Association studies, ACE polymorphisms and renal disease

P. N. Harden1,2, K. P. J. O’Kane1, S. Ueda1, J. M. C. Connell1 and A. G. Jardine1

1Department of Medicine and Therapeutics, University of Glasgow, and 2Renal Unit, Western Infirmary, Glasgow, UK

Introduction

The genetic contribution to major disorders is increasingly recognized. This usually reflects interaction at a number of different loci to determine the eventual clinical phenotype, and dissecting out the role of individual components is a complex task. However, a clear understanding of the genetic factors involved in diseases such as hypertension, atherosclerosis, diabetes and glomerulonephritis is important as it will allow us to identify high-risk groups (those more likely to develop end-organ damage or progressive disease), and to target therapy at those most likely to benefit.

ACE as a paradigm

The angiotensin-converting enzyme (ACE) gene provides a useful paradigm. ACE converts angiotensin I to angiotensin II (Ang II), and is widely distributed in tissues [1]. Plasma and tissue ACE levels vary in the population; the variability being partly familial [2]. Following the identification of a bi-allelic polymorphism in intron 16 of the ACE gene (identified by the presence or absence of a 287 bp region of DNA) by Rigat et al. [3], it was reported that this genetic marker accounts for more than 50% of the variation in plasma ACE levels [3]. Subsequent studies have examined whether this "deletion/insertion" (D/I) polymorphism has associations with disease. An initial investigation suggested that subjects homozygous for the D allele were at increased risk of myocardial infarction [4]. Subsequently, several small studies confirmed this observation and extended it to demonstrate increased frequency of the D allele in subjects with a family history of ischaemic heart disease, and an increased risk of ventricular dilatation and death following myocardial infarction in D homozygotes [5–10]. However, a large study has since cast doubt on the association between ACE and ischaemic heart disease with the demonstration of negative findings in a sample of 1250 North American physicians [11]. Although low statistical power of the early studies may explain contradictory findings, an alternative explanation could be the presence of other discrete loci which contribute to ACE variability but are not in linkage with the D/I polymorphism [12]. The observation by Cambien that ACE levels provide a better marker of cardiac risk than the ACE polymorphism is consistent with this notion [13].

In other small studies, the DD genotype has also been associated with increased risk of sudden death in patients with hypertrophic [14] and ischaemic [8] cardiomyopathy, and with the development of left ventricular hypertrophy (LVH) in a general population and hypertensive subjects [15,16]. There is a general consensus that ACE is not implicated in essential hypertension [17,18], although positive associations were initially reported in small population studies [19].

If ACE activity (and ACE genotype) are implicated in cardiovascular disease these effects are likely to be mediated by increased generation of Ang II and the action of Ang II on growth and matrix production by vascular smooth-muscle cells (VSMC) in atherosclerotic plaques. In favour of this hypothesis, proliferation of VSMC is increased following transfection with the gene for ACE [20]. In man, subjects homozygous for the D allele have been shown to have an exaggerated pressor response to Ang I, indicating a functional role for the genotype [21]. At a tissue level, expression of the ACE gene in myocardium is also increased in subjects homozygous for the D allele [22].

Preliminary studies in renal disease

What evidence is there for the influence of ACE genotype in renal disease? Ang II is potentially important as a local regulator of cell growth and matrix production and as a pressor hormone. If there is increased Ang II generation one might expect altered intrarenal haemodynamics and increased cell growth and matrix production—factors known to be central to the progression of glomerulonephritis and the development of glomerulosclerosis [23]. The beneficial effects of ACE inhibitors in renal disease also suggest that increased ACE activity may be deleterious [23]. It is of interest that two studies in diabetic nephropathy
have suggested an association between DD genotype and the development of nephropathy [24,25], although a larger cross-sectional study found no association in either type I or type II diabetics [26]. In IgA nephropathy we were unable to show altered allele frequency. However, patients homozygous for the D allele did have a significantly greater rate of decline of GFR, and tended both to present at a younger age and to require renal replacement therapy earlier [27]. A similar report from Japan noted no difference in ACE genotype frequency in patients with IgA nephropathy in general but an increased frequency of DD genotype in patients requiring dialysis [28], again consistent with more severe disease in this group. These observations in renal disease are exciting but, particularly in view of the conflicting studies in cardiac disease, a large population study is urgently required. Moreover, in view of the high incidence of cardiovascular disease in renal failure patients, the relationship between ACE genotype and cardiovascular mortality or left ventricular hypertrophy in these patients are important questions which need to be addressed. In addition to the ACE gene, several other polymorphic markers are available including lipoproteins [29], angiotensinogen [30] and cytokines [31] for which a role in renal and associated cardiovascular disease might readily be tested.

Problems to test

Two major issues arise in renal disease. Firstly, which genetic factors predispose to the development of particular conditions, and secondly, which genes influence the highly variable natural history and rate of progression of most of the common forms of renal diseases [23]. It may be that these are related questions but it is highly likely that, as in essential hypertension, several genes will determine progression, whilst it is conceivable that single genes, perhaps in the MHC or basement membrane proteins, might confer susceptibility to some forms of primary renal disease. Identification of genetic markers for progression is not simply an intellectual exercise. If the findings on ACE genotype and diabetic nephropathy or IgA nephropathy are confirmed, it would be reasonable to target patients with the DD genotype with antihypertensive therapy, most probably with an ACE inhibitor, before any of the conventional indications such as hypertension or proteinuria occur. Even if this only delays the need for dialysis in individual patients by perhaps a year, the economic and human benefits would be considerable, and easily enough to justify the cost of early genotyping and intervention.

Proposals

Association studies using large populations of patients and of controls are the only practical way of addressing these issues in renal disease. Since no single centre is likely to have a sufficiently large population, this implies a need for multicentre collaboration in assembling a patient cohort. The need to recruit a large population from different centres gives rise to problems of genetic heterogeneity and racial mix and difficulty in assembling suitable control populations. The experience with the ECTIM study shows that such collaborations are possible [4]. Indeed, considerable interest derives in comparing interracial variation in genetic predisposition. We suggest that it is timely and appropriate to establish a European collaboration on genetic factors that affect the occurrence, progression and outcome of renal disease. Such a study needs to address the practical and ethical problems implicit in large-scale accumulation of patient data and information. In the first instance we suggest that interested groups agree common protocols for patient identification, inclusion and phenotyping DNA should be extracted and stored by individual centres, although exchange of material may be possible as the collaboration proceeds. Collaborating centres could agree to examine particular candidate genes, at which stage the question of assay standardization would need to be addressed. In some centres the hard work (extracting DNA and collecting clinical data) has probably already been done, since many workers have published on small DNA collections, and it is likely that there are many more unpublished negative studies. Furthermore, tissue typing laboratories have, for many years, been storing DNA from patients on transplant waiting lists. We propose to establish a central register of these resources to be published as a newsletter and on the internet at http://www.gla.ac.uk/acad/medther/dept/renal.html. Interested groups with DNA collections could advertise their availability here. The onus to establish collaborative studies would then lie with individuals. Contributing groups would retain the main DNA samples and patient data, circumventing ethical and data protection issues, which would continue to be addressed at a local level, allowing small samples to be used for analysis either locally or centrally (with the participating centres retaining patient data and sample coding). A worker with an interest in a given genetic marker would then establish a group from within the network to answer a particular question, for example, does ACE genotype affect progression of membranous glomerulonephritis? Finally, we should consider including DNA collection as part of the protocol for any large interventional studies in renal disease. As long as the protocol is fixed, response to treatment or differences in natural history could be reassessed by genotype either using currently available markers or future reanalysis using novel markers.

It is necessary to establish this collaborative approach now. The rapid pace of development of new genetic markers and of the human genome map means that the availability of candidate loci will not be rate limiting. The lessons from cardiovascular disease where multiple small studies have produced conflicting results illustrates the need for large, well-characterized, data-
sideroblastic anaemia, thrombocytopenia, neutropenia, pancreatic insufficiency, renal tubular dysfunction and hepatic dysfunction accompanied by lactic acidosis. However, the systemic symptoms may be misleading: retinopathy, blindness (which may be associated with various kidney diseases, including nephropathiasis in the Senior-Loken syndrome), deafness (suggesting Alport syndrome), diabetes mellitus, or intestinal villous atrophy. In addition, the clinical manifestations may not occur synchronously. In the case studied by Szabolcs et al. [5], the disease was revealed by megaloblastic anaemia, growth retardation, partial Fanconi syndrome and renal failure, while external ophthalmoplegia developed only later.

First-line biochemical screening for genetic defects of oxidative phosphorylation includes the determination of lactate, pyruvate and ketone bodies and their molar ratio in both fasted and fed subjects. The finding of hyperlactataemia points to a defect of oxidative phosphorylation. However, serum lactate concentration may be normal in some patients with mitochondrial cytopathies and renal involvement. This can be ascribed to proximal dysfunction which contributes to lowering blood lactate. Therefore, normal lactataemia does not rule out a mitochondrial disorder in a patient with Fanconi syndrome. Gas chromatography mass spectrometry can detect urinary lactate and citric acid cycle intermediates. Identification of the mtDNA mutation is the next step, taking into account the phenomenon of heteroplasmy, as discussed above.

Lastly, nephrologists are already exposed to one of the most common mitochondrial disorders, diabetes mellitus. Indeed, approximately 1 to 2% of cases of diabetes mellitus, often noninsulin-dependent, but sometimes insulin-dependent, are of mitochondrial origin [14]. Diabetes is often associated with sensorineuronal hearing loss. In rarer instances, multisystemic involvement [15] accompanies diabetes mellitus, such as in Wolfram syndrome in which diabetes insipidus, optic atrophy and deafness are also present, and which can be due to mtDNA mutations in some families [16]. These diabetic patients may progress to diabetic nephropathy. Some more common (hot-spot) mtDNA mutations such as the A 3243 G mutation of the mitochondrial tRNA^Leu (UUR) gene, have been reported in families with various phenotypic manifestations, often including diabetes mellitus. The variable clinical expression of a similar mutation could be ascribed to random segregation of mutated mitochondria in the different tissues, to a particular mtDNA haplotype or to the nuclear genetic background.

References