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Angiotensin Converting Enzyme Gene Insertion/Deletion Polymorphism and Cardiovascular Disease

Therapeutic Implications

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Abstract

Cardiovascular disease is the major cause of morbidity and mortality in Westernised societies. It is well known that the aetiology of this devastating disorder involves both genetic and environmental factors. Sequence variants of the components of the renin-angiotensin-aldosterone system and the kallikrein-kinin system are suggested to have significant influences on cardiovascular homeostasis.

Both gene targeting and transgenic studies in mice have clearly suggested a critical role of the angiotensin converting enzyme (ACE) gene in blood pressure regulation. Furthermore, an up-regulation of myocardial ACE gene expression has been observed in patients with heart failure. Thus, the ACE gene has been recognised as a top candidate gene for cardiovascular research.

Over the past decade, the insertion/deletion (I/D) polymorphism of a 287-bp Alu element in intron 16 of the ACE gene has attracted significant attention and has been extensively investigated in a spectrum of cardiovascular phenotypes, because of its correlation with serum ACE activity. A large majority of previous studies have shown a positive association between the DD genotype and an increased risk of myocardial infarction, but results in hypertension, left ventricular hypertrophy, cardiomyopathy and restenosis after percutaneous transluminal coronary angioplasty remain quite controversial.

Since ACE inhibitors are widely used in hypertension and congestive heart failure, we also review the literature on the relationship of ACE I/D polymorphism with ACE inhibitor response. It appears that this polymorphism has some moderate impact on the cardiovascular response to ACE inhibitors but there is no consensus as to which allele confers a more pronounced effect. In addition, previous data are suggestive of an association between the ACE I allele and a greater risk of increased occurrence of ACE inhibitor-induced cough, but such a relationship needs further confirmation. Overall, since ACE I/D is only an intronic marker, the true locus that controls the ACE enzyme activity remains to be identified, and could be located within either the ACE gene or another nearby gene

such as the human growth hormone gene. We note that since associations tend to vary across different gender or ethnic groups, or across different socio-ecological settings, consideration of potential gene-gene and gene-environment interactions should be made. Furthermore, the dissection of the genetic underpinning of cardiovascular disease needs delineation of all molecular variants of the key physiological pathways that influence cardiovascular function.

Both the renin-angiotensin-aldosterone system (RAAS) and the kallikrein-kinin system (KKS) play significant roles in cardiovascular pathophysiology (figure 1). The angiotensin converting enzyme (ACE) [kinase, dipeptidyl carboxypeptide I; EC 3.4.15.1, (MIM 106180)], which acts primarily as a dipeptidyl carboxypeptidase, not only generates the potent vasoconstrictor angiotensin II from angiotensin I, but also inactivates the vasodilator bradykinin by sequential removal of two carboxyl-terminal dipeptides (figure 1).[1] ACE is a monomeric zinc metallopeptidase that occurs predominantly as a single molecular form of 170 kDa in humans. It is an extensively glycosylated protein[2] and is a membrane-bound enzyme that has catalytic sites on the extracellular surface of the cell.

The ACE gene is one of the top candidates in genetic epidemiological studies of cardiovascular outcomes and recently, the insertion/deletion (I/D) polymorphism of the ACE gene has attracted a lot of attention.[3,4] In this article, we provide an extensive review of the ACE gene I/D genotype and cardiovascular diseases as well as its implications in the therapeutic response to ACE inhibitors.

1. Molecular Biology of the ACE Gene

1.1 ACE Gene Structure and Protein Function

The human ACE gene contains 26 exons and is located on chromosome 17q23. The structure of the human ACE gene provides support for the duplication of an ancestral ACE gene. Exons 4-11 and 17-24, which encode the two homologous domains of the ACE molecule, are highly similar both in size and in sequence[5] indicating a gene duplication event during evolution that may have occurred more than 600 million years ago. The functional significance of somatic ACE versus circulating plasma ACE was first investigated by Ng and Vane,[6] who showed that most of the conversion of angiotensin I to angiotensin II occurred in the lung.

The majority of ACE enzyme activity is bound to cell membranes and found in the endothelium of vasculature, lung, kidney, intestine, cerebrum, heart and adrenal glands.[7] ACE is bound to the plasma membrane by a hydrophobic membrane-spanning domain near the C-terminus.[8] Protease activity cleaves the vascular form into blood, which yields a measurable plasma ACE activity.

Serum/plasma ACE is probably derived from vascular endothelial cells by a post-translational event of solubilisation. The soluble form is released by a membrane-bound ACE-secreting enzyme, which cuts off the carboxyl terminus of ACE. The resultant serum form is not recognised by a specific antibody directed against a peptide corresponding to this part of ACE.[9] It was shown in conscious rabbits that the hypotensive effect of ACE inhibitor is correlated with the inhibition of the activity of ACE expressed in pulmonary endothelium but not correlated with plasma ACE inhibition.[10]

1.2 Somatic and Testicular Forms of ACE

The ACE gene is expressed in a tissue-specific manner to somatic and testicular isoforms through the use of alternative promoters and differential splicing.[11,12] The somatic ACE mRNA is transcribed from exons 1 to 26, but exon 13 is spliced out. The somatic ACE protein comprises 1306 amino acids, including a 33 amino-acid-long signal peptide at the amino terminus and a potential membrane-anchoring domain near the carboxyl terminus. Both amino and carboxyl catalytic domains are present in somatic ACE. Each catalytic domain

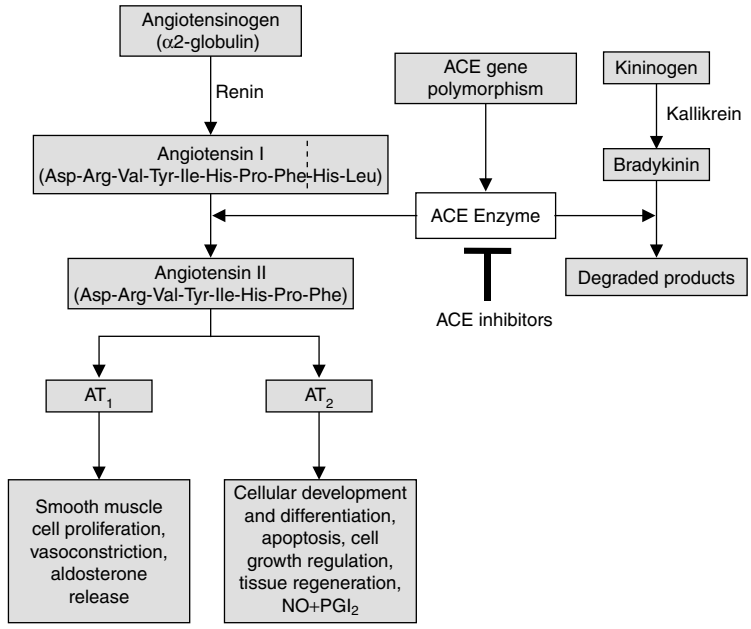

Fig 1. A schematic diagram depicting the dual role of the angiotensin converting enzyme (ACE) gene in the renin-angiotensin-aldosterone system and the kallikrein-kinin system. **AT_1** = angiotensin II type 1 receptor; **AT_2** = angiotensin II type 2 receptor; **NO** = nitric oxide; **PGI_2** = prostaglandin I_2

contains the same zinc-binding motif (His-Glu-X-Y-His).[13] The zinc binding domain is highly conserved across the fruit fly, rabbit, human, rat and mouse (figure 2). The testicular mRNA is transcribed from exons 13 to 26 (figure 3) using a testis-specific promoter in intron 12.[14] Thus, testicular ACE (containing 732 amino acids) is about half the size of the somatic ACE with just one catalytic domain. It has been shown that testicular ACE plays an important role in male fertility[15] and is only expressed in developing male germ cells.[16-18]

Somatic and testicular ACE proteins share 665 common residues at their C-termini. By contrast, at the N-termini, somatic and testicular forms of ACE contain 664 and 72 unique residues, respectively.[20] The evolutionary conservation of these two forms of ACE indicates that both are functionally indispensable.[21]

1.3 Functional Analysis of the ACE Gene in Experimental Models

Gene targeting and transgenic experiments are powerful approaches for identifying the physiological role of genes. In this section, we briefly summarise the results from ACE knock-out and transgenic rodent models, which are critical to understanding the biological effects of the ACE gene.

1.3.1 Knock-Out Experiments

Esther et al.[22] synthesised a replacement DNA construct and introduced it into the mouse germ line via homologous recombination to disrupt the ACE gene. Homozygous ace-null animals have no detectable activity of either somatic or testicular ACE, and the systolic blood pressure (SBP) of male and female ace -/- mice were comparable, both of which were significantly lower than ace +/- or ace +/+ animals. Nevertheless, ace +/- mice that have a 50% reduction in ACE activity do not have

Fig. 2. Cross-species conservation of the zinc-binding domain of the angiotensin converting enzyme (ACE) gene in the buffalo fly (SWISS-PROT Acc. No. Q10715), fruit fly (SWISS-PROT Acc. No. Q10714), rabbit (SWISS-PROT Acc. No. P12822), human (SWISS-PROT Acc. No. P12821), mouse (SWISS-PROT Acc. No. P09470), rat (SWISS-PROT Acc. No. P47820), and chicken (SWISS-PROT Acc. No. Q10751). Multiple sequence alignment was carried out using CLUSTAL X version 1.81.[19]

reduced blood pressure.[22] Moreover, another strain of mice expressing only secreted forms of ACE (since the C-terminal domain is eliminated by introducing a DNA construct with an inserted in-frame stop-codon) has been created.[23] It was found that although the circulating ACE activity remains in the homozygous mutant animals, the SBP was significantly lower than those of heterozygous or wild-type animals, which suggests that circulating ACE plays a relative minor role in the control of SBP. Together, these data suggest that the tissue-bound ACE might have a more pronounced impact on blood pressure regulation than the secreted ACE. It is also noted that these homozygous mutant mice cannot generate sufficient angiotensin II despite significant (≈34%) plasma ACE activity; which is consistent with the finding showing that the bulk of angiotensin II formation occurs in tissues, such as the lung.[6]

1.3.2 Tansgenic Experiments

Ramaraj et al.[24] constructed a sperm-specific transcriptional promoter to generate transgenic mice that express the testicular ACE only in sperm. Basically, the transgene contains the promoter from the human phosphoglycerate kinase-2 gene,[25] which has been shown to be testis-specific, a rabbit testicular ACE cDNA and a polyadenylation signal from the bovine growth hormone (GH) gene. In order to generate the experimental mice of the genotype ace -/- (a/a), transgene$^+$/transgene$^+$ (B/B), they cross-bred transgenic mice with ace-null mice. They showed that the exclusive expression of transgenic somatic ACE in vascular endothelial cells of ace -/- mice was sufficient for restoring its normal blood pressure.

By creating transgenic mice that express somatic ACE exclusively in ACE-deficient sperm, Kessler et al.[26] demonstrated that each ACE isoform is functionally distinct and sperm surface-bound somatic ACE failed to restore male fertility in those mice deficient of the testicular ACE. By contrast, female ace -/- mice are normally fertile.[27] Increasing ACE levels by increasing the number of functional ace gene copies does not affect blood pressure. Krege et al.[28] studied mice containing 1 to 3 functional copies of the ace gene at its normal chromosomal location. The blood pressures of the mice possessing different numbers

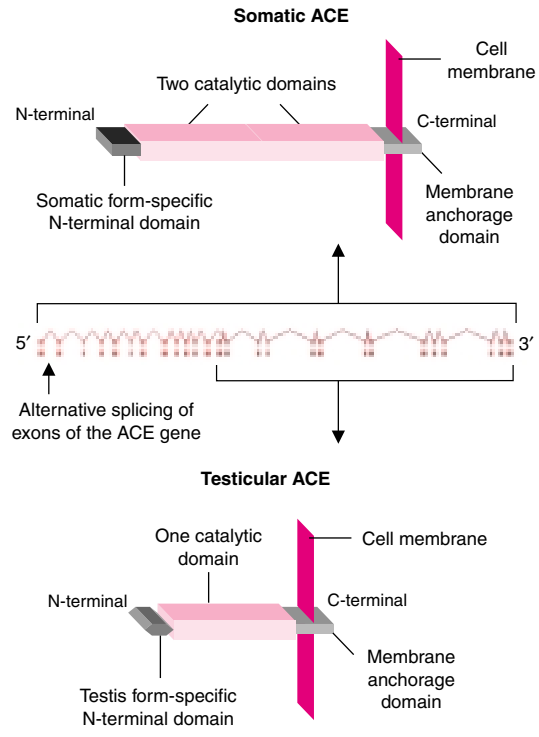

Fig. 3. A schematic diagram illustrating the somatic and testicular angiotensin converting enzyme (ACE) isoforms generated through alternative exon splicing.

of copies of the ace gene did not differ significantly, but other phenotypes such as the heart rates, heart weights and renal tubulointerstitial volumes decreased significantly with increasing copies of the ace gene.

Overall, gene targeting and transgenic studies clearly point to a critical role of the ACE gene in blood pressure regulation and in male fertility.

1.4 ACE Gene Expression and ACE Activity in Myocardium

The mRNA expression of ACE can be quantified using reverse transcription - polymerase chain reaction (PCR).[29] In human heart failure, an upregulation of myocardial ACE mRNA levels, an increase in the number of ACE binding sites and an increase in ACE activity are simultaneously observed.[30,31] However, it remains unknown which mechanism is responsible for the upregulation of myocardial ACE gene expression. The ACE I/D polymorphism has been hypothesised to influence ACE expression in human heart. Davis et al.[29] observed that ACE gene expression in the left ventricle varied with the ACE genotype in patients with ischaemic heart disease undergoing coronary artery bypass graft surgery (CABG). Danser et al.[32] also found that postmortem cardiac ACE activity in human left ventricles is higher in DD than in II genotypes. These limited data suggest that the ACE I/D polymorphism may be correlated with the myocardial ACE mRNA and protein expression.

1.5 ACE Gene I/D Polymorphism and its Potential Biological Effects

A total of 78 molecular variants were identified for the ACE gene.[33] Among the 78 polymorphisms, the most prominent one is the insertion or deletion of a 287-bp sequence in intron 16 of the gene. This Alu element was inferred to be an insertion (rather than a deletion) because its sequence has closest similarity to other human-specific Alu elements[34] and PCR of chimpanzee DNA revealed a fragment consistent with a lack of an Alu.[35] These data suggest that the Alu insertion occurred after the human-chimpanzee split.

Since the I/D polymorphism is an intronic marker, it may be functionally neutral but is in strong linkage disequilibrium with another unobserved functional mutation within the ACE gene. In particular, Tiret et al.[36] first showed that ACE activity is significantly influenced by a quantitative trait locus (QTL) either within or very close to the ACE gene. Zhu et al.[37] revealed two potential ancestral breakpoints in a 9-kb region of the 3′portion of the ACE that probably harbours a functional variant. Their study, together with other reports, suggests that the ACE I/D polymorphism may not be the functional QTL and the use of trans-ethnic mapping may help isolate the true functional QTL. The different degrees of linkage disequilibrium between the QTL and I/D polymorphism in different ethnic populations (e.g. Afro-Caribbeans, Caucasians, Chinese, Japanese) may account for some negative results in studies of ACE I/D genotype and cardiovascular disorders.[38]

The intra-individual serum level of ACE is rather stable. However, the inter-individual variation is high, which may be attributed to molecular variants of ACE.[39,40] ACE polymorphism has attracted much attention in recent years. Up to now, probably the only unequivocal result is the linkage between the I/D genotype and serum ACE level. It was reported that the serum ACE level is determined by the ACE I/D polymorphism in the following order: DD > ID > II. Furthermore, the I/D polymorphism may account for 47% of the total phenotype variance of serum ACE.[41] This result has been confirmed in diverse populations including French centenarians,[42] Pima Indians[43] and Whites (but not Blacks) of the United States.[44] Since the I/D polymorphism is the most widely used marker for studies of cardiovascular diseases, we have exclusively focused on this marker in this article.

1.6 ACE Gene I/D Polymorphism Genotyping

It has been noted that the conventional method of Rigat et al.[45] may result in the preferential am-

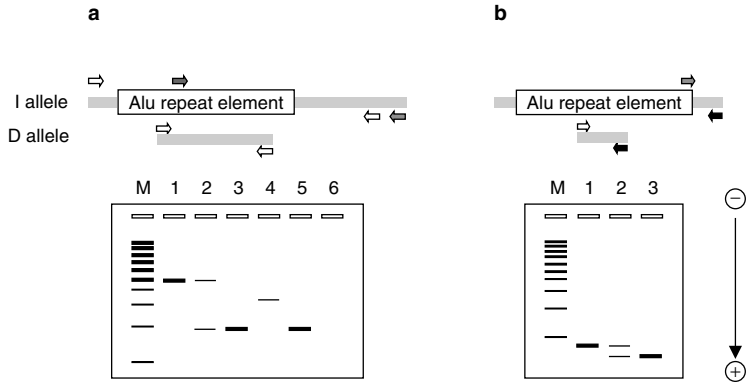

Fig. 4. The reliable genotyping methods of the angiotensin converting enzyme (ACE) insertion/deletion (I/D) polymorphism. (**a**) Upper panel is an illustration of the genotyping method of using an initial set of primers flanking the insertion region (open arrows) described by Rigat et al.[45] followed by polymerase chain reaction (PCR) employing a second set of insertion-specific primer pairs (shaded arrows) in DD individuals.[51] Lower panel is a schematic depiction of the agarose gel electrophoresis of the PCR products. Lane M, 100 bp ladder marker; Lane 1, II genotype; Lane 2, ID II genotype; Lanes 3 and 4, an initially mistyped DD genotype which is later confirmed to be the ID genotype using the second set of insertion-specific primer pairs; Lanes 5 and 6, DD genotype confirmed by PCR using the second set of insertion-specific primer pairs. (**b**) Upper panel is an illustration of the triprimer genotyping method described by Evans et al.[52] Only one round of PCR is needed for this method. Lower panel is a schematic depiction of the agarose gel electrophoresis of the PCR products. Lane M, 100 bp ladder marker; Lane 1, DD genotype; Lane 2, ID genotype; Lanes 3, II genotype.

plification of the D allele, such that a portion of ID heterozygotes could be mistyped as DD homozygotes, with an estimated error rate of approximately 5%.[46-48] Several modified techniques have been proposed to address this issue. The multiplex PCR put forward by Shanmugam et al.[49] uses a third PCR primer inside the I allele, but was found to be still troublesome.[50] One better solution is to use a second, independent set of primer pairs that recognises the insertion-specific sequence, which has been described by Lindpaintner et al.[46] and Odawara et al.[51] This can identify those ID individuals who have been mistyped as DD individuals (figure 4). Evans et al.[52] and Ueda et al.[48] used the triple-primer method using standard PCR conditions with addition of 5% dimethyl sulfoxide (DMSO) into the reaction cocktail and an initial 'hot-start' (i.e. a pre-amplification heating at 95°C for 2 min) procedure. The addition of DMSO improves denaturation of the double-stranded DNA molecules and a 'hot-start' enhances the stringency of primer annealing.[53] Both of these two procedures may improve the sensitivity of the genotyping and minimise genotyping errors.

In summary, ACE I/D mistyping may partially account for the discrepancies among the different series regarding the ACE I/D polymorphism and cardiovascular disease, especially when sample sizes were relatively small, or when neither the DMSO nor the 'hot-start' procedures were used. Thus, routine genotyping of I/D polymorphism should employ reliable methodologies such as the triprimer method or inclusion of a secondary PCR for DD individuals using the insertion-specific primer (figure 4).

2. ACE I/D Polymorphism and Cardiovascular Diseases

2.1 Essential Hypertension

Hypertension is a prominent risk factor for cardiovascular diseases, affects about 60 million Americans,[54] and is present in about two thirds of all individuals aged 65 years or older.[55,56] Despite the influence of environmental factors, genetic

variants could play a pivotal role in contributing to a substantial proportion of the variance of blood pressure in the general population.[57] ACE gene is linked to high blood pressure in salt-loaded stroke-prone spontaneously hypertensive rats.[58,59] Using gene targeting, Krege et al.[15] constructed ace -/- (i.e. homozygous null) mice and found that the blood pressure values of heterozygous male mice were 15 to 20mm Hg less than normal (i.e. ace +/+). Because of its integral role in water and electrolyte homeostasis, there is a large body of literature examining the relationship between the ACE I/D polymorphism and risk of essential hypertension. Staessen et al.[4] compiled a comprehensive computerised database of published reports and conducted a thorough meta-analysis on the association of the ACE I/D genotype with hypertension. Our goal here is to summarise the results of those studies conducted in a diverse set of ethnic populations worldwide to pinpoint any convergence or divergence of results obtained in these distinct groups that may reveal any racial concordances or discordances in the genotype-phenotype correlations.

Zee et al.[60] found that the ACE I/D polymorphism was associated with essential hypertension in an Australian population. However, no association between ACE polymorphism and essential hypertension was found in many countries including Belgium,[61] Denmark,[62] Greece[63] and the United Arab Emirates.[64] Barley et al.[65] found there was no significant association between the ACE genotype and high blood pressure in Whites of the UK, but within the UK Black group of Afro-Caribbean descent, there was a positive association between the frequency of the D allele and increased blood pressure. Similarly, a positive association of the frequency of the D allele with elevated blood pressure was found in two studies in African Americans.[66,67] Although a linkage study of affected sibpairs in Caucasian Americans in Utah found no evidence to support linkage between the ACE locus and hypertension, in the Framingham Heart Study, of which most participants are Caucasians, O'Donnell et al.[68] found evidence for association and genetic linkage of the ACE I/D polymorphism with hypertension and blood pressure only in men. In a Sikh population, Mastana and Nunn[69] also found a signification association between the ACE I/D polymorphism and hypertension.

In Japanese, Suwazono et al.[70] did not find an association between the ACE I/D polymorphism and blood pressure in 196 men, and neither did a large Japanese study conducted by Zaman and coworkers[71] demonstrate a significant association between the ACE I/D polymorphism and blood pressure. A case-control study[72] in 701 Japanese men (387 hypertensive and 314 normotensive individuals) and 542 Japanese women (324 hypertensive and 218 normotensive individuals) also found no significant association between I/D genotype and hypertension when men and women were analysed separately or together. However, Uemura et al.[73] detected a significant association of the polymorphism with SBP and diastolic blood pressure (DBP). Another Japanese study,[74] with findings similar to the study conducted by O'Donnell and coworkers[68] in Caucasian Americans, reported a significant association between the ACE I/D polymorphism and essential hypertension only in men.

In Chinese populations, Jeng et al.[75] found a marginally significant association between the DD genotype and hypertension ($p = 0.07$). Similarly, Liu et al.[76] observed a significant association between the D allele of the ACE gene and hypertension. By contrast, Thomas et al.[77] did not find a significant association between the ACE I/D polymorphism and hypertension in Hong Kong Chinese.

On the whole, studies in different countries give divergent results. Nevertheless, it appears that the association between the ACE DD genotype and an increased risk of hypertension is consistently shown in populations of African descent, and there might be a gender-specific association in Caucasians and Japanese.

2.2 Left Ventricular Hypertrophy

Left ventricular hypertrophy (LVH) is a common condition that profoundly affects morbidity and mortality from cardiovascular diseases, including myocardial infarction (MI), congestive heart failure and stroke.[78] Since cardiac mass might be influenced by an action of plasma and/or tissue angiotensin II, it has been hypothesised that the ACE gene I/D polymorphism may be related to the development of LVH. Several studies provide support for this hypothesis.[79,80] However, there is also a lack of agreement in the literature (reviewed in West et al.[81]). In the Framingham Heart Study, Lindpaintner et al.[82] found no evidence of an association between the ACE polymorphism and echocardiographically determined left ventricular mass or LVH. Two recent studies[83,84] also found no correlations between the ACE I/D polymorphism and LVH in essential hypertension.

Kuznetsova et al.[85] conducted a meta-analysis across 23 studies with 5438 participants and concluded that overall, LVH was not associated with the D allele. However, if untreated hypertensive patients were analysed separately, echocardiographic left ventricular mass was found to be approximately 10% higher in DD individuals than in an II reference group ($p = 0.001$).

Overall, since the majority of positive association studies between the ACE DD genotype and elevated cardiac mass are based on patients under stressful conditions such as exercise,[86] hypertension,[87] haemolysis[88] or ischaemic heart disease,[29] it is possible that the DD genotype only plays a permissive role in influencing LVH.

2.3 Cardiomyopathies

2.3.1 Idiopathic Dilated Cardiomyopathy

Idiopathic dilated cardiomyopathy (IDC), a disorder in which left ventricular dilation and dysfunction leads to congestive heart failure, is inherited in over 30% of individuals.[89] Three studies[90-92] did not find evidence for involvement of the ACE I/D polymorphism in the susceptibility to IDC. However, a cross-sectional study found a significant association between the ACE polymorphism and IDC.[93]

2.3.2 Hypertrophic Cardiomyopathy

Hypertrophic cardiomyopathy (HCM), is a serious disorder characterised by hypertrophy of the interventricular septum and left ventricular wall,[89] hypercontractile systolic function with diastolic dysfunction, and in some individuals, left ventricular outflow tract obstruction. The findings of Yoneya et al.[94] suggest that HCM may be partially determined by the ACE I/D polymorphism. Findings imply that the ACE D allele is one of the genetic contributing factors associated with cardiac hypertrophy in HCM. An excess of the DD genotype was seen in patients with HCM, particularly in families with a high incidence of sudden heart death.[95]

2.4 Myocardial Infarction

MI is a life-threatening syndrome resulting from partial blockade of coronary perfusion of the heart. It has been suggested that the coronary thrombosis and bleeding that occur after atheroma plaque rupture may occlude the lumen of the blood vessel and lead to acute MI.[96,97] A higher serum ACE activity associated with the DD genotype might be linked to a higher risk of coronary thrombosis, which may result in a greater risk of MI.[98]

Cambien et al.[99] first reported a significant association between the ACE DD genotype and an increased risk for MI among 610 cases versus 733 controls from Ireland and France. However, Lindpaintner et al.[46] could not confirm such an association in 387 patients versus 1475 controls selected from the US. Samani et al.[3] conducted a meta-analysis of 15 published studies containing 3394 MI cases and 5479 controls. The mean odds ratio (OR) of MI for DD versus ID/II genotypes across all studies was 1.26 [95% confidence interval (CI), 1.15-1.39; $p < 0.0001$], and in Japanese populations, the OR is reported to be 2.55 (95% CI, 1.75-3.70), which supports an association of the ACE D allele with MI risk. Recently, Anderson et al.[100] found a modest association of the ACE DD genotype with MI in women. Espinosa et al.[101]

found an association between the ACE I/D polymorphism and MI in a Spanish population. A more-updated meta-analysis that included over 10 000 participants concluded that the DD genotype confers a 10% increased risk of MI.[102]

It appears that most reports suggest an association of the ACE DD genotype with a higher risk of MI.

2.5 Restenosis After Percutaneous Transluminal Coronary Angioplasty

Percutaneous transluminal coronary angioplasty (PTCA) is a procedure to widen narrowed coronary arteries without the need for CABG. However, the therapeutic benefit of PTCA is limited by restenosis in about 30% of patients.[103] The underlying mechanisms are yet to be deciphered. Several previous studies suggest that the ACE I/D polymorphism was related to restenosis after PTCA.[104-106]

Amant et al.[107] declared that the D allele of the ACE gene was a major risk factor for restenosis after coronary stenting. This initial finding has attracted a favourable editorial comment as the new frontier in interventional cardiology in *Circulation*.[108] Although this conclusion was supported by Ribichini et al.,[109] it was discrepant with Koch et al.,[110] who concluded that the I/D polymorphism of ACE gene is not associated with restenosis after coronary stent placement. Similarly, the Multicenter European Research Trial with Cilazapril after Angioplasty to Prevent Transluminal Coronary Obstruction and Restenosis (MERCATOR) study group[111,112] failed to show any beneficial effects of ACE inhibition on post-PTCA restenosis. Finally, the PARIS study by Meurice et al.[113] also did not display successful prevention of in-stent restenosis by ACE inhibition either.

- There are many potential reasons for the lack of an effect of ACE inhibitor on restenosis after PTCA despite the postulated link between the I/D polymorphism and restenosis.
- Although animal studies suggest that ACE inhibition lowers plasma angiotensin II levels, which reduces intimal hyperplasia after vascular injury,[114] restenosis after balloon angioplasty in humans may be mainly caused by vessel remodeling at the site of angioplasty, and that neointimal proliferation may play only a minor role.[115]
- Restenosis might be a multifactorial process, and attempts to prevent it by using ACE inhibitor as the only agent may be inadequate.
- The dosage effect may account for the negative results. Experimental data suggest that high doses of ACE inhibitor are necessary to prevent restenosis,[116] and the doses used in the trials with negative findings may be insufficient to inhibit intimal hyperplasia.
- Timing of drug administration could also be a critical determinant in reducing restenosis after PTCA.[111]

3. ACE I/D Polymorphism and Therapeutic Response to ACE Inhibitors

3.1 Cardiovascular Function Response

ACE inhibitors are widely used in the treatment of hypertension because they lower blood pressure and reduce cardiovascular events and in treatment of congestive heart failure because they reduce morbidity and mortality. The reduction of circulating angiotensin II levels by ACE inhibitors has been shown to be only a transient effect[117] and therefore other mechanisms may also account for the hypotensive effects of ACE inhibitors. A summary of relevant literature on the ACE I/D polymorphism and ACE inhibitor response is presented in table I.

One placebo-controlled study provided no support for the hypothesis that the ACE polymorphism could predict treatment responses to atenolol, lisinopril or nifedipine.[120] Sasaki et al. [121] found that enalapril-induced regression of LVH and improvement in left ventricular impaired diastolic filling were significantly greater in the DD genotype group than they were in the ID and II genotype groups. Serum ACE activity was significantly related to the ACE genotype at each time-point after enalapril administration, whereas falls in mean ar-

Table I. Genetic susceptibility to angiotensin converting enzyme (ACE) inhibitor responses

Drug	Phenotype	Association	Reference
ACE inhibitor (not specified)	Blood pressure response to ACE inhibitor	Negative	118
Enalapril	The percent reduction of ACE activity	Positive (the effect is greater in II genotype group)	119
Atenolol, lisinopril, nifedipine	Blood pressure response to ACE inhibitor	Negative	120
Enalapril	Enalapril-induced regression of left ventricular hypertrophy and improvement in left ventricular impaired diastolic filling	Positive (the effect is greater in the DD genotype group)	121
Imidapril	Reduction and the percent reduction in DBP	Positive (the effect is greater in II genotype group)	122
Captopril	The increase in effective renal plasma flow and the fall in renal vascular resistance in response to captopril	Positive (the effect is reduced in DD genotype group)	123
Captopril	Blood pressure response to ACE inhibitor	Negative	124
Lisinopril, captopril	Change in mean arterial pressure	Positive (the effect is greater in II genotype group; captopril only)	125
Fosinopril	Reductions in SBP and DBP	Positive (the effect is greater in the DD genotype group)	126

DBP = diastolic blood pressure; **SBP** = systolic blood pressure.

terial pressure in response to enalapril were not significantly related to the ACE genotype. Mizuiri et al.[123] found that the increase in effective renal plasma flow and the fall in renal vascular resistance in response to captopril were significantly less in individuals with the DD genotype than in those with the other genotypes. These data suggest that intrarenal ACE inhibition by captopril differs according to the ACE gene I/D polymorphism in healthy individuals. Todd et al.[119] reported that the percent reduction of ACE activity was larger in the II genotype than in the DD genotype after enalapril therapy.

Hingorani et al.[118] reported that the ACE genotype had no effect on the response of blood pressure to treatment with an ACE inhibitor in hypertensive individuals. In a prospective study of Japanese hypertensive patients, Ohmichi et al.[122] investigated whether the response to the ACE inhibitor imidapril varied according to the ACE genotype. They found that both the reduction and the percent reduction in DBP (but not SBP) tended to be higher in patients with the II genotype, and the reduction in DBP was inversely associated with plasma ACE activity. Stavroulakis et al.[126] conducted a prospective investigation to assess whether the response to the ACE inhibitor fosinopril varied according to the ACE genotype. It was found that the reduction in SBP and DBP was significantly greater in patients carrying the DD genotype compared with II or ID. In a randomised, double-blind, crossover study in patients with heart failure, O'Toole et al.[125] found a significant relationship between the ACE genotype and change in mean arterial pressure with captopril but not with lisinopril. Their results contrast with those of Nakano et al.,[124] and such discrepancy might be due to differences in ethnicity, study design and age range.

A report from China confirmed an association between the ACE gene polymorphism and therapeutic responsiveness to ACE inhibitor in patients with diabetic nephropathy. In a study of 89 patients with type 2 (non-insulin-dependent) diabetes mellitus, it was shown that the therapeutic efficacy of ACE inhibitor was greatest in those with the DD genotype and worst in those with the II genotype.[127]

In summary, it remains controversial whether the I/D genotype is correlated with ACE inhibitor response and further studies on the nature of the link are needed.

3.2 ACE Inhibitor-Induced Cough

Persistent dry cough is the most common adverse effect of ACE inhibitors, with a frequency of approximately 5 to 15%. The mechanism is linked to the accumulation of bradykinin and tachykinins resulting from ACE inhibition in the airway with consequent stimulation of vagal afferents.[128] It was found that the frequency of genotype II in patients with cough was increased by 74% greater than that in patients without such cough. Therefore, it was suggested that a greater I allele frequency may increase genetic susceptibility to ACE inhibitor-induced cough.[129] This provides one possible explanation for the observation that the Chinese experience more cough induced by ACE inhibitors (captopril and enalapril) than Caucasians.[130-132] However, Zee et al.[133] evaluated three candidate genes including ACE, and found no association between the genetic polymorphisms examined and ACE inhibitor-induced cough. Moreover, McGarvey et al.[134] found no association between the ACE I/D polymorphism and susceptibility to ACE inhibitor-induced cough either. Thus, the link between the I allele and an increased risk for ACE-induced cough remains to be established.

4. Considerations of Gene-Gene and Gene-Environmental Interactions

4.1 Gene-Gene Interactions

In polygenetic disorders such as cardiovascular disease, the investigation of gene-gene interactions rather than determination of single gene effects is crucial to a better understanding of the contribution of genetic factors. However, only a few studies have addressed this critical subject.

Kamitani et al.[135] found that the risk of MI calculated by combining the angiotensinogen-TT and ACE-DD genotypes (OR, 11.2) was considerably higher than when the two genotypes were analysed separately. In a group of 585 patients with coronary artery disease (CAD) with (n = 270) or without (n = 315) previous MI, Naber et al.[136] demonstrated a significant interaction between the ACE D allele and the GNB3 T825 allele ($p < 0.001$). The risk for MI associated with the T825 allele was not increased in carriers of the ACE II genotype (OR, 0.5; $p = 0.09$), but was significantly elevated in carriers of the ACE ID genotype (OR, 1.9; $p = 0.01$), and further increased in individuals with the ACE DD genotype (OR, 2.4; $p = 0.02$). The highest OR was found in those who were homozygous for both GNB3 T825 allele and ACE D alleles (OR, 7.5; $p = 0.006$). Furthermore, a synergistic contribution of ACE and AT1R polymorphisms to the risk of CAD was found.[137] Similarly, van Bockxmeer et al.[138] observed that the ACE genotype had no obvious impact on restenosis, but a significant interaction between ACE and ApoE genotypes was shown. Carriers of both the ACE D and ApoE ε4 alleles had an increased risk of restenosis. Taken together, gene-gene interactions should be more broadly assessed in future investigations.

4.2 Gene-Environment Interactions

The major environmental risk factors for cardiovascular disease include dietary intake of fat, fibre or electrolytes, cigarette smoking, alcohol consumption, physical activity and stress. Different individuals have different degrees of genetic susceptibility to the adverse effects of environmental influences.[139] It remains possible that previous studies have not sufficiently controlled for confounding factors that might explain a portion of the residual associations in multivariate analyses. To date, most genetic epidemiological studies have not addressed the role of ACE I/D genotype-environment interaction in cardiovascular phenotypes.

Cambien et al.[99] conducted a multicentre case-control study of 1300 individuals for MI, and the DD genotype was found significantly more prevalent in patients with a history of MI (OR, 1.3). The association was found to be stronger (OR, 3.2) in patients considered to be at low risk for conventional risk factors (body mass index <26 kg/m^2 and plasma apolipoprotein B level <1.25 g/L), suggest-

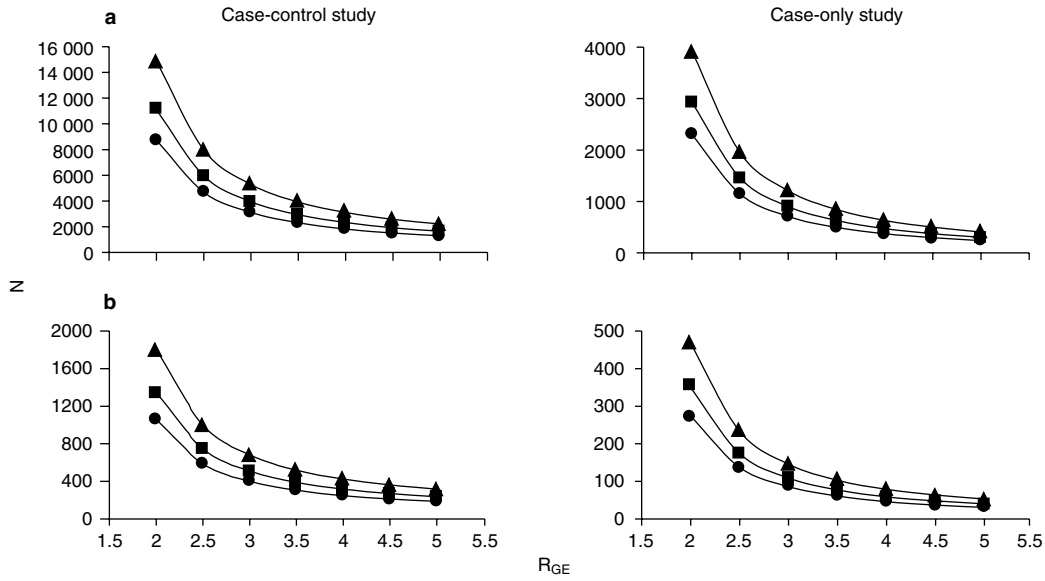

Fig. 5. Sample size calculations for testing the gene-environment interaction. Left and right panels represent case-control and case-only designs, respectively. The prevalence of the environmental exposure is 0.1. The population risk is 0.01. The main effects associated with the environmental exposure (R_E) and with the disease allele (R_G) are both 1.5. All calculations assume a log-additive effect of the disease alleles. The required sample sizes are calculated to achieve a power of 70% (filled circles), 80% (filled squares), and 90% (filled triangles) for detecting various effects of gene-environment interaction (R_{GE}) at a 2-sided type I error rate of 0.05. (**a**) Disease allele frequency is 0.01; (**b**) disease allele frequency is 0.1. **N** = sample size.

ing a potential role of a gene-environment interaction.

Case-control and case-only study designs are two popular strategies for assessing the interactive effects of genetic and environmental risk factors.[140] Sample size calculations have been made for case-control and case-only studies under different parameter settings (figure 5). It appears that the case-only design is more efficient than the case-control design.

In summary, elucidation of gene-environment interaction is crucial to disentangling the role of genetic and environmental influences.

5. Conclusion and Future Directions

Over the years, great strides have been made in understanding the physiological function of ACE using knockout and transgenic animal models. It was shown that the DD genotype is related to an increased local cardiac ACE activity and might be implicated in cardiovascular dysfunctions.[32] According to our comprehensive review, the results obtained so far are quite divergent and there are many possible issues which relate to this.[47]

One is the use of different methods for the assessment and diagnosis of cardiovascular diseases, and another is the use of different experimental protocols for I/D genotype determination. Moreover, selection bias cannot be ignored. There have been striking differences in terms of the DD genotype frequency across studies and different selection procedures may have had a dramatic impact. Furthermore, the discordance of the results across distinctive populations may be attributable to inter-ethnic differences of the ACE gene DD genotype frequencies.[141] Finally, the association between the ACE I/D polymorphism and cardiovascular phenotypes may be caused by its linkage disequi-

librium with a mutation of a nearby gene - the human GH (hGH) gene. For example, Julier et al.[142] found the hGH marker gave strongly significant results for linkage to hypertension when considered as a qualitative trait. O'Donnell et al.[68] also found a significant linkage with DBP for hGH ($p<0.05$) in males. Since an increased risk of cardiovascular morbidity and mortality has been documented with both GH excess or deficiency,[143] the common polymorphisms of the hGH gene should be examined in future analyses.

In summary, we should consider the current data on the ACE I/D genotype as interesting progress toward elucidation of its role in the pathogenesis of cardiovascular disease and in pharmacogenomic response to ACE inhibitor therapy. After all, the ACE enzyme is only one component of the RAAS and the KKS, and the I/D variant may exert a modest individual impact. With considerable advances in molecular genetic techniques, the era of 'genomic epidemiology' has come of age. Elucidation of the genetic basis underlying cardiovascular phenotypes and therapeutic response awaits more comprehensive dissection of the molecular variations of key candidates of all major physiological pathways implicated in cardiovascular functions as well as the interaction between the human genome and the environment.

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