene with left ventricle dysfunction in coronary artery disease patients. *PLoS One*. 2011;6:e24123.
22. Pilbrow AP, Palmer BR, Frampton CM, et al. Angiotensinogen m235t and t174m gene polymorphisms in combination doubles the risk of mortality in heart failure. *Hypertension*. 2007;49:322–327.
23. van Geel PP, Pinto YM, Voors AA, et al. Angiotensin ii type 1 receptor a1166c gene polymorphism is associated with an increased response to angiotensin ii in human arteries. *Hypertension*. 2000;35:717–721.
24. Sethupathy P, Borel C, Gagnebin M, et al. Human microrna-155 on chromosome 21 differentially interacts with its polymorphic target in the agtr1 3' untranslated region: a mechanism for functional single-nucleotide polymorphisms related to phenotypes. *Am J Hum Genet*. 2007;81:405–413.
25. Maczewski M, Maczewska J, Duda M. Hypercholesterolaemia exacerbates ventricular remodelling after myocardial infarction in the rat: role of angiotensin ii type 1 receptors. *Br J Pharmacol*. 2008;154:1640–1648.
26. Alvarez R, Reguero JR, Batalla A, et al. Angiotensin-converting enzyme and angiotensin ii receptor 1 polymorphisms: association with early coronary disease. *Cardiovasc Res*. 1998;40:375–379.
27. Canavy I, Henry M, Morange PE, et al. Genetic polymorphisms and coronary artery disease in the south of France. *Thromb Haemost*. 2000;83:212–216.
28. Andrikopoulos GK, Richter DJ, Needham EW, et al. The paradoxical association of common polymorphisms of the renin-angiotensin system genes with risk of myocardial infarction. *Eur J Cardiovasc Prev Rehabil*. 2004;11:477–483.
29. Araujo MA, Menezes BS, Lourenco C, Cordeiro ER, Gatti RR, Goulart LR. The a1166c polymorphism of the angiotensin ii type-1 receptor in acute myocardial infarction. *Arq Bras Cardiol*. 2004;83:409–413, 404-408.
30. Andersson B, Blange I, Sylven C. Angiotensin-ii type 1 receptor gene polymorphism and long-term survival in patients with idiopathic congestive heart failure. *Eur J Heart Fail*. 1999;1:363–369.
31. Hamon M, Amant C, Bauters C, et al. Association of angiotensin converting enzyme and angiotensin ii type 1 receptor genotypes with left ventricular function and mass in patients with angiographically normal coronary arteries. *Heart*. 1997;77:502–505.
32. Mishra A, Srivastava A, Mittal T, Garg N, Mittal B. Role of inflammatory gene polymorphisms in left ventricular dysfunction (lvd) susceptibility in coronary artery disease (cad) patients. *Cytokine*. 2013;61:856–861.
33. Mishra A, Srivastava A, Mittal T, Garg N, Mittal B. Association of matrix metalloproteinases (mmp2, mmp7 and mmp9) genetic variants with left ventricular dysfunction in coronary artery disease patients. *Clin Chim Acta*. 2012;413:1668–1674.