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The renin angiotensin system from conception to old age: the good, the bad and the ugly.

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Summary

1. The renin-angiotensin system (RAS) plays a critical role in placentation and nephrogenesis. Failure to thrive during intrauterine life, possibly related to placental dysfunction and impaired expression of the renal RAS, as well as prematurity, results in smaller kidneys at birth and reduced nephron number. The remaining nephrons are therefore hyperfiltering from birth. Hyperfiltration, infections and Type II diabetes cause glomerular and tubular fibrosis leading to further reductions in nephron number.
2. The intrarenal RAS plays a key role in promoting tubulointerstitial fibrosis. Low birth weight and a high incidence of preterm birth program Indigenous children for early onset renal disease in adult life. Indigenous Australians have 404,000 fewer nephrons than non Indigenous Australians. This, coupled with the high incidence of infectious diseases (particularly acute post-streptococcal glomerulonephritis) and the increasing prevalence of Type II diabetes, explain why end-stage renal disease is of epidemic proportion in Indigenous Australians.
3. The existence of RAS gene polymorphisms and inflammatory cytokines might further potentiate susceptibility to renal disease in these people.

1 Introduction:

2 Susceptibility to disease is the result of the interaction between the individual's unique
3 genotype, the resulting phenotype and the environment. Recently it has been recognised
4 that the environment affects genotypes in two ways: direct effects on cellular processes
5 and functions and influences on expression of genes through epigenetic regulation of
6 transcription and translation. This results in 'heritability' of altered patterns of gene
7 expression within a cell line and possibly across several generations. Such 'epigenetic'
8 influences form the scientific basis of the "Barker Hypothesis", the concept of the
9 'developmental origins of health and disease' (DOHAD).¹

10 Two tissue renin-angiotensin systems (RASs) might play a role in influencing
11 nephrogenesis: the placental RAS because it is important in placentation and therefore in
12 fetal growth and the intrarenal RAS because it directly affects renal development. The
13 levels of expression of individual components of the intrarenal RAS during intrauterine
14 life could be the major pathway through which inadequate placentation and/or maternal
15 undernutrition cause oligonephronia.

16 This review describes the roles of these two RASs. The significance of the two systems
17 from the perspective of 'lifetime health' arises because all the nephrons that an individual
18 has are formed during intrauterine life. In humans, nephrogenesis is complete before birth.
19 Should an infant be born before 34 weeks gestation, nephrogenesis continues *ex utero* but
20 the outer, most recently formed cortical nephrons are likely to show cystic degeneration,
21 which also results in fewer nephrons.²

22 Glomerular filtration, the first step in the formation of urine, is related to body surface
23 area. Therefore with fewer nephrons a greater single nephron filtration rate results.
24 Individuals born small for gestation age (SGA) have fewer nephrons.³⁻⁵ Hyperfiltration
25 leads to glomerular cell injury and ultimately to nephrosclerosis and a further decline in
26 nephron number. Individuals born SGA or premature or both run the risk of earlier onset
27 of chronic kidney disease and ultimately end-stage renal disease (ESRD) than
28 appropriately born infants, especially if they are exposed to a lifestyle that adversely
29 affects renal health.

30 Indigenous Australians are a population more likely to have fewer nephrons at birth and
31 more likely to have like Type II diabetes and acute post streptococcal nephritis (APSGN).
32 Therefore, it is not surprising that the incidence of chronic kidney disease (CKD) in
33 Indigenous populations is of 'epidemic proportion'.⁶ CKD is a major risk factor for
34 hypertension and cardiovascular disease (CVD).⁷ Since CVD is the major cause of

mortality in all populations, the reduction in life expectancy of Indigenous Australians depends not only directly on the high incidence of ESRD but indirectly on the effects of CKD on the incidence of CVD.

It follows that early recognition and prevention of CKD is of paramount importance in improving the health of Indigenous Australians. This review, as well as describing the role of the RAS in placentation and renal development when its activity is essential for normal placental and renal organogenesis, reviews the ‘ugly’ aspect of the activity of the RAS in adult life in accelerating the rate of deterioration in CKD, whether the result of diabetic nephropathy or other causes.

It is well known that blockade of the RAS reduces the rate of progression of CKD and diabetic nephropathy, but drugs currently in use in Australia are not completely effective.⁸ Intervention early in life in women of reproductive age is not feasible because the developing embryo and fetus require intact RASs for normal placental and renal development; all drugs currently used to block the RAS cross the placenta.⁹

The renin angiotensin system (Figure 1)

For many years the RAS was regarded solely as a circulatory ‘endocrine’ system. Tissue renin-angiotensin systems seemed to be regarded as of lesser importance, but tissue RASs are ubiquitous.

Apart from the kidney, which secretes active renin, all other tissue RASs only produce prorenin. The major components of tissue RASs are described in Figure 1. Prorenin is inactive unless activated by proteases or by binding to the prorenin receptor ((P)RR) discovered in 2002.¹⁰⁻¹² Prorenin and renin bound to (P)RR can have actions independent of the formation of Ang II though stimulation of ERK1/2 pathways and Wnt signalling.¹² ¹³ Prorenin bound to the (P)RR and active renin catalyse angiotensinogen, yielding the decapeptide, angiotensin I (Ang I). Ang I is converted by angiotensin converting enzyme (ACE) to Ang II, the most well described of the Ang peptides. Ang I can also be converted to Ang (1-7) through conversion to Ang (1-9) via ACE2 (a homolog of ACE), which is then converted by ACE to Ang (1-7). Alternatively, Ang I can be directly converted to Ang (1-7) via neutral endopeptidase (NEP) or prolylendopeptidase (PEP). Ang (1-7) can also be formed from Ang II by ACE2, which acts at a much faster rate than other Ang (1-7) forming pathways.¹⁴ Ang II can be converted by aminopeptidase (AMP) to Ang III, a peptide with similar actions to Ang II or to Ang IV by dipeptidyl AMP. Ang II can act via the Ang II type I receptor (AT₁R) or via its type II receptor (AT₂R). Acting

via the AT₁R, Ang II exerts most of its well known actions, e.g. effects on blood pressure and aldosterone secretion. Of particular importance for tissue RASs, the Ang II/AT₁R promotes cell growth and angiogenesis, while Ang II acting via the AT₂R and Ang (1-7) acting via its Mas Receptor (MasR) have opposite effects to Ang II/AT₁R mediated actions; i.e. they are anti angiogenic and anti proliferative.^{15, 16}

Placentation

The human placenta develops from the outermost cells of the developing blastocyst, the trophoctoderm. The trophoblast cells proliferate and differentiate to form an inner cytotrophoblast cell layer and an outer syncytiotrophoblast layer of the placental villi. Some cytotrophoblast cells break through the overlying syncytium to form cell columns and further differentiate into the extravillous trophoblast, which invades the maternal decidua, and endovascular trophoblast, which plugs maternal spiral arterioles and creates a hypoxic milieu that is essential for normal placentation. These trophoblast cells also cause remodeling of the maternal spiral arterioles so they become spacious conduits opening into the intervillous space. After about 12 weeks gestation, the villi, the terminal ramifications of the fetal placenta which are composed of two layers of trophoblast enclosing the fetal capillaries, are bathed by maternal blood spurting from the now remodeled spiral arterial conduits into the extravillous space. Fetal capillaries from the umbilicoplacental vasculature form branching networks within the villi. Exchange of oxygen, water and nutrients, occurs across 2 layers of trophoblast and the fetal endothelium. Inadequate placentation has been implicated in several complications of pregnancy including preeclampsia, intrauterine growth restriction (IUGR) and pre-term labour with intact membranes.¹⁷⁻¹⁹ IUGR has been shown to be associated with a reduction in nephron number and placental insufficiency has been suggested as a key factor in the developmental origins of adult disease.^{4, 5, 20} So what is the role of the placental RAS in placental development? Could inadequate placentation be the result of early dysfunction of the placental RAS?

Angiotensin peptides: angiogenesis and cell proliferation.

Ang II stimulates angiogenesis in the chorioallantoic membrane of the chick embryo.²¹ Activation of the AT₁R by Ang II leads to potent induction of a variety of factors including vascular endothelial growth factor VEGF.²²⁻²⁴ Both basal and Ang II stimulated angiogenesis is depressed when AT₁Rs are blocked.^{25, 26} Ang II stimulated VEGF in

human umbilical vein endothelial cells (HUVEC), depressed angiotensin 1 but had no effect on angiotensin 2. These patterns of gene expression resulting from Ang II stimulation caused endothelial proliferation, which was blocked by the AT₁R antagonist, candesartan.²³

VEGF is thought to act locally via its receptors, VEGFR-1 (also known as Flt-1) and VEGFR-2, to establish the fetoplacental circulation. VEGFR-1 and -2 and Angiotensin receptors are found in fetal endothelial cells in both humans and sheep.^{23, 27-29} Enhanced VEGF production due to Ang II might be due to stabilization of the transcription factor hypoxia inducible factor-1 α (HIF-1 α).³⁰ Indeed, HIF-1 α knockout mice have impaired placental vascularization as do AT₁R knockout mice.^{30, 31} Thus in early gestation, hypoxia, which induces HIF-1 α , and Ang II, which stabilises it, act in concert to stimulate angiogenesis. Ang (1-7) on the other hand, which acts via the MasR, has been shown to inhibit tumour angiogenesis via decreased VEGF mRNA and protein levels.³²

Ang II acting via the AT₁R also promotes cellular hyperplasia by epidermal growth factor (EGF) receptor transactivation; this activates metalloproteinases yielding the mature EGF ligand that binds and activates EGF receptor initiated cell signalling. EGF receptor transactivation mediates ERK activation, c-fos induction and cell proliferation. Metalloproteinase-dependent shedding of EGF ligands is important for Ang II/AT₁R mediated growth.³³

In contrast, Ang II acting via the AT₂R inhibits Ang II/AT₁R induced activation of MAPKs.¹⁴ In cultured tumour cells the actions of Ang II via AT₁R and AT₂R are antagonistic when it comes to cell growth and angiogenesis. The Ang 1-7/Mas receptor axis is also anti-proliferative via inhibition of the ERK1/ERK2 pathway as well as anti-angiogenic.^{16, 32, 34, 35} This indicates that local production of ACE2 (Figure 2) also determines whether RAS signalling is pro- or anti-proliferative.

The role of the RAS in placentation

If the placental RAS is involved in placental growth, angiogenesis and invasion, it would be anticipated that it would be most active in early gestation when the placenta is established. This is in fact the case. As Figure 2 shows, placental renin, the prorenin receptor and the AT₁R receptor have their highest levels of expression in very early gestation, with angiotensinogen and ACE2 having a similar pattern.³⁶ The RAS proteins are localised to the invading extravillous cytotrophoblast and to the syncytiotrophoblast.³⁶

Expression of VEGF is similar and there are strong correlations between renin, prorenin receptor and the AT₁R and VEGF expression, indicative of the role of the RAS in placental angiogenesis. By late gestation the expression of the placental RAS is down regulated, except for placental ACE, the only component of the placental RAS that is more highly expressed in late gestation. Interestingly placental ACE is localised to the fetal capillary endothelium.³⁶ Lining the fetal capillaries, ACE is ideally situated to catalyse the conversion of Ang I generated by the intracorporeal fetal RAS to Ang II, as has been shown in animal models.³⁷

Regulation of the expression and activity of the placental RAS

In the primary human placental trophoblast cell line (HTR-8/SVneo), which expresses key genes of the placental RAS, the sex steroids estradiol and medroxyprogesterone acetate in combination stimulate prorenin gene expression and prorenin secretion.³⁸ They also stimulate the expression of (P)RR and the AT₁R.³⁹ Unlike freshly obtained human placental tissue, this cell line does not express AT₂R, ACE2 nor the MasR.^{36, 38} These cells are also responsive to cAMP, a known stimulus for renal renin secretion as it is for placental trophoblast renin.^{40, 41} Angiotensinogen gene expression as well as *REN* mRNA and prorenin secretion is increased by cAMP.³⁹

Epigenetic Regulation

Epigenetic regulation of gene expression alters the heritability of cell characteristics without changing the structure of DNA. Genes are regulated by the degree of methylation of CpG islands in their promoter region. Their activity can also be affected by changes to histones. Acetylation, methylation and other changes to histones alter the packaging of DNA and so alter access to the promoter regions of the genes. As well, small non-coding RNAs that target the specific mRNAs block protein production, either by increasing destruction or blocking translation of the mRNA.

Genes that have a high density of CpG doublets in the promoter region, known as differentially methylated regions (DMRs) or CpG islands, are silenced when the cytosine residue is methylated. Three genes in the RAS pathway, *ATP6AP2* ((P)RR), *ACE* and *AGTR1* (AT₁R) have a DMR in their promoter region. These genes are however, hypomethylated in both early and late gestation placentae (Sykes, Lumbers *et. al.*, unpublished observations) so their expression is not inhibited. Perhaps, not surprisingly, the chemical 5'-aza-2'-deoxycytidine, that causes global hypomethylation did not affect

the expression of these 3 genes.³⁹ It did however cause increased *AGT* and *REN* expression and prorenin secretion.

It is possible that renin gene expression in the placenta is affected by the level of methylation of the placental genome. A low protein diet during pregnancy reduces the methylation of Ang II receptor genes in rat offspring and incubation of preimplantation embryos inhibits fetal growth and reduces the methylation of susceptible genes.^{42, 43} In fact maternal diet has been shown to affect renal *REN* expression in offspring as described below.

Global hypomethylation affects not only the DMRs but also other cytosines within the genome as well as histones. The effects of histone modification on placental renin expression have not been studied, but inhibitors of histone deacetylases stimulate *REN* expression. Forskolin, which increases intracellular cAMP, stimulates histone acetylation and renin expression in renal artery smooth muscle cell cultures.⁴⁴ Global hypomethylation of trophoblast HTR-8/SVneo cells likewise amplifies *REN* expression and increases responsiveness to cAMP.³⁹

Another possible pathway for regulation of the placental RAS gene is via non-coding RNAs (in particular miRNAs) that suppress translation and inhibit protein expression. Goyal *et al.* have shown that there are miRNAs in the mouse placenta that target placental *REN* mRNA (miR-199b), placental *ACE* mRNA (miR-27a) and *AGTRI* mRNA (miR-468). These mi-RNAs in the placenta and also the lung are down regulated by maternal hypoxia.⁴² We postulate that the low oxygen tension occurring during the hypoxic phase of normal placental development suppresses mi-RNAs that target placental RAS mRNAs and so the placental RAS is very active. At the end of the first trimester when maternal blood flow to the placenta commences and the oxygen tension rises steeply from <20 mmHg at 8 weeks of gestation to >50 mmHg at 12 weeks the expression of mi-RNAs is stimulated and so the activity of the placental RAS is suppressed.⁴⁵

Thus failure to maintain an early hypoxic milieu could result in under expression of the placental RAS and impaired placental function as has been shown in the AT₁R knockout mouse and lead indirectly to IUGR and low birth weight infants.

The RAS and the developing kidney

The RAS is essential for normal renal development. In 1995 Tufro-McReddie *et al.* showed that losartan, a drug that blocks AT₁Rs, caused severe renal abnormalities when given to newborn rats, which continue nephrogenesis during the first week of postnatal

1 life.⁴⁶ Nephron growth was delayed resulting in reduced nephron size and number.
2 Maturation and development of the intrarenal vasculature was impaired leading to a
3 hypoplastic distorted renal vascular architecture and tubular dilation. AT₁R blocking
4 drugs given to tadpoles had even more profound effects on nephrogenesis indicating that
5 the RAS plays a critical role in nephrogenesis across a wide phylogenetic spectrum.
6 Using developing metanephroi, Tufro-McReddie *et al.* later found that a critical low
7 oxygen environment (3%) was optimal for expression of VEGF and other angiogenic
8 factors as well as tubular, epithelial cell and endothelial cell proliferation.⁴⁷ At 20%
9 oxygen, VEGF expression and cell growth were limited.

10 If renin synthesizing cells are ablated, the kidney is small and 25% of glomeruli are
11 atrophic.⁴⁸ Renin synthesizing cells have the capacity to differentiate into non-renin
12 synthesizing cells such as smooth muscle cells, mesangial and epithelial cells. These cells
13 retain the capacity to revert to renin-synthesizing cells when required. This has
14 implications for recruitment of the intrarenal RAS in chronic kidney disease.⁴⁹

15 Thus development of the kidney and placenta are similar, in that both require a local RAS
16 and a low and probably critical oxygen environment at an early stage for optimal
17 development because of the role of HIF-1 α induced VEGF in angiogenesis. The
18 intraocular RAS serves as a model for both the placenta and kidney in terms of its role in
19 regulating angiogenesis. VEGF promotes the retinopathy of prematurity and in this model
20 of neovascularisation, the ocular RAS plays a significant role in stimulating its
21 production.⁵⁰ Briefly, exposure of preterm neonatal animals to very high oxygen levels
22 causes cessation of vessel growth in the inner retina of the eye. When the animals are
23 returned to room air, the retina becomes hypoxic, and neovascularization occurs. Both the
24 ocular RAS and ocular VEGF are upregulated by this ischaemia. VEGF expression and
25 its associated ocular neovascularization are blocked by drugs that block the RAS.⁵⁰ Thus
26 hypoxia/ischaemia induces the ocular RAS, which causes angiogenesis. We suggest that
27 there is a similar linkage between hypoxia, the expression of the placental and renal RASs,
28 VEGF and angiogenesis in the developing kidney and the developing human placenta.
29 We postulate that, in part, the activities of both the placental RAS (as described above)
30 and the renal RAS are regulated, in part, by oxygen dependent miRNAs.

31 There is good evidence that renal development can be affected by maternal undernutrition,
32 and this is associated with altered regulation of renin expression within the developing
33 kidney. Woods *et al.* showed, as have others, that maternal undernutrition (specifically a
34 low protein diet of 8.5%) in pregnant rats resulted in offspring that later developed

hypertension.⁵¹ She showed that this was associated with reduced nephron number and reduced expression of renal renin. These experiments directly link maternal diet to reduced activity of the renal RAS in the offspring, and to impaired renal development and high blood pressure in adult life.

Finally, in late gestation and in the early neonatal period, blockade of the RAS causes acute renal failure and oligohydramnios. This is due in part to fetal hypotension but also to failure to maintain efferent arteriolar tone and hence to sustain a glomerular hydrostatic pressure sufficient to maintain GFR.⁵² This could further add to nephron deficit.

In conclusion during development, deficiencies in the activity of developing renal and placental RASs play a role in determining fetal growth, renal development and function. Through effects on the placenta and kidney, affected individuals are programmed for an increased risk of hypertension, Type II diabetes mellitus and renal disease in adult life.

The role of the renal RAS in adult life in the pathogenesis of chronic renal disease

In adult life, the RAS plays a major role in the day to day regulation of salt and water balance because the system is exquisitely responsive to neural and humoral control and to flow past the macula densa. Because of the direct effects of Ang II and aldosterone on sodium reabsorption the RAS plays a critical role in survival when fluid and electrolyte balance are compromised. But in hypertension, diabetes and chronic renal disease the intrarenal RAS, contributing through multiple pathways accelerates progression of glomerular and tubular interstitial fibrosis (Figure 3). Blockade of the RAS by drugs that stop the formation of Ang II (ACE inhibitors and renin inhibitors) or block AT₁Rs are routinely used to treat chronic kidney disease and diabetic nephropathy. The activity of the intrarenal RAS accelerates progression of renal and cardiac pathologies in those age related diseases of adult life, susceptibility to which was programmed by the intrauterine environment.

A reduction in glomerular filtration rate (GFR) indicates functional renal impairment. Albuminuria and proteinuria are other markers of impaired renal function. Even mild impairment of renal function is a risk factor for cardiovascular disease and the presence of albuminuria represents a risk factor for CVD in diabetic and non-diabetic subjects.^{7, 53}

Activation of the intrarenal RAS as well as the circulating RAS and aldosterone are involved in the progression of CKD (Figure 3).

1 The intrarenal RAS can be driven by circulating Ang II or angiotensinogen.^{54, 55} While
2 Ang II inhibits renin secretion from juxtaglomerular cells, increased levels of Ang II
3 stimulate prorenin secretion in the more distal portions of the renal tubule.^{54, 55}

4 Angiotensinogen is formed in the proximal convoluted tubule (PCT) and its reaction with
5 renin in tubular fluid generates Ang II because ACE is located in renal tubular cells.⁵⁴
6 Ang II acting via AT₁Rs located along renal tubule, promotes sodium reabsorption.⁵⁶
7 Selective knockout of these renal AT₁Rs lowers blood pressure to the same extent as that
8 seen in animals in which there has been total body knockout of AT₁R. Since PCT AT₁aR
9 knockout mice have lower blood pressures and lower proximal fractional sodium
10 reabsorption, the renal tubular actions of Ang II are essential for maintenance of Ang II
11 dependent blood pressure.⁵⁶⁻⁵⁸

12 Within the kidney the RAS has other actions apart from stimulation of sodium
13 reabsorption (see Figure 3).⁵⁹ All these actions of the intrarenal RAS, including those that
14 are independent of the formation of Ang II (i.e. the direct result of prorenin/(P)RR
15 interactions) lead to glomerular and tubular fibrosis and hence to exacerbation of CKD⁵⁹
16 and impaired ability to regulate extracellular fluid volume. Briefly, intrarenal Ang II
17 causes increases the generation of free radicals, increases glomerular capillary pressure
18 and promotes the release of proinflammatory mediators, all of which, via various
19 pathways cause glomerular cell injury and ultimately glomerular and interstitial fibrosis
20 (as shown in Figure 3 and excellently reviewed by Siragy and Carey).⁵⁹ Ang II induced
21 formation of aldosterone from deoxycorticosterone (CYP11 β -2) has been identified in rat
22 glomerular cells and podocytes and is upregulated in diabetes.^{60, 61} Aldosterone is pro-
23 inflammatory and profibrotic through activation of many of the factors cited in Figure 3.^{62,}

24 ⁶³ The profibrotic actions of Ang II are enhanced by the angiotensin independent
25 profibrotic actions of (Pro)renin receptor activation following renin binding and in
26 diabetes by activation of a succinate receptor (GPR91) that causes renin release. High
27 levels of succinate generated in the TCA cycle by high glucose levels stimulate renin
28 release.^{12, 64}

29 Significantly, measures of the activity of the intrarenal RAS, such as the urinary
30 angiotensinogen (AGT)/creatinine ratio, correlate well with urinary albumin/creatinine
31 and urinary protein/creatinine ratios and are elevated in hypertension; levels are predictive
32 of the rate of annual decline in GFR in diabetic patients.⁶⁵⁻⁶⁷ The role of the intrarenal
33 RAS in these associations is demonstrated by RAS blockade resulting in fall in urinary
34 AGT/creatinine in both hypertensive subjects and in those subjects who have chronic

glomerulonephritis.^{68, 69} In an animal model, the urinary AGT level was an earlier predictor of the decline in renal function due to streptozocin induced diabetes than urinary albumin.⁷⁰

CKD and Indigenous Australians

Indigenous Australians have the highest incidence of ESRD. Rates of new cases are up to 15 times higher than non-Indigenous Australians.^{6, 71} Compared to non-Indigenous Australians the ratios of the rates of ESRD are particularly high for people aged 35-44 years (8.9), 45-54 years (17.8) and 55-64 years (15.2).⁶ Over 90% of patients on dialysis in the Northern Territory are Indigenous Australians.

There are a number of factors associated with this epidemic. First there is developmental programming of the kidney. Indigenous Australian women have higher rates of low birth weight babies (12.3% compared with 5.9%) and preterm babies (14.8% compared with 7.6%) than non-Indigenous women.^{71, 72} Indigenous Australians therefore have fewer nephrons from birth (see above) resulting in hyperfiltration, enlarged glomerular volumes and, ultimately glomerulosclerosis (Figure 3). Indigenous Australian adults have about 404,000 fewer nephrons per person.⁷³ From the time of birth nephrons of low birth weight babies are hyperfiltering.³ Prematurity has been shown to be associated with cystic degeneration of outer glomeruli in human and animal models.² Coupled with these findings there are observations from animal studies that impaired maternal renal function during pregnancy leads to hyperfiltration by the fetus and neonate and to glomerulomegaly.⁷⁴ From early adult life, albuminuria is pervasive in Indigenous communities.⁷⁵ Therefore, as part of a cycle, there is evidence of renal damage in Aboriginal women of childbearing age. As well as the physiological impact of maternal renal dysfunction on the fetal kidney described above, mothers with renal disease also have an increased risk of preterm and low birth weight deliveries with risks increasing with increasing severity of renal disease.⁷⁵⁻⁷⁸

Thus there is good evidence that an Indigenous infant is programmed for early onset of renal disease and consequent effects on the incidence of hypertension and cardiovascular disease in adult life.⁵³ The Indigenous child is also exposed to a high risk of infections and streptococcal infections are particularly toxic to renal health because they cause acute post streptococcal glomerulonephritis. As well these children have a high incidence of urinary urolithiasis while the incidence of urinary tract infections in pregnancy is about 30% and the incidence of Type II diabetes is 3.4 times higher than in non Indigenous

1 people and rising.^{6, 79} All of these factors compound the probability of early onset of
2 ESRD.

3 The Europeanization of Australia is partly responsible for the exacerbation of some of
4 these risk factors because dispossession from tribal lands and destruction of cultural and
5 social norms had profound effects on diet and the incidence of alcohol and drug abuse.
6 Furthermore, exposure to diseases carried by Europeans coupled with the invasion of
7 tribal lands and relocation of peoples caused high levels of infectious diseases. Diet,
8 alcohol and drug abuse and undernutrition are identified as key factors in the
9 intergenerational cycle of disease by the PMSEIC Working Group on Aboriginal &
10 Torres Strait Islander health focusing on maternal, fetal and post-natal health.⁸⁰ But there
11 could also be genetic factors because although the renin-angiotensin system plays vital
12 roles in fetal life in placental and renal development, it also accelerates progression of
13 CKD. Therefore it is worth considering any potential effects of novel gain-of-function
14 polymorphisms on the renal health of Australian Aboriginals.

15 The Indigenous Australian of the central desert regions of Australia has, until European
16 invasion, lived well in an arid environment for 40,000 years or more. The ability to
17 survive and reproduce in such an environment depends on a healthy cardiovascular
18 system, and renal function. Under these environmental pressures, the RAAS would be
19 critical for maintenance of salt and water homeostasis and it could be argued that there
20 might have been selection for polymorphisms of key components in the RAS pathway
21 leading to enhanced production of RAS proteins. Little work has been done in this area. It
22 is known that an insertion deletion (I/D) polymorphism of the ACE gene, common in
23 Europeans is nearly absent in Tiwi and Central Australian Aboriginals.⁸¹ This
24 polymorphism is associated with increased ACE levels and is known as a risk factor in a
25 wide range of non-Indigenous populations for diabetic nephropathy, CKD and CVD.⁸²⁻⁸⁴
26 Interestingly while the ACE I/D polymorphism occurs in only about 2% of healthy
27 Indigenous Australians it occurs in 14% of those suffering from ESRD, suggesting that
28 when present it exacerbates CKD. It is certainly associated with a much higher incidence
29 of albuminuria in Indigenous Australians (W.E. Hoy pers. comm).

30 We have identified, however, identified a high prevalence of other ACE SNPs in central
31 desert Aboriginal Australians. The incidence of the TT genotype of A240T (rs 4292) is
32 52.3% in this population compared with 13.7% in Caucasians (unpublished observations).
33 Since this SNP occurs in the promoter region and is associated with increased levels of
34 ACE, it could, like the ACE I/D polymorphism, contribute to an increased risk of CKD.⁸⁵

1 In addition, the prevalence of other polymorphisms in Indigenous Australians that
2 enhance activity of the RAAS is totally unexplored. For example, 88% of Indigenous
3 Australians have a G174-C polymorphism for the cytokine interleukin-6 (IL-6) associated
4 with high levels of responses of this inflammatory mediator.⁸⁶ IL-6, a cytokine, is
5 upregulated in chronic inflammatory conditions. IL-6, like Ang II, stimulates production
6 of angiotensinogen by the liver and the kidney.⁸⁷ Since angiotensinogen is present in
7 blood in rate limiting concentrations (i.e. plasma levels are similar to the *K_m* for the
8 renin-AGT reaction) and Ang II drives the activity of the intrarenal RAS, Ang II and IL-6
9 act in synchrony to enhance the activity of both the circulating and the intrarenal renin-
10 angiotensin systems.⁸⁸⁻⁹⁰ The significance of the high incidence of the gain-of function
11 mutation, i.e. the IL-6 G174-C polymorphism, in influencing levels of AGT in blood and
12 urine of Indigenous Australians is not known but in view of the very high incidence of
13 chronic infections which would further stimulate IL-6 production, it could be a very
14 important risk factor for CKD in Indigenous Australians.

15 In conclusion, throughout life tissue and circulating RASs play key roles in health and
16 disease. Tissue RASs control normal development and so are liable to intrauterine
17 programming while in adult life the activity of the same system can increase the
18 morbidity and mortality rates from CKD. The roles of the RASs during development and
19 in accelerating the progression of renal disease is of particular importance for Indigenous
20 people. Preventing CKD not only prevents ESRD but it also reduces CVD. Research into
21 the factors regulating the activity of the RAS in Indigenous Australians would help
22 promote an increase in life expectancy; a key feature of the “Close the Gap” campaign.
23 The “Close the Gap” campaign is a commitment by the Australian government to
24 improve the lives of all Indigenous Australians and in particular provide a better future
25 for Indigenous children within a generation.

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Legends to Figures:

Figure 1. Pathways for the formation of Angiotensin peptides in the placental and renal renin-angiotensin systems. ACE, angiotensin converting enzyme; NEP, neutral endopeptidase; PEP, prolylendopeptidase; AMP, aminopeptidase; diAMP, dipeptidylaminopeptidase. For more comprehensive description see ⁹¹.

Figure 2. Expression of the human placental renin-angiotensin system throughout gestation. Renin (*REN*), (P)RR (*ATP6AP2*), angiotensinogen (*AGT*), AT₁R (*AGTRI*) and ACE2 (*ACE2*) mRNAs are highest in early gestation while ACE (*ACE*) is highest at term. Different superscripts denote differences between groups, $P < 0.05$. N=5-11 per gestational age group. * denotes significant difference to early gestation placentae (6-16 weeks; $P = 0.022$). Modified from ³⁶.

Figure 3. Pathways via which the intrarenal renin-angiotensin system accelerates the progression of chronic kidney disease (modified from Siragy and Carey⁵⁹). AGT is angiotensinogen. Mesangial cells are contractile cells that are intraglomerular; podocytes are specialised epithelial cells, their shape and charge determine the filtration barrier, proteinuria is indicative of podocyte damage⁹². TGF- β (transforming growth factor), PAI-1 (plasminogen activator inhibitor), and ECM (extracellular matrix) are all involved in tubular and glomerular fibrosis. NF κ -B is a transcription factor which when activated translocates to the nucleus and initiates inflammatory and immune cellular responses.