ANGIOTENSIN CONVERTING ENZYME 2 IN THE KIDNEY

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SUMMARY

1. Angiotensin converting enzyme 2 (ACE2) is an important homeostatic component of the renin angiotensin system (RAS). ACE2 both degrades the vasoconstrictor, angiotensin II and generates the potent vasodilator peptide, angiotensin 1–7. These actions counterbalance those of ACE.

2. ACE2 is highly expressed in the healthy kidney, particularly in the proximal tubules, where it colocalizes with ACE and angiotensin receptors.

3. Kidney disease and subtotal nephrectomy is associated with a reduction in renal ACE2 expression, possibly facilitating the damaging effects of angiotensin II in the failing kidney. Acquired or genetic ACE2 deficiency also appears to exacerbate renal damage and albuminuria in experimental models, supporting this hypothesis.

4. ACE2 also has an important role in blood pressure control. Many models of hypertension are associated with reduced ACE2 expression. Although *ACE2* KO animals are normotensive, in states associated with activation of the RAS, ACE2 overexpression improves blood pressure control and reduces angiotensin responsiveness.

Key words: angiotensin 1–7, angiotensin II, angiotensin converting enzyme 2, diabetes, hypertension, renal impairment, rennin-angiotensin system.

RENIN-ANGIOTENSIN SYSTEM

The rennin-angiotensin system (RAS) is a pivotal mediator of renal and vascular homeostasis.[1] Its systemic actions include the regulation of blood pressure, natriuresis and blood volume control. The RAS also plays an important role at a local level, regulating regional blood flow and controlling trophic and fibrotic responses to a range of stimuli. The RAS is composed of a number of different regulatory components and effector molecules that facilitate the dynamic control of renal function, in both health and disease. Many of these components have opposing functions to accommodate a rapid response with regards to the balance of vasoconstriction and vasodilatation in response to specific triggers (Fig. 1). For example, angiotensin I

(Ang I) is metabolised by the dipeptide carboxypeptidase, angiotensin converting enzyme (ACE) to form angiotensin II (Ang II), a peptide considered to be the major effector of the RAS. At the same time both Ang I and Ang II may be metabolised by the carboxypeptidase ACE2, producing, respectively, angiotensin$_{(1–9)}$ (Ang 1–9) and the vasodilator, angiotensin$_{(1–7)}$ (Ang 1–7).[2-4] ACE is also the major enzyme involved in the extracellular degradation of Ang 1–7, further emphasizing that the balance of synthesis and degradation occurs at many levels within the RAS. This review will examine the effects of ACE2 in the kidney, in both health and various disease states.

Angiotensin converting enzyme 2

Angiotensin converting enzyme 2 (ACE2) is a type 1 integral membrane glycoprotein comprising 805 amino acids.[5] The human *ACE2* gene maps to the chromosomal location Xp22.[5] Analysis of the genomic sequence has identified that *ACE2* contains 18 exons, of which several have similarity with the first 17 exons of human ACE[5] suggesting a common origin. ACE2-like enzymes are also seen in non-vertebrate species, including the fruit fly *Drosophila melanogaster*, where they have an important role in cardiac development.[6]

ACE2 is expressed in many tissues, with the highest expression observed in the kidney, cardiovascular tissues and the testis.[2,5] Lower levels of ACE2 expression have also been documented in a range of other tissues, including the gastrointestinal tract,[7] liver,[8] retina,[9] central nervous system, lymphoid tissues[7] bone marrow[10] and pancreas.[11] Like ACE, the extracellular surface of the human ACE2 enzyme contains a catalytic metallopeptidase domain (residues 19–611) that shares 60% homology with the testis ACE isoform and 42% sequence identity and 61% sequence similarity with the N-terminal catalytic domain of somatic ACE.[2] However, unlike ACE, it contains only one active enzymatic site and is not susceptible to ACE inhibitors.[4] In addition, ACE2 functions as a carboxypeptidase, distinct and complementary to the dipeptidyl carboxypeptidase activity of ACE. The substrate affinity of ACE2 is also not confined to the angiotensin peptides. For example, ACE2 is able to cleave the C-terminal amino acid from vasoactive bradykinin (1–8) (also known as des-Arg⁹-bradykinin) to form the inactive peptide, bradykinin (1–7) and metabolize des-Arg-kallidin (also known as des-Arg¹⁰ Lys-bradykinin).[2] Other peptide compounds including dynorphin A, casamorphin, neurotensin 1–13, apelin 13, apelin-36 and kinetensin may also be hydrolyzed by ACE2 *in vitro*.[12] ACE2 also appears to be a functional receptor for the novel coronavirus implicated as the causative agent of Severe Acute Respiratory Syndrome.[13]

Angiotensin peptides in the healthy kidney

Most intrarenal Ang II is generated within the kidney[14] particularly by the proximal tubular cells.[14] This results in Ang II levels that are
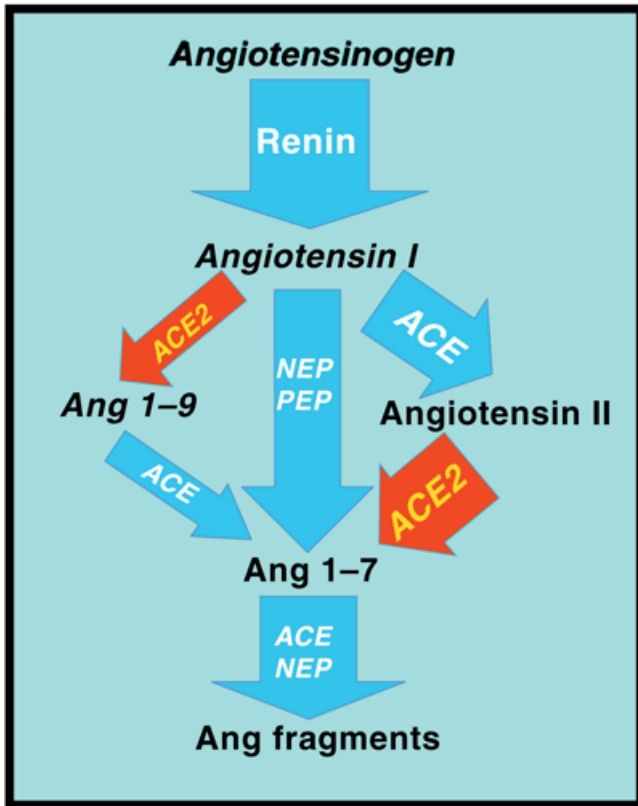
Fig. 1 Schematic representation of the proteolytic components of the renin–angiotensin system. ACE, angiotensin converting enzyme; ACE2, angiotensin converting enzyme 2; NEP, neutral endopeptidase-24.11; PEP, prolyl endopeptidase.
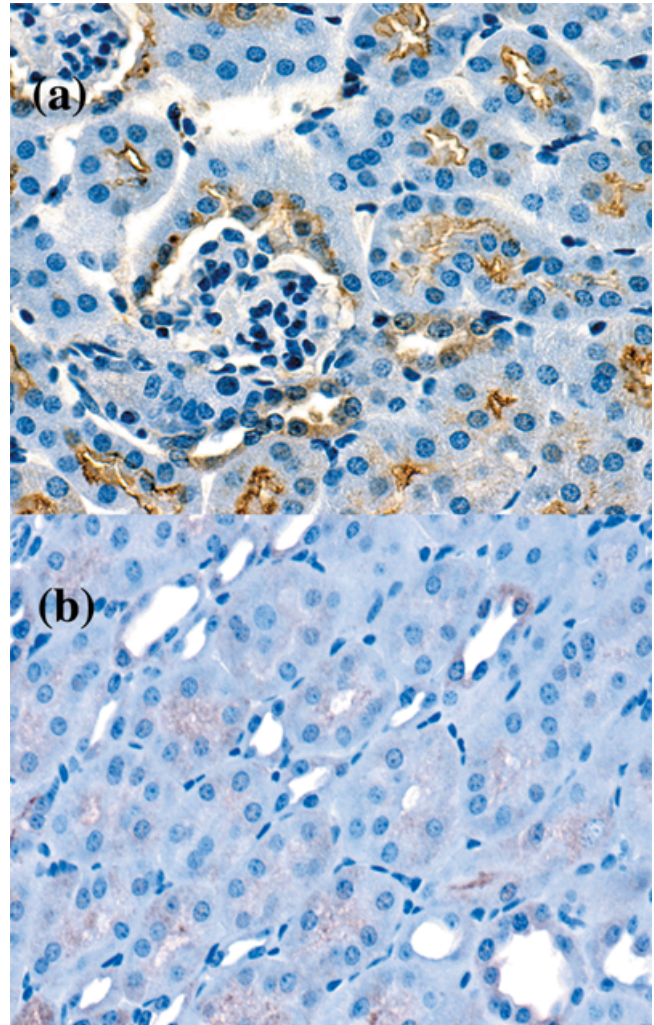

Fig. 2 The renal expression of angiotensin converting enzyme 2 (ACE2) in cortical sections from control (a) is modified following the induction of experimental diabetes in male C57Bl6 mice (b). Original magnification ×40.

1000-fold higher in the renal interstitium than in the circulation. Ang II modulates glomerular filtration by influencing intraglomerular capillaries through effects on both afferent and efferent arterial tone. Ang II also influences glomerular perm-selectivity by direct effects on mesangial cell contraction[15] and on the glomerular sieving coefficient.[16] In addition, Ang II stimulates renal salt retention *via* direct actions on the proximal tubule as well as stimulation of aldosterone secretion.

Ang 1–7 has an important counter-regulatory role in the homeostasis of normal renal function. The renal vasculature is highly sensitive to the vasodilator effects of Ang 1–7, particularly at the afferent arteriole[17] leading to an increase in glomerular filtration rate following an infusion of ang 1–7.[18] At physiological concentrations, Ang 1–7 also influences glomerular filtration pressure[19,20] sodium balance, bicarbonate and water reabsorption, opposing many of the actions of Ang II.[21]

Other angiotensin peptides are also present in the kidney, some of which maintain a certain degree of biological activity.[22] For example, Ang 1–7 undergoes further processing by ACE, which cleaves two more amino acids from its C-terminal, resulting in Ang 1–5,[2] which is itself further degraded to Ang 3–5[23] or Ang 1–4. These peptide 'fragments' may also have some vasodilatory and trophic actions. Angiotensinogen itself may also have renal effects of its own. For example, angiotensinogen overexpressing mice demonstrate inhibited growth of renal artery walls.[24] However, the biological significance of these other angiotensin peptides remains to be fully elucidated.

ACE2 in the healthy kidney

As noted above, ACE2 is present in high levels in the adult kidney.[2] ACE2 is predominantly expressed in the proximal tubule, where it is co-localized with ACE to the luminal brush border (Fig. 2). This tubular distribution is also similar to the distribution of Ang 1–7, its major product.[25] ACE2 expression has also been documented at other parts of the nephron, including expression in glomerular podocytes[26] and the endothelium of renal capillaries.[27] Epithelial cells from the distal tubules and collecting ducts have also been reported to show weak cytoplasmic staining for ACE2,[28] although cross-reactivity with the ACE2-homolog, collectrin which is expressed at these sites within the kidney,[29] may have contributed to this finding.

The major actions of ACE2 in the kidney reflect its major role in the efficient generation of renal Ang 1–7. The catalytic efficiency of ACE2 for the generation of Ang 1–7 is high, with a k_{cat}/K_m of 2.2×10^6/M per s. Notably, this activity is greater than that of neutral endopeptidase (NEP) or even ACE for their respective angiotensin peptides.[12] Its catalytic ability is also significantly higher than that of the prolyl-peptidases and other enzymes implicated in the genesis of Ang 1–7, suggesting that ACE2 is the dominant renal source of

Ang 1–7.[12] Recent studies have confirmed this hypothesis, showing that ACE2 inhibition completely blocks the generation of Ang 1–7 using rodent isolated proximal straight tubules.[30]

Although the angiotensinase activity of ACE2 in the healthy kidney is well understood, its role in the maintenance of normal physiology remains to be determined. Notably, both kidney function and renal development are normal in the *ACE2* knockout (KO) mouse, despite the presence of unopposed ACE activity and elevated Ang II levels.[31] By comparison, *ACE* KO mice have abnormal tubuloglomerular feedback responses and display a number of alterations in kidney morphology. Alterations in the renal architecture have also been described for the mice lacking *angiotensinogen* or receptors for Ang II.[32] Although no renal abnormalities are detected in the *ACE2* KO mice at 3 months of age, more recently, it has been suggested that the same mice by 12 months of age show a degree of glomerulosclerosis, with mesangial matrix expansion, extensive foci of segmental hyalinosis and deposition of fibrillar collagen.[33] While it is possible that these changes may have occurred as a result of the downstream effect of poor cardiac function that has been described in these animals[31] it is also possible that activation of the RAS associated with ageing may require the actions of ACE2 to maintain normal renal function/glomerular pressure.

RAS in kidney disease

The importance of RAS to the development and progression of kidney disease is well established. While, in the short term, activation of RAS is crucial for maintaining renal homeostasis, particularly in states of effective or absolute volume depletion, this occurs at the expense of glomerular hypertension. Ultimately, activation of the intrarenal RAS becomes counter-productive, resulting in progressive nephron *burnout* placing even more 'pressure' on the remaining nephron mass.[34] The same phenomenon occurs in the renal tubule as compensatory (i.e. Ang II-mediated) hypertrophy and leads to tubular damage, tubular drop-out and interstitial fibrosis. In addition, Ang II directly stimulates the increased synthesis and accumulation of glomerular and interstitial matrix proteins, such as collagen and fibronectin, *via* activation of TGF-β and other fibrogenic cytokines.[35] The pivotal importance of the RAS in kidney disease is best illustrated by the clear efficacy of agents that block the RAS, over and above effects on systemic blood pressure.[36,37]

Diabetic kidney disease is associated with significant changes in the intrarenal RAS.[28] For example, following the induction of experimental (streptozotocin) diabetes in Sprague Dawley (SD) rats, the expression of ACE protein is upregulated in glomeruli[38] but reduced in the proximal tubule, reflecting the general loss of brush border proteins associated with albuminuria. In renal cortical biopsies from patients with type 2 diabetes the expression of *ACE* mRNA is also upregulated.[39]

ACE2 and the diabetic kidney

Recent data suggest that similar changes are also observed in the expression of ACE2. For example, expression of ACE2 is down-regulated in tubules from streptozotocin-induced diabetic Sprague Dawley rats, while at same time the expression of *ACE2* mRNA in diabetic glomeruli is increased.[28] This site-specific disruption of ACE2 expression in the diabetic kidney is similar to that observed in spontaneously hypertensive rats, where glomerular and tubular ACE2 *mRNA* expression are also differentially affected.[40]
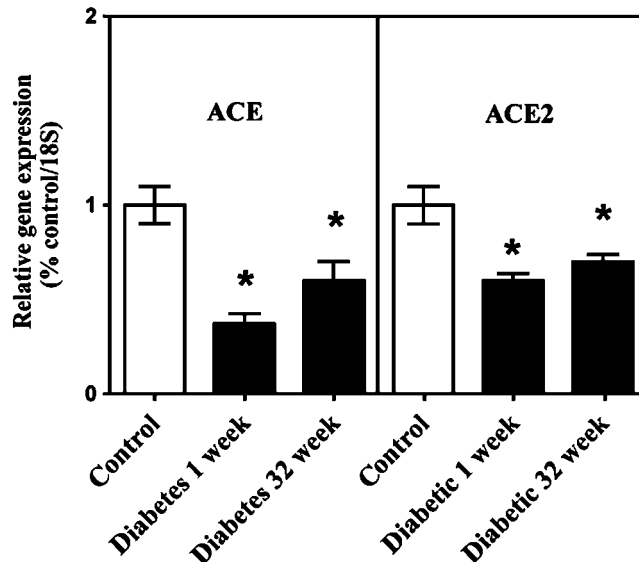

Fig. 3 Expression of angiotensin converting enzyme (ACE) and ACE2 gene as measured by real-time reverse transcription-polymerase chain reaction (RT-PCR) in cortical homogenates from control and diabetic (1 and 32 weeks) Sprague-Dawley rats. Data are shown as mean percentage control ± SEM; *$P < 0.05$ versus control.

The role of ACE2 in the development and progression of diabetic kidney disease is a matter of ongoing investigation. In our original study, we observed *ACE* and *ACE2* mRNA to be decreased in the diabetic SD kidney at 24 weeks of diabetes.[28] However, it appears that changes in the expression of ACE2 precede the development of nephropathy. For example, reduced renal expression of ACE2 may be observed after as little as 1-week of experimental diabetes, before any detectable change in albuminuria or tubular injury (Fig. 3). In addition, two recent studies have also confirmed that changes in ACE2 expression are able to influence injury in the diabetic kidney. First, in the streptozotocin diabetic mouse, a selective ACE2 inhibitor led to increased albuminuria in association with glomerular matrix expansion.[41] More recently, Wong *et al.* demonstrated that when an Akita mouse, a model of type 1 diabetes, was crossed with an ACE2 KO mouse there was a twofold increase in albuminuria, which was accompanied by pathological changes in the kidney including an increase in mesangial matrix and glomerular basement thickening.[42] Although both these studies suggest that the loss of ACE2 increases renal injury in diabetes,[42] whether this is directly due to reduced ACE2 expression or increased shedase activity in diabetes remains to be established.

ACE2 and non-diabetic kidney disease

While the expression of ACE2 in the diabetic kidney has been extensively investigated, there has been little research into non-diabetic kidney disease, although the RAS is known to play an important role in the pathogenesis of these non-diabetic forms of nephropathy.[43] Local increases in Ang II levels have been reported in various experimental models of progressive renal disease, including renal ablation, Heymann nephritis, anti-Thy1 glomerulonephritis, anti-glomerular basement membrane (GBM) nephritis.[44] For example, in the subtotal nephrectomy model[45] we have recently observed that renal ACE2 gene expression is markedly reduced (Fig. 4), potentially
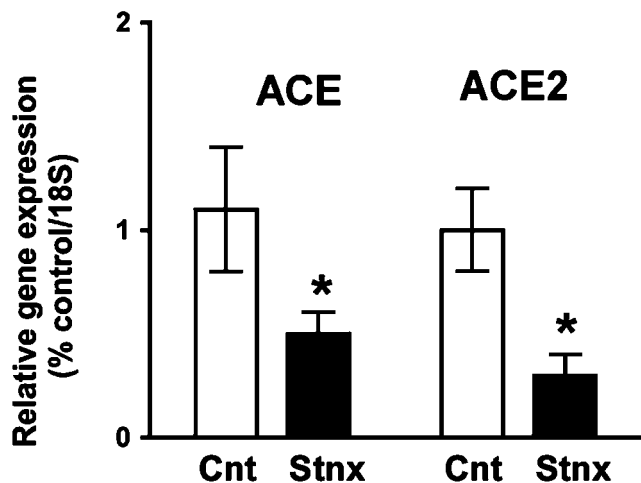
Fig. 4 Gene expression of angiotensin converting enzyme (ACE) and ACE2 gene as measured by real-time reverse transcription-polymerase chain reaction (RT-PCR) in cortical homogenates from control (cnt) and 12-week subtotal nephrectomized (stnx) Sprague Dawley rats. Data are shown as mean percentage control ± SEM, *$P < 0.05$ versus control.

contributing to the local increase in tubular Ang II in this model.[46] Interestingly, in a renal biopsy study of patients with diverse primary and secondary renal diseases, including hypertensive nephropathy, immunoglobulin A (IgA) glomerulopathy, minimal change nephropathy, focal glomerulosclerosis, vasculitis and membranous glomerulopathy, ACE2 expression was found to be uniformly increased in glomeruli.[27] Again, this differential expression pattern of glomerular and tubular ACE2 appears to be an important determinant for progressive renal disease.

ACE2 and blood pressure regulation

Hypertension has an important role in the development and progression of kidney disease. The RAS is key regulator of blood pressure, both in health and disease. For example, Ang II acts to increase systemic blood pressure levels through a number of mechanisms, including direct vasoconstriction, the stimulation of vasopressin release and the enhancement of aldosterone production leading to renal salt and water retention. To the extent that ACE2 reduces the local concentration of Ang II, by hydrolysing this constrictor peptide to Ang 1–7, ACE2 is able to indirectly antagonize these pro-hypertensive processes. Furthermore, as noted above, Ang 1–7 generated by ACE2 is a vasodilatory peptide, with actions on blood pressure that act as a counterbalance to the effects of Ang II. For example, Ang 1–7 also stimulates the production of nitric oxide[47] and vasodilator prostaglandins[48,49] and potentiates bradykinin synthesis[22] and activity of bradykinin at the B$_2$ receptor.[50,51] However, the importance of Ang 1–7 in normal physiology and particularly in blood pressure control, is unclear, as both Ang 1–7 and Ang 1–7-mimetic compounds fail to lower systemic blood pressure levels in healthy individuals. This contrasts strikingly with disease states, such as diabetes, hypertension or volume overload. In these conditions, where the RAS is activated, Ang 1–7 has potent antihypertensive actions.[52] The potential importance of Ang 1–7 to the management of hypertension is further illustrated by the finding that the vasodilatory effects of both ACE inhibition and blockade of the AT1 receptor in experimental animals are attenuated by depletion of Ang 1–7.[53]

It was initially hypothesized that disruption of the balance between ACE and ACE2 would result in abnormal blood pressure control[54] such that ACE2 overexpression might protect against increases in blood pressure and conversely ACE2 deficiency might lead to hypertension. However, blood pressure levels in ACE2 KO mice are normal or only mildly elevated[55] despite increased Ang II plasma and tissue levels. The unaltered blood pressure is not explained by any compensatory changes in ACE, as blood pressures in the *ace/ace2* double null mice were similar to that of *ace* single mutant mice.[31] It is possible that other homeostatic mechanisms, such as the apelin system or nitric oxide metabolism, are able to compensate *in utero* for loss of ACE2, thereby resulting in normal foetal development and basal blood pressure levels in the ACE2 knockout mice. An alternative hypothesis is that ACE2, like Ang 1–7 confers an antihypertensive action only when the RAS is activated. For example, ACE2 KO mice demonstrate a heightened hypertensive response to Ang II infusion, above that observed in wild type controls.[55] ACE2 expression levels are lower in the kidneys of salt-sensitive Sabra hypertensive rats when compared with normotensive salt-resistant Sabra normotensive rats. Furthermore, the additional increase in blood pressure that follows treatment with deoxycorticosterone acetate in salt-sensitive rats was also correlated with a further decrease in ACE2 protein expression.[31] In spontaneously hypertensive rats (SHR), where the intrarenal and systemic RAS are also activated, ACE2 also plays an important role in blood pressure regulation.[40] For example, ACE2 was significantly reduced in hypertensive SHR, but when ACE2 expression was restored with all-*trans* retinoic acid[56] or a lentiviral vector containing ACE2,[57] there was a significant attenuation in hypertension in these animals. It is also interesting to note that in this model at birth, when SHR rats are still normotensive, the renal expression and activity of ACE2 was significantly increased when compared with normotensive Wistar Kyoto (WKY) rats assessed at the same time point.[40] This further supports the notion that renal ACE2 is a compensatory mechanism, which can offset stimuli that promote hypertension, but ultimately the loss of ACE2 expression in the SHR leads to the full manifestation of its phenotype with the development of adult-onset hypertension.

The association between ACE2 and hypertension has also been observed in epidemiological studies. For example, in a Chinese cohort study consisting of 745 patients with essential hypertension and 362 healthy controls, it was demonstrated that one single nucleotide polymorphism, G8790A, was associated with essential hypertension.[58] Another study, carried out in 3408 hypertensive patients randomized to various antihypertensive treatments, also identified a positive association between the *ACE2* T allele, the high risk for hypertension and reduced antihypertensive response to ACE inhibitors.[59] However, other studies have failed to find evidence for an association of *ACE2* polymorphisms with essential hypertension.[60] In another study, albeit in diabetic nephropathy, ACE2 gene polymorphisms were also examined but no clear relationship between an ACE2 gene polymorphism and development of renal disease was observed in that population.[61]

CONCLUSION

Angiotensin converting enzyme 2 is an integral component of the RAS. It is highly expressed in the kidney tissues where its actions balance those of ACE, by degrading Ang II and generating the physiological antagonist Ang 1–7. While its role in the maintenance

of normal kidney function of healthy kidney remains to be established, its actions appear critical in a variety of disease states, including hypertension, diabetes, ageing, renal impairment and cardiovascular disease, where activation of the RAS requires the counter-balancing actions of ACE2. Studies using ACE2 inhibitors and knockout animals suggest that the loss of these compensatory mechanisms may be an important factor in the development and progression of kidney disease. Equally, augmentation of ACE2 expression, particularly at a tubular level may have important benefits relevant in the treatment of a range of kidney diseases. However, more information is still required about the diverse actions of Ang 1–7, at both a local and systemic level, for such interventions to be appropriately targeted and achieve their full clinical potential.

ACKNOWLEDGEMENTS

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