

Proteinuria and Angiotensin Converting Enzyme (ACE) Gene Polymorphism

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Abstract

Introduction: Although proteinuria is traditionally regarded as the result of glomerular damage, recent evidence suggests that proteinuria can directly cause renal damage through lysosomal injury, growth factor induced tubular fibrosis and transcriptase genes which trigger vasoactive and inflammatory renal damage. The renin angiotensin system which releases angiotensin, a most powerful vasoconstrictor and mediator of mesangial cell proliferation and matrix expansion, has been associated with progressive renal injury. This paper reviews the role of angiotensin converting enzyme (ACE) gene polymorphisms in the genesis of progressive renal failure, as well as their role in the response of proteinuria to ACE inhibitor (ACEI) or angiotensin receptor antagonist (ATRA) therapy. **Methodology:** The deletion/insertion polymorphism of the ACE gene has 3 genotypes DD, ID and II. Reported studies on the association of ACE gene polymorphisms with patients with IgA nephritis and progressive renal failure, including their response to ACEI or ATRA therapy, were reviewed. **Results:** Individuals with the DD genotypes have a higher risk of declining renal function with progressive IgA nephritis and more rapid deterioration to renal failure. Therapy with ACEI and or ATRA may be beneficial in patients with IgA nephritis with renal impairment and non-selective proteinuria as such patients may respond to therapy with improvement in protein selectivity, decrease in proteinuria and improvement in renal function. **Conclusion:** ACE genotypes appear to predict antiproteinuric response to ACEI therapy. ACEI/ATRA therapy probably modifies pore size distribution by reducing the radius of large non-selective pores, causing the shunt pathway to become less pronounced resulting in less leakage of protein into the urine.

Ann Acad Med Singapore 2000; 29:383-7

Key words: Genotypes, Glomerulonephritis, Protein selectivity, Renal injury, Therapy

Introduction

Proteinuria is considered a marker of renal disease.¹ Traditionally it has been thought that proteinuria is the result of diseased glomeruli. As the disease progressed, proteinuria worsens. Patients with glomerulonephritis (GN) of more than 1 g of protein excretion per day in the urine are more likely to have glomerulosclerosis or scarring of the kidney on renal biopsy and those exceeding 2 g a day, a higher incidence of progression to renal failure on follow up.²

Proteinuria and Disease Progression

More severe proteinuria is associated with faster progression of the renal disease, and this correlation is stronger for those patients with nephrotic range proteinuria.³ In essential hypertension, the onset of *de novo* proteinuria after a number of years of adequate blood pressure control is a marker of subsequent decline in renal function.⁴ Diabetic patients with persistent microalbuminuria have about twenty

times the risk of developing diabetic nephropathy.⁵ Albumin excretion rate (AER) has been shown to correlate with the rate of decline in glomerular filtration rate (GFR) in both insulin-dependent diabetes mellitus (IDDM) and non insulin-dependent diabetes mellitus (NIDDM) patients. However, at the stage of incipient nephropathy (AER 20 to 200 ug/ml), it is more difficult to relate levels of microalbuminuria to subsequent progression. In a study of IDDM patients with long duration of diabetes mellitus (>15 years), only 5 out of 18 progressed to clinical nephropathy in 10 years.⁶ Therefore, albuminuria alone may not predict change in GFR in all diabetic patients. In a recent study of 176 patients studied over a median of 5 to 8 years, baseline AER, male sex, presence of retinopathy, serum cholesterol and HbA1C were the strongest predictors for development of incipient nephropathy.⁷ A complicating factor in the natural history of diabetes mellitus is that a subgroup of patients showed a decline in renal function without increasing AER.

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Intrinsic Toxicity of Filtered Proteins

Hitherto, it was believed that proteinuria is the result of damage to the kidneys but recently, evidence suggests that the converse is also true, that proteinuria can also directly cause renal damage.

When there is excessive leakage of protein in the renal tubules, the proximal tubular cells (PTC) become overloaded with protein. Lysosomes present in the PTC when they engulf excessive proteins, would swell, rupture and release injurious lysosomal enzymes which cause tubulointerstitial damage and fibrosis and with time, give rise to renal failure. In the second mechanism, protein overload of the PTC also triggers the release of certain growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF- β) which are mitogenic to the PTC. They cause excessive production of collagen as well as interstitial cell proliferation, eventually leading to fibrosis and renal failure. Finally, protein overloading of the PTC causes the activation of transcriptase genes which in turn trigger genes encoding vasoactive and inflammatory mediators; the release of these lead to vasoconstriction and inflammation of the renal tissue with injury and renal failure.⁸

ACE Gene Polymorphism

Individuals are prone to certain diseases because of disease susceptibility genes. In many instances, such genes are present at birth, while in other situations, a particular set of genes could be modified by environmental factors, predisposing the individual to development of a certain disease.

The renin angiotensin system (RAS) which regulates salt and blood pressure within our body comprises genes which have several forms of polymorphism. One such polymorphism is the deletion/insertion polymorphism of the angiotensin converting enzyme (ACE) gene. The ACE gene is made up of 26 exons and spans 21 kb on chromosome 17.⁹ A polymorphism exists within intron 16, consisting of the presence or absence of a 287 base pair fragment. This deletion polymorphism is associated with raised serum and cellular ACE levels and may be associated with hypertension, left ventricular hypertrophy and ischaemic heart disease. However, this association varies depending on the genetic make-up and environmental factors affecting individuals.^{10,11} Angiotensin II is a most powerful vasoconstrictor within the body. It promotes hypertrophy and collagen accumulation within the heart leading to ischaemic heart disease and heart failure. Within the kidney, it causes mesangial cell proliferation, matrix expansion leading to progressive renal injury with eventual renal failure.

IgA nephritis is one disease where the role of renal injury related to the RAS is well documented. Yoshida et al¹²

studied 53 patients with IgA nephritis who had at least 10 years of follow up since onset of symptoms, in relation to reduction in creatinine clearance over 5 years and performed genotype testing on them to determine the insertion/deletion locus of the ACE gene. The frequency of 3 genotypes was documented. The genotypes were:

- 1) homozygote for deletion allele (DD);
- 2) heterozygote (ID);
- 3) homozygote for insertion allele (II).

The DD genotype was more frequently associated with declining creatinine clearance and had 4-fold risk of progression to renal failure compared to those with ID or II genotype. The increased risk of renal failure with the ACE DD genotype was a risk factor independent of the risk factor of hypertension. It was found that the ACE DD genotype was significantly higher in frequency among patients with declining renal function even in the absence of hypertension. These authors¹² further found that, patients with glomerular hypertrophy at the time of biopsy with the DD genotype (7/9) developed loss of renal function 10 years later compared to patients (0/4) with no glomerular hypertrophy. Also patients with the DD or ID genotype had more glomerulosclerosis compared to those with II genotype.

Among Caucasian series, the ACE DD genotype was also associated with progressive decline of renal function in IgA nephritis,^{13,14} indicating that the ACE I/D genotype crosses geographical and racial boundaries.

Basis for Treatment of Proteinuria using ACEI

Local generation of angiotensin II in the kidney, formed in excessive amounts in response to haemodynamic injury to the endothelium in glomerular hyperfiltration, shifts glomerular non-selective pores to larger dimensions (i.e. patients with non-selective proteinuria become even more non-selective). Experimental and human evidence suggest that ACEI tends to improve non-selective proteinuria, but how they do so is still speculative. The improvement in the selective properties of the glomerular capillary wall induced by ACEI could be the result of changes in the morphometry, as reflected by changes in the macromolecular organisation of the protein matrix in the glomerular basement membrane (GBM) or the slit-diaphragm of the podocytes. This is consistent with recent findings that ACEI reduces RNA expression for extracellular matrix components in experimental diabetes,¹⁵ as well as in an immunological rat model with massive proteinuria.¹⁶

ACE inhibitors in animals and humans reduce the log-normal component of the assumed pore size distribution¹⁷ and more importantly, cause the shunt pathway to become less pronounced by reducing the radius of large non-selective pores.¹⁸

Progression of Renal Disease and Protein Selectivity

There is evidence that in certain renal diseases like focal and segmental glomerulosclerosis, IgA nephritis, lupus nephritis and diabetic nephropathy, the degree of interstitial mononuclear cell infiltrates correlate with the rate of renal functional decline.¹⁹ The presence of chronic tubulo-interstitial lesions in these diseases is correlated with declining renal function.²⁰ What these diseases have in common is non-selective proteinuria. In IgA nephritis, the non-selective proteinuria was correlated with tubulo-interstitial lesions and a higher incidence of renal failure.²¹

In contrast to the above diseases, interstitial infiltrates seldom develop in minimal change disease (MCD) despite heavy proteinuria. The obvious reason is that the period of heavy protein excretion in the renal tubules is of short duration since MCD responds promptly to steroids. The other reason is the difference in the quality of the proteinuria. In MCD, patients have selective proteinuria and excrete albumin, which is relatively innocuous compared to the larger immunoglobulins like IgG in non-selective proteinuria, which can alter expression of tubular activation molecules much more than albumin. In addition, non-selective proteinuria normally includes complement components C 5 to 9, which enter tubular fluid and allow complement-mediated injury resulting in tubulo-interstitial inflammation and injury as in focal and segmental glomerulosclerosis and diabetic nephropathy.

ACE I/D Polymorphism and Response to ACEI/ATRA Therapy

Since ACE inhibitors decrease ACE activity, ACE inhibitor therapy in patients with IgA nephritis would be expected to reduce renal injury in these patients. We have shown that ACEI therapy does lead to decreased proteinuria and retardation of the progression of renal failure in IgA nephritis. In our study we also showed that angiotensin II receptor antagonist (ATRA) is as effective as ACEI in decreasing proteinuria and preservation of renal function.²²

It has been postulated that ACEI/ATRA (ACE inhibitor/angiotensin receptor antagonist) may decrease proteinuria in patients with glomerulonephritis by its action on the glomerular basement membrane. We performed a study²² to examine the relationship between the response of patients with IgA nephritis (IgA Nx) to ACEI/ATRA therapy by decreasing proteinuria and its effect on the selectivity index (SI) in these patients. Forty-one patients with biopsy proven IgA Nx entered a control trial with 21 in the treatment group and 20 in the control group. The entry criteria included proteinuria of 1 g or more and/or renal impairment. Patients in the treatment group received ACEI /ATRA or both with 3 monthly increase in dosage. In the control group, hypertension was treated with atenolol,

hydralazine or methyldopa. After a mean duration of therapy of 13 ± 5 months, it was noted in the treatment group, there was no significant change in serum creatinine, proteinuria or SI; but in the control group, serum creatinine deteriorated from 1.8 ± 0.8 to 2.3 ± 1.1 mg/dL ($P < 0.05$). Among the 21 patients in the treatment group, 10 responded to ACEI/ATRA therapy determined as decrease in proteinuria by 30% (responders) and the other 11 did not (non responders). Among the responders, SI improved from a mean of 0.26 ± 0.07 to 0.18 ± 0.07 ($P < 0.001$) indicating a tendency towards selective proteinuria. This was associated with improvement in serum creatinine from mean 1.7 ± 0.6 to 1.5 ± 0.6 mg/dL ($P < 0.02$) and decrease in proteinuria from mean of 2.3 ± 1.1 g/day to 0.7 ± 0.5 g/day ($P < 0.001$).

After treatment, proteinuria in the treatment group (1.8 ± 1.6 g/day) was significantly less than in the control group (2.9 ± 1.8 g/day) ($P < 0.05$). The post-treatment SI in the responder group (0.18 ± 0.07) was better than that of the non responder group (0.33 ± 0.11) ($P < 0.002$). Eight out of 21 patients in the treatment group who had documented renal impairment had improvement in their renal function compared to 2 in the control group ($\chi^2 = 4.4$, $P < 0.05$). Of the 8 patients in the treatment group who improved their renal function, 3 normalised their renal function.

Our study suggests that ACEI/ATRA therapy may be beneficial in patients with IgA Nx with renal impairment and non-selective proteinuria, as such patients may respond to therapy with improvement in protein selectivity, decrease in proteinuria and improvement in renal function. ACEI/ATRA therapy probably modifies pore size distribution by reducing the radius of large non-selective pores, causing the shunt pathway to become less pronounced and resulting in less leakage of protein into the urine.

Individual antiproteinuric response to ACEI/ATRA therapy varies depending on ACE gene polymorphism as those with the DD genotype respond better to the antiproteinuric effect of ACEI/ATRA therapy.^{12,23} Yoshida et al,²⁴ in a study examining the role of the deletion polymorphism of the ACE gene in the progression and therapeutic responsiveness of IgA nephropathy using the ACEI lisinopril, reported that the ACEI lisinopril significantly decreased proteinuria in the DD genotype patients but not in the II or ID genotype. Similar findings have also been reported by Moriyama et al.²⁵

In a third series,²³ the DD genotype was found to predict a stronger antiproteinuric response to ACEI therapy in a subgroup of patients in the series where patients had non-nephrotic range proteinuria exceeding 1 g/day, not on diuretics. However, two other studies from Europe^{26,27} in patients with a variety of renal diseases with proteinuria found that those with the DD genotype were less responsive

to ACEI therapy. There could be a genetic explanation to this.

The underlying mechanism for the association of the DD genotype with the antiproteinuric efficacy of ACEI therapy is related to the serum and tissue levels of ACE.^{28,29} Individuals with DD genotype have higher serum ACE levels than those with II genotype, whereas those with ID genotype have intermediate values. There is more ACE activity to inhibit in those with the DD genotype since they have the highest levels and therefore, such patients have the most dramatic decrease in proteinuria with ACEI therapy.

The antiproteinuric effect of ACEI is independent of its effect on blood pressure.³⁰ Various studies^{24,25} have shown that there was no association of the I/D polymorphism of the ACE gene with blood pressure response to ACEI in the presence of an association with the antiproteinuric effect.

Whilst patients with the DD genotype with renal disease have better response to the antiproteinuric effect of ACEI/ATRA as they have very high serum and tissue ACE levels, it also means that those patients are also the ones more likely to develop progressive renal disease leading to renal failure. In diabetic nephropathy, patients with the DD genotype had a significantly shorter time interval from onset of diabetes to initiation of dialysis compared to those with ID and II genotype.³¹ The clinical course of the disease is accelerated in patients with the DD genotype.

There are at this time some unanswered questions with regards to ACEI/ATRA therapy in these DD genotype patients. In view of their high ACE levels, does it mean that they will require a larger dose of ACEI/ATRA to reduce proteinuria? In other words, a small dose may not be effective in these patients. One may have to increase the dose of ACEI/ATRA progressively until the maximum tolerable dose is prescribed. The comforting thought is that, as long as ACE levels are inhibited, renal damage could be contained. In some of the patients in our trial, ACEI/ATRA therapy has restored normal renal function amongst those with renal impairment. Hopefully, for as long as these patients are on ACEI/ATRA therapy, renal function can remain normal and renal damage repaired if not contained. We have embarked on our own studies of ACE gene polymorphism in our patients and would attempt to find answers to some of these questions.

Recent studies have also demonstrated that ACEI, apart from remodelling the GBM with the improvement in pore selectivity being one of the end results, may also have beneficial effects on the mesangial cells by decreasing TGF- β production, thereby decreasing mesangial matrix production and ameliorating the disease process in IgA Nx where there is mesangial cell proliferation.³² In addition, ATRA has been shown to exert an antiproliferative effect on mesangial proliferative GN.³³ This could help to further

explain the improvement in renal function in patients with IgA Nx on ACEI/ATRA therapy. In order for this to occur, the injury must still be at the stage where it is possible for amelioration of the renal lesions and possibly remodelling of the renal architecture. Patients with more advanced renal disease with glomerulosclerosis and thickening of the GBM are less likely to respond to therapy with improvement in SI and serum creatinine. In this respect, we found that patients with serum creatinine exceeding 3 mg/dL are less likely to recover renal function, though therapy with ACEI/ATRA may retard their long-term progression to end-stage renal failure (ESRF).

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