The Renin Angiotensin System and the Metabolic Syndrome

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Abstract: The renin angiotensin system (RAS) is important for fluid and blood pressure regulation. Recent studies suggest that an overactive RAS is involved in the metabolic syndrome. This article discusses recent advances on how genetic alteration of the RAS affects cardiovascular and metabolic phenotypes, with a special emphasis on the potential role of angiotensin-independent effects of renin.

Keywords: (Pro) renin receptor, body fat, fecal fat, thermogenesis.

INTRODUCTION

The metabolic syndrome is a widely accepted concept that identifies the centrally obese patients with increased risk of cardiovascular diseases and diabetes. The syndrome has a rising prevalence worldwide, which related largely to increasing obesity and sedentary lifestyles [1]. In the US approximately 34% of adults meet the criteria for the metabolic syndrome, and the syndrome is 3 times more prevalent in males and females 40-59 years of age compared to those 20-39 years of age [2]. Individuals with the metabolic syndrome have twice the risk of developing cardiovascular disease over the next 5 to 10 years relative to individuals without the syndrome [1]. The syndrome confers 5-fold risk for type 2 diabetes [1]. The metabolic syndrome is clinically characterized by several inter-related symptoms including obesity, dyslipidemia, high BP, insulin resistance, impaired glucose tolerance or diabetes. Each of these is a risk factor of cardiovascular disease and diabetes, but combinations of them greatly increase the risk of cardiovascular diseases [3]. But there were many definitions of the metabolic syndrome, which led to confusion regarding how to identify patients with the syndrome [4, 5], leading to a confusion in clinical practice. The main difference was that The International Diabetes Federation (IDF) had a threshold value for waist circumference as obligatory, whereas American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) had it one of the factors but not obligatory. Accordingly, IDF and AHA/NHLBI, jointed by the World Heart Federation, International Atherosclerosis Society, and International Association for the Study of Obesity recently developed one unified definition [1] (Table **1**): there should be no obligatory component, but waist measurement would continue to be a useful screening tool. Three abnormal findings out of 5 would qualify a person for the metabolic syndrome. A single set of cut points would be used for all components except waist circumference. Yet, because the relation between waist circumference and cardiovascular disease and diabetes risk differs globally, the definition for waist circumference remains unsettled.

High blood pressure is a major factor of the metabolic syndrome, and genetic factors that regulate blood pressure are likely important as a cause of the metabolic syndrome. However, progress has been slow with regard to the identification of genetic factors affecting blood pressure and the metabolic syndrome. This is because hypertension and the metabolic syndrome are probably caused by combinations of a variety of small quantitative changes in the expression of many genes plus a similarly diverse collection of environmental factors.

Current efforts in humans using single nucleotide polymorphisms (SNPs) to identify susceptibility loci containing one or more QTLs (quantitative trait loci) relevant to blood pressure and the metabolic syndrome has the potential to be informative. False-positive and false-negative results are, however, inevitable because the genotype–phenotype associations with QTLs are weaker than those observed with the more direct and severe Mendelian traits, and because non-genomic environmental factors can easily obscure any association. Additionally, even when an association is strong, the possibility remains that the cause of an observed difference in blood pressure and the metabolic syndrome is a linked but unrecognized genetic difference rather than the recognized polymorphic factor.

Using animal models have the potential to overcome some of the genetic and environmental complexities of human studies because we can use homogenous genetic background and environmental condition. Above all generating genetically engineered animals especially using gene targeting technique in mice will allow us to directly study the effects of changing the gene of interest without being obscured by genetic background and environmental factors such as diet. The mouse models generated by gene targeting can establish a causative link between differences in blood pressure or the metabolic syndrome and changes in gene expression in animals that are otherwise unaffected (i.e. wild type) and have all their homeostatic mechanisms intact. The results are often predictive of the effects of genetic polymorphisms in humans. In the reciprocal direction, once naturally occurring

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1876-5262/10

Table 1. Criteria for Clinical Diagnosis of the Metabolic Syndrome

	Categorical cutpoints
Increased waist circumference*	Population-specific and country-specific definitions
Increased triglycerides (drug treatment for elevated TG is alternate indicator†)	≥150 mg/dL (1.7mmol/L)
Reduced HDL cholesterol (drug treatment for reduced HDL cholesterol is alternate indicator†)	<40 mg/dL (1.0 mmol/L) in men <50 mg/dL (1.3 mmol/L) in women
Increased blood pressure (antihypertensive drug treatment in patient with history of hypertension is alternate indicator)	Systolic ≥130 and/or diastolic ≥85 mm Hg
Increased fasting glucose‡ (drug treatment of increased glucose is alternate indicator)	> 100 mg/dL (5.5 mmol/L)

*It is recommended that the IDF cutpoints be used for non-Europeans and either the IDF or AHA/NHLBI cutpoints used for people of European origin until more data are available. †Most commonly used drugs for increased triglycerides and reduced HDL cholesterol are fibrates and nicotinic acid. A patient on one of these drugs can be presumed to have high triglycerides and low HDL. Use of high-dose ω-3 fatty acids presumes high triglycerides. ‡Most patients with type 2 diabetes will have the metabolic syndrome by the proposed criteria.

candidate allelic variations have been detected in humans, gene targeting can be used to test whether the variations cause differences in blood pressure or the metabolic syndrome in mice. A common feature to all mouse experiments is the ability to change the genetic background of the mutant mice by generating a backcross with a different strain. This type of experiment is valuable because the detection of a difference in the severity of a phenotype in different genetic backgrounds indicates the presence of modifier gene(s) in which expression differs from one strain to another. Identifying such modifier genes can then help uncover genetic differences in humans that have similar effects on the phenotype severity.

The renin angiotensin system (RAS) is one of the most important systems that regulate cardiovascular and fluid homeostasis (Fig. **1**). Angiotensins can also act as neurotransmitters, regulating blood pressure (BP), memory, cognition and stress [6-8]. Renin, released from the kidney juxtaglomerular (JG) cells, is a rate-limiting enzyme in angiotensin II (Ang II) production. Renin cleaves the liver- and adipose-tissue-derived angiotensinogen (AGT) to form inactive angiotensin I (Ang I) [9]. The angiotensin-converting enzyme (ACE), which is mainly expressed in the endothelium, then removes two C-terminal amino acids and generates active peptide Ang II. Interestingly, the human *ACE* insertion/deletion (ID) polymorphism does not affect BP despite the powerful antihypertensive effects of ACE inhibitors (reviewed in [10, 11]), although mild increase in ACE causes exacerbation of diabetic nephropathy (see the section on ACE).

The majority of the biological effects of Ang II, including BP elevation, are mediated by the type 1 Ang II receptor (AT1R). An Ang II negative-feedback mechanism inhibits the expression and secretion of renin. Treatments of hypertension often involve the inhibition of RAS with Ang receptor blockers (ARB), ACE inhibitors, or renin inhibitors, all of which disrupt this negative-feedback mechanism and increase renin levels.

In addition to these classical RAS components, several new molecules have been discovered in recent years. A homolog of ACE, ACE2, was discovered and shown to convert Ang II to Ang(1-7) [12]. Santos *et al.* discovered that the receptor for Ang(1-7) is the Mas proto-oncogene, and that this ACE2–Ang(1-7)-Mas pathway counteracts the effects of Ang II [13]. Furthermore, Nguyen *et al.* discovered the (pro)renin receptor (PRR) in 2002 [14, 15]. This receptor is expressed in many tissues including brain, adipose tissue, endothelial cells, vascular smooth muscle cells, and kidney. PRR binds and activates prorenin. This newly discovered

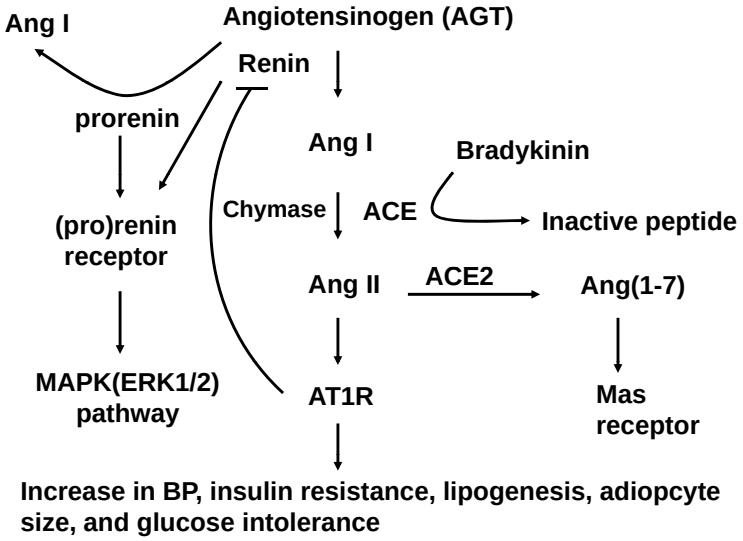

Fig. (1). The Renin-angiotensin system (RAS). Angiotensin II effects are predominately mediated by Ang II type 1 receptor (AT1R). In addition to the classical RAS, ACE2 and (pro)renin receptor are new players of this system. See text in detail.

RAS component likely impacts local Ang II effects in cardiovascular diseases and the metabolic syndrome.

The RAS recently has been implicated in the metabolic syndrome. Ang II induces adipogenesis (differentiation into adipocytes) [16, 17] and lipogenesis (triglyceride storage in adipocytes) *in vitro* [18]. However, the *in vivo* role of RAS in the metabolic syndrome has been unclear. The effects of Ang II on adipose tissue are mediated by Ang II type 1 and type 2 receptors.

Accordingly, we will focus on the roles of genetic changes in RAS in mice, rats, and in humans. Although much of the data available are from animal studies, because much of the genes and physiology between mice, rats, and humans are quite similar, it is important to understand information available from animal experiment to better treat human patients with the metabolic syndrome.

Angiotensinogen (AGT)

AGT is produced in several tissues: liver (the main source of circulating AGT), adipose tissue, kidney, heart, and brain. Using linkage analysis of the variants of the angiotensinogen gene (*AGT*), Jeunemaitre *et al.* found that M235T was observed more frequently in hypertensive versus normotensive individuals. In sibling studies, M235T segregates with the hypertensive condition [19]. In addition, individuals with the M235T variant had a higher plasma AGT concentration than individuals with 235M. Subsequently, Inoue *et al.* demonstrated that the 235 polymorphism is tightly linked with a single polymorphic change in its promoter region (G at –6 in 235M, and A at –6 in 235T). Promoter regions with A at –6 were associated with a greater concentration of plasma AGT [20]. Jain *et al.* identified an A/G polymorphism at -217 in the *AGT* gene promoter [21], and showed that, particularly in African-American individuals, an A at -217 is strongly associated with essential hypertension due to increased glucocorticoid binding to the *AGT* promoter. The association between *AGT* polymorphism and the metabolic syndrome is not clear.

Mice lacking AGT (*Agt-/-*) have severe hypotension and overexpress renin [22-24]. These mice also have a high rate of neonatal mortality associated with an impaired ability to concentrate urine, and have severe renal abnormalities including hydronephrosis, and hypertrophy of renal arteries [23, 25]. Administration of Ang II to the brain can correct hydronephrosis and partially correct the renal dysfunction seen in these mice [25]. In response to mild quantitative changes in AGT by altering the gene copy number of *Agt*, BP levels in 1-, 2-, 3-, and 4-copy mice were progressively increased [22]. This is consistent with what is observed in humans with *AGT* polymorphisms.

Mice lacking AGT are lean [26]. Although their food intake and fecal fat excretion were similar to those of wild-type mice, the *Agt-/-* mice have significantly increased energy expenditure due to increased locomotor activity. In addition, the uncoupling proteins (UCP-1, 2, 3), which dissipate heat instead of producing ATP and are found in the mitochondria of brown adipose tissue (BAT), epididymal fat, and skeletal muscle, respectively, did not differ between *Agt-/-* and wild-type mice [26]. The *Agt-/-* mice exhibited depressed adipose tissue development, which is associated with decreased fatty acid synthase (FAS) activity.

Renin

Renin is an important enzyme for regulation of BP and cardiovascular homeostasis. Cleavage of AGT to Ang I by renin is a rate-limiting step of Ang II production [27]. Renin is secreted from juxtaglomerular cells, which are modified smooth muscle cells of the afferent arterioles in the kidney. In Spanish populations, individuals with the GG genotype at the rs5707 intron 4 polymorphism of renin have significantly higher BP compared to those with the TT or TG genotype [28]. Individuals with GG genotype of the missense mutation in exon 9 (G1051A) have higher plasma renin activity, and this polymorphism may be involved in the etiology of hypertension [29].

Renin is encoded by a single gene in humans, rats, and some strains of laboratory mice such as C57BL/6, BALB/c, and C3 (Fig. **2**). However, some laboratory mouse strains (eg. 129, DBA/2J) have 2 renin genes (*Ren2* and *Ren1d*) in tandem on the same chromosome due to naturally happened duplication of the gene. The regulation of expression and tissue specificity of *Ren1d* and *Ren*2 are different [30], which complicates renin studies. Mice lacking either *Ren1d* or *Ren*2 exhibited no change in BP or any other apparent

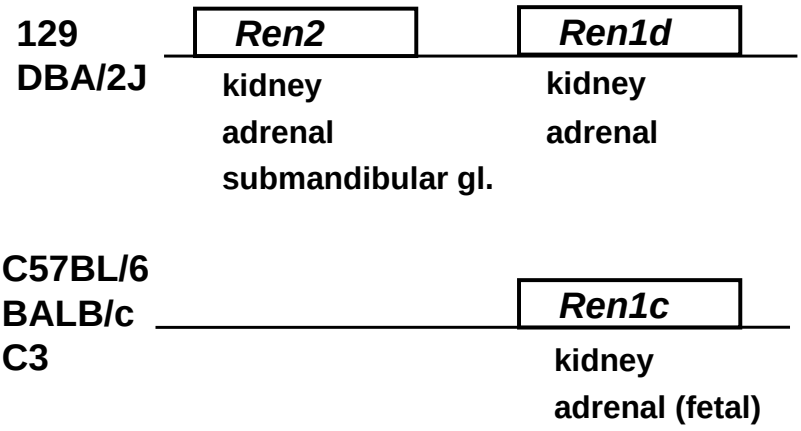

Fig. (2). Mouse renin genes. Renin is encoded by a single gene in humans, rats, and some strains of laboratory mice such as C57BL/6, BALB/c, and C3. However, some laboratory mouse strains (eg. 129, DBA/2J) have 2 renin genes (*Ren2* and *Ren1d*) in tandem on the same chromosome through natural duplication. Tissue expression of these renin genes is different.

phenotype [31, 32], although homozygous mice with *Ren1d* replaced with GFP had lower BP [33].

Since C57BL/6 mice, like humans, have only one renin gene, *Ren1c*, and are susceptible to diet-induced obesity, they are more suitable for research involving changes in renin expression and the metabolic syndrome [30]. Because of the difficulty in generating mice by gene targeting with C57BL/6 embryonic stem cells, Yanai *et al.* disrupted the *Ren1c* gene using TT2 ES cells derived from an F1 hybrid between C57BL/6 and CBA [34]. The authors demonstrated that these homozygous mutant mice had undetectable levels of plasma renin activity and plasma Ang I. In addition, the BP of these animals was lower than wild type by 20-30 mm Hg. The knockouts also had increased urine and drinking water volume and hydronephrosis, as observed in the *Agt*-/- mice. Abnormal granular cell layers in the hippocampus, as observed in the *Agt*-/- mice [35], were absent in mice lacking renin [34].

We have independently generated mice lacking *Ren1c* using embryonic stem cells from C57BL/6N mice [27]. Our *Ren1c*-/- mice have low BP and undetectable levels of plasma renin, Ang I, and Ang II [27]. Similar to the *Ace*-/- mice, our *Ren1c*-/- mice are anemic, as reflected by their low hematocrit (32 ± 3% vs. 45 ± 5 % in wild-type litter mates). Administration of Ang II (200 ng/kg/min) using an osmotic minipump restored BP levels in the *Ren1c*-/- mice to wild-type levels, but preexisting damage to the kidney medulla by hydronephrosis prevented restoration of the ability to concentrate urine. Heterozygous *Ren1c*+/- mice are normal with wild type levels of BP, and their kidney renin mRNA expression and plasma renin concentration are indistinguishable from those of wild type control mice [27].

Metabolic Phenotype of Mice Lacking Renin

We have recently investigated the role of renin in the metabolic syndrome and demonstrated that the *Ren1c*-/- mice have less than 50% of wild-type adipose tissue weight, which is consistent with the reduced fat volume of the knockouts analyzed by MRI (Fig. **3**). These mice were resistant to diet-induced obesity, although there were no changes in food intake [36]. The *Ren1c*-/- mice had smaller adipocytes in all adipose tissue tested, including white adipose tissue (WAT) and brown adipose tissue (BAT), and reduced hepatic triglyceride. Based on energy balance, the lean phenotype seen in these mice should be due to either increased energy expenditure and/or increased fecal energy loss (Fig. **4**). Indeed, the null mice displayed gastrointestinal loss of dietary fat as indicated by a higher steatocrit and a lower daily fat absorption.

Indirect calorimetry showed that, although physical activity was the same in the *Ren1c*-/- and wild-type mice, the *Renc1*-/- mice displayed increased heat generation and lipid combustion, as indicated by their low respiratory quotient (RQ). These mice require a smaller amount of insulin to maintain the same or lower plasma glucose levels relative to wild types. These results suggest a role for renin in the pathogenesis of diet-induced obesity and insulin resistance. Inhibition of renin may provide a good therapeutic target in the metabolic syndrome.

Other investigators have demonstrated that mice lacking *Agt* or *Ace* also have low BP, lean body type, and resistance

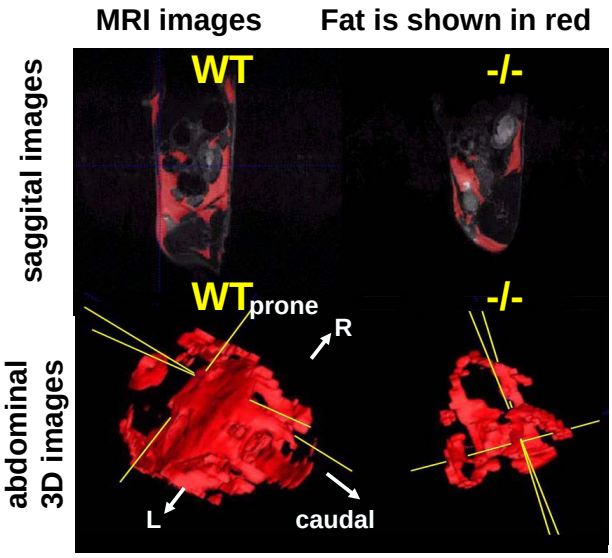

Fig. (3). Reduced adipose tissue in mice lacking renin. The *Ren1c*-/- mice have less than 50% of wild-type adipose tissue weight, as analyzed by MRI. Top panels are saggital sections. Bottom panels are 3D images showing the adipose tissue caudal to the diaphragm constructed from saggital images.

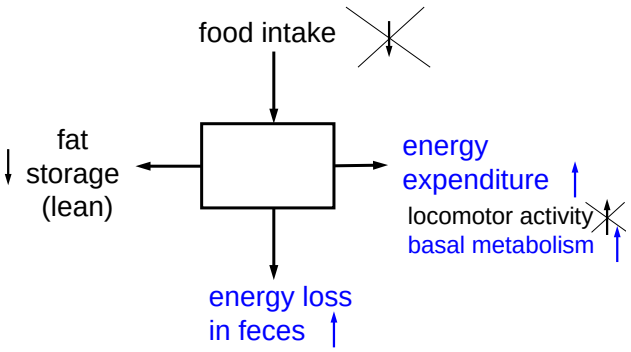

Fig. (4). The energy flow of the *Ren1c*-/- mice. Mice lacking renin are lean and resistant to diet-induced obesity due to increased energy expenditure and increased fecal energy loss.

to diet-induced obesity. Interestingly, however, these mice are not as lean as mice lacking renin (Table **2**).

The *Ren1c*-/- mice also display a unique increase in fecal fat excretion, heat generation, uncoupling protein (UCP1) expression in brown adipose tissue (BAT), and a reduced respiratory quotient (RQ), which were not observed in the *Agt*-/- and the *Ace*-/- mice (Table **2**). Because BAT is required for most non-shivering thermogenesis in rodents, these data suggest that the *Ren1c*-/- mice generate more heat by combusting fat in BAT. The *Agt*-/-, *Ace*-/- and *Ren1c*-/- mice have no detectable levels of Ang II, but different from the *Ren1c*-/- mice, both *Agt*-/- and *Ace*-/- mice have very high levels of renin due to disruption of a negative feedback mechanism. Accordingly, the lack of Ang-independent effects of renin likely contributes to decreased adiposity by decreasing dietary fat absorption and increasing heat generation. This possibility is currently being investigated.

Human Renin Transgenic Mice

Consistent with the Ang-independent effects of renin on adiposity, human renin transgenic (*hREN Tg)* mice weigh

Table 2. Metabolic Phenotypes of Mice Lacking Renin, AGT and ACE

	% Body Fat (%WT)	Food Intake	Fecal Fat	Locomotor Activity	Heat Generation	RQ	UCP-1	Renin
Ren1c-/- [36]	45%	NS	High	NS	High	Low	High	absent
Agt-/- [26]	76%	NS	NS	High	NS		NS	High
Ace-/- [37]	53%	NS	NS	NS	NS	NS	NS	High

RQ: respiratory quotient, UCP1: uncoupling protein 1, NS, not significantly different from wild type controls.

twice as much as wild-type mice at 60 weeks of age and display normal BP [38]. BP and plasma Ang II levels in *hREN Tg* mice are indistinguishable from wild-type mice, while double transgenic mice harboring both *hREN Tg* and *hAGT Tg*, have high BP and Ang II levels [39], suggesting that human renin does not generate Ang I from mouse AGT. Indeed, when incubated with plasma from our *Ren1c*-/- mice, which contains high levels of mouse AGT, exogenous recombinant human renin ($10^{-12\sim-8}$ M) does not cause Ang I production even at a concentration 10,000 times higher than normal physiological levels of mouse renin (Fig. **5**), although both human and mouse renin equally activates ERK1/2 in mouse vascular smooth muscle cells. Thus, the obesity phenotype observed in human renin transgenic mice underscores the importance of an Ang-independent role of renin in regulating body weight possibly *via* (pro)renin receptor.

Human Renin Transgenic Rats

Human renin transgenic rats developed moderate obesity mainly due to increased food intake [40]. However, when they were calorie restricted and pair-fed, h*REN Tg* rats became even leaner than non-transgenic control rats because they have increased energy expenditure, exercise, thermogenesis and lipid oxidation. Because this transgene is highly expressed in the hypothalamus, the metabolic phenotype of h*REN Tg* rats might not reflect Ang II-independent effects of endogenous renin.

Mouse Renin Transgenic Mice

Caron and Smithies generated three lines of unique mouse renin transgenic mice using the albumin promoter/enhancer, and targeting the transgene into the ApoA1-C3 locus [41-43]. The transgenes have a furin cleavage site and the active renin is generated and secreted from the liver without feedback regulation. The three mouse lines have different levels of renin, BP, and cardiac and renal fibrosis.

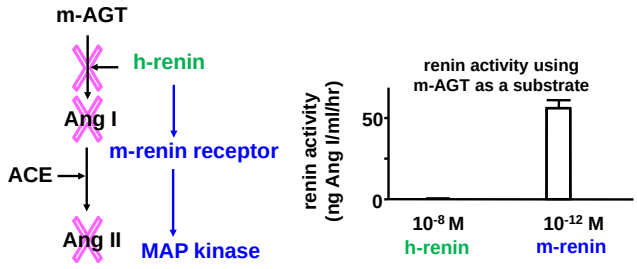

Fig. (5). Human (h-) renin does not generate Ang I from mouse (m-) AGT. Human renin does not generate Ang I from mouse AGT.

Prorenin and Renin Receptor

The (pro)renin receptor (PRR) is a single transmembrane protein of 350 amino acids. This specific renin receptor, discovered by Nguyen and colleagues in 2002, has dual functions (Fig. **6**): (i) increases catalytic activity of the PRR-bound prorenin to produce Ang I [44] (Fig. **1**); and (ii) activates MAP kinases independently of Ang II [45]. When prorenin binds PRR, a conformational change occurs in the prorenin molecule conferring full enzymatic activity and the ability to produce Ang I without undergoing proteolytic cleavage [reviewed in [46]]. Prorenin makes up 70-90% of total circulating renin [47], but it will not be converted to renin [48], which is synthesized only in the kidney. The binding of prorenin and renin to the cell surface in tissues is of pivotal importance in the physiology of local RAS in organs, since it provides a mechanism to generate Ang II locally in excess of the Ang II that is produced in plasma. Nguyen and colleagues also demonstrated that the binding of renin to its receptor increases the conversion rate of AGT to Ang I by four-fold [45]. In addition, both renin and prorenin bind to the PRR with similar affinity and activate the MAP kinase pathway [45, 47, 49].

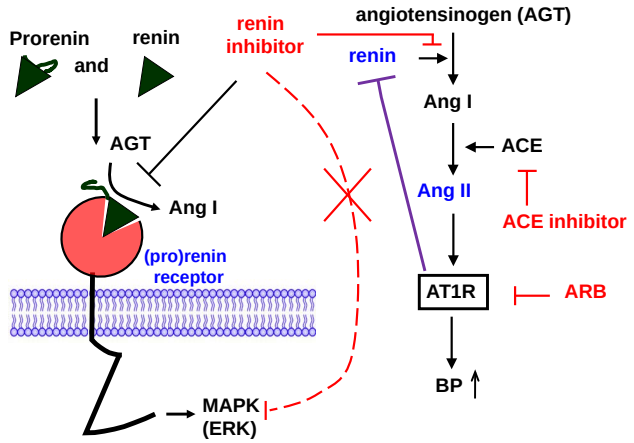

Fig. (6). Classical RAS pathway and novel effects of (pro)renin mediated by (pro)renin receptor. Prorenin and renin effects *via* PRR include Ang II-dependent and independent actions. Ang II-dependent actions involve increased catalytic activity of the PRR-bound prorenin to generate Ang I from AGT. Ang II-independent action involves intracellular signaling that triggers activation of an extracellular signal-related protein kinase (ERK1/2) pathway.

Abnormal signaling from the (pro)renin receptor is involved in cardiac fibrosis [50], nephrosclerosis [51], and microvascular complications [52]. Binding of PRR to its receptor in renal glomerular mesangial cells and cardiomyo-

cytes activates ERK and p38, respectively, independent of Ang II [45, 53]. The (pro)renin receptor is highly conserved in human, mouse, rat, and other species [47], and our preliminary data show that human renin and mouse renin are equally effective at activating MAPK in mouse cells.

A silent transition at 321C>T in exon 4 of PRR causes inefficient inclusion of exon 4 and impairment of ERK1/2 activation, which presents in patients with X-linked mental retardation [54]. In addition, T allele carriers of the PRR gene intervening sequence polymorphism in intron 5 [(IVS)5+169C>T] have higher BP in Japanese men than C allele carriers [55].

A renin inhibitor, aliskiren, inhibits Ang I synthesis and is widely used for treatment of hypertension [56]. Ichihara and colleagues proposed using a 10-amino acid sequence of the prorenin prosegment, termed "handle region peptide (HRP)", and demonstrated that HRP blocks binding of prorenin to PRR [52]. These authors showed that subcutaneous administration of HRP to streptozotocin-induced diabetic rats decreased renal content of Ang I and Ang II, and inhibition of diabetic nephropathy [52]. However, Feldt *et al.* were not able to block prorenin and renin-induced ERK1/2 activation by HRP or aliskiren [49]. It is possible that HRP efficacy *in vivo* depends on an undefined mechanism rather than competitive antagonism for the PRR.

The properties and binding specificities of the PRR, and its function in disease states such as the metabolic syndrome, remain a matter of debate.

Angiotensin Converting Enzyme (ACE)

ACE, mainly expressed in endothelial cells, removes two C-terminal amino acids from Ang I to generate Ang II [57]. ACE inhibitors are widely used and potent anti-hypertensive drugs. *ACE* I/D polymorphism in intron 16 is associated with altered plasma ACE levels, ranging from 75% in I/I, to 100% in I/D (by definition) and 125% in D/D individuals [58]. Yet, unlike the *AGT* polymorphism, the *ACE* I/D polymorphism does not significantly affect BP [59]. Mice lacking ACE have a striking reduction in BP, inability to concentrate urine, anemia, and a marked reduction in male fertility [60, 61]. Renal papilla is markedly reduced in these mice, and the intrarenal arteries exhibit vascular hyperplasia associated with a perivascular inflammatory infiltrate [12, 60]. The tail-cuff BP of the *Ace-/-* mice is ~73 mmHg, over 30 mmHg less than what is seen in wild-type mice [61]. This observation is similar to the phenotype of the *Agt-/-* mice [22-24], *Ren1c-/-* mice [27], and the *Agtr1a-/-; Agtr1b-/-* double-null mice [62, 63], suggesting that the reduction of BP observed in *Ace*-knockout mice is due to the lack of Ang II production.

Heterozygous disruption of *Ace*, which brought ACE levels to 50% of wild-type, did not affect BP [64], unlike what is seen in response to mild genetic alteration of *Agt* expression [65]. Similarly, when the *Ace* gene was duplicated, BP levels of three-copy heterozygotes and four-copy homozygotes were normal despite their increased plasma ACE (1.5 times and 2 times wild-type levels, respectively) [64]. This is consistent with the finding that humans with the ACE D/D genotype have elevated serum and tissue ACE, but no significant increases in plasma Ang II and BP. However, these individuals have a reduction in the vasodilator bradykinin [66]. This reduction in bradykinin could explain why individuals that are homozygous for the D allele have a poor prognosis for several cardiac and renal conditions, whereas the I allele is associated with enhanced endurance performance in elite distance runners, rowers, and mountaineers [67-69]. Developing computer simulation was very useful to understand the apparent paradox between the effects of genetic and drug-induced changes in ACE activity [11]. The simulation shows that Ang I (substrate of ACE) decreases with mild increase in ACE, and the effects of the increase in ACE activity are offset, and Ang II (product of ACE) and BP stay at the same levels, but bradykinin (substrate of ACE) decreases. If ACE mildly decreases, the level of Ang I increases and that of bradykinin increases [66]. However, the plasma Ang I cannot increase indefinitely; it eventually plateaus with more substantial inhibition of ACE with ACE inhibitors. Once Ang I has plateaued, further inhibition of ACE decreases Ang II and therefore BP. There are reports demonstrating the association of ACE D/D genotype and the metabolic syndrome [70, 71], but the absence of association is also reported as expected in human studies [72, 73].

Mice with 3 copies of *Ace* that were made diabetic by streptozotocin developed more severe diabetic nephropathy relative to wild type diabetic mice [74]. The ACE D/D genotype is associated with impaired insulin sensitivity and glucose tolerance, obesity [75, 76], and increased susceptibility to type 2 diabetes mellitus [77]. ACE D/D genotype is also associated with development of diabetic nephropathy [78-82], most likely due to decreased bradykinin. Indeed we have shown that lack of type 2 bradykinin receptor (B2R) exacerbates diabetic nephropathy [83]. The D allele of the ACE gene also may be a risk factor for the development of wall thickening of the carotid artery in NIDDM patients [84]. These observations suggest that ACE inhibition is beneficial for the treatment of diabetes and obesity. Mice lacking ACE have lower body weight and reduced fat mass with improved glucose clearance [37]. Although their food intake and fecal fat excretion are indistinguishable from those of wild-type mice, the *Ace-/-* mice have higher energy expenditure due to increased metabolism of fatty acids in the liver [37]. Pharmacological inhibition of ACE does not have the same effect on body composition, however [85, 86]. Reports are inconsistent with regard to the effect of pharmacological inhibition of ACE on body weight [87, 88]. Heimann *et al.* found that mice with 3 copies of *Ace* that were fed a high-fat diet had lower body weight than 1- and 2-copy mice [89]. In addition, these 3-copy mice have lower blood glucose and insulin levels, suggesting improved insulin sensitivity compared with mice that have 1 or 2 copies of the *Ace* gene [89]. Further investigation is needed into the effects of mild changes in ACE expression on obesity and diabetes.

Bradykinin and its Receptors

In addition to converting Ang I to Ang II, ACE (also known as kininase II) converts the active vasodilator kinins bradykinin (1-9) and kallidin (1-10) into inactive peptides bradykinin (1-7) and kallidin (1-8), respectively [see review [90] for detail]. The K_m and k_{cat}/K_m between ACE and the kinins are 30 times lower and 10 times higher, respectively, than between ACE and Ang I, indicating ACE has higher affinity and efficiency for kinins than for Ang I [91]. Modest quantitative changes in ACE expression do not significantly affect BP in humans or mice [59, 64]. Nevertheless, the D

allele, which causes higher levels of ACE, confers increased risk of a wide constellation of diseases, including diabetic nephropathy, congestive heart failure, and Alzheimer's disease [92-94], possibly due to decreased kinin levels. The kinins, bradykinin and kallidin in humans and rodents, are generated from kininogens by kallikreins. Humans have one kininogen gene while rodents have two closely linked kininogen genes [95, 96]. Kallidin can be converted into bradykinin by a plasma aminopeptidase.

Two receptors for kinins have been identified: B1R and B2R. Both of these receptors are G protein-coupled receptors with seven transmembrane domains [97]. B2R is constitutively expressed, whereas B1R is inducible by inflammation and by inhibition of B2R [98]. All the kinins are strong agonists of B2R, with less affinity for B1R [96]. Mice lacking B2R are normotensive, but have increased BP in response to a high-salt diet [99]. The B1R-/- mice have reduced BP [100], and antisense oligodeoxynucleotides targeted to the B1 receptor mRNA decreased BP in spontaneously hypertensive rats (SHR) [101]. In contrast, a B1R antagonist increased BP in the B2R-/- mice (but not in wild-type mice), suggesting that in the absence of the B2R the vascular B1R adopts B2K-like hemodynamic function [99]. In the presence of B2R, inhibition or removal of B1R upregulated B2R [102] and the protective BP lowering phenotype is likely due to augmented B2R effects.

Bradykinin through its receptors sequentially activates PI3-kinase, phosphorylates Akt and endothelial nitric oxide synthase (eNOS). B2R forms a complex with eNOS, from which the active enzyme is released following receptor activation [103]. Bradykinin also increases the association of heat-shock protein 90 with eNOS, which is required for nitric oxide (NO) formation [104]. eNOS is involved in the insulin signaling pathway. Because lack or decrease in eNOS expression leads to the metabolic syndrome [105, 106], it is likely that decreased expression of bradykinin receptors would also lead to the metabolic syndrome. Bradykinin receptors have three B2R polymorphisms (the C/T-58 promoter polymorphism, and the exon 2 (C/T181) and exon 1 (+/-9bp) polymorphisms) and one B1R promoter polymorphism (G/C-699) [107-109]. The B2R exon 1 (-9bp) polymorphism is a susceptibility marker for diabetic nephropathy [107].

The B1R-/- mice are lean and resistant against high-fat diet-induced weight gain, and have increased leptin sensitivity demonstrated by a reduction in food intake and body weight after leptin administration [110]. Thus, selective antagonism of the inducible B1R subtype may constitute a novel therapeutic approach for the treatment of obesity and diabetes [111]. The B2R-/-mice, or blockage of B2R by HOE140 in mice have shown to reduce clearance of glucose [112]. As in the case of BP phenotype, in the presence of B2R, inhibition or removal of B1R upregulates B2R expression [113] and the lean phenotype of B1R-/- mice is likely due to augmented B2R effects. In contrast, in the absence of B2R, B1R is upregulated [83], but it is thought that the effect on metabolic function is not strong enough to compensate for the absence of B2R. This hypothesis is being tested by investigating whether inhibition of either one or both of the bradykinin receptors (genetically and pharmacologically) confers obesity and insulin resistance. Blocking B1R, or increasing B2R activity, may provide a therapeutic strategy for the metabolic syndrome.

Angiotensin-Converting Enzyme 2

ACE2, discovered in 2000, is a homologue of ACE and a key player in the conversion of Ang II to Ang(1-7) and Ang I to Ang(1-9) (Fig. **7**). In contrast with ACE, ACE2 does not convert Ang I to Ang II and is not inhibited by ACE inhibitors [114]. ACE2 increases degradation of Ang II and reduces Ang II formation by competitively stimulating alternative pathways for Ang I degradation [114]. ACE2 is expressed on the surface of certain endothelial cell populations. Compared with ACE, the expression pattern of ACE2 is more limited, with highest expression in the kidney, followed by the heart and the testis. Several groups have created mice lacking ACE2. Crackower *et al.* reported that *Ace2* gene ablation severely impairs cardiac contractility and causes mild ventricular dilation and increased Ang II levels, but not alterations in BP [115]. Ablation of both *Ace* and *Ace2* prevented these cardiac abnormalities and the increase in Ang II formation. Thus, ACE and ACE2 probably counteract each other in terms of enzymatic activity and function [115]. In contrast, Gurley & Coffman demonstrated enhanced susceptibility of the *Ace2*-/- mice to Ang II-induced hypertension without detectable alteration in cardiac structure and function [116]. Yamamoto and colleagues reported normal baseline cardiac function in their *Ace2*-/- mouse, but found an accelerated cardiac dysfunction after aortic banding associated with enhanced accumulation of Ang II in the heart [117]. More detailed similarities and differences in these studies are reviewed in [118].

Mice lacking the Mas receptor have increased BP and exhibit increased anxiety, while ACE2 transgenic rats have low BP and cardioprotective effects [119]. In primary cultures of human cardiomyocytes, Ang(1-7) and Ang II have opposite and antagonistic effects on transcriptional regulation of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) [120]. This suggests a potential role for Ang(1-7) in attenuating cardiac damage in Ang II-induced extracellular matrix remodeling. The mechanism of the antiproliferative effect of Ang(1-7) in

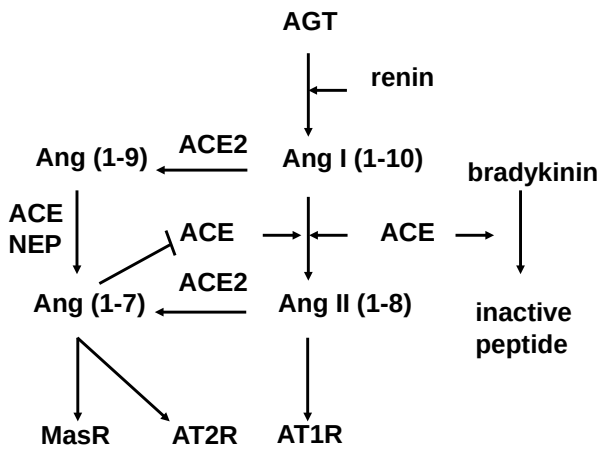

Fig. (7). Role of ACE2 in the RAS. ACE2 is a key player in the conversion of Ang II to Ang(1-7) and Ang I to Ang(1-9), and counteract the effects of Ang II. Different from ACE ACE2 does not convert Ang I to Ang II, or regulate plasma bradykinin levels.

the heart could be mediated by inhibition of Ang II-induced extracellular signal-regulated kinase 1 and 2 (ERK1/2) signaling [121]. ACE2 levels in the cardiovascular system are critical for the vasodilatory, antiproliferative effects of the ACE2-Ang(1-7)-MasR pathway.

Male *Ace2* KO mice displayed a selective decrease in first-phase insulin secretion in response to glucose and a progressive impairment of glucose tolerance compared with wild-type mice [122]. Mice lacking ACE2 also exhibit accelerated diabetic nephropathy with increased urinary albumin excretion, mesangial matrix scores, and glomerular basement membrane thicknesses [123].

Chymase

ACE is generally considered to be the main Ang II-producing enzyme in the systemic circulation. In tissues, however, many serine proteases, such as cathepsin G and chymases, appear to be capable of producing Ang II. Chymases are primarily in mast cells [124], but are also found in endothelial and mesenchymal cells [125], and in the human heart as a mechanism of ACE-independent Ang II production [126]. ACE inhibition does not block chymase generation of Ang II. Studies using a chymase inhibitor, and transgenic mice overexpressing human chymase, indicate that chymase causes hypercholesterolemia and atherosclerosis [127, 128]. Increased chymase activity is strongly associated with various cardiac diseases, such as myocardial ischemia, volume overload cardiac failure, cardiomyopathy, and viral myocarditis, suggesting that increased cardiac chymase activity is involved in cardiac remodeling. Chymase plays an important role in diabetes; the protein is markedly upregulated in the diabetic kidney and may be associated with the development of diabetic nephropathy [129]. The chymase inhibitor TY-51469 protects against pancreatic islet disorganization caused by streptozotocin [130]. Blocking alternative Ang II-producing pathways, such as chymases, may be beneficial in treating diabetes.

Ang II Receptors

Ang II has two receptors, the Ang II type I receptor (AT1R) and the Ang II type II receptor (AT2R). Both receptors are G-protein-coupled receptors with seven transmembrane domains [131]. The AT1R and AT2R subtypes have similar Ang II-binding properties but different genomic structure and localization, as well as tissue-specific expression and regulation. The AT1R is responsible for the classical actions of Ang II such as vasoconstriction, aldosterone release from the adrenal zona glomerulosa, salt retention in the renal tubules, and stimulation of the sympathetic nervous system. AT2R plays an important role in the growth, differentiation, and regeneration of neuronal tissue [132, 133].

The A1166C polymorphism in the 3'UTR of AT1R is strongly associated with the incidence of essential hypertension and increased coronary artery vasoconstriction [134], cardiac hypertrophy [135], and diabetic nephropathy [136], and predicts the development of the metabolic syndrome[137, 138]. Humans have only one AT1R, but mice have two AT1R subtypes: AT1aR and AT1bR (*Agtr1a* and *Agtr1b*) [139]. Their signaling mechanisms are almost identical but the regulation of their expression differs. AT1aR is dominant in most tissues relevant to the cardiovascular system, while AT1bR is only expressed in pituitary glands, testes, and adrenal gland [140, 141]. The type 2 receptor is highly and widely expressed during fetal development; but in adults, its expression is confined to the adrenal medulla, uterus, ovary, vascular endothelium and certain areas of the brain. AT2R appears to counterbalance some of the effects of AT1R by inducing vasodilatation, growth arrest and apoptosis (Fig. **8**).

Deletion of AT1aR causes hypotension and an increase in renin which leads to a profound increase in Ang II. Interestingly, *Agtr1b-/-* mice exhibit normal BP and pressor response to Ang II [142], whereas mice lacking both AT1aR and AT1bR have low BP similar to *Agt-/-* mice [143]. The dual null *Agtr1a-/-; Agtr1b-/-* mice also develop abnormal renal phenotypes identical to those observed in the *Agt-/-*, *Ace-/-*, and *Ren1c-/-* mice. In contrast, mice lacking the AT2R gene do not exhibit these phenotypes. BP in the *Agtr2-/-* mice is approximately 3-17 mmHg higher than in wild-type mice [144].

When the *Agtr2-/-* mice were treated with deoxycorticosterone acetate (DOCA) and salt, it became obvious that they are salt sensitive [144]. The *Agtr2-/-* mice show congenital

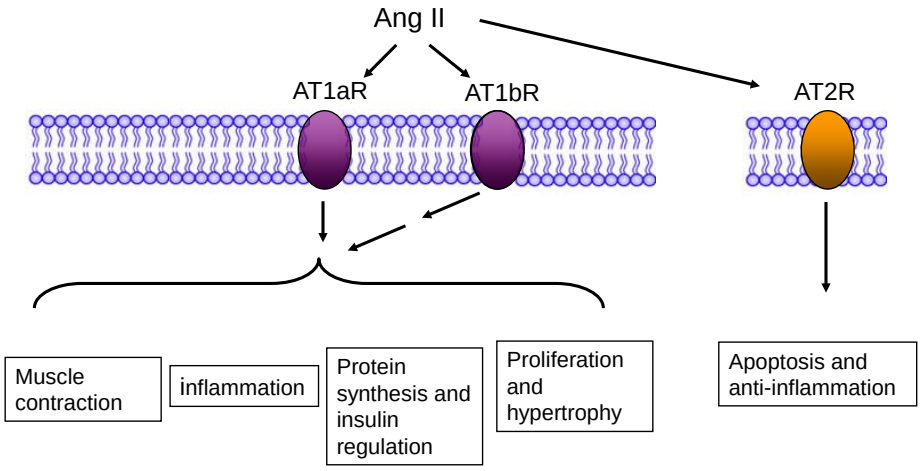

Fig. (8). Ang II receptor. Type 1 Ang II receptor (AT1R, AT1aR and AT1bR in mice) mediates the majority of Ang II effects, and regulates muscle contraction, inflammation, insulin levels and cell proliferation, while AT2R regulates apoptosis.

anomalies of the kidney and urinary tract (CAKUT) as observed in humans, namely lack of interstitial fibrosis at birth, with some hypoplastic, cystic, and/or dysplastic parenchyma [145]. They also identified a single nucleotide transition (A-1332G) in intron 1 of the *AGTR2* gene in patients with CAKUT, which decreases the amount of *AGTR2* mRNA expression.

Kouyama *et al.* have shown that the *Agtr1a-/-* mice are lean, resistant to diet-induced obesity, with improved insulin sensitivity [146]. These authors also found that these mutant mice have increased sympathetic activity and energy expenditure, probably due to the activation of AT1bR. These observations suggest that *Agtr1a-/-* mice are protected from some components of the metabolic syndrome. In addition, blocking AT1R with losartan activates the insulin-mediated IRS1/PI3/GLUT4 cascade in skeletal muscle and white adipose tissue, leading to improved glucose tolerance and insulin sensitivity [147]. AT2R is expressed in the heart, kidney, brain, uterus, and adipose tissue [148]. AT2R expression in adipose tissue is low, but AT2R seems to mediate Ang II stimulation of adipose tissue development [149]. AT2R can induce production and release of prostacyclin from adipocytes, which in turn stimulates differentiation of preadipocytes [150]. Mice lacking *Agtr2* have increased glucose uptake in adipose tissues [150]. Laurent *et al.* have shown that the $Agtr2^{y/-}$ mice (AT2R gene is on the X chromosome) have normal adiposity but display small adipocytes at an increased number [151]. These authors found that mice lacking *Agtr2* have increased lipid oxidation, which is caused by increased expression of fatty acid translocase, uncoupling protein-3, peroxisome proliferation activated receptor (α, δ), and carnitine palmitoyl transferase-1 (CPT-1). In agreement with their lean phenotype, the $Agtr2^{y/-}$ mice have decreased food intake and increased total energy expenditure. These studies indicate that inhibition of AT1R and AT2R improves insulin sensitivity and causes fat loss, which is in agreement with the involvement of Ang II in the insulin signaling pathway and in the control of adipose tissue metabolism.

Pharmacological Inhibition of RAS

Blocking RAS with ACE inhibitors or Ang receptor blockers (ARB) has become a crucial element of cardiovascular and renal medicine. Clinical studies indicate that ARBs and ACE inhibitors prevent the onset of diabetes and improve insulin sensitivity [152, 153]. In mice, blocking AT1R improves insulin sensitivity and other diabetes symptoms due to increased glucose uptake in skeletal muscle and white adipose tissue [154]. This is due to enhanced insulin signaling and GLUT4 translocation to the plasma membrane [154]. Treatment with ARBs (losartan, or candesartan) does not reverse diabetes, but it effectively improves glucose tolerance and protects β-cell function by attenuating oxidative stress, islet fibrosis, sparse blood supply, and disruption of ultrastructure in a dose-dependent and BP-independent manner [155]. In addition, ARBs may protect the pancreas in type II diabetic animal models with enhanced insulin secretion [156]. Furthermore, in diabetic models, some ARBs induce adipocyte differentiation by increasing peroxisome proliferator-activated receptor-gamma (PPARγ) in adipose tissue [157]. Accordingly, ARBs could be a novel form of treatment for the metabolic syndrome and associated pathological disorders.

Moreover, aliskiren is a novel renin inhibitor for the treatment of hypertension [158]. This renin inhibitor has protective effects on endothelial function and atherosclerotic changes. Lu *et al.* showed that aliskiren resulted in striking reductions of atherosclerotic lesion size in both the aortic arch and the root in fat-fed LDL receptor-deficient ($Ldlr^{-/-}$) mice [159]. Furthermore, cotreatment with aliskiren and an ARB has additive protective effects [160]. Thus, aliskiren may also be a promising protective drug for patients with hypertension and diabetes [161].

Summary and Future Perspectives

In the past decade we have seen a dramatic improvement of our understanding of RAS. An overactive RAS has been shown to be associated with the metabolic syndrome using gene targeting approaches in animal models. Clinical trials have shown that ACE inhibitors and ARBs have beneficial effects on insulin sensitivity. However, uncovering the physiological roles of the Ang-independent effects of renin, PRR, and ACE2 will require additional study. The challenge over the next decade will be to gain a better understanding of the physiological and pathophysiological roles of RAS to identify new therapeutics for the metabolic syndrome.

ACKNOWLEDGMENTS

The authors thank Drs. Guido Geric, Hyung-Suk Kim, Weili Lin, Nobuyo Maeda and Oliver Smithies for their assistance.

This work is supported by grants from the National Institutes of Health (HL 077145, DK076131, HL49277, P30DK56350), American Heart Association (0855335E), and UNC Department of Pathology and Laboratory Medicine.

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Received: February 12, 2010 Revised: April 08, 2010 Accepted: April 19, 2010