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The effect of Angiotensin-Converting Enzyme gene variants on Heart Failure

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ABSTRACT

Polymorphism in the angiotensin-converting enzyme (ACE) gene has been identified as a potential contributor to the pathophysiology of heart failure (HF). Several studies have demonstrated a link between genetic variations in the ACE gene and an increased risk of HF, even in individuals with otherwise low risk factors. This review aims to examine the effect of the ACE gene Insertion-Deletion (I/D) polymorphism on the development of HF. While the ACE genetic variations do not appear to significantly contribute to the development of hypertension, they may affect the mass of the left ventricles by altering the production of angiotensin II, a potent coronary vasoconstrictor. This is evident in individuals who also have other cardiovascular conditions such as hypertension and cardiomyopathy. From a therapeutic perspective, the connection between the ACE gene and angiotensin II type-1 receptors may have clinical implications for the management and prevention of HF. Screening for genetic risk may be beneficial if drugs such as ACE inhibitors and angiotensin II receptor blockers (ARB) can counteract the effects of the ACE gene and its connection to angiotensin II type-1 receptors. The effects of angiotensin II on HF are complex, as it serves both as a cellular growth modulator and vasoactive agent, thus its impact on HF can be analysed at various stages of the disease. Future studies may confirm genotypic associations and facilitate the classification of patients into groups at risk for developing HF.

Keywords: *Angiotensin-converting enzyme, genetic variations, DD genotype, left ventricular hypertrophy, heart failure*

INTRODUCTION

The renin-angiotensin system plays a crucial role in maintaining the equilibrium of blood pressure (BP). It includes the enzyme angiotensin-converting enzyme (ACE), which catalyzes the conversion of angiotensin I to angiotensin II. The mechanism of action of peptidyl dipeptidase ACE is the release of C-terminal dipeptides from substrates. Plasma ACE levels remain within a narrow range in an individual but there may be inter-patient variability due to age and gender. The plasma level of the ACE enzyme is controlled at the genetic level by the presence of an Alu Insertion-Deletion (I/D)

variant found in the 16th intron of the 23rd chromosome on the ACE gene, a 287-base pair Alu repeat sequence (1-3). In addition, the renin angiotensin system plays a key role in the molecular pathogenesis of heart failure (HF).

There is a connection between certain genetic variations, such as the homozygous deletion (DD) genotype, and various cardiovascular diseases such as left ventricular enlargement, HF and cardiac insufficiency (4, 5). More recently, the ACE genetic variants have been shown to be involved in the pathophysiology of HF. Multiple studies have revealed a correlation between the DD genotype and progressive dilation of the left ventricular (LV), increased deterioration in LV systolic performance, and elevated rates in death among patients with HF (4, 6). Several prior studies have classified this genetic polymorphism as a risk factor for the development of hypertension (7-10). However, variations exist in the results of these studies (4, 11-15). Furthermore, serum and tissue ACE levels and consequently their effect on several cardiovascular diseases have been linked to the ACE gene (16). It has been demonstrated that the DD genotype is linked to higher levels of ACE enzyme in the blood, followed by the ID genotype which results in a moderate increase in ACE enzyme levels. On the other hand, the II genotype has been documented to result in lower relative ACE serum levels (17). Although all papers have not reached the same conclusion (5, 18-22), a meta-analysis has demonstrated that individuals having the DD genotype have a statistically significant higher chance of developing coronary artery disease in some nationalities or communities rather than others (23). Therefore, the purpose of the current review is to study the evidence of the role of ACE gene polymorphism in the aetiology and progression of HF.

ACE expression and its variability

The level of plasma ACE in healthy adults remains stable within a narrow range. However, there may be inter-patient variability. This difference is independent of hormonal or environmental factors. However, it has been found that men have a slightly higher level of plasma ACE in comparison to women. ACE levels were measured using the circulatory soluble form of ACE. Moreover, ACE expression differs depending on an individual's age. For example, ACE expression tends to be higher in as children tend to get older until they reach puberty where it gradually tends to decline. ACE levels differ by only roughly 10% when isolating age as a variable (24). The reason for why this may be so is partially due to hormonal regulations which develop during puberty. Human blood cells that contained a single nucleus were investigated in order to determine the level of activity of ACE. The investigations concluded that T lymphocytes contained 30 times higher levels of activity of ACE in comparison to that found in monocytes.

Since the variation is relatively low in one patient as they get older but variations are higher between individuals, we may infer that genetics play a role in affecting plasma ACE levels. To date, many studies have shown significant associations between ACE scores among family members, but no pattern of similarity was found among random individuals within the sample group (25). Gene transmission from parents to offspring provides a rational theory towards this correlation. It is unlikely that this transmission is polygenic or that environmental variables affect ACE levels.

Currently there is a lot of debate regarding the source of plasma ACE. This debate includes whether it is formed via a proteolytic process (26) or whether it is translated into its soluble form via mRNA (27). From this arises two main ideas concerning ACE expression in the plasma. The first theory states that ACE expression is indirectly related to the gene responsible for ACE expression while the second theory states that a mutation affects the ACE proteolytic cleavage or mRNA-based soluble ACE synthesis. We can now use technology to detect variations in the ACE gene between patients. Individuals with the DD or II genotype can be identified. Scholarly work has investigated the I/D variation of ACE and how it impacts ACE levels in the blood plasma. In a study whereby ACE plasma levels were measured in a sample size of patients, a strong relationship was found between ACE gene polymorphism variants and plasma ACE levels (28). The presence of the co-dominant gene in

affected patients explained why plasma ACE levels differed between individuals. Furthermore, a statistically significant correlation between ACE levels in T lymphocytes, myocardium, and the blood plasma was established (29). Moreover, cellular T lymphocyte ACE levels where higher in patients with the D allele than those without. Therefore, it has been established that intracellular and plasma ACE levels are affected mostly by ACE genetic polymorphisms in an individual's genome (30). Individual differences in plasma levels were explained by I/D and S/s polymorphism genetic variants to a respective extent of 28% and 44%. The 16th intron of the I/D polymorphism genetic variant can cause the 287 bp DNA fragment to be either present or absent. The ACE I/D polymorphism accounts for as much as a 50% variation in plasma ACE levels (31).

Identification of angiotensin converting enzyme genotypes

The polymorphism of ACE can be determined by cloning the cDNA of ACE (32). The insertion/deletion (I/D) genetic variant was detected using a cDNA probe. In this variant, the 16th intron of the gene is missing the 287-bp fragment. Polymerase chain-reactions (PCR) are utilized to determine the presence of D and I alleles and gel electrophoresis is applied to visualize their fraction sizes. Firstly, genomic DNA is extracted from whole peripheral blood. Then by employing spectrophotometry and Nanodrop, the concentration and purity of the eluted genomic DNA are determined.

Using oligoprimers, the human ACE I/D region in the gene were amplified. Figure 1 shows the sense 5'GCCCTGCAGGTGTGCAGCATGT-3' and the antisense primer 5'GGATGGCTCCCCCTTGTCTC-3' were used to generate 319 and 597 bp amplicons for the D and I alleles, respectively (33). 0.5 M primers were utilized in a volume of 25-microliters. Human genomic DNA was also added to the process (either as 100 or 200 micrograms). The reaction was run for 35 cycles in a PCR. The cycle included a 94° C denaturation step for 30 seconds, followed by a 56° C annealing step for 45 seconds and a 72° C extension step for 2 minutes. The cycle ended with a 72° C final extension step for 7 minutes. A 1.5 or 2% agarose gel electrophoresis was used to analyze and semi-quantify the D and I allele amplification results. Via 300-nm ultraviolet transillumination, the amplified sample containing the D and I alleles were distinguished as separate thick lines or bands. A third band, assumed to be a heteroduplex DNA product, was commonly seen in heterozygous samples. Samples discovered to contain the DD genotype were amplified once more using an independent PCR reaction due to the heterozygous nature of the sample and the D allele's preferential amplification rates (34). The two primer pairs, 5'TGGGACCACAGCCCGCCACTAC-3' (antisense) and 5'TCGCCAGCCCTCCCATGCCCATAA-3' (sense), recognize a sequence specific to an insertion. The second PCR reaction was similar to the first reaction except that the annealing step was performed at 70°C. It was determined that a 335-bp amplicon sample was only produced in instances where an I allele was present within the sample under examination. Conversely, no discernible results were obtained when the sample exhibited homozygosity for the DD genotype. Utilizing primers specifically designed to span insertions, the 4-5% of samples that exhibit the DI genotype and are frequently misclassified as DD were successfully identified (33).

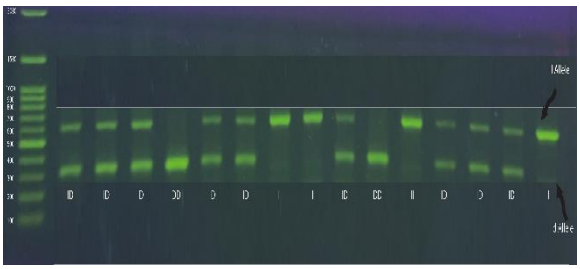

FIGURE 1: Detection of angiotensin converting enzyme polymorphism by PCR amplification and 1.5% agarose gel electrophoresis. DD: homozygous deletion for the ACE gene. ID: heterozygous insertion/deletion for ACE gene. II: homozygous insertion for the ACE gene.

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Pathophysiology of ACE gene polymorphism in HF

Angiotensin I is a biologically inactive decapeptide that has been identified on the outer regions of endothelial cells and within the bloodstream. Angiotensin I undergoes a transformation through the action of ACE into the highly vasoactive and aldosterone stimulating agent known as Angiotensin II. This latter compound exerts a powerful vasoconstrictive effect (35, 36) on coronary vessels and plays a significant role in the growth of vascular smooth muscle and the development of myointimal hyperplasia following endothelial injury (37). It is capable of affecting structure of the arterial walls and plays a role in the pathogenesis of arteriosclerosis by inducing synthesis of the extracellular matrix and stimulating cell proliferation. The enzyme angiotensin converting enzyme (ACE) has also been found to act upon the substrate known as bradykinin, which exhibits a potent vasodilation effect and inhibit the proliferation of vascular smooth muscle cells. It achieves this by binding to bradykinin (B2) receptors which in turn leads to endothelial factors such as prostacyclin and nitric oxide being released. ACE is responsible for proteolytically deactivating bradykinin and thereby causing vascular smooth muscle cells to grow. This could explain in part one of the advantages of ACE inhibitors and harmful side-effects of high ACE expression (38).

A significant predictor of HF morbidity and mortality is the presence of LVH. Additionally, ACE inhibitors are effective medications for managing and avoiding complications arising from HF (39). These findings imply that a high ACE expression may be more predisposed for cardiac hypertrophy. In a previous study conducted in Germany, a total of 290 individuals (comprising of 149 males and 141 females), who possessed electrocardiogram (ECG) evidence of LVH, were selected for comparison with a group of appropriately matched controls (40). In a study population consisting of individuals diagnosed with LVH, it was determined that the prevalence of ACE DD homozygotes was disproportionately high in comparison to control groups (36.6% in the LVH group versus 14.8% in control groups, with a statistical significance of $P=0.005$). Furthermore, this association was observed exclusively in male patients ($P<0.001$) and was unlikely to be present in female subjects. Due to an overabundance of heterozygotes, the genotype distribution in patients from the control group deviated from the Hardy-Weinberg expectation ($P<0.01$). This may be partially attributed to patients with ECG-LVH being excluded from this group. According to the researchers' findings, men with normotensive rather than hypertensive blood pressure tended to have a stronger link with the ACE DD genotype and the presence of LVH demonstrated via an ECG. However, the data does not regularly demonstrate a greater effect in normotensive patients as this discovery was associated with a significant excess of heterozygotes in male patients with blood pressures in the normal range in the control group (57 heterozygotes for 29 homozygotes).

There are a few additional examples for how gene polymorphism may impact physiological processes within the body. For example, the hormone noradrenaline is released at a higher rate from sympathetic nerves with the presence of the deletion polymorphism. Another example would be the presence of the Arg 389 beta1-adrenergic receptor gene that modulates signalling of the receptors. Cardiac adrenergic signalling is modulated by the effect of G-protein receptor kinases (GRKs). In HF, GRK 5 upregulation is caused by polymorphism due to a substituted leucine amino acid for a wild type glutamine at the 41st position (GRK5-Leu41) (41). ACE expression is increased with the presence of the ACE DD genotype whilst CLCNKA Gly83 may lead to an increase in renin as demonstrated in figure 2. The synergistic effect of the two results in an augmented influence of the renin-angiotensin-aldosterone system (RAAS) (42, 43). Furthermore, a prior study has shown that variations in the CLCNKA gene (rs10927887 which encodes for Arg83Gly) was associated as a risk factor of HF progression (44). An important implication is that this may lead to myocardial impairment due to reactive LVH. Therefore, patients with the ACE genetic variation might possess the DD genotype that make them predisposed to being at a higher risk for disease progression.

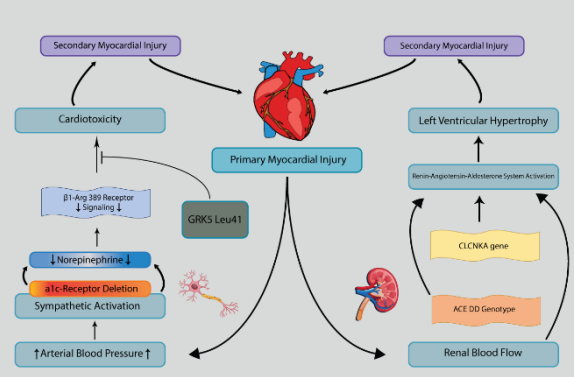

FIGURE 2: Suggested influences of a known polymorphism on neurohormonal stimulation in HF. ACE: angiotensin-converting enzyme. DD: homozygous deletion for the ACE. CLCKA gene: chloride voltage-gated channel Ka. GRK5 Leu41: G-protein receptor kinases substituting leucine amino acid at position 41.

Certain genetic factors may predispose individuals to one of a myriad of chronic cardiovascular diseases such as atherosclerosis, hypertension, cardiac and vascular hypertrophy, and endothelial dysfunction. Furthermore, it may adversely affect individuals on an acute scale with illnesses such as thrombosis or plaque rupture. The kallikrein-kinin and the renin-angiotensin system both contribute to a number of pathophysiological mechanisms leading to cardiovascular diseases. Renin-angiotensin-aldosterone signalling is increased and catecholamine is released from sympathetic nerves upon myocardial injury which may occur due to cardiac damage or haemodynamic stress. The presence of these mechanisms in conjunction with well-established genetic polymorphisms leads to the expression of numerous phenotypes. The RESOLVD study has found that gene expression plays a role in patient being concurrently treated with angiotensin II receptor blockers (ARB) and ACE inhibitors (45).

The I/D polymorphism genetic variant's effect on HF

Prior research has suggested that the ACE I/D polymorphism may play a role in the histopathology of HF (46). However, this topic is regarded to be controversial. Our literature scanned for the DD genotype records amongst control groups regardless of the affected disease patients had. This gap was between 2.8 to 65.1 percent (47-50). One of the reasons could be the sample size. Another could be due to differences in gender. Men detected with DD polymorphism recorded stronger link to LVH as appeared in electrocardiographic measurements, while this link was not detected among women (4). A prior study that was conducted linked the DD genotype polymorphism to hypertension (31).

As has been mentioned previously, the I/D alu fragments in the 16 intron of the ACE gene which is located at 17q23 has been determined to decide the genetic component for ACE plasma levels. Furthermore, the I/D alleles account for a portion of the differences in inter-patient ACE variability in the blood stream. Patients that have the II genotype typically have reduced ACE levels in the plasma, whilst DD genotype carrying patients have elevated levels of plasma ACE. Patients carrying the ID genetic variant have been found to have intermediate levels of plasma ACE (28). The I/D polymorphism itself is not responsible for the change in ACE. However, it most likely has a relationship with one or more ACE polymorphisms. The mechanisms responsible for increased plasma ACE levels in patients with respect to the ACE deletion polymorphism have not been fully elucidated yet. It is crucial to highlight that the ACE in/del polymorphism is unlikely to be the reason for the change in plasma ACE activity, and may instead serve as a quantifiable marker for one or more additional ACE polymorphisms with which it shares a close functional linkage-disequilibrium (51).

The I/D genotype has been found to have little impact on hypertension. However, a relationship with HF and cardiac hypertrophy has been established (52, 53). The underlying molecular and cellular mechanisms behind the increased expression of ACE due to the ACE deletion polymorphism are yet to be identified. However, a study previously published by Bahramali et. al has found that patients with cardiomyopathy tended to have a higher chance of carrying the DD genotype in comparison to patients in the control group (54). More evidence is appearing on the adverse impact that the DD genotype has on patients with HF. This impact has been found to increase the rate at which HF progresses

through the stages. In a study that was conducted by Chen et al. it was shown that the DD polymorphism was linked to higher rates of sudden cardiac death in subjects with cardiovascular diseases (6). The rate of occurrence of the D allele did not differ significantly between the sample group and the control group. However, HF patients with the DD genotype had worse survival rates and larger left ventricular mass (55). Because there is a link between LVH and angiotensin II, it has been hypothesized that the existence of the ACE DD genotype influences the progression of LVH. This demonstrates that the D allele is a modulator for illness progression rather than a risk factor, indicating a link between this polymorphism variant and structural cardiac abnormalities (56). The study also concluded based on a subsequent evaluation that an interaction between genes of the ACE DD polymorphism genotype and polymorphism in the 3rd region of the Angiotensin II type 1 receptor gene also contributed to the advancement of HF. Another study found that in 443 diseased subjects that excluded patients with myocardial or valvular heart disease found that the presence of the DD genotype affected the weight of the patients' heart (57). However, the study also stated that other independent risk factors such as hypertension had a greater level of impact (58). These findings have been supported by other small studies that investigated the relationship between the presence of the DD genotype and LVH (54, 59). Furthermore, this genotype has been associated with a reduced survival rate of patients in ischemic and non-ischemic HF (60). A prior study has also demonstrated a pharmacogenomic reaction between beta blockers and the ACE genotype. Individuals with the ACE D allele had a lower survival rate after a transplant but patients with the DD genotype had a greater survival rate when treated with beta blockers and ACE inhibitor (61). A combination of multiple risk factors such as hypertension and renal failure alongside the presence of the DD genotype generally leads to an acceleration in the deterioration of HF in patients (62, 63).

Interestingly, the ACE DD genotype has shown the strongest association with LVH at normal blood pressure levels. It is assumed that the DD genotype demonstrates a stronger effect on the extent of hypertrophy on the left ventricles in the absence of cumulative factors such as left ventricular pressure overload (64). However, in patients with idiopathic dilated cardiomyopathy, there may be a considerable connection between left ventricular chamber size and systolic output and ACE genotype via its impact on arterial pressure. The direct correlation between left ventricular ejection and the negative correlation between left ventricular end-diastolic diameter and systolic blood pressure suggests that better systolic performance can lead to increased systolic blood pressure, without being adversely affected by systolic performance in idiopathic dilated cardiomyopathy (65). Therefore, even if the link between exercise or stress-induced hypertension cannot be judged, the interaction of the ACE DD genotype and LVH potentially consists of mechanisms other than blood pressure alone.

CONCLUSION

Through direct myocardial or load-induced effects, the increased tissue and circulatory levels of angiotensin II associated with the ACE gene deletion polymorphism may affect LV remodelling. Because of the therapeutic benefits of ACE inhibitors in decreasing morbidity and mortality associated with HF, the renin-angiotensin system is assumed to play a substantial role in determining the severity of HF. However, the associated mechanisms remain undiscovered so far. Despite the fact that current research does not relate the pathogenicity of ACE gene polymorphism to the severity of HF, the ACE insertion-deletion polymorphism does not serve as a useful marker to assess the risk of HF.

The literature on the association between ACE gene polymorphism and heart failure has been reviewed in this paper. However, the combination of pathophysiological mechanisms has not been fully studied yet. For example, the gene-gene polymorphism interaction on the prevalence and progression of the disease has received less focus. Nonetheless, due to the nature of most cardiovascular diseases being multi-genetic and multi-factorial, defining these association are more complex than previously

thought. HF is more heterogenous in terms of its risk factors, mechanisms and complications. Therefore, more studies are needed to outline this heterogenicity and find clues to better future clinical treatments.

It has been increasingly realised that no single factor influences the progression of HF. The importance of underlining the combined effects such as ACE DD genotype and β adrenergic receptor polymorphisms on the pathophysiology of HF must be realised in future studies to allow the detection of appropriate risk factor of the disease. This approach will also facilitate the discovery of personalised treatments for individuals with specific medicinal needs based on their genetic backgrounds. Therefore, future work would need to be conducted in the form of a metanalysis that combines all published studies on the DD genotype concentration in control groups against that of HF cases in order to reach a conclusion.

Other studies should direct their focus on determining the effect of ACE serum and tissue levels on the pathophysiology of HF. These experiments should study the ACE levels in response to acute and chronic physiological stresses. Moreover, whether ACE genotypes have the same influence on ACE levels in all organs. Furthermore, how ACE serum levels mirrors their tissue levels. To do so, several considerations should be taken into account. Such considerations are whether ACE is stable as well as the feasibility of using human tissue explants in vitro. With combined efforts we will be better able to answer whether future diagnostic tools should employ ACE genotype detection or the circulating ACE phenotype to aid in the management of HF.

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