Renin-Angiotensin System and Cardiovascular Functions

Chia-Hua Wu,* Shayan Mohammadmoradi,* Jeff Z. Chen,* Hisashi Sawada,* Alan Daugherty, Hong S. Lu

The renin-angiotensin system plays critical roles in maintaining normal cardiovascular functions and contributes to a spectrum of cardiovascular diseases. Classically, the renin-angiotensin system is composed of AGT (angiotensinogen), renin, angiotensin-converting enzyme (ACE), Ang II (angiotensin II), and 2 Ang II receptors (AT1 and AT2 receptors).1,2 AGT, a protein with 452 amino acids, is cleaved by renin to produce Ang I. Ang I is a decapeptide, which is then cleaved by ACE to produce Ang II. Ang II is an octapeptide, acting through binding to its receptors, AT1 and AT2 receptors. AT1 receptor is the major receptor for Ang II to regulate many physiological and pathophysiological functions.3–6 In mice, AT1 receptor has 2 subtypes, AT1a and AT1b, which have >90% sequence homology, but distinctive distributions and functions.4,7–12 AT1a receptor is important for blood pressure regulation and contributes to atherosclerosis and aortic aneurysms,5,13,14 whereas AT1b receptor has no evident contribution to these functions15 but is associated with vasculature contractility.16–18 AT2 receptor is abundant during fetal development but becomes low in most tissues after birth.18

In the past 2 decades, many new components in this system have been discovered. These include ACE2, a homologue of ACE, which converts Ang II to Ang(1–7) or converts Ang I to Ang(1–9).19,20 The G protein–coupled receptor Mas1 was identified as the receptor of Ang(1–7).21

This review highlights some recent publications in ATVB that have provided insights into understanding the classic components of the renin-angiotensin system and its alternative components contributing to cardiovascular functions. We will focus on effects of this hormonal system on cardiac dysfunction, hypertension, atherosclerosis, and aortic aneurysms.22–29

Angiotensinogen

AGT is the only known substrate of the renin-angiotensin system to produce all downstream angiotensin peptides. AGT regulates blood pressure as demonstrated by multiple mouse models, including global AGT-deficient mouse model and human AGT and renin transgenic mouse model.30–33 AGT was also implicated in atherosclerosis using a transgenic mouse model expressing both human angiotensinogen (Agt) and renin genes.34 Two recent studies have provided direct evidence that AGT regulates blood pressure and contributes to atherosclerosis through Ang II–mediated mechanisms.35,36 These studies used multiple genetic manipulations, including AGT hypomorphic mice, bone marrow transplantation, hepatocyte-specific AGT-deficient mouse model, and adeno-associated viral infection to repopulate the manipulated Agt in vivo. These studies demonstrate that hepatocyte-derived AGT is the predominant source to regulate blood pressure and promote atherosclerosis. A pharmacological approach using antisense oligonucleotides has also opened a door to directly target AGT for preventing high blood pressure and atherosclerosis.36

Renin

Renin is the rate-limiting enzyme of the renin-angiotensin system and the only enzyme known to cleave AGT. These properties make renin a potentially attractive target to inhibit the renin-angiotensin cascade and improve Ang II–mediated cardiovascular dysfunctions.37,38 Inhibition of renin reduces blood pressure and atherosclerosis in animal models.6,36,39–43 Unfortunately, renin inhibitors in patients with cardiovascular diseases have not provided superior beneficial effects beyond the well-established ACE inhibitors or AT1 receptor blockers.44

Despite some disappointing findings in human studies of renin inhibition, it has not discouraged research to understand renin-related mechanisms of cardiovascular diseases. The juxtaglomerular cells of the kidney are the major source of renin production and secretion. As an important organ in blood pressure regulation and cardiovascular functions, renal denervation aiming to reduce sympathetic nerve activity has drawn significant attention, although there are conflicting findings that need further research.45–46 A recent study using pigs discovered that this approach reduced blood pressure and improved cardiovascular functions through its influence on kidney-brain-heart axis with profound changes of plasma renin activity, implicating the involvement of the renal renin-angiotensin system regulation in the process.49

Angiotensin-Converting Enzymes

In contrast to the rate-limiting and substrate-specific properties of renin, ACE is not sensitive to Ang II concentration changes, and it is an enzyme that cleaves not only Ang I but also many other substrates including bradykinin (a vasodilator) and N-acetyl-Ser-Asp-Lys-Pro (a hemoregulatory peptide).50–55 There is a highly consistent literature demonstrating that ACE inhibition reduces blood pressure and atherosclerosis in animal models.6,54,55 ACE inhibitors are one major class for treatment of hypertension, cardiovascular dysfunctions, and diabetic nephropathy in patients.56–60 Recent studies have also added new mechanistic insights into guiding the use of ACE inhibitors. It was found that high serum concentration of

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From the Saha Cardiovascular Research Center (C.-H.W., S.M., J.Z.C., H.S., A.D., H.S.L.), Department of Pharmacology and Nutritional Sciences (C.-H.W., S.M., A.D., H.S.), and Department of Physiology (J.Z.C., A.D., H.S.L.), University of Kentucky, Lexington.

*These authors contributed equally to this article.

Correspondence to Hong S. Lu, MD, PhD, Saha Cardiovascular Research Center, University of Kentucky, BBSRB Room B249, 741 S Limestone, Lexington, KY 40503. E-mail hong.lu@uky.edu

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homocysteine decreased antihypertensive effect of enalapril, an ACE inhibitor, in chronic hypertensive patients.61

ACE is ubiquitously present in many cell types, tissues, and organs.62,63 Leukocyte or smooth muscle cell–derived ACE contributed to atherosclerosis as demonstrated by bone marrow transplantation and cell-specific depletion of ACE, respectively, in mouse models,54,64 although their effects were less potent than pharmacological inhibition of ACE systemically.7 ACE is abundant in endothelial cells.65 However, depletion of ACE in this cell type had no effects on atherosclerosis.64 Global genetic depletion or pharmacological inhibition of ACE reduced blood pressure.6,66 but depletion of ACE in leukocyte, endothelial cells, or smooth muscle cells did not affect blood pressure.54,64 Despite a well-known enzyme discovered half century ago67,68 with impressive success of its inhibitors in clinical patients,69 it is still a long road to define mechanisms by which ACE contributes to multiple cardiovascular functions, including its cellular source that influences blood pressure regulation.

Angiotensin II

As the major bioactive peptide of the renin-angiotensin system, there are broad views of mechanistic insights into understanding how Ang II contributes to multiple cardiovascular physiological and pathophysiological functions. We provide a brief review of the following diseases published recently in ATVB. For most of these studies, the approach used was chronic subcutaneous infusion of Ang II.70,71

Cardiac Dysfunction

Ang II induces several forms of cardiac dysfunction including hypertrophy, arrhythmia, and ventricle function failure.72,73 Basigin is a transmembrane glycoprotein that has multiple functions.74 In a mouse model of transverse aortic constriction, genetic reduction of basigin led to less cardiac hypertrophy, fibrosis, and heart failure.75 Deficiency of smooth muscle stromal interaction molecule 1, an endoplasmic reticulum Ca2+ sensor, also prevented Ang II–induced cardiac hypertrophy.76 These findings are consistent with renin-angiotensin inhibition is crucial for improving cardiac dysfunction.

Hypertension

There are many factors contributing to hypertension.77–79 Salt intake is believed to be a critical factor for high blood pressure.80 Ang II is also a well-recognized contributor to high blood pressure.81,82 However, high salt intake suppresses the renin-angiotensin system, whereas low dietary salt increases Ang II production.83,84 In accord with the paradox between salt intake and the renin-angiotensin regulation, dietary salt intake in blood pressure regulation and its consequent cardiovascular events have also been inconsistent, as reported in both human studies and animal models,85–91 implicating complex molecular mechanisms involved in salt versus Ang II–mediated hypertension and related cardiovascular dysfunctions.

Batchu et al92 found that Axl, a receptor tyrosine kinase, in T lymphocytes exerted a significant role in Ang II–mediated blood pressure regulation. This finding is consistent with reports by Guzik et al92 and Norlander et al93 that T-lymphocyte–mediated immune response contributed to Ang II–induced high blood pressure, although this needs to be validated in human studies. In addition to immune cells, smooth muscle cells are a critical cell type in Ang II–mediated blood pressure regulation. Smooth muscle 22α is a cytoskeleton-associated protein in smooth muscle cells. Smooth muscle 22α deficiency in mice reduced Ang II–induced high blood pressure and senescence of vascular smooth muscle cells.93,94 These phenotypes were proposed to be associated with many mediators including p53-dependent pathway.95 Activation of the α7 subtype of nicotinic acetylcholine receptors (α7nAchR) inhibited Ang II–induced senescence in cultured vascular smooth muscle cells and wild-type mice, but not in mice with α7nAchR deficiency. This effect was associated with sirtuin 1 activity because inhibition of sirtuin 1 abrogated this effect.96 microRNA-143 and 145 are abundant in vascular smooth muscle cells and regulate myogenic tone.97 Depletion of these 2 microRNAs did not affect Ang II–induced high blood pressure but caused more severe arterial wall disruption, vascular remodeling, and inflammation.98 Another recent study identified cellular repressor of E1A-stimulated genes as a mediator of Ang II–induced vascular remodeling.99 From these recent studies, we can gather that Ang II–mediated hypertension is a complex process that involves a large spectrum of molecules and many cell types.

Atherosclerosis

Atherosclerosis is a complex disease involving diverse mechanisms including disordered lipoprotein metabolism, inflammation, endothelial dysfunction, reactive oxygen species, and endoplasmic reticulum stress.29,100–103 Animal models are a common tool to study these mechanisms and exploring potential therapeutic targets. For example, application of drugs using nanoparticles holds promise to optimize drug delivery and efficacy. In apolipoprotein E–deficient (ApoE–/–) mice fed a high-fat diet and infused with Ang II, nanoparticles containing pioglitazone, an antidiabetic drug that also had peroxisome proliferator–activated receptor-γ agonistic effects, was injected intravenously on a weekly basis for 4 weeks. Although pioglitazone administration did not change atherosclerotic lesion size and macrophage content, it reduced Ly-6C high monocytes, matrix metalloproteinase activity, and cathepsin activity.104

In addition to mouse models, rabbits have been frequently used to study atherosclerosis. In one study, infusion of Ang II to Watanabe heritable hyperlipidemic rabbits led to high death rate (50% for Ang II 100 ng/kg per minute and 92% for Ang II 200 ng/kg per minute) because of acute myocardial infarction with coronary plaque erosion, rupture, and thrombosis.105 Because plaque rupture and thrombosis are high-risk complications in humans,106 this model would be optimal to study mechanisms related to the human disease. In another study, Honda et al107 infused Ang II to Japanese White rabbits when they were fed a high-cholesterol diet and injured using balloon catheter to femoral arteries. This procedure also led to atherothrombotic occlusions. Ezetimibe, a lipid-lowering drug used in patients, profoundly decreased this fatal pathology, providing rationale to determine its extended effects in patients.107

Thoracic Aortic Aneurysms

Thoracic aortic aneurysms (TAA) manifest as profound dilation of the thoracic aorta, accompanied by compromise of
Aortic wall integrity, dissection, or rupture. Many genetic disorders are involved in this disease process including fibrillin-1, TGF (transforming growth factor)-β ligands and receptors, smooth muscle cell–specific isoforms of α-actin (encoded by Acta2), and myosin heavy chain (encoded by Myh11). In addition to these genetic manipulations, infusion of Ang II also leads to TAA, predominantly localized to the ascending aortic region.

The aortic wall is composed of the intima, media, and adventitia. Among the cell types of the aorta, smooth muscle cells are the most abundant cell type and have been the most frequently studied cell type in the development of TAA. Vascular smooth muscle cell phenotypes are associated with aortic aneurysm formation and its pathological process.

Components of TGF-β signaling pathways are important for maintaining aortic wall integrity. However, its effects on TAA and abdominal aortic aneurysm (AAA) formation are controversial. Inhibition of TGF-β by neutralizing antibodies augmented aortic rupture rate and aortic dilation in both abdominal and thoracic aortic regions in Ang II–infused mice but attenuated development of TAA in a Marfan mouse model. To explore the conflicting findings in different mouse models and different locations of aortic aneurysms, a recent study determined mechanisms of TGF-β signaling in Ang II–induced TAA and AAA, combined with smooth muscle cell–specific TGF-β receptor 2 deficiency. Systemic TGF-β neutralization augmented AAA but had no effects on TAA. In contrast, smooth muscle cell–specific TGF-β receptor 2 deficiency augmented TAA but had no apparent effects on the abdominal aorta. This study emphasizes the distinctive mechanisms between TAA and AAA.

MicroRNA-21 was identified as a critical modulator of proliferation and apoptosis of smooth muscle cells during development of AAA. Overexpression of microRNA-21 reduced AAA, and inhibition of this microRNA augmented AAA in 2 common mouse models. A recent study discovered that in mice with Smad3 heterozygous background, aortic micR-21 expression was increased by Ang II infusion, and a recent study reported that NLRP3 or caspase-1 deficiency in mice significantly reduced Ang II–induced contractile protein degradation and aortic aneurysm formation in both thoracic and abdominal aortic regions.

Abdominal Aortic Aneurysms

AAA is defined as pathological dilation of the abdominal aorta. Same as individuals afflicted with TAA, aortic rupture is a fatal consequence of AAA. There are three commonly used mouse models to study AAA: perfusion of elastase into the infrarenal aorta, periaortic application of calcium chloride, or subcutaneous infusion of Ang II. Modifications of these mouse models have also provided mechanistic insights. For example, coadministration of β-aminopropionitrile with Ang II, coadministration of TGF-β–neutralizing antibody with Ang II, administration of TGF-β–neutralizing antibody to mice with elastase-induced AAA, or application of calcium chloride with phosphate-buffered saline onto the infrarenal aorta.

Hypercholesterolemia augments Ang II–induced AAA. Therefore, Apoε−/− mice and low-density lipoprotein receptor–deficient mice are the 2 commonly used mouse models for Ang II–induced AAA studies. Although Ang II–infused mouse model has become a popular model to study AAA, breeding mice to a hypercholesterolemic background has hampered its more broad use. A recent study provided a rapid approach for increasing plasma cholesterol and Ang II–induced AAA incidence in C57BL/6 mice by applying a gain-of-function mutation of mouse PCSK9 protein using an adeno-associated viral method, which was also frequently used in atherosclerosis studies.

Inflammation and extracellular matrix disruption and remodeling are important features of Ang II–induced AAA. Publications describing Ang II–induced AAA were featured in a recent ATVB Highlights, including molecules that promote inflammation involving not only macrophages but also T and B lymphocytes, oxidative stress, and many other factors.

In addition to extensive studies to define molecular mechanisms of AAA, some recent studies have emphasized the importance of studying sex differences. One study used the 4 core mouse model to generate gonadal male mice with XX or XY chromosomes. This study found that gonadal male mice with an XY chromosome complement exhibited diffuse aortic aneurysms, whereas XX chromosome complement exhibited focal aortic dilation. Orchiectomy attenuated Ang II–induced TAA and AAA in male mice.

Angiotensin II Receptors

AT1a Receptor

AT1a receptor, a subtype of Ang II receptor, is the major receptor for Ang II–mediated cardiovascular functions in mice. Global deficiency of AT1a receptor ablates atherosclerosis and attenuates Ang II–induced TAA and AAA. This effect was not attributed to the presence of AT1a receptor on leukocytes or smooth muscle cells, whereas endothelial cell–specific depletion of AT1a receptor had modest protective effects on Ang II–induced TAA but not AAA and
atherosclerosis. In agreement with these previous studies, using a well-established Marfan mouse model with genetic disruption of fibrillin-1 expression, Galatioto et al. found that endothelial cell–specific deletion, but not smooth muscle cell–specific deficiency, of AT1a receptor modestly attenuated TAA development and related aortic rupture.

**AT2 Receptor**

Although AT2 receptor remains low in most tissues and organs postnatally, many studies have reported increased presence of AT2 receptor under certain pathophysiological conditions as reviewed in a recent article. Genetic deletion of AT2 receptor in mice had no effects on general health and development but promoted angiogenesis within ischemic muscle. A diabetic mouse model with a spontaneous mutation in the insulin 2 gene (Ins2+/-C96Y) was bred with AT2 receptor–deficient mouse model. Hindlimb ischemia was induced by ligating femoral artery. Depletion of AT2 receptor augmented blood flow reperfusion and collateral vessel formation that were associated with SH2 domain-containing phosphatase 1 activity and vascular endothelial growth factor action.

**Alternative Pathways**

This section introduces an enzyme, a bioactive peptide, and a receptor beyond the classic renin–angiotensin components.

**Angiotensin-Converting Enzyme 2**

ACE2 prevents atherosclerosis and aortic aneurysms, as demonstrated by deficiency of ACE2 accelerating atherosclerosis and Ang II–induced AAA in hypercholesterolemic mice. Recently, Moran et al. reported that ACE2 deficiency in Apoe−/− mice augmented incidence of AAA and aortic rupture rate. Of note, deficiency of ACE2 also led to spontaneous AAA formation in the absence of Ang II. Resveratrol, a class of compounds produced by many plants, increased ACE2 and inhibited AAA growth in Ang II–infused mice.

**Angiotensin (1–7) and Mas1**

Recent studies have implicated that Ang(1–7) has protective effects on multiple cardiovascular functions through its interaction with Mas1. Many studies reported that Ang(1–7)/Mas1-mediated actions counteracted actions of Ang II. For example, Ang(1–7) had vasodilatory effect that was mediated by Mas1, whereas Ang II had potent vasoconstrictor effect. One study reported that Ang(1–7)–induced NO–mediated vasodilation and increased telomerase activity of endothelial cells. In another study, low dose of Ang(1–7) increased angiogenesis and vasodilation through its interaction with Mas1, which had equivalent effects as same low dose of Ang II. Among potential mechanisms, ERK1/2 was essential for Ang(1–7)–induced angiogenesis and vasodilation.

**Summary**

Although the major renin-angiotensin members were discovered more than a half century ago, this system still attracts a large number of research work in different fields. This implicates the importance of this hormonal system in physiological and pathophysiological functions but also notes that there are many unknowns and conundrums of this system in our knowledge that require more extensive research work.

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None.

**References**

1. Wu C, Lu H, Cassis LA, Daugherty A. Molecular and pathophysiological features of angiotensinogen: a mini review. *Ann N Y Acad Sci* (Boston). 2011;1218:190.
2. Lu H, Cassis LA, Kooi CW, Daugherty A. Structure and functions of angiotensins. *Hypertens. Res*. 2016;39:492–500. doi: 10.1038/hr.2016.17.
3. Ito M, Oliverio MI, Mannon PJ, Best CF, Maeda N, Smithies O, Coffman TM. Regulation of blood pressure by the type 1A angiotensin II receptor gene. *Proc Natl Acad Sci USA*. 1995;92:3521–3525.
4. Oliverio MI, Best CF, Kim HS, Arendshorst WJ, Smithies O, Coffman TM. Angiotensin II responses in AT1A receptor–deficient mice: a role for AT1B receptors in blood pressure regulation. *Am J Physiol*. 1997;272(4 pt 2):F515–F520. doi: 10.1152/ajprenal.1997.272.4.F515.
5. Daugherty A, Rateri DL, Lu H, Inagami T, Cassis LA. Hypercholesterolemia stimulates angiotensin peptide synthesis and contributes to atherosclerosis through the AT1A receptor. *Circulation*. 2004;110:3849–3857. doi: 10.1161/01.CIR.0000150540.54220.C4.
6. Lu H, Balakrishnan A, Howatt DA, Wu C, Charnigo R, Liu G, Cassis LA, Daugherty A. Comparative effects of different modes of renin angiotensin system inhibition on hypercholesterolemia–induced atherosclerosis. *Br J Pharmacol*. 2012;165:2000–2008. doi: 10.1111/j.1476-5381.2011.01712.x.
7. Burson JM, Aguiler G, Gross KW, Sigmund CM. Differential expression of angiotensin receptor 1A and 1B in mouse. *Am J Physiol*. 1994;267(2 pt 1):E260–E267. doi: 10.1152/ajpcell.1994.267.2.E260.
8. Gasc JM, Shannumug S, Sibony M, Corvol P. Tissue-specific expression of type 1 angiotensin II receptor subtypes. An in situ hybridization study. *Hypertension*. 1994;24:531–537.
9. Sugaya T, Nishimatsu S, Tanimoto K, Takimoto E, Yamagishi T, Imamura K, Goto S, Imaizumi K, Hisada Y, Otsuka A. Angiotensin II type 1a receptor-deficient mice with hypotension and hyperreninemia. *J Biol Chem*. 1995;270:18719–18722.
10. Chen X, Li W, Yoshida H, Tsuchida S, Nishimura H, Takimoto E, Sibony M, Corvol P. Tissue-specific expression of renin angiotensin system inhibition on hypercholesterolaemia–induced atherosclerosis. *Br J Pharmacol*. 1998;126:15496–15501.
F1000Res. 2016;5: doi: 10.12688/f1000research.7508.v1. eCollection 2016.

Bernstein KE, Khan Z, Gianfi JF, Cao DY, Bernstein EA, Shen XZ. Angiotsin-converting enzyme in innate and adaptive immunity. Nat Rev Nephrol. 2016;12:323–336. doi: 10.1038/nrneph.2016.15.

Chien X, Li H, Zhao M, Tashiro K, Cassis LA, Daugherty A. Contributions of leukocyte angiotsin-converting enzyme to development of atherosclerosis. Arterioscler Thromb Vasc Biol. 2013;33:2075–2080. doi: 10.1161/ATVBAHA.113.301777.

Lu H, Cassis LA, Daugherty A. Atherosclerosis and arterial blood pressure in mice. Curr Drug Targets. 2007;8:1181–1189.

Yasuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. N Engl J Med. 2000;342:145–153.

Svensson P, de Faire U, Sleight P, Osterberg S. Comparative outcomes: results of the ONTARGET trial. J Hypertens. 2013;31:414–421. doi: 10.1097/HJH.0b013e32835bf700.

Jin SM, Han KA, Yu JM, et al. Probucol in albuminuric type 2 diabetes. J Cardiovasc Med (Hagerstown). 2016;10:146–147. doi: 10.1097/JCM.0000000000000518.

Mann JF, Anderson C, Gao P, Gerstein HC, Boehm M, Rydén L, Sleight P, et al. Basigin promotes cardiac fibrosis and failure in response to chronic pressure overload in mice. Arterioscler Thromb Vasc Biol. 2016;36:636–646. doi: 10.1161/ATVBAHA.116.306566.

Madsen A, Ophoff RA, Strohschein LM, Yamazaki N, Wang Y, Schuster SC. Role of renin-angiotensin system–an endocrine and paracrine system. Endocrinology. 2003;144:2179–2183. doi: 10.1210/en.2003-0150.

Lu H, Cassis LA, Daugherty A, Lu H. Angiotensin-converting enzyme in smooth muscle cells promotes atherosclerosis–brief report. Arterioscler Thromb Vasc Biol. 2016;36:1085–1089. doi: 10.1161/ATVBAHA.115.307038.

Dzau VJ, Bernstein K, Celermajer D, et al. Pathophysiological and therapeutic importance of tissue ACE: a consensus report. Cardiov Drugs Ther. 2002;16:149–160.

Chen X, Lu H, Zhao M, Tashiro K, Cassis LA, Daugherty A, Lu H. Angiotsin-converting enzyme inhibitors in hypertensive patients. Arterioscler Thromb Vasc Biol. 2017;37:166–172. doi: 10.1161/ATVBAHA.116.308515.

Shen XZ, Xiao HD, Li P, Billlet S, Lin CX, Fuchs S, Bernstein KE. Tissue specific expression of angiotsin converting enzyme: a new way to study an old friend. Int Immunopharmacol. 2008;8:171–176. doi: 10.1016/j.intimp.2007.08.010.

Kesson M, Ait-Aissa K, Radwan E, Mali V, Haddox S, Gabani M, Zhang W, Belmadani S, Irani K, Trebak M, Matrougui K. Essential role of smooth muscle STIM1 in hypertension and cardiovascular dysfunction. Arterioscler Thromb Vasc Biol. 2016;36:1900–1909. doi: 10.1161/ATVBAHA.116.307869.

Sun J, Canton G, Balu N, Hippe DS, Xu D, Liu J, Hatakasa TS, Yuan C. Blood pressure is a major modifiable risk factor implicated in pathogenesis of intraplaque hemorrhage: an in vivo magnetic resonance imaging study. Arterioscler Thromb Vasc Biol. 2016;36:743–749. doi: 10.1161/ATVBAHA.115.307048.

Lavoie JL, Sigmund CD. Minireview: overview of the renin-angiotensin system—an endocrine and paracrine system. Endocriology. 2003;144:2179–2183. doi: 10.1210/en.2003-0150.

Jin SM, Han KA, Yu JM, et al. Probucol in albuminuric type 2 diabetes mellitus patients on renin-angiotensin system blockade: a 16-week, randomized, double-blind, placebo-controlled trial. Arterioscler Thromb Vasc Biol. 2016;36:2108–2114. doi: 10.1161/ATVBAHA.116.308034.

Lu H, Cassis LA, Daugherty A, Lu H. Angiotsin-converting enzyme in smooth muscle cells promotes atherosclerosis—brief report. Arterioscler Thromb Vasc Biol. 2016;36:1085–1089. doi: 10.1161/ATVBAHA.115.307038.

Ng KK, Vane JR. The conversion of angiotensin I to angiotensin II in vivo. Naunyn Schmiedebergs Arch Exp Pathol Pharmacol. 1968;259:189–198.

Ketonen J, Merasto S, Paakkari I, Mervaala EM. High sodium intake increases plasma lipoprotein and inflammatory marker concentrations to urinary sodium excretion. Atherosclerosis. 2008;200:410–416. doi: 10.1016/j.atherosclerosis.2007.12.034.

Milei J. Angiotsin-converting enzyme in smooth muscle cells promotes atherosclerosis—brief report. Arterioscler Thromb Vasc Biol. 2016;36:1085–1089. doi: 10.1161/ATVBAHA.115.307038.

Ketonen J, Merasto S, Paakkari I, Mervaala EM. High sodium intake increases plasma lipoprotein and inflammatory marker concentrations to urinary sodium excretion. Atherosclerosis. 2008;200:410–416. doi: 1.1016/j.atherosclerosis.2007.12.034.

Alderman MH, Cohen H, Madhavan S. Dietary sodium intake and mortality: the National health and nutrition examination survey (NHANES I). Lancet. 1998;351:781–785. doi: 10.1016/S0140-6736(97)00992-2.
91. Lu H, Wu C, Howatt DA, Balakrishnan A, Charnigo RJ Jr, Cassis LA, Daugherty A. Differential effects of dietary sodium intake on blood pressure and atherosclerosis in hypercholesterolemic mice. *J Nutr Biochem.* 2013;24:49–53. doi: 10.1016/j.nutbio.2012.03.001.

92. Guzik TJ, Hoch NE, Brown KA, McCann LA, Rahman A, Dikalov S, Goronyz J, Weyand C, Harrison DG. Role of the T cell in the genesis of angiostatin II induced hypertension and vascular dysfunction. *J Exp Med.* 2007;204:2449–2460. doi: 10.1084/jem.20070657.

93. Norlander AE, Saleh MA, Pandey AK, Itani HA, Wu J, Xiao L, Kang J, Dale BL, Goleva SB, Laroumiane F, Du L, Harrison DG, Madhur MS. A salt-sensing kinase in T lymphocytes, SGK1, drives hypertension and hyperpertensive end-organ damage. *JCI Insight.* 2017;2. doi: 10.1172/jci.insight.92801.

94. Kunieda T, Minaminoto T, Nishi J, Tateno K, Oyama T, Katsuno T, Miyauchi H, Orimo M, Okada S, Takamura M, Nagai T, Kaneko S, Komuro I. Angiostatin II induces premature senescence of vascular smooth muscle cells and accelerates the development of atherosclerosis via a β2-dependent pathway. *Circulation.* 2006;114:953–960. doi: 10.1161/CIRCULATIONAHA.106.626606.

95. Miao SB, Xie XL, Yin YJ, Zhao LL, Zhang F, Shu YN, Chen R, Chen P, Dong LH, Lin YL, Lv P, Zhang DD, Nie X, Xue ZY, Han M. Accumulation of smooth muscle α2α protein accelerates senescence of vascular smooth muscle cells via stabilization of p53 in vitro and in vivo. *Arterioscler Thromb Vasc Biol.* 2017;37:1849–1859. doi: 10.1161/ATVBAHA.170.309378.

96. Lu H, Daugherty A, Fu H, Zhang LS, Shen FM. α7 Nicotinic acetylcholine receptor relieves angiostatin II-induced senescence in vascular smooth muscle cells by raising nicotinamide adenine dinucleotide-dependent SIRT1 activity. *Arterioscler Thromb Vasc Biol.* 2016;36:1566–1576. doi: 10.1161/ATVBAHA.117.307157.

97. Boettger T, Beetz N, Kostin S, Schneider J, Krüger M, Hein L, Braun T. Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the Mir143/145 gene cluster. *J Clin Invest.* 2009;119:2634–2647. doi: 10.1172/JCI38864.

98. Holmberg J, Bhattacharjya A, Alajbegovic A, Rippe C, Ekman M, Dahan D, Hien TT, Boettger T, Braun T, Swärd K, Hellstrand P, Albinsson S. Loss of vascular myogenic tone in miR-143/145 knockout mice is associated with hypertension-induced vascular lesions in small mesenteric arteries. *Arterioscler Thromb Vasc Biol.* 2018;38:414–424. doi: 10.1161/ATVBAHA.117.310499.

99. Li Y, Liu Y, Tian X, Zhang Y, Song H, Liu M, Zhang X, Liu H, Zhang J, Zhang Q, Liu D, Peng C, Yan C, Han Y. Cellulor repressor of E1A-stimulated genes is a critical determinant of vascular remodeling in response to angiostatin II. *Arterioscler Thromb Vasc Biol.* 2017;37:485–494. doi: 10.1161/ATVBAHA.116.308794.

100. Tall AR, Yvan-Charvet L, Westerterp M, Murphy AJ. Cholesterol efflux: role of cytokines in atherosclerosis. *Circulation.* 2012;32:2045–2051. doi: 10.1161/CIRCULATIONAHA.110.179705.

101. Libby P. Inflammation in atherosclerosis. *Nature.* 2011;473:308–316. doi: 10.1038/nature10145.

102. Ait-Oufella H, Taleb S, Mallat Z, Tedgui A. Recent advances on the pathogenesis of aneurysms. *Arterioscler Thromb Vasc Biol.* 2011;31:969–979. doi: 10.1161/ATVBAHA.110.207415.

103. Rateri DL, Moorleghen JJ, Balakrishnan A, Cassis LA, Daugherty A. Depletion of endothelial or smooth muscle α7 Nicotinic acetylcholine receptors ameliorates aortic aneurysm and hypertensive end-organ damage. *JCI Insight.* 2017;2. doi: 10.1172/jci.insight.92801.

104. Li S, Wang YN, Niimi M, Ning B, Chen Y, Kang D, Wang Z, Yu Q, Li J, Wu Q, Yuan H, Li Y, Shi H, Zheng J, Wang Z, Wu Y, Zhang J, Zhang M, Zhang D, Liu H, Zhang J, Gu X, Shu Y, Chen H, Li T, Liang W, Wu Z, Xia C, Li X, Lu Y, Pan W, Wang Y, Zhang J, Liu J, Wang J, Xue J, Guo B, Wang T, Biery NJ, Dietz HC, Sakai LY, Ramírez F. Pathogenetic sequence for aneurysm revealed in mice underexpressing fibrillin-1. *Proc Natl Acad Sci USA.* 1999;96:3819–3823.

105. Habashi JP, Judge DP, Holm TM, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. *Science.* 2006;312:117–121. doi: 10.1126/science.1124287.

106. Mizuguchi T, Collod-Beroud G, Akiyama T, et al. Heterozygous TGFBR2 mutations in Marfan syndrome. *Nat Genet.* 2004;36:855–860. doi: 10.1038/ng1392.

107. Loeyes BL, Schwarze U, Holm T, et al. Aneurysm syndromes caused by mutations in the TGF-β receptor. *N Engl J Med.* 2006;355:788–798. doi: 10.1056/NEJMoa055695.

108. Lindsay ME, Dietz HC. Lessons on the pathogenesis of aneurysm from heritable conditions. *Nature.* 2011;473:308–316. doi: 10.1038/nature10145.

109. Panhu F, Fadulou VT, Chang J, Lafton A, Hasham SN, Sparks E, Giampietro PF, Zaleski C, Estrada AR, Safi JH, Shete S, Willing MC, Raman CS, Milewicz DM. Mutations in transforming growth factor-beta receptor type II cause familial thoracic aortic aneurysms and dissections. *Circulation.* 2005;112:513–520. doi: 10.1161/CIRCULATIONAHA.105.537340.

110. Boileau C, Guo DC, Hanna N, et al. National Heart, Lung, and Blood Institute (NHLBI) Go Exome Sequencing Project. TGFBR2 mutations cause familial thoracic aortic aneurysms and dissections associated with mild systemic features of Marfan syndrome. *Nat Genet.* 2012;44:916–921. doi: 10.1038/ng.2348.

111. Bertoli-Avella AM, Gillis E, Morisaki H, et al. Mutations in a TGF-β ligand, TGFBR3, cause mild systemic features of Marfan syndrome. *J Am Coll Cardiol.* 2015;65:2508–2517. doi: 10.1016/j.jacc.2015.01.040.

112. Li S, Wang YN, Niimi M, Ning B, Chen Y, Kang J, Wang D, Wang Z, Yu Q, Waag AB, Liu E, Zhang J, Shiomi M, Chen YE, Fan J. Angiotensin II infusions promote ascending aortic aneurysms: attenuation by CCR2 deficiency in apoE−/− mice. *Clin Sci (Lond).* 2010;118:681–689. doi: 10.1042/CS20090372.

113. Rateri DL, Moorleghen JJ, Knight V, Balakrishnan A, Howatt DA, Cassis LA, Daugherty A. Depletion of endothelial or smooth muscle cell-specific angiostatin II type 1a receptors does not influence aortic aneurysms or atherosclerosis in LDL receptor deficient mice. *PLoS One.* 2012;7:e51483. doi: 10.1371/journal.pone.0051483.

114. Rateri DL, Davis FM, Balakrishnan A, Howatt DA, Moorleghen JJ, O’Connor WN, Charnigo R, Cassis LA, Daugherty A. Angiostatin II induces region-specific medial disruption during evolution of ascending aortic aneurysms: attenuation from CR2 deficiency in apoE−/− mice. *Circ Clin Sci (Lond).* 2010;118:681–689. doi: 10.1042/CS20090372.

115. Trachet B, Piersigilli A, Fraga-Silva RA, Aslanidou L, Sordet-Dessimoz T, Cassis LA, Daugherty A. TGF-β neutralization enhances angII-induced aortic rupture and aneurysm in both thoracic and abdominal regions. *PLoS One.* 2011;6:e15381. doi: 10.1371/journal.pone.0153811.
127. Chen X, Lu H, Rateri DL, Cassis LA, Daugherty A. Conundrum of angiotensin II and TGF-β interactions in aortic aneurysms. *Curr Opin Pharmacol*. 2013;13:180–185. doi: 10.1016/j.coph.2013.01.002.

128. Angelov SN, Hu JH, Wei H, Airhart N, Shi M, Dichek DA. TGF-β (Transforming growth factor-β) signaling protects the thoracic and abdominal aorta from angiotensin II-induced pathology by distinct mechanisms. *Arterioscler Thromb Vasc Biol*. 2017;37:2102–2113. doi: 10.1161/ATVBAHA.117.309401.

129. Daugherty A, Chen Z, Sawada H, Rateri DL, Sheppard MB. Transforming growth factor-beta in thoracic aortic aneurysms: good, bad, or irrelevant? *J Am Heart Assoc*. 2017;6:e005221.

130. Majesky MW. Developmental basis of vascular smooth muscle diversity. *Arterioscler Thromb Vasc Biol*. 2007;27:1248–1258. doi: 10.1161/ATVBAHA.106.140669.

131. Sawada H, Rateri DL, Moorleghen JJ, Majesky MW, Daugherty A. Smooth muscle cells derived from second heart field and cardiac neural crest reside in spatially distinct domains in the media of the ascending aorta-brief report. *Arterioscler Thromb Vasc Biol*. 2017;37:1722–1726. doi: 10.1161/ATVBAHA.117.309599.

132. Wu D, Ren P, Zheng Y, Zhang L, Xu G, Xie W, Lloyd EE, Zhang S, Sawada H, Rateri DL, Moorleghen JJ, Majesky MW, Daugherty A. Hypercholesterolemia induced by a PCSK9 gain-of-function mutation augments angiotensin II-induced abdominal aortic aneurysm development and nicotine-augmented expansion. *Sci Transl Med*. 2012;4:122ra22. doi: 10.1126/scitranslmed.3003441.

133. Roche-Molina M, Sanz-Rosa D, Cruz FM, García-Prieto J, López S, Abia R, Muriana FJ, Fuster V, Ibáñez B, Bernal JA. Inhibition of sustained hypercholesterolemia by single adeno-associated virus-medi- ated gene transfer of mutant hPCSK9. *Arterioscler Thromb Vasc Biol*. 2015;35:50–59. doi: 10.1161/ATVBAHA.114.306317.

134. Davis FM, Rateri DL, Daugherty A. Mechanisms of aortic aneurysm formation: translating preclinical studies into clinical therapies. *Heart*. 2014;100:1498–1505. doi: 10.1136/heartjnl-2014-305648.

135. Davis FM, Rateri DL, Daugherty A. Abdominal aortic aneurysm: novel mechanisms and therapies. *Curr Opin Cardiol*. 2015;30:566–573. doi: 10.1097/HCC.0000000000000216.

136. Ijaz T, Sun H, Pinchuk IV, Milewicz DM, Tilton RG, Brasier AR. Deletion of NF-κB RelA in angiotensin II-sensitive mesenchymal cells blocks aortic vascular inflammation and abdominal aortic aneurysm formation. *Arterioscler Thromb Vasc Biol*. 2015;35:1826–1834. doi: 10.1161/ATVBAHA.114.307751.

137. Bajaj D, Paolini DM, Ceballos T, Tontonoz P, Wang K, Wu C, Ding X, Ye P, Xia J. MicroRNA-21 knockout exacerbates angiotensin II-induced thoracic aortic aneurysm and dissec- tion in mice with abnormal transforming growth factor-β-SMAD3 signaling. *Arterioscler Thromb Vasc Biol*. 2018;38:1086–1101. doi: 10.1161/ATVBAHA.117.310694.

138. Goettsch C, Hutcheson JD, Hagita S, Rogers MA, Creager MD, Tham T, Choi J, Mynarich AK, Pieper B, Kbjolly M, Aikawa M, Aikawa E. A single injection of gain-of-function mutant PCSK9 adeno- associated virus vector induces cardiovascular calcification in mice with no genetic modification. *Atherosclerosis*. 2016;251:109–118. doi: 10.1016/j.atherosclerosis.2016.06.011.

139. Daugherty A, Cassis LA, Lu H. Complex pathologies of angioten- sin II-induced abdominal aortic aneurysms. *J Zhejiang Univ Sci B*. 2011;12:624–628. doi: 10.1631/jzus.B1101002.

140. Rateri DL, Howatt DA, Moorleghen JJ, Charmigo R, Cassis LA. Daugherty A. Prolonged infusion of angiotensin II in apoE(−/−) mice promotes macrophage recruitment with continued expansion of abdominal aortic aneurysm. *Am J Pathol*. 2011;179:1542–1548. doi: 10.1016/j.ajpath.2011.05.049.

141. Davis FM, Rateri DL, Daugherty A. Mechanisms of aortic aneurysm formation: translating preclinical studies into clinical therapies. *Heart*. 2014;100:1498–1505. doi: 10.1136/heartjnl-2014-305648.

142. Kusters PJJ, Seijkens TTP, Beckers L, Lievens D, WinkelS h, de Waard V, Duijvestijn A, Lindquist Liljegqvist M, Roy J, Daugherty A, Newby A, Verdes N, Latour Y. hPCSK9 deficiency protects against aortic aneurysm formation. *Arterioscler Thromb Vasc Biol*. 2016;36:2191–2200. doi: 10.1161/ATVBAHA.115.307595.

143. Krishna SM, Seto SW, Jose RJ, Li J, Morton SK, Biros E, Wang Y, Nsengiyumva V, Lindeman JH, Loots GG, Rush CM, Craig JM, Grollede J. Wnt signaling pathway inhibitor sclerostin inhibits angioten- sin II-induced aortic aneurysm and atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2017;37:553–566. doi: 10.1161/ATVBAHA.116.308723.
dissecting abdominal aortic aneurysm in apolipoprotein E-knockout mice. *Arterioscler Thromb Vasc Biol*. 2016;36:1587–1597. doi: 10.1161/ATVBAHA.116.307530.

162. Nakao T, Horie T, Baba O, et al. Genetic ablation of microRNA-33 attenuates inflammation and abdominal aortic aneurysm formation via several anti-inflammatory pathways. *Arterioscler Thromb Vasc Biol*. 2017;37:2161–2170. doi: 10.1161/ATVBAHA.117.309768.

163. Liu CL, Wang Y, Liao M, et al. Allergic lung inflammation aggravates angiotensin II-induced abdominal aortic aneurysms in mice. *Arterioscler Thromb Vasc Biol*. 2016;36:69–77. doi: 10.1161/ATVBAHA.115.309911.

164. Moran CS, Rush CM, Dougan T, Jose RJ, Biros E, Norman PE, Gera L, Golledge J. Modulation of kinin B2 receptor signaling controls aortic dilatation and rupture in the angiotensin II-infused apolipoprotein E-deficient mouse. *Arterioscler Thromb Vasc Biol*. 2016;36:898–907. doi: 10.1161/ATVBAHA.115.306945.

165. Lu WW, Jia LX, Ni XQ, Zhao L, Zhang JG, Zhang JS, Hou YL, Zhu Y, Guan YF, Yu YR, Du J, Tang CS, Qi YF. Intermedin-1-53 attenuates abdominal aortic aneurysm by inhibiting oxidative stress. *Arterioscler Thromb Vasc Biol*. 2016;36:2170–2190. doi: 10.1161/ATVBAHA.116.307825.

166. Yan YF, Pei JF, Zhang Y, Zhang R, Wang F, Gao P, Zhang ZQ, Wang TT, She ZG, Chen HZ, Liu DP. The paraoxonase gene cluster protects against abdominal aortic aneurysm formation. *Arterioscler Thromb Vasc Biol*. 2017;37:291–300. doi: 10.1161/ATVBAHA.116.308684.

167. Umebayashi R, Uchida HA, Kakio Y, Subramanian V, Daugherty A, Wada J. Cilostazol attenuates angiotensin II-induced abdominal aortic aneurysms but not atherosclerosis in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*. 2018;38:903–912. doi: 10.1161/ATVBAHA.117.309707.

168. Lutshumba J, Liu S, Zhong Y, Hou T, Daugherty A, Lu H, Guo Z, Gong MC. Deletion of BMAL1 in smooth muscle cells protects mice from abdominal aortic aneurysms. *Arterioscler Thromb Vasc Biol*. 2018;38:1063–1075. doi: 10.1161/ATVBAHA.117.310153.

169. Robinet P, Milewicz DM, Cassis LA, Leeper NJ, Lu HS, Smith JD. Consideration of sex differences in design and reporting of experimental arterial pathology studies—statement from ATVB council. *Arterioscler Thromb Vasc Biol*. 2018;38:292–303. doi: 10.1161/ATVBAHA.117.309524.

170. Arnold AP, Cassis LA, Eghbali M, Reue K, Sandberg K. Sex hormones and sex chromosomes cause sex differences in the development of cardiovascular diseases. *Arterioscler Thromb Vasc Biol*. 2017;37:746–756. doi: 10.1161/ATVBAHA.116.307301.

171. Chenu C, Adlannerini M, Boudou F, Chantaltal E, Guibot AL, Toutain C, Raymond-Levron I, Vincedo P, Gadeau AP, Henrion D, Arnal JF, Lenfant F. Testosterone prevents cutaneous ischemia and necrosis in males through complementary estrogen and androgenic actions. *Arterioscler Thromb Vasc Biol*. 2017;37:909–919. doi: 10.1161/ATVBAHA.117.309219.

172. Alsiraj Y, Thatcher SE, Blalock E, Fleenor B, Daugherty A, Cassis LA. Sex chromosome complement defines: differences versus focal angiotensin II-induced aortic pathology. *Arterioscler Thromb Vasc Biol*. 2018;38:143–153. doi: 10.1161/ATVBAHA.117.310035.

173. Wassmann S, Czech T, van Eickels M, Fleming I, Böhm M, Nickenig G. Inhibition of diet-induced atherosclerosis and endothelial dysfunction in apolipoprotein E/angiotensin II type 1A receptor double-knockout mice. *Circulation*. 2004;110:3062–3067. doi: 10.1161/01.CIR.0000137970.47771.AF.

174. Cassis LA, Rateri DL, Lu H, Daugherty A. Bone marrow transplantation reveals that recipient AT1a receptors are required to initiate angiotensin II-induced atherosclerosis and aneurysms. *Arterioscler Thromb Vasc Biol*. 2007;27:380–386. doi: 10.1161/01.ATV.0000254680.71485.92.

175. Galatioti J, Caescu CI, Hansen J, Cook JR, Miramontes I, Iyengar R, Ramirez F. Cell-type-specific contributions of the angiotensin II type 1a receptor to aorta homeostasis and atherosclerotic disease—A minireview. *Arterioscler Thromb Vasc Biol*. doi: 10.1161/01.ATV.116.307778.

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