

## Renoprotection: Clues from knockout models of rare diseases

Glomerulopathies due to defective known genes are becoming a central source of information in the field of mechanisms of progression of renal disease. Among hereditary diseases, Alport syndrome of glomerulopathy and hearing loss has long been the best recognized one and yet the disease remains a major challenge for both basic and clinical scientists. Mutations of the gene encoding for the  $\alpha 5$  chain of type IV collagen are the molecular basis in the classic form, inherited in an X-linked dominant pattern (85% of cases). Genetic and phenotypic heterogeneity, however, is large and other patients have autosomal-recessive or, most rarely, autosomal forms resulting from mutations of  $\alpha 3$  or  $\alpha 4$ (IV) collagen chain genes. The target organs reflect the sites where these collagen chains are normally highly expressed and assembled into functional networks. Thus, the abnormal  $\alpha 5$  chain appears to prevent incorporation of  $\alpha 3$  or  $\alpha 4$  chains into the triple helical collagen monomers of the glomerular membrane. Common to different Alport forms are the typical splitting of the glomerular basement membrane, persistent hematuria, and variable degrees of proteinuria. End-stage renal failure almost inevitably develops in male patients, as well as in patients with autosomal-recessive Alport syndrome in the long term. Despite advances in defining the molecular lesion, mechanisms underlying the progressive injury in Alport syndrome have remained obscure. Furthermore, there is no accepted treatment that may delay the time of dialysis or a kidney transplant. Cyclosporine treatment given for 7 to 10 years to eight patients with Alport syndrome (on the theoretical basis of a favorable hemodynamic action) reduced proteinuria and was associated with no aggravation of lesions present at the first biopsy [1]. Angiotensin I-converting enzyme (ACE) inhibitors have been attempted recently and few preliminary results are published. Proesmans, Knockaert, and Trouet [2] administered enalapril at a starting dose of 0.1 mg/kg body weight per day to five children with the classical form and two siblings with the autosomal-recessive form. Overall, proteinuria and protein/creatinine ratio decreased at 6 months and reached nadirs at 18 months (approximately one fifth and one tenth from the baseline levels of 40 mg/kg/day and 2.5). This effect, which was observed in five patients,

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partially or completely reversed at 24 months without clear change in creatinine clearance. Other reports described no effects of ACE inhibitor in few sporadic cases.

In this issue of *Kidney International*, Gross et al [3] compared the effects of ramipril given since early stage versus more advanced disease on renal structure and function in homozygous  $\alpha 3$ (IV) chain (COL4A3) knockout mice, a model for autosomal-recessive Alport syndrome. The ACE inhibitor given to mice since 4 weeks of life markedly delayed the onset of proteinuria, progressive renal damage, and uremia. Remarkably, the treatment also prolonged their lifespan by several weeks, up to 150 days on average instead of 71 days if continued until death. Ramipril showed no apparent protective effect against the ultrastructural changes of the glomerular basement membrane. Conversely, drug treatment exerted antifibrotic effects attributable to inhibition of excess transforming growth factor  $\beta$  (TGF- $\beta$ ) production. ACE inhibitor beginning at 7 weeks of age, when the animals were already heavily proteinuric, had no effect on renal damage and survival. The main findings in the paper by Gross et al [3] that the prolonged treatment with the ACE inhibitor, and, to a lesser extent, a treatment given for a shorter period postponed the onset of uremia and prolonged life revives hopes that patients with Alport syndrome may have their kidney function preserved to a significant extent. It was already known that the ACE inhibitor could have beneficial effects against proteinuria, renal function deterioration, and survival in Samoyed dogs, a model of X-linked hereditary nephropathy closely mimicking human Alport syndrome [4]. However, factor(s) that may be affected by ACE inhibitor therapy have remained elusive. In the study by Gross et al [3], a role for increased glomerular capillary pressure could not be formally excluded and the experimental protocol lacked a group of mice treated with other antihypertensive drugs to achieve comparable levels of blood pressure. Nevertheless, hypertension usually occurs late and does not seem to play a primary pathogenic role. On the other hand, the reduction of proteinuria has been established as an important strategy to retard or prevent the loss of renal function in progressive nephropathy. In this respect, the effect of ACE inhibitor in the COL4A3 knockout mouse, as well as in the dog model, was no exception. The knockout mice studied by Gross et al uniformly displayed a peculiar onset of disease at 4 weeks and rapid development of severe proteinuria (>5g/L) by 8 weeks eventuating in end-stage renal

failure. Therefore, it is not surprising that the delayed ACE inhibitor treatment given from 7 weeks, despite transient amelioration of the nephrotic syndrome (from 6.5g/L to 3.0 g/L) and reduction in TGF- $\beta$  levels (50%) by Western blot [3], failed to protect the mice from both further worsening of proteinuria and progressive injury. Thus, although factors inherent to the Alport model might play a role, the continued exposure of the renal parenchyma to deleterious effects of glomerular barrier dysfunction, possibly including excess TGF- $\beta$  both prior to and during the delayed treatment, would explain the resistance to therapy.

The recent discovery of key molecules of the slit diaphragm has conclusively led to recognize that the podocyte maintains the integrity of the normal glomerular barrier to macromolecules in such a way that inherited abnormalities in podocyte genes may have devastating consequences on glomerular and tubular cell biology. Impressively, no studies have been published on pathway(s) leading to proteinuria in Alport disease. Thus, putative mechanisms of antiproteinuric action by ACE were left completely unaddressed. We only know that the ACE inhibitor treatment in the dog model caused a transient reduction of in the splitting of glomerular basement membrane [4], whereas ramipril appears to fail to do so in COL4A3 $-/-$  mice [3]. The drug is not expected to affect substantially the inherited molecular lesion of abnormal collagen network within the glomerular basement membrane. In the COL4A3 $-/-$  mice, however, the observation that ramipril preserved the slit diaphragms and podocyte morphology is a hint that suggests that the podocyte component of the permselective barrier was the primary target of renoprotective therapy. Such mode of the drug's action would be in agreement with findings that the ACE inhibitor prevented glomerular redistribution of ZO1 [5] and the reduction in synthesis of nephrin [6] in spontaneous or immune models. We are now also learning from knockout models of genetic glomerulopathies, for example, in *Nphs2* $-/-$  mice deficient for podocin and in  *$\alpha$ -actinin 4* $-/-$  mice (abstracts; Roselli et al, *J Am Soc Nephrol* 13:17A, 2002; Kos et al, *J Am Soc Nephrol* 13:17A, 2002), that the lack of slit diaphragm- or foot process-associated molecules promotes both progressive proteinuria and glomerulosclerosis. Of interest, COL4A3 and podocin synthesis was severely reduced in mice deficient for a transcription factor, LMX1B, and mutations in LMX1B cause nail patella syndrome, a related hereditary glomerulopathy [7]. It will become crucial to establish whether and how angiotensin II and ACE inhibitors may influence the expression of transcription factors and genes of the barrier both in cultured podocyte models and by in vivo studies. Rare diseases due to defective glomerular genes can be a powerful tool to understand the mechanisms of the drugs' action and resistance and to identify novel molecular

targets of therapy in progressive nephropathy. For example, in Alport syndrome, where the long-term stability of the ultrafiltration barrier is lacking due to defective  $\alpha 3$ - $\alpha 4$ - $\alpha 5$  networks, the ACE inhibitor may initially stabilize the barrier by maintaining slit membrane function, but the inability to normalize the basement membrane network, particularly if the network is already severely compromised, might ultimately account for abnormal permeability of the membrane and long-term failure of therapy. Finally, regardless of etiology, angiotensin II is most likely to play a pivotal role in loss of glomerular permselectivity and progressive renal damage. In isolated rat kidney preparations, the perfusion with angiotensin II directly impairs the barrier, resulting in increases in clearance of dextran macromolecular probes and protein excretion rate amenable to abrogation by ACE inhibitor therapy. Excess protein uptake can also be deleterious in itself by promoting TGF- $\beta$  synthesis and podocyte dedifferentiation [8]. The simultaneous inhibition of these pathways may greatly contribute to the beneficial effect of the ACE inhibitor.

Gene replacement therapy appears to be a feasible prospect for Alport syndrome [9]. In the meantime, alternative approaches should pursue the goal of preserving the glomerular barrier. Combining ACE inhibitors and angiotensin II receptor antagonists may offer a better therapeutic effect than the treatment with an ACE inhibitor alone. An additional strategy may be aimed at minimizing the consequences of permselective barrier dysfunction. For example, rats with passive Heymann nephritis were protected by further combining a statin that may act by inhibition of interstitial fibrogenic reactions [10]. Gross et al point to specific inhibition of TGF- $\beta$ , a target also shown to be effective in other models. In Alport syndrome, the early detection of patients at risk might be needed even before signs of renal disease develop. Only well-designed clinical trials can indicate which will be the best way to prevent end-stage renal failure.

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