Is genotype or phenotype the better tool for investigating the role of ACE in human cardiovascular disease?

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Introduction

As part of the systemic circulating human renin–angiotensin system, angiotensin I-converting enzyme (ACE) degrades (vasodilator) kinins and generates (vasoconstrictor) angiotensin II. Angiotensin II also drives adrenal aldosterone production, leading to salt and water retention. Pharmacological inhibition of ACE activity has thus found its place in the management of both heart failure and hypertension, with a cumulative worldwide experience of more than 200 million patient-years of treatment. However, the reductions in cardiovascular mortality and morbidity associated with such therapy far exceed those anticipated for a mere vasodilating natriuretic,[1] benefits which have yet to be adequately mechanistically explained. In recent years, however, it has become increasingly clear that local tissue renin–angiotensin systems also exist in diverse tissues[2–7], whose influence on tissue physiology and pathophysiology has become the subject of intense research efforts. It is postulated that a reduction in tissue ACE activity, rather than that in the circulation, might underlie the beneficial effects of ACE inhibitor therapy.

ACE activity in both circulating (endocrine) and local (autocrine or paracrine) systems is under strong genetic influence. The existence of a single major genetic influence on circulating ACE levels was first suggested by Cambien and colleagues in 1988[8]. Only 2 years later, and this genetic influence was shown to be the ACE gene itself: the absence (deletion, D) rather than the presence (insertion, I) of a 287 base pair marker was associated with significantly higher circulating ACE levels[9]. Not long afterwards, the ACE I/D polymorphism was shown to be associated with ACE levels in human cardiac tissue[10] and lymphocytes[11], suggesting that ACE activity in both tissue systems and the circulation were under similar genetic control.

Given the identification of such a marker of tissue ACE activity, a plethora of studies followed in which ACE genotype was used as a tool, through association studies, to investigate the role of tissue ACE in human disease pathogenesis. Indeed, there have been more than 1000 such papers published within the last 8 years. However, whilst consistent associations are identified for some disease states[12–14], the data for others have been inconsistent, leading some to question, for instance, the association of ACE genotype (and hence, by inference, ACE itself) with myocardial infarction[15].

Such inconsistency may, of course, have several underlying causes. Firstly, it may relate to a publication bias in which small studies of negative outcome are deemed ‘underpowered’ and are rejected, whilst similarly underpowered positive studies are published. Indeed, it has previously been noted that the strength of statistical association of disease with the D allele falls as the size of the study increases[16]. Secondly, the strength of association may be race-dependent. Thus, whilst ACE levels are strongly correlated with ACE I/D genotype in European populations, this may not hold true in other races. Such racial differences have been proposed as one explanation for the wealth of positive association studies amongst Japanese and European population samples, and their relative paucity amongst US samples[17].

Nonetheless, many of the ‘negative’ data (in which an association of the D allele with clinical phenotypes is absent) conflict with those from pharmacological studies, in which relatively low doses of ACE-inhibitors may yield substantial reductions in cardiovascular risk[18]. What, then, might account for such negative findings? Perhaps such inconsistencies are explicable by the fact that a substantial degree of variation in both tissue and circulating ACE levels remains unaccounted for by the I/D polymorphism[6,19]. Indeed, there is significant residual heritability in circulating ACE activity...
when the variance attributed to the I/D polymorphism (28%) is allowed for, meaning that some 70–80% must be accounted for by other variations\(^\text{[19]}\). To date, more than 78 different polymorphisms have been identified at the ACE locus, of which 61 were not in linkage disequilibrium with the ACE I/D polymorphism\(^\text{[20]}\). Several are likely to have substantial effects on ACE activity\(^\text{[21–23]}\). Given the fact that so much of the variance in circulating ACE levels is unaccounted for by the I/D polymorphism, is it appropriate that a role for ACE in some diseases might be dismissed through lack of such genotype-association? Furthermore, racial variation in allele frequency might make the ACE I/D polymorphism more (or less) useful in the study of some populations than others\(^\text{[24–26]}\). In these cases, and where a disease pathogenesis is less strongly ACE-dependent, might not ACE phenotype rather than genotype be more strongly disease-associated? Might correlation with a continuous phenotype, rather than association with one of only three discrete genotypes, have worth? Would we actually be better off using plasma ACE levels rather than ACE genotype as our investigative tool? In order to be used in this way, we would need to be reassured on several issues.

**How consistent and stable are plasma ACE levels?**

Firstly, it would need to be assumed that much of the variation in ACE activity between individuals was indeed largely due to genetic variation and the influence of the unique ‘internal mileux’ of each individual. Data (above) do suggest this to be the case. Further support for such a concept would come from finding marked stability of ACE activity over time. In fact, although changing somewhat with age\(^\text{[27]}\), basal ACE activity does seem remarkably stable within any given individual over periods of up to 6 months\(^\text{[28]}\). It also seems remarkably unaffected by external environmental and hormonal factors\(^\text{[29]}\) including, in the shorter term, the ingestion of meals\(^\text{[30]}\), prolonged intensive exercise\(^\text{[31]}\), and even normal labour\(^\text{[32]}\) which have little short-term effect on plasma ACE activity. However, circulating ACE activity is influenced by some environmental factors such as salt intake. Thus, plasma ACE levels increase by a mean of 29% after only 4 days of a low-salt diet, with a concurrent fall in circulating concentration of plasma kinins and kallikrein\(^\text{[33]}\). Similarly, disease states (such as sarcoidosis) may alter circulating ACE activity. Further, although basal and 6-month levels of ACE activity correlate highly (with an r-value of 0.87 and \(P<0.001\)\(^\text{[33]}\)), values differ by more than 100% in some individuals over this timescale\(^\text{[28]}\). Thus, the data simply do not yet exist to support a categorical statement that ‘ACE activity is stable in normal individuals’. It is even less clear to what extent ACE levels are altered by the disease states in which association studies are sought.

**Does basal plasma ACE activity mirror tissue ACE activity?**

Secondly, we would need to be reassured that circulating and tissue ACE activity are closely related if we were to use the former as a more reliable guide than ACE genotype to the latter. However, levels of circulating ACE may be separately regulated from even membrane-bound ACE, let alone tissue ACE. ACE is anchored to the cell membrane by a single hydrophobic transmembrane polypeptide near its carboxyterminal end. The soluble form of ACE in plasma is derived through post-translational cleavage of the anchor, a process under quite separate control from that of synthesis itself\(^\text{[34,35]}\).

Whilst there is a marked variation in ACE activity between different human tissues\(^\text{[36]}\), the ACE I/D polymorphism does influence both circulating and (some) tissue ACE activity\(^\text{[3,13]}\). However, there have been no papers published which address the positive predictive power of plasma ACE activity for tissue ACE activity in any, let alone a multitude of, different tissues. Further, there is reason to believe that circulating and tissue ACE activity might not correlate perfectly in any one individual. Although circulating ACE activity seems co-dominantly influenced by the I and D alleles\(^\text{[39]}\), this may not hold true for either T-lymphocytes\(^\text{[11]}\) or cardiac tissue\(^\text{[10]}\), where only the presence of two D alleles seems to be associated with elevation of ACE levels (Fig. 1). These studies, however, were both small, and it is possible that this lack of allele codominance is an artifact of small sample size. Thus, the exploration of tissue physiology using genotype and circulating ACE phenotype as tools may have weaknesses in both cases: circulating ACE levels may not predict tissue levels, and the relationship between basal ACE activity and ACE genotype is unclear in most tissues. Indeed, in some complex systems such as the human atherosclerotic plaque, ACE activity may be unrelated to ACE I/D genotype\(^\text{[37]}\).

**Does plasma ACE activity mirror that in ‘activated’ tissue systems?**

Finally, we would need to know that basal circulating ACE levels predict not only basal tissue ACE levels, but their response to physiological and pathophysiological stimuli. Tissue ACE activity increases in response to a variety of stimuli (such as those which drive growth)\(^\text{[38]}\), and may indeed transduce such stimuli\(^\text{[39]}\). However, if the strength of the association of circulating with tissue ACE activity is unknown, the strength of its association with stimulated tissue ACE activity is equally unknown. Certainly, it seems that circulating and tissue renin–angiotensin systems are under quite separate control, producing different responses even to one stimulus\(^\text{[33]}\). In addition, ACE activity may alter radically in different tissues even in one pathophysiological state. Thus,
in animals with aortic banding and left ventricular hypertrophy, pulmonary ACE activity falls dramatically whilst LV ACE activity rises\[40\]. If mirrored in human pathophysiological states, plasma ACE activity could not correlate with the activity in all tissues.

Few, if any, studies have considered these factors. Although several papers have demonstrated an association of plasma ACE activity with clinico-pathological outcomes (such as in-stent restenosis\[28\], left ventricular mass\[41\], and post-infarct left ventricular dilatation\[42\]), few have addressed whether such association exists within ACE genotype groups or even independently of them. That ACE genotype distribution may be similar amongst patients with non-insulin dependent diabetes and microalbuminuria and amongst those without whilst ACE activity is elevated in the former group\[43\] at least suggests that this might be the case.

Thus, the investigation of the role of tissue ACE in human physiology and pathophysiology has been greatly aided by the discovery of the human ACE I/D polymorphism. However, we are now greatly in need of further studies. Such studies cannot be performed in animals, given that the I/D polymorphism is unique to humans. How truly stable are both circulating and tissue ACE levels? Long-term studies need to be performed. So too do studies of ACE activity in response to a variety of acute physiological stressors. Does ACE genotype influence ACE levels in all organs to a similar degree? How good a marker is circulating ACE activity of tissue activity? Studies in explant human hearts\[10\] should be repeated with measures of ACE activity in blood and in a variety of other tissues in order to answer such questions. Is the association of ACE genotype with ACE activity consistent in both basal and stimulated conditions? Such studies may be best performed using human tissue in in vitro experiments. Only when such questions are answered will we truly know whether our association studies should use genotype, or would be better powered by using circulating ACE phenotype as a marker of tissue ACE activity. Initially, however, studies in which positive association of the D allele with a given phenotype has been identified might be repeated, but with plasma ACE levels being additionally documented. In this way, within-genotype correlation of ACE activity with phenotype might be directly studied.

In any event, there is an urgent need to perform these studies, as we may yet be failing to identify powerful roles for ACE in human pathophysiology, or inferring roles for ACE through a spurious association with genotype (and unproven association with ACE phenotype or activity). Until such studies are complete, association studies should wherever possible utilise both genotype and measures of ACE activity.

References


