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# Cardiovascular Diseases: A Molecular Diagnostic Approach

**Nitu Nigam[1*], Suhasini Bhatnagar[2], Harish Gupta[3]**

[1]Assistant professor, Cytogenetics unit , Department of CFAR, KGMU, Lucknow

[2]Rus Industries, 178, Nitikhand III, Indirapuram , Ghaziabad, 201014.UP

[3]Associate professor, Department of medicine, KGMU, lucknow

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***Corresponding author:** Nitu Nigam, Assistant professor, Cytogenetics unit, Department of CFAR, KGMU, Lucknow.

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## Abstract

The findings of various studies have led to the conclusion that multifactorial diseases such as hypertension, diabetes, cardiovascular diseases, obesity etc are also controlled at genetic level with respect to the environmental factors. Cardiovascular disease is the major cause of morbidity and mortality in Westernized societies. It is well known that the etiology of this devastating disorder involves both genetic and environmental factors. Sequence variants of the components of the renin-angiotensin-aldosterone system suggested to have significant influences on cardiovascular homeostasis. Polymorphisms of the genes encoding Angiotensin-1 converting enzyme, MTHFR and VDR represent an area of intense research for cardiovascular disease associations, with promising, although sometimes contradictory findings.

**Key Words:** cardiovascular disease; ace1; mthfr; vdr; polymorphism

## Introduction:

The global burden of cardiovascular disease is increasing as the world's population ages and the lifestyle in lower and middle income countries become more similar to wealthier nations [1]. Cardiovascular diseases(CVDs) are a group of disorders of the heart and blood vessels and they include coronary heart diseases, peripheral arterial diseases, congenital heart diseases etc. The concept of cardiovascular risk continuum was first proposed by Dzau and Braunwald as a new paradigm for cardiovascular disease pathogenesis [2]. Modifiable risk factors of cardiovascular disease such as hypertension, abdominal obesity, abnormal lipids, smoking, diabetes mellitus as well as stress, low consumption of fruits and vegetables and lack of regular physical activity are the important risk factors and contribute to >90% of all myocardial infarctions[3]. Cardiovascular risk factors show a continuous association with overall cardiovascular risk with no minimum threshold for disease [4]. Risk factors rarely occur in isolation and instead tend to cluster in individuals. The risk factors act synergistically to increase the cardiovascular disease risk by multiple times[5]. Today over 80% of the world's death occur from CVDs. According to the facts of WHO (World Health Organization) CVDs are the number one causes of death globally: more people die annually from CVDs than from any other cause. An estimated 17.3 million people die from CVD in 2008, representing 30% of all global deaths. The The number of people who died from CVDs mainly heart diseases and stroke will increase to reach 23.3 million by 2030. 9.4 million deaths each year or 16.5% of all deaths can be attributed to high blood pressure. CVDs are projected to remain the single leading cause of death.

Researchers have identified more than 250 genes that play role in CVDs. As we know that CVD often results from the blended effects of multiple genes known as polygenic effects mean that the genetics of CVD are extremely complicated, with many different genes influencing a person's risk.

**Angiotensin-1 converting enzyme (ACE) gene: A Major culprit in Cardiovascular diseases:**

Polymorphism at intron 16 of the angiotensin-1 converting enzyme (ACE) gene, located at chromosome 17q23, has been implicated in various disease etiologies, including coronary artery disease[6], myocardial infarction [7], left ventricular hypertrophy [8], diabetes [9], hypertension[10],venous thrombosis [11], diabetic nephropathy [12],

coronary restenosis [13], Alzheimer [14], and ischemic stroke [15], and in a number of such physiological events such as athletic mechanical efficiency and in performance endurance [16, 17] and in senescence [18]. However, other studies have suggested that there is no association of disease etiology with ACE I/D gene polymorphism [19-23]. The ACE polymorphism identified in 1990 by Rigat and co-workers is one of the best-researched polymorphisms. This polymorphism of the ACE gene is based on the presence or absence of a 287-bp element on intron 16 on chromosome 17. Rigat et al.[24] have shown that the level of circulating ACE enzymes depends on the insertion/ deletion (I/D) polymorphism. Angiotensin I-converting enzyme (ACE, CD143, EC 3.4.15.1), a zinc-metallopeptidase, is a key regulator of blood pressure participating in the development of vascular pathology and remodeling[25-27]. ANGIOTENSIN-converting enzyme (EC 3.4.15.1; dipeptidyl carboxypeptidase) is a zinc metallopeptidase which cleaves the C-terminal dipeptide (His–Leu) from angiotensin 1 and generates a vasoconstrictor[28], angiotensin II. Through protease activity it also inactivates bradykinin, which is a potent vasodilator. Due to its role in the renin-angiotensin–aldosterone system, human vascular tone and blood salt/water balance have been maintained. The gene for angiotensin converting enzyme (ACE) comprises 26 exons and 25 introns[29-30]. The activity of ACE was strongly influenced by a quantitative trait locus which is in linkage disequilibrium with the *Alu* insertion/deletion (I/D) marker [24, 31-33] in intron 16. A relationship between D-allele dose and enzymatic levels was established for both circulating and cellular ACE [34-39]. Numerous studies reported association of D-allele with cardiovascular diseases [40-43]. However, this association was not observed in all the studies[44-49]. Thus, there has been a considerable controversy over the association of ACE (I/D) polymorphism and disease status. The insertion deletion (I/D) polymorphism in this gene refers to an *Alu* repetitive sequence 287 bp long, in intron 16, resulting in three genotypes, *DD* and *II* homozygotes and *ID* heterozygotes. The I/D polymorphism is reported to determine circulating and tissue *ACE* levels, such that individuals homozygous for the *D* allele have higher tissue and plasma *ACE* concentrations than heterozygotes and *II* homozygotes [24,50]. The I/D polymorphism is associated with cardiovascular diseases [51-54] as well as chronic renal diseases[55,56]. The *DD* genotype is known as an independent risk factor in several cardiovascular diseases such as hypertrophic cardiomyopathy[52], myocardial infarction [51, 54] and ventricular hypertrophy [53], as well as chronic renal diseases such as IgA nephropathy [57], diabetic nephropathy [58], renal scarring [59; 56,60] and congenital urological anomalies [55] *Alu* insertion polymorphisms, like *ACE* I/D polymorphism, are also suitable markers for studying genetic variation in human populations. They can be easily detected by PCR amplification and gel electrophoresis and they are stable markers that represent a unique evolutionary event. The distribution of the *ACE* genotypes differs between races and it is used as a marker in population structure analyses [61].

**MTHFR gene: Candidate gene polymorphism in Cardiovascular diseases**

About 30 years ago, McCully postulated that mildly elevated homocysteine concentrations could increase the risk of cardiovascular disease[62] after observing artery wall lesions in two different metabolic disorders of methionine metabolism which resulted in elevated plasma homocysteine concentrations[63]. Since then many studies have been conducted to investigate whether elevated plasma homocysteine concentrations are associated with an increased risk of cardiovascular disease. A modest elevation of plasma homocysteine concentration, commonly referred to as hyperhomocysteinaemia, is generally[64, 65] although not universally[66, 67]accepted as an independent and graded risk factor for both arterial occlusive diseases and venous thrombosis[68, 69]. In 1988, Kang *et al.*[70] detected a variant of the MTHFR enzyme which was associated with decreased enzyme activity, reduced stability after heating at 46°C and increased homocysteine concentrations. A few years later these authors demonstrated that this thermolabile form of the MTHFR enzyme was more common among CVD patients (17%) than among controls (5%)[71]. In many studies this thermolabile MTHFR enzyme was identified in patients with different forms of premature vascular disease and was associated with fasting as well as post-methionine-load homocysteine concentrations[72]. In 1995, Frosst *et al.*[73] identified the single base pair substitution of C toT at nucleotide 677 to be responsible for this thermolabile MTHFR enzyme. Since then, numerous studies have been reported which investigated this MTHFR variant and its association with homocysteine concentrations and CVD risk [74]. Although an association between the 677C!T variant and elevated homocysteine concentrations was universally found[75,76,73] an increased risk for CVD was found in only some studies[75, 76]. The association between the 677C!T variant and elevated homocysteine concentrations was reported to exist only in individuals with low folate status[78,79]. In 1998, the hypothesis that this variant is associated with altered distribution of RBC folates was tested by a chromatographic method *in vitro* [84]. This method involves the analysis of RBC folates by affinity/high-performance liquid chromatography with electrochemical (coulometric) detection [85]. Probably due to the reduced MTHFR enzyme activity, formylated tetrahydrofolate polyglutamates were present at the expense of methyl-THF in most 677TT individuals. Thermolabile MTHFR accounts for 25% of the mild hyperhomocysteinaemia observed in patients with vascular disease [72], indicating that additional mutations in the MTHFR gene or other genes may also affect homocysteine concentrations. Moreover, it appears that the 677TT genotype is associated with increased homocysteine concentrations only in individuals with low folate status [78,79]. Thus, possible gene-environment interactions also play an important role in modulating plasma homocysteine concentrations. In 1998, a second common polymorphism in the MTHFR gene was described, the1298A!C transition, which mandates an amino acid substitution of glutamate by alanine [86]. This variant was observed only in *trans* with the 677C!T variant and was associated with decreased MTHFR enzyme activity. We have described the associations of this 1298A!C variant with MTHFR enzyme activity, plasma homocysteine concentrations and risk of CVD [87]. We again detected a decrease in enzyme activity in individuals with the 1298AC and1298CC genotypes, but noted no effect on the thermostability of the enzyme or on plasma homocysteine concentrations.100 Although all studies confirm that the 1298A!C variant is associated with decreased MTHFR activity [86-89] supported by expression analysis in *Escherichia coli* [90] an association with homocysteine concentrations has not been detected [88,89,91-96]. Probably, other factors that affect

homocysteine concentrations, such as nutritional status, play a role, or the decreased MTHFR enzyme activity must reach a certain threshold below which increased plasma homocysteine concentrations result.

**VDR gene polymorphism in Cardiovascular diseases**

The active form of vitamin D, 1,25 dihydroxyvitamin D or calcitriol, is the end product of two hydroxylation steps of vitamin D: a hepatic 25-hydroxylation and a subsequent renal 1a-hydroxylation. Calcitriol exerts genomic and non genomic effects through a cytosolic vitamin D receptor (VDR) and a membrane bound receptor. VDRs have been found in almost all human tissues and cells, among them cardiomyocytes, endothelial cells, and vascular smooth muscle cells. Several tissues also possess an enzymatically active 25-hydroxyvitamin D-1a-hydroxylase system, among them vascular smooth muscle cells [97]. Studies have revealed that the biologically active metabolite of vitamin D—1,25 dihydroxy-vitamin D (1,25[OH]2D)— can modulate various processes involved in the pathogenesis of cardiovascular disease (CVD) through its role in calcium homeostasis and through the participation of its receptor—a steroid hormone nuclear receptor—in the regulation of gene transcription. Its effects appear to support normal myocardial contractility, vasomotor activity, and nitric oxide production, while reducing the risk of cardiac hypertrophy and atherosclerosis. Thus, vitamin D may be beneficial in patients with heart failure, arrhythmias, ischemic heart disease, or hypertension. Briefly, it is a steroid hormone whose primary function is to maintain calcium homeostasis by enhancing calcium absorption from the intestinal tract, promoting osteoblast differentiation, and inhibiting osteoclast activity. By supporting calcium homeostasis, vitamin D inhibits substances that are activated by low serum calcium levels—including parathyroid hormone (PTH)—most of which promote bone resorption as a means of restoring normal calcium levels. Its biologically active metabolite, 1,25 dihydroxy-vitamin D (1,25[OH]2D), binds with the vitamin D receptor (VDR), a steroid hormone nuclear receptor that participates in the regulation of gene transcription. Because of the virtually ubiquitous nature of the VDR, vitamin D can affect a myriad of functions in body tissues, including intracellular signaling pathways that block cell proliferation, promote cell differentiation, modulate immune activity, and influence blood pressure (BP). Its potential cardiovascular benefits are associated with its ability to inhibit PTH,2 which is involved in the pathogenesis of several conditions that increase the risk for heart disease (HD). This study will elucidate the role of VDR gene polymorphism in cardiovascular patients. It is found that genetic variants in VDR gene were associated with an increased risk for stroke and other myocardial infraction especially in Vitamin D deficient subjects. This finding could contribute to the development of strategies for the prevention of cardiovascular diseases [98,99].

## Conclusion

Although there are several mendelian disorders that contribute to CVD, most common forms of CVD are believed to be multifactorial and to result from many genes, each with a relatively small effect working alone or in combination with modifier genes and/or environmental factors. The identification and the characterization of these genes and their modifiers would enhance prediction of CVD risk and improve prevention, treatment, and quality of care. This scientific statement describes the approaches researchers are using to advance understanding of the genetic basis of CVD and details the current state of knowledge regarding the genetics of myocardial infarction, atherosclerotic CVD, hypercholesterolemia, and hypertension

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