Analysis of gene-environment interactions by "stressing-the-genotype" studies: the angiotensin converting enzyme and exercise-induced left ventricular hypertrophy as an example

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The human angiotensin converting enzyme (ACE) gene contains a length polymorphism consisting of the presence (insertion, I) or absence (deletion, D) of a 287 base pair "Alu" repeat sequence in intron 16, with the D allele being associated with higher ACE levels than the I allele in plasma and in tissues. We have carried out several studies to examine the relationship between this polymorphism and cardiovascular health, and have examined the hypothesis that if renin-angiotensin systems regulate left ventricular (LV) growth, individuals of DD genotype might show a greater hypertrophic response than those of II genotype. A strategy was used involving screening over 1200 male military recruits to select only subjects homozygous for the I or D allele for the expensive and time-consuming but extremely accurate method of LV mass determination by magnetic resonance imaging. LV dimensions and mass were compared at the start and end of a 10-week physical training period. LV mass increased with training by 8.4 g overall ($p < 0.0001$), but with DD men showing roughly 3 fold greater growth than II men ($p < 0.001$). When indexed to lean body mass, LV growth in II subjects was essentially negligible whilst remaining significant in DD subjects (-0.022 vs +0.131 g/kg respectively, $p = 0.0009$). Although the precise molecular mechanism of this effect remains to be elucidated it clearly demonstrates the importance of the ACE-renin-angiotensin system in determining LV dimensions in situations of high cardiac demand, which may also be important in pathology such as hypertension and heart failure. The use of these "stress-the-genotype" approaches to explore gene-environment interactions are likely to be the key to understanding the causes determining both coronary artery disease and other multi-factorial disorders.

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Introduction: homeostasis and coronary artery disease

In the context of a cell (or even an organ or organism), genes can be considered to code for the synthesis of proteins which allow the maintenance of intracellular homeostasis in the face of extracellular or environmental changes. Cells require oxygen, energy and various chemicals, in order to survive and divide and, in a Darwinian sense, to pass on their genes to the next generation. Naturally occurring genetic variation means that some individuals are better able to maintain homeostasis than others given the same environmental challenge. In the western culture people now experience many challenges to maintaining cardiovascular health, such as a high fat diet, high levels of smoking and alcohol intake, and reduced physical exercise leading to obesity. Even with all these environmental "insults", some individuals maintain cardiovascular health into old age, while others, with a different genetic make-up, fail to maintain homeostasis (e.g. plasma levels of cholesterol or fibrinogen or cardiac dimensions and arterial tone within an optimal range), and thus develop atherosclerosis. Identifying the genes involved in maintaining cardiovascular homeostasis despite environmental challenge should thus lead to progress in understanding pathophysiology and aetiology, as well as in genetic risk prediction, since mutations in such genes are likely to be strongly predisposing to or protecting from coronary artery disease (CAD).

"Stressing-the-genotype" analyses

The basic strategy is to genotype a large number of subjects, for example a group of

healthy men from a population-based sample, and then recruit for "stress" investigations only those subjects homozygous for the rare allele matched (for age or other relevant confounders) with an equal number of common allele homozygotes. If required, an equal number of heterozygote carriers can be included although these may not give particularly useful mechanistic insight. By definition, the rarest group in a population sample are those homozygous for the rare allele, and this is usually the group that will give the greatest mechanistic information, while the genotype-and-select strategy gives a balanced design. Also many of these "stress" tests used are time-consuming for volunteers and investigator, as well as requiring costly assays for the measures. Obtaining these measures on a whole population-based sample of several hundred subjects (in order to include the necessary number of rare homozygotes for adequate power) may be prohibitively expensive.

Some of the stress situations that have been used by us and others in CAD genetic research are shown in table I[1-20], and include a fatty meal or a dietary manipulation or supplements, strenuous exercise to mimic an injury, or surgical traumas. The example that will be presented in more detail here is based on work we have done with the angiotensin converting enzyme (ACE) gene polymorphism.

The angiotensin converting enzyme gene. Exercise, and left ventricular hypertrophy

The human ACE gene is found on chromosome 17 and contains a restriction fragment length polymorphism consisting of the presence (insertion, I) or absence (deletion, D) of a 287 base pair "Alu" repeat sequence in intron 16[21]. These have been based on the data that the D allele is associated with higher ACE levels than the I allele in plasma[21] and in tissues[22]. The association of the I allele with lower ACE activity in both serum and tissues has ramifications throughout the renin-angiotensin system and kallikrein-kinin system and has stimulated much fascinating work in regard to various pathological and physiological states. In 1992, the ACE gene I/D polymorphism was reported to be associated with risk of myocardial infarction[23], and this effect, though of a considerably smaller size than originally reported, has been confirmed in a meta-analysis[24] and in a recent large study[25]. Since then, we have carried out several studies to examine the relationship between this polymorphism and cardiovascular health, and have examined the hypothesis that if renin-angiotensin systems regulate left ventricular (LV) growth, individuals of DD genotype might show a greater hypertrophic response than those of II genotype.

The theoretical basis for this is shown in figure 1. The protease renin is released from the cells of the juxta-glomerular apparatus in the kidney under conditions of salt or volume loss or sympathetic activation. Renin cleaves angiotensinogen (synthesised in the liver) to generate the non-pressor decapeptide angiotensin I. The octapeptide angiotensin II is then derived primarily by the action of the dipeptidyl-carboxypeptidase, ACE, which is responsible for the hydrolytic cleavage of dipeptides from the carboxyl terminus his-leu dipeptide. ACE also catalyses inactivation of the nonapeptide bradykinin by two sequential dipeptide hydrolytic steps and in this context, is known as kininase II. By these two additive mechanisms ACE thus has a direct effect on controlling blood pressure. Tissue ACE is released from the cell membrane by a carboxypeptidase that cleaves the protein between Arg-663 and Ser-664 to generate circulating ACE[26]. However the rate-limiting step in the production of angiotensin II appears to be levels of ACE itself, and ACE inhibition, by any of a number of well-known therapeutic agents, has a powerful effect on lowering blood pressure and reducing cardiac mortality[27]. It has however been recognised that the reduction in mortality associated with ACE inhibitor therapy cannot be explained simply by the observed fall in blood pressure, suggesting the ACE inhibition may be having additional beneficial effects. Demonstration of a reduction in LV hypertrophy by lowering ACE levels, by the mechanism indicated in figure 1, would be of great clinical interest.

Table I. Examples of coronary artery disease (CAD) risk traits where "stressing-the-genotype" studies have been analysed.

Plasma CAD risk trait	Environment stressor	Candidate genes	Reference
Cholesterol	Dietary fat	*APOE/CETP/LPL*	1,2
		APOA4	3
Triglyceride	High fat meal	*LPL/APOC3*	4,5,6
Fibrinogen	Injury/acute exercise	*FIBB*	7
Fibrinogen	Surgery	*FIBB*	8,9
Factor VII	Dietary triglyceride/meal	*FVII*	10,11,12,13
Interleukin-6	Injury/surgery	*IL-6*	14
Homocysteine	Low folate diet	*MTHFR*	15,16,17,18
Left ventricular mass	Hypertension/exercise	*ACE*	19,20

ACE = angiotensin converting enzyme; CETP = colesteryl ester transfer protein; FIBB = beta fibrinogen gene; LPL = lipoprotein lipase; MTHFR = methylenetetrahydrofolate reductase.

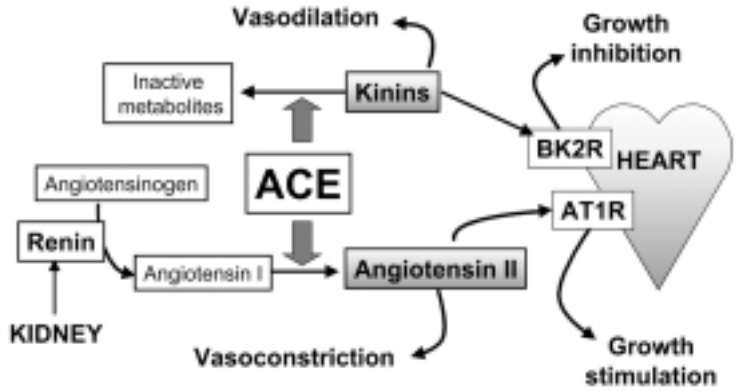

Figure 1. *Biochemical pathways by which angiotensin converting enzyme (ACE) affects blood pressure and left ventricular hypertrophy – the renin-angiotensin/kallikrein-kinin system. Because it is the rate-limiting step in the pathway, higher plasma ACE, as is found in individuals lacking the Alu insertion (i.e. the DD genotype), leads to a higher conversion of vaso-inactive precursor to the vasoconstrictor product (angiotensin II), and higher breakdown of the vasodilatory kinins to inactive metabolites. Both of these contribute to a rapid fall of blood pressure by action on vascular motor tone. In addition, angiotensin II stimulates angiotensin type 1 receptors on cardiac tissue which leads to growth stimulation, while the lower levels of kinins leads to less stimulation of the bradykinin 2 receptor (BK2R) and less growth inhibition. Overall this leads to greater cardiac muscle growth in the presence of high ACE (i.e. the DD genotype) compared to those with low ACE (i.e. II genotype).*

In 1997 we reported results of a study of changes in LV mass upon exercise training[19]. Echocardiographically determined LV dimensions and mass, and frequency of LV hypertrophy were compared at the start and end of a 10-week physical training period in 156 male military recruits. Overall, LV mass increased by 18% ($p < 0.0001$), but response magnitude was strongly associated with ACE genotype: mean LV mass altered by +2.0, +38.5 and +42.3 g in II, ID and DD respectively ($p < 0.0001$).

We have now replicated this observation in a second independent study of army recruits[20]. Here a strategy was used involving screening over 1200 recruits to select only subjects homozygous for the I or D allele for the expensive and time-consuming (but considerably more accurate) method of LV mass determination by magnetic resonance imaging. Since the LV hypertrophic responses may be mediated through either increased activity of the cellular growth factor angiotensin II on the angiotensin type 1 receptor (AT_1), or increased degradation of growth-inhibiting kinins (Fig. 1), the study was also designed to clarify the role of the AT_1 receptor in this association. The recruits were randomised to receive placebo or a well-known and well-tolerated antihypertensive drug, losartan, throughout their 10-week physical training programme. Subjects received a subhypotensive dose (25 mg/day), sufficient to inhibit tissue AT_1 receptors, and thus if LV growth occurs in the presence of the drug it would implicate the other pathways in the ACE gene effect. As before[19], LV mass was not different by genotype at baseline in either group, showing that in healthy non-trained subjects ACE genotype is not "rate-limiting" for cardiac size. LV mass increased with training by 8.4 g overall ($p < 0.0001$), but with a highly significant difference in men with different ACE genotypes. As shown in figure 2 the increase in the placebo limb was 12.1 vs 4.8 g for DD vs II genotype ($p = 0.022$). Interestingly, LV growth was similar in the losartan arm, being 11.0 vs 3.7 g for DD vs II genotypes ($p = 0.034$). When indexed to lean

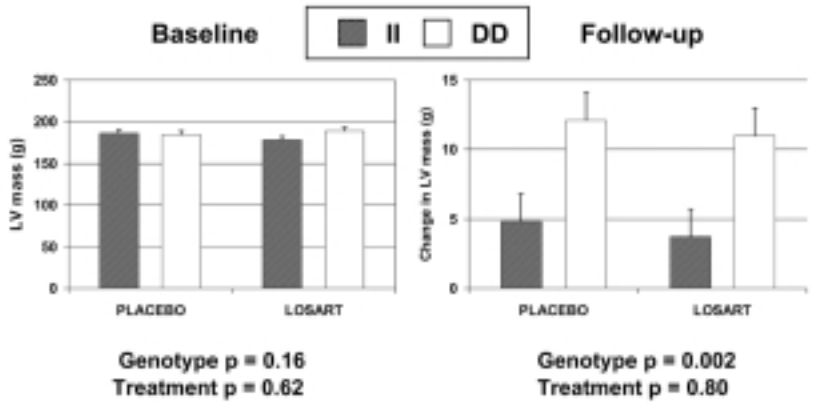

Figure 2. *Baseline and change in left ventricular (LV) mass by angiotensin converting enzyme genotype and treatment group in army recruits. Data adjusted for height, age, systolic blood pressure (and for change also for pre-training LV mass). Number of subjects in each group were DD placebo 41, losartan 38, II placebo 34, losartan 28. Data from Myerson et al.[20].*

body mass, LV growth in II subjects was abolished whilst remaining in DD subjects (-0.022 vs +0.131 g/kg respectively, p = 0.0009).

Thus the ACE genotype-dependence of exercise-induced LV hypertrophy seen in the earlier study was strongly confirmed. These effects were not influenced by AT_1-receptor antagonism using losartan, suggesting that the 2.4-fold greater LV growth in DD men may be due either to angiotensin II effects on other receptors (e.g. AT_4), or lower degradation of growth-inhibitory kinins. It is of relevance that in both of these studies with regard both to LV mass and skeletal muscle efficiency, there was no significant effect associated with ACE genotype in "unstressed" subjects at recruitment but only after the 10-week "stress" of the training period. This is clearly an example of gene-environment interaction, suggesting ACE genotype (and ACE levels) will not be a predictor of LV hypertrophy in unstressed subjects. These results also point to a potential novel mechanism of the ACE gene effect on CAD seen in previous studies, but indicate that risk of disease may be confined to those where LV hypertrophy may have been induced (e.g. by hypertension).

Mechanism of the gene-environment effect

The precise mechanism by which the ACE genotype may be having its effect is under investigation and may be through both or either the pathway although which ACE influences circulatory homeostasis through the degradation of vasodilator kinins or the formation of vasopressor angiotensin II. However, local tissue-based renin-angiotensin system also exists in human myocardium[28] and it is likely that ACE levels expressed in the myocardium would be high in DD and low in II subjects. In support of both pathways, increased ACE gene expression and ACE activity in the myocardium[29] significantly increase the rate of local conversion of angiotensin I to angiotensin II. Furthermore, the ACE DD genotype itself tends to show increased conversion of infused angiotensin I to angiotensin II in humans[30]. This study also revealed a significant inverse relationship between the half-life of bradykinin and both serum ACE activity and the conversion of angiotensin I to angiotensin II, confirming that the ACE genotype influences bradykinin degradation. The role of bradykinin in tissue metabolism and vasodilatation is now recognised as being endothelium-dependent. Data from studies on isolated perfused rat hearts (that still have an intact endothelium) provide strong evidence of an improvement in myocardial metabolic efficiency mediated by bradykinin[31]. Despite this evidence it remains possible that the ACE gene mediates its observed effects on endurance independent of the renin-angiotensin system or indeed that the ACE gene is not directly responsible but other gene or genes in linkage disequilibrium with the ACE locus.

Conclusions and future avenues of research

These controlled stress-induced gene-environment interactions are likely to be the key to understanding the "causes" determining both CAD and other multi-factorial disorders. Many associations have proved to be robust, and for example the stress-induced greater fibrinogen-raising effect associated with the β-fibrinogen -455 A-allele (Table I) has now been replicated and therefore is very unlikely to have been observed by chance alone, although the precise molecular mechanism of these effects remains to be elucidated. One area where environmental manipulation may be available to confirm gene-environment interaction observed in an association study would be to remove the environment (or the individual from the environment) and observe the decay of the induced phenotype effect over a period of time. Although this could not easily be done for the phenotype of atherosclerosis, requiring invasive serial coronary angiography for example, it would be possible for some environments such as smoking on plasma traits such as fibrinogen, or on the consequence of hypertension on LV hypertrophy after antihypertensive therapy. The potential for this area of research has yet to be examined in detail.

Once the effect has been confirmed by replication, such genetic-environment information would be useful for inclusion into a CAD-risk algorithm, such has been prepared by Framingham[32] using essentially non-genetic factors. This will enable us to give genotype-specific life-style advice, or to tailor clinical and therapeutic decisions to an individual's genotype. Finally, once the mechanism of such gene-environment interactions has been understood at the molecular level it may also point to novel therapeutic possibilities, for example to block inflammatory processes during stress situations, or the novel use of available drugs such as ACE-inhibitors, to prevent disease in a molecularly rational manner. Since there are many widely used and well tolerated drugs which alter ACE activity, this raises the exciting possibility of the use of these drugs in order to maintain human health in hitherto unexplored ways.

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References

1. Ordovas JM, Cupples LA, Corella D, et al. Association of cholesteryl ester transfer protein-TaqIB polymorphism with variations in lipoprotein subclasses and coronary heart disease risk: the Framingham study. Arterioscler Thromb Vasc Biol 2000; 20: 1323-9.

2. Wallace AJ, Mann JI, Sutherland WHF, et al. Variants in the cholesterol ester transfer protein and lipoprotein lipase genes are predictors of plasma cholesterol response to dietary change. Atherosclerosis 2000; 15: 327-36.
3. Ordovas JM, Schaefer EJ. Genes, variation of cholesterol and fat intake and serum lipids. Curr Opin Lipidol 1999; 10: 15-22.
4. Gerdes C, Fisher RM, Nicaud V, et al, on behalf of the EARS Group. Lipoprotein lipase variants D9N and N291S are associated with increased plasma triglyceride and lower high-density lipoprotein cholesterol concentrations: studies in the fasting and post-prandial states. The European Atherosclerosis Research Studies. Circulation 1997; 96: 733-40.
5. Humphries SE, Nicaud V, Margalef J, et al, for the EARS Group. Lipoprotein lipase gene variation is associated with a paternal history of premature coronary artery disease and fasting and postprandial plasma triglycerides. Arterioscler Thromb Vasc Biol 1998; 18: 526-34.
6. Waterworth DM, Ribalta J, Nicaud V, Dallongeville J, Humphries SE, Talmud P. ApoCIII gene variants modulate postprandial response to both glucose and fat tolerance tests. Circulation 1999; 99: 1872-7.
7. Montgomery HE, Clarkson P, Nwose OM, et al. The acute rise in serum fibrinogen concentration with exercise is influenced by the G-453-A polymorphism of the beta-fibrinogen gene. Arterioscler Thromb Vasc Biol 1996; 16: 386-91.
8. Gardemann A, Schwartz O, Haberbosch W, et al. Positive association of the fibrinogen H1/H2 gene variation to basal fibrinogen levels and to the increase in fibrinogen concentration during acute phase reaction but not to coronary artery disease and myocardial infarction. Thromb Haemost 1997; 77: 1120-6.
9. Cotton JM, Webb KE, Mathur A, Martin JF, Humphries SE. Impact of the -455G>A promoter polymorphism in the β-fibrinogen gene on stimulated fibrinogen production following bypass surgery. Thromb Haemost 2000; 84: 926-7.
10. Miller GJ, Stirling Y, Howarth DJ, et al. Dietary fat intake and plasma factor VII antigen concentration. Thromb Haemost 1995; 73: 890-5.
11. Ghaddar HM, Folsom AR, Aleksic N, et al. Correlation of factor VIIa values with factor VII gene polymorphism, fasting and postprandial triglyceride levels, and subclinical carotid atherosclerosis. Circulation 1998; 98: 2815-21.
12. Mennen LI, de Maat MP, Schouten EG, et al. Dietary effects on coagulation factor VII vary across genotypes of the R/Q353 polymorphism in elderly people. J Nutr 1998; 128: 870-4.
13. Sanders TAB, de Grassi T, Miller GJ, Humphries SE. Dietary oleic and palmitic acids and postprandial factor VII in middle-aged men heterozygous and homozygous for factor VII R353Q polymorphism. Am J Clin Nutr 1999; 69: 220-5.
14. Brull DJ, Montgomery HE, Sanders J, et al. Interleukin-6 gene -174G>C and -572G>C promoter polymorphisms are strong predictors of plasma interleukin-6 levels after coronary artery bypass surgery. Arterioscler Thromb Vasc Biol 2001; 21: 1458-63.
15. Ma J, Stampfer MJ, Hennekens CH, et al. Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians. Circulation 1996; 94: 2410-6.
16. Verhoef P, Kok FJ, Kluijtmans LA, et al. The 677C→T mutation in the methylenetetrahydrofolate reductase gene: associations with plasma total homocysteine levels and risk of coronary atherosclerotic disease. Atherosclerosis 1997; 32: 105-13.
17. Candito M, Bedoucha P, Gibelin P, et al. Fasting, postprandial, and post-methionine-load homocysteinaemia and methylenetetrahydrofolate reductase polymorphism in vascular disease. J Inherit Metab Dis 1999; 22: 588-92.
18. Van den Berg M, de Jong SC, Deville W, et al. Variability of fasting and post-methionine plasma homocysteine levels in normo- and hyperhomocysteinaemic individuals. Neth J Med 1999; 55: 29-38.
19. Montgomery H, Clarkson P, Dollery CM, et al. Association of angiotensin-converting enzyme gene I/D polymorphism with change in left ventricular mass in response to physical training. Circulation 1997; 96: 741-7.
20. Myerson SG, Montgomery HE, Whittingham M, et al. Left ventricular hypertrophy with exercise and the angiotensin converting enzyme gene I/D polymorphism: a randomized controlled trial with losartan. Circulation 2001; 103: 226-30.
21. Rigat B, Hubert C, Alhenc-Gelas F, et al. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990; 86: 1343-6.
22. Danser AH, Schalekamp MA, Bax WA, et al. Angiotensin-converting enzyme in the human heart. Effect of the deletion/insertion polymorphism. Circulation 1995; 92: 1387-8.
23. Cambien F, Poirier O, Lecerf L, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature 1992; 359: 641-4.
24. Samani NJ, Thompson JR, O'Toole L, et al. A meta-analysis of the association of the deletion allele of the angiotensin-converting enzyme gene with myocardial infarction. Circulation 1996; 94: 708-12.
25. Keavney B, McKenzie C, Delepine M, et al. Large-scale test of hypothesised associations between the angiotensin-converting-enzyme insertion/deletion polymorphism and myocardial infarction in about 5000 cases and 6000 controls. Lancet 2000; 355: 434-42.
26. Zisman LS. Inhibiting tissue angiotensin-converting enzyme. A pound of flesh without the blood? Circulation 1998; 98: 2788-90.
27. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. N Engl J Med 2000; 342: 145-53.
28. Dzau VJ. Circulating vs local renin-angiotensin system in cardiovascular homeostasis. Circulation 1988; 77 (Suppl 1): 4-13.
29. Ohmichi N, Iwai N, Kinoshita M. Expression of angiotensin converting enzyme and chymase in human atria. J Hypertens 1997; 15: 935-43.
30. Brown NJ, Blais C, Gandhi SK, Adam A. ACE insertion/deletion genotype affects bradykinin metabolism. J Cardiovasc Pharmacol 1998; 32: 373-7.
31. Martorana P, Kettenbach B, Breipohl G, et al. Reduction of infarct size by local angiotensin-converting enzyme inhibition is abolished by a bradykinin antagonist. Eur J Pharmacol 1990; 182: 395-6.
32. Wilson PW, D'Agostino RB, Levy D, et al. Prediction of coronary heart disease using risk factor categories. Circulation 1998; 97: 1837-47.