AHA Scientific Statement:

Animal Models of Hypertension

A Scientific Statement From the American Heart Association

Lilach O. Lerman, MD, PhD, FAHA, Chair; Theodore W. Kurtz, MD, FAHA; Rhian M. Touyz, BSc, MBBCh, MSc, PhD, FAHA; David H. Ellison MD, FAHA; Alejandro R. Chade, MD, FAHA; Steven D. Crowley, MD, FAHA; David L. Mattson, PhD, FAHA; John J. Mullins, PhD, FRSE; Jeffrey Osborn, PhD, FAHA; Alfonso Eirin, MD; Jane F. Reckelhoff, PhD, FAHA; Costantino Iadecola, MD; Thomas M. Coffman, MD, FAHA, Co-Chair

On behalf of the American Heart Association Council on Hypertension

Mayo Clinic, Rochester, Minnesota

University of California, San Francisco, California

Glasgow Cardiovascular Research Centre, Glasgow, United Kingdom

Oregon Health & Science University, Portland, OR

University of Mississippi Medical Center, Jackson, Mississippi

Duke University Medical Center, Durham, North Carolina

Medical College of Wisconsin, Milwaukee, Wisconsin

The University of Edinburgh, United Kingdom

University of Kentucky Lexington, Kentucky

Weill Cornell Medicine, New York, NY

Duke-NUS Medical School, Singapore

Correspondence:

Lilach O. Lerman, MD, PhD, Division of Nephrology and Hypertension, Mayo Clinic, 200 First Street SW, Rochester, MN 55905.

Fax: (507)-266-9316 Phone: (507)-266-9376 Email: lerman.lilach@mayo.edu

ABSTRACT

Hypertension is the most common chronic disease in the world, yet the precise cause of elevated blood pressure ~~(BP)~~ often cannot be determined. Animal models have been useful for unraveling the pathogenesis of hypertension and for testing novel therapeutic strategies. The utility of animal models for improving understanding of the pathogenesis, prevention, and treatment of hypertension and its comorbidities depends on their validity for representing human forms of hypertension, including responses to therapy, as well as on the quality of studies in those models (such as reproducibility and experimental design). Important unmet needs in this field include development of models that mimic the discrete hypertensive syndromes that now populate the clinic ~~(such as primary aldosteronism)~~, a necessity to resolve ongoing controversies regarding the pathogenesis of hypertension, and developing new avenues for preventing and treating hypertension and its complications. Animal models may indeed be useful for addressing these unmet needs.

CLINICAL PROBLEM AND UNMET NEEDS

Hypertension is the most common chronic disease in the world, and produces substantial morbidity and mortality. Yet, in the majority of individuals, the precise cause of elevated blood pressure (BP) cannot be determined. Risk factors for primary (formerly called 'essential') hypertension include advancing age, obesity, high dietary NaCl consumption, and low dietary potassium intake, however although these appear to contribute to, but not cause, hypertension. Renin-sodium profiling has been used to classify primary hypertension, suggesting that the phenotype is highly variable, but treatment remains largely empirical, and influenced by race and comorbid disease.

A number of hypertensive subtypes also exist, and while they may comprise only a small percentage fraction of individuals with hypertension, they can nonetheless be relatively common, given the broad prevalence of hypertension itself. Malignant hypertension is related to, but pathophysiologically distinct from, primary hypertension, as is the syndrome of preeclampsia. Genetic forms of hypertension with Mendelian inheritance are rare, but have helped to identify important blood pressure (BP) regulating pathways. Secondary causes may involve the renal vasculature, endocrine organs, and the kidney, and may comprise up to 20% of cases of resistant hypertension. Finally, an increasing number of drugs used to treat cancer and other conditions are now recognized as causing hypertension, which is often severe. Genetic forms of hypertension with Mendelian inheritance are rare, but have helped to identify important blood pressure (BP) regulating pathways. Over the past 20 years, some the most important scientific breakthroughs have emanated from discovering the basis of rare subtypes of human hypertension. Among these are the solution of nearly all the monogenic causes of hypertension; identification of discrete somatic mutations that cause primary aldosteronism; the discovery that polymorphisms in the *APOL1* gene underlie some racial disparities in hypertensive kidney disease; the discovery that placental insufficiency generates placental growth factor and soluble fms-like tyrosine kinase-1 (sFlt-1), factors that mark and contribute to preeclampsia; and finally, the recognition that certain anti-cancer drugs commonly cause hypertension by disrupting impairing the function of the vascular endothelium and the glomerulus.

The initial animal models of hypertension to be developed involved constriction of renal arteries ('Goldblatt kidney') or parenchyma (Page Kidneys); the pathophysiology closely mimicked their human analogs. Yet, renovascular hypertension and 'Page kidneys' represent

only a small fraction of human hypertension. Most experimental studies of hypertension using animals, therefore, have focused on understanding mechanisms of primary hypertension.

While excellent animal models with good human fidelity have been developed for many of these rare causes of hypertension (1, 2), models of primary hypertension have been more difficult to develop, largely because the causes of the human disorder are ~~not~~ unclear. Of NIH-sponsored hypertension research, studies using angiotensin (ANG)-~~-~~II infusion comprise a disproportionate share (nearly 50%) (3). Only 4% of studies focus on aging, and 4% on deoxycorticosterone acetate (DOCA)-salt hypertension (which ~~even~~ itself does not model primary aldosteronism). Thus, an important unmet need is to develop better animal models that more closely mimic the discrete hypertensive syndromes that now populate the clinic, such as primary aldosteronism. A corollary would be that the portfolio of hypertension research might more closely mimic the spectrum of human hypertension.

A second important unmet need is to resolve ongoing controversies regarding pathogenesis. Proponents for individual pathways including the primacy of the nervous system, kidney, and vasculature in development of hypertension typically focused on their own views and interests, often independent of considerations of heritability, environmental exposure, and developmental programming. Despite more than 50 years of work, there is no consensus integrating this range of contributing causative factors. This persistent lack of convergence slows bona fide progress and can limit the impact of the field. Addressing this unmet need will require that we bring together diverse teams, with competing views, who are committed to this common goal.

THE UTILITY AND VALIDITY OF ANIMAL MODELS OF HYPERTENSION

Across a range of human diseases including hypertension, animal models have been useful for unraveling disease pathogenesis, providing incisive experimental strategies not possible in human studies. In hypertension, the utility of animal models for improving understanding of the pathogenesis, prevention, and treatment of hypertension and its comorbidities depends on: 1) their validity for representing human forms of hypertension, including responses to therapy, and 2) the quality of studies in those models. Recently, the utility of animal studies in translational medical research has come under increasing scrutiny because of low study reproducibility and problems such as bias, poor experimental design and execution, analytical and logical errors, and incomplete reporting- (4-8). Published recommendations on ways to mitigate these issues should be considered for any studies utilizing animal models. It should be noted that over 1000 scientific

journals have endorsed guidelines designed to improve the reporting of animal experiments. Nonetheless, these should be applied cautiously, as excessive regulation may also hinder studies in animals.

Various criteria have been used to assess the utility of animal models in translational medical research, including "face" validity, "construct" validity, and "predictive" validity (9).~~(9)~~ By conventional definition, each animal model of hypertension has at least some rudimentary degree of "face" validity in that each demonstrates the primary diagnostic feature, an increase in BP compared to a level deemed to be normal. However, some models may have greater face validity than others with respect to other phenotypic aspects of hypertension, like age of onset, temporal course, severity, variability, and associated comorbidities. Given the clinical importance of hypertension-related target organ damage, it is noteworthy that models are also available exhibiting face validity with respect to risk for hypertension-related disturbances, such as left ventricular hypertrophy (LVH), metabolic abnormalities, heart failure, renal damage, and stroke (e.g., spontaneously hypertensive rats [SHR], Dahl salt sensitive ([DSS] rats).(10-16) However, other hypertension-associated conditions, such as spontaneous development of atherosclerosis~~, angina, and~~or acute myocardial infarction, are not typically observed in current models.

While all typical animal models uniformly exhibit increased BP, the models vary considerably with respect to "construct" validity, defined by how faithfully they recapitulate key features of human hypertension such as genetic and environmental triggers or key pathophysiologic mechanisms. As in other fields, there is no ideal animal model of human hypertension, as all have inherent limitations in construct validity. For example, there are striking differences between humans and animals with respect to factors that influence BP, including genetics, physiology, anatomy, behavior, environmental conditions and triggers, etc. The nature of these differences, particularly between humans and rodents, has motivated efforts to study hypertension and related disorders in larger animals and non-human primates. In addition, it should be emphasized that the validity or utility of BP studies in animal models may be compromised by using BP measurement techniques involving anesthesia or other forms of stress, or those that do not allow for adequate assessment of BP over 24 hours and of key features of the BP wave form, including both systolic and diastolic pressure, as these may influence risk for cardiovascular events (17-19).

Predictive Validity and the Primary Reason for using Animal Models in Hypertension Research

The main goal of studying animal models of hypertension is to help develop improved approaches to preventing and treating high BP and its complications. Therefore, from a practical perspective, the most important aspect of such a model is "predictive" validity, defined by its value for guiding development of effective preventive or therapeutic interventions in humans. This raises several corollaries. What are the major obstacles and unmet needs for effectively preventing and treating hypertension and its complications? How useful are studies in a particular animal model(s) of hypertension for addressing these unmet needs? Some of the obstacles to achieving effective BP control and reduction of associated cardiovascular risk are related to behavioral issues leading to poor adherence to therapies or preventive measures, where application of animal models is unlikely to be productive.

On the other hand, the problem of resistant hypertension is an area where animal models could have significant utility. Even in patients thought to be taking the requisite antihypertensive drugs as prescribed, the prevalence of treatment-resistant hypertension is estimated to be in the range of 10-15% (20). While this may well be an overestimate, many individuals could still benefit from availability of new therapies, particularly since this group of patients is at high risk for complications of hypertension. Development of successful vaccines or device-based therapies could be particularly helpful for improving BP control in patients who cannot be controlled with conventional therapies, are not adherent to antihypertensive treatments, or in those being prescribed suboptimal therapies. It is also conceivable that new antihypertensive therapies might reduce the sizeable cardiovascular risk that persists in treated hypertensive patients with seemingly good BP control and other determinants of cardiovascular disease. While traditional antihypertensive agents do not necessarily completely abolish the cardiovascular risk of the treated hypertensive patient (21), further research is required to determine the extent to which such residual risk is related to unrecognized inadequate BP control, or to some underlying mechanism of hypertension that is conferring increased cardiovascular risk beyond just effects of elevated BP. While better approaches addressing the issue of suboptimal BP control are required, important unmet needs exist for developing new avenues for treating hypertension and its complications (19-22). ~~(19-22)~~ Animal models may indeed be useful for addressing these unmet needs.

Predictive Value of Animal Models for Improving Management of Hypertension

The utility of animal models for developing better approaches to the prevention and treatment

of human disease has been controversial. A major concern is the poor success rate for new drugs advanced to clinical trials on the basis of pre-clinical studies in animal models (9).~~(9)~~ ~~However,~~ On the other hand, animal models have a verified track record in some human disorders, including hypertension, where all clinically effective antihypertensive drugs lower BP (9). In this regard, all major classes of antihypertensive drugs in use today have been demonstrated to substantially reduce BP in one or more of the most commonly used animal models of hypertension (SHR, DSS rat, renal artery stenosis, mineralocorticoid-salt model) (10, 22).~~(10, 22)~~ For example, the SHR responds to the antihypertensive effects of almost all classes of drugs approved for treatment of hypertension. Because hypertension is a multifactorial heterogeneous disorder, and pharmacokinetic/pharmacodynamic variables may also vary among models, the magnitude of the BP response to a given antihypertensive treatment can vary greatly among animal models, just as among different patient subgroups. The availability of a wide variety of animal models is advantageous for generating hypotheses regarding the pathogenesis, prevention, and treatment of different forms of high BP in humans. Nonetheless, some approaches to lowering BP were apparently first tested in humans (e.g., ablation of sympathetic nerves, diet therapies, weight reduction, or supplemental potassium, and various drugs) questioning the need for research on animal models of human disorders~~,~~ (23). Finally, because the main goal of treating hypertension is reducing risk for devastating cardiovascular complications, one could argue that the most valuable animal models should provide insights into prevention and treatment of these complications.

Since understanding of the pathogenesis of human hypertension remains obscure, attempts to generalize study observations from a single animal model to the human circumstance should be viewed with considerable skepticism. Ideally, studies in multiple models may be most helpful in providing a more complete view of the potential clinical relevance of mechanisms and therapeutic responses observed in experimental studies of hypertension, and in stimulating hypotheses about responses in subsets of hypertensive humans Nevertheless, studies in animal models of hypertension have successfully tested important hypotheses relevant to human hypertension, and have motivated clinical research studies leading to significant improvements in clinical management and outcomes, such as key applications of angiotensin converting enzyme inhibitors~~,~~ (24, 25), dihydropyridine calcium channel blockers, angiotensin receptor blockers, and renin inhibitors to hypertension treatment. The history of the development of renin inhibitors

illustrates both the value and potential pitfalls of using animal models of hypertension to predict BP lowering effects of new molecules in hypertensive humans. Because of species differences in drug pharmacokinetics and in the amino acid sequence of renin especially in rodents, results in both non-primate and primate models were critical for defining applications of these agents in humans. (26-28). Overall, translational research in animal models of hypertension has largely been a success story in of modern medicine. We suggest that judicious use of such models will continue to guide successful identification and advancement of interventions.

LARGE VS. SMALL ANIMAL MODELS OF HYPERTENSION

In selecting the most appropriate model of hypertension, one of the first decisions facing researchers is the choice between small and large animal models. Several factors must be considered, including the research scope and objectives, institutional resources, experimental cost, animal welfare, and practical suitability. The pros and cons of these models need to be thoroughly evaluated to select the best model to meet a particular research purpose (Table A).

Small animal models are most commonly employed to study hypertension, providing useful insights. For example, these models may target specific factors implicated in the pathogenesis of human hypertension, including salt sensitivity, activation of the renin-angiotensin-aldosterone system (RAAS), and genetic factors. Rats and mice offer several advantages over larger animal models, like cost effectiveness, short gestation period, and tractability for genetic manipulation. However, reliable measurement of BP is challenging in small animals, surgical procedures are technically difficult, and the amount of sample available, particularly plasma and urine, may be limiting. Nevertheless, recent advances in imaging and surgical interventions have addressed some of these issues and have greatly streamlined the assessment of target organ damage (29).

One of the most significant advantages of rodent models is ready availability of techniques for precise genetic alterations through whole-body or cell-specific gene deletions (knockout) or gene editing, allowing mechanistic studies to elucidate molecular mechanisms and identify novel targets for therapy, which are further enhanced by the relatively larger availability of specific antibodies for molecular studies compared to large animals. One great advantage of the rat is the existence of numerous genetic strains exhibiting robust spontaneous hypertensive phenotypes at baseline or through induction by environmental conditions. In addition, the rat is easy and cost effective to maintain, house and breed, yet large enough for most analytical studies, including long term, dynamic cardiovascular monitoring, and blood and tissue sampling. Since these rat

models exhibit many phenotypic characteristics observed in human hypertension, they have been widely used to examine both the genetic and the mechanistic basis of hypertension. In recent years, many of these physiological monitoring techniques have been adapted to mice, which bear lower experimental and maintenance costs compared to rats. In addition, there is a very wide range of specific antibodies available commercially for mouse that can be used for monitoring and in vivo treatment studies.

Major advantages of large animal models, such as pig and primate, are their anatomical, physiological, and hemodynamic similarities to humans, combined with developmental pathophysiology in general, and specifically of hypertension, that may also more closely resemble humans compared to small animal models. They are also~~The major advantage of large animal models, such as pig and primate, are their anatomical, physiological, and hemodynamic similarities to humans, where pathophysiology of hypertension may also more closely resemble humans, compared to small animal models (Table A). Large animals are~~ particularly suitable for linear studies of hemodynamic consequences of long-term elevation of BP, with the added advantage of opportunities for repeated sampling of plasma and abundant tissues in which to quantify and often follow functional and structural injury in target organs *in vivo* and *ex vivo*~~functional and structural injury in target organs~~. Hence, integrated longitudinal data may be obtained in the same animal. A major disadvantage, however, is limited availability of genetically modified large animal models of hypertension compared to the breadth of genetically modified rodents. This is largely related to the higher costs of maintenance, longer reproductive cycles, and labor-intensive experiments in large animals. Along with lack of other reagents such as specific antibodies, this restricts the mechanistic depth of some studies using large animals. Finally, ethical issues have been raised for studies utilizing non-human primates.

The most frequently used large animals for hypertension studies are the swine, non-human primates, sheep, and to a declining extent, dogs. The means to induce hypertension generally require pharmacological or surgical approaches. Pharmacological interventions using chronic infusions of Ang II, glucocorticoids, or DOCA (with and without high-salt diet) in pigs or dogs (30-33) are less frequently used than in smaller animals, partly due to the high cost of body-size titrated doses of drugs required over prolonged periods of time. On the other hand, surgical induction of hypertension is relatively simple, widely used, well tolerated, and carries a low risk for surgery-related mortality. These interventions include constriction of the aorta by extra-

vascular banding (34), implantable adjustable occluders in the supra-renal aorta (35, 36) or renal arteries (37, 38), or intra-vascular devices in the renal arteries (39). These methods provide reliable models of chronic hypertension primarily of renovascular origin. The use of adjustable occluders to restrict blood flow afford controlling the degree of insult leading to hypertension, which provides opportunities for determining the extent of BP elevation required to trigger target organ injury and understanding how the process of end-organ damage unfolds. Intra-arterial devices such as coils (39, 40) that induce a progressive narrowing of the renal arterial lumen may mirror the obstructive role of plaques in human renal artery stenosis, and thus more closely recapitulate the pathophysiology of this well-documented clinical condition. Unlike adjustable occluders, the resulting degree of obstruction and target organ injury achieved by intra-vascular devices is often variable, again mimicking the clinical course of disease development. Finally, recent data show the potential of the African green monkey as a model of spontaneous hypertension (41). Hypertension in this model seems to develop without the need of external interventions, exacerbates with aging, and is associated with target organ injury, which may offer a new avenue for translational hypertension research.

PLATFORMS OF EXPERIMENTAL HYPERTENSION: GENETIC

Genetic Rat Models of Hypertension

The complex nature of hypertensive phenotypes in combination with the polygenic mode of inheritance of hypertension requires appropriate models amenable to study. Great insight has been gained from genetic studies of human hypertension and from mechanistic studies in experimental animal models of hypertension, summarized in other sections in this Statement.

A number of rat genetic models of hypertension that have been utilized in genetic, (patho)-physiological, and pharmacological studies (42-46). Rat strains exhibiting genetic hypertension include the SHR, DSS rat, the Fawn Hooded Hypertensive rat (FHH), the Milan Hypertensive Strain (MHS), the Lyon Hypertensive (LH) rat, the Sabra Hypertensive (SH) rat, the Genetically Hypertensive (GH) rat, and the Inherited Stress-Induced Arterial Hypertension rats (ISIAH) model. Of these, the most commonly studied is the SHR; in the past 10 years, over 4500 articles were indexed in PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) under the term "Spontaneously Hypertensive Rats". In contrast, the next most commonly cited model, the "Dahl Salt Sensitive Rat", was indexed 585 times over the same time span, while the other genetic rat strains were indexed less frequently. In addition to the above-described inbred strains,

there are a number of congenic and transgenic animals exhibiting hypertensive phenotypes. While too numerous to conveniently list in this space, these other strains are commonly based upon the genetic background of the major strains listed above. The following section provides a brief overview of the origin of a number of these strains, their general experimental applications, considerations for choosing a rat genetic model of hypertension, and the advantages and limitations of rat models.

Commonly Utilized Strains: The majority of the genetically hypertensive rats have been derived from outbred Wistar or Sprague-Dawley breeding stock with selection for hypertension-related traits. These models provide reliable and reproducible phenotypes that are often representative of clinical observations. The severity of hypertension and of related phenotypes is different among strains and can be a consideration when choosing an animal for study; a number of reviews and resources provide comprehensive information (42-46). Moreover, direct comparisons of the hypertensive phenotypes and general body characteristics of several of the genetic strains provide insight into the different degree of disease attained under similar conditions.

SHR: The SHR rat strain originated in Kyoto, Japan, from the cross of an outbred Wistar male rat, which exhibited spontaneously elevated BP, and a female with slightly elevated BP (47). Subsequent brother-sister mating was continued with selection for animals with systolic BP over 150mmHg. The inbred strain was subsequently established in the US in the late 1960's after 20 generations of inbreeding at NIH (48) and spontaneously develops hypertension as adult animals. The SHR is widely used in different studies as a rat model of primary or essential hypertension. This strain, or substrains such as the Stroke-Prone SHR (49, 50), has proven useful in studies of stroke, vascular function, autonomic regulation, renal function, therapeutic interventions, and the genetics of ~~essential~~primary hypertension.

Dahl Salt-Sensitive Rats: DSS rats were developed by Lewis Dahl, who observed the beneficial effects of low sodium-containing diets in the 1950's (51), and examined the influence of different salt diets on BP in outbred Sprague-Dawley (SD) rats. Selective breeding of those rats fed high salt that developed hypertension led to the inbred DSS rats, which are often used in experiments examining the kidney, vasculature, and genetics in hypertension (51). Selective breeding of rats resistant to salt-sensitive hypertension led to the inbred Dahl salt resistant rats.

Fawn Hooded Hypertensive Rat: The FHH model was derived by inbreeding the outbred

fawn-hooded (or fawn-headed) rat originally characterized for its bleeding disorder (52). The outbred rats were demonstrated to have an elevated mean arterial pressure in comparison to Wistar rats and were subsequently inbred by Provoost to produce two strains designated the hypertensive FHH and the normotensive fawn-hooded (also known as FHL) rat (53). The FHH has been useful to address the genetics of hypertension and chronic kidney disease.

Milan Hypertensive Strain: The MHS rats were derived from Wistar rats observed to have elevated BP (54, 55); the rats were inbred for multiple generations to establish a strain that spontaneously develops hypertension soon after weaning and plateaus at 7-8 weeks of age. MHS rats have been used to study ~~essential~~primary hypertension. The Milan Normotensive Strain has also recently proved useful for investigating genetic mechanisms mediating impaired myogenic responses and susceptibility to development of proteinuria and renal injury (56).

Lyon Hypertensive Rats: In the late 1960's, a group in France selected outbred SD rats for elevated, normal, or decreased BP, and that were subsequently inbred for multiple generations into strains with low, normal, or high BP (57, 58). A separation of BP between the strains is evident at a relatively early age (5 weeks), and is subsequently sustained. These strains have been used for renal, metabolic, autonomic, cardiac and genetic studies (59).

Sabra Hypertensive Rats: SBH rats were originally derived from Sabra outbred rats by brother/sister mating and selection for high BP following unilateral nephrectomy and treatment with DOCA and dietary sodium chloride (60, 61). A secondary round of inbreeding more recently re-derived the Sabra hypertension prone (SBH/y) and resistant (SBN/y) rat models, which are also sensitive or resistant to DOCA-salt treatment (62). The SBH rats have therefore been useful for genetic studies examining environmental interactions on BP.

Experimental Use And Considerations: The most distinct advantage of the rat genetic models of hypertension are the similarities of the BP/hypertension phenotypes to those observed in patients and the genetic basis of disease development that occurs in these animals. In combination with the relatively low costs associated with maintenance of rat colonies, the ease of experimental studies, and their relatively rapid growth and reproductive rate, the genetic rat models have been popular for experimental studies of hypertension.

Genetics of hypertension: The inbred genetic rats have served as useful model systems to identify BP quantitative trait loci (QTL), regions on the genome that contribute to the elevation

of BP. With the development and application of polymorphic microsatellite markers to identify BP QTL, linkage-mapping approaches were extensively used to study inheritance of hypertension in rat models. Subsequent studies to validate these QTL were performed through the generation of congenic or consomic strains (46, 63). The subsequent identification of genes within these QTL has been dependent upon complementary approaches including transcriptomic analyses, gene sequencing, and gene editing approaches. Perhaps the ultimate question related to the use of rat genetic models is the applicability of findings to human hypertension. Encouragingly, comparative mapping strategies have identified overlap of QTL's detected in rat and human linkage studies (64), indicating that findings in rat models of hypertension may be translatable to human disease.

Phenotypes and mechanism of hypertension: In the past 10 years, a major technological breakthrough occurred with the use of gene-editing technology to manipulate the rat genome. The zinc finger nuclease methodology for gene manipulation, pioneered by Geurts and Jacob (65), has permitted assessment of function of genes identified in human association studies to be examined in animals with a hypertensive genetic background (45, 66). This approach has permitted elucidation of the function of a number of genes that associate with hypertension in human genome-wide association studies (GWAS). Of note, the functional importance of various gene products with previously unrecognized function in the regulation of BP (e.g., an adherens junction protein, immune signaling proteins, a secreted metalloproteinase) have been revealed through the use of this technology in genetically hypertensive rats. Similar studies are also underway using the more recently developed methods of gene manipulation involving CRISPR-Cas system based technologies.

Considerations: Important considerations to take into account with the use of genetic rat models include the choice of the appropriate model for a particular research question, the need to ensure an appropriate genetic background of the experimental and control rats, and the requirement for a controlled environment. It should be noted that the basic genetic architecture of the SHR, and of other inbred strains in which the animals are homozygous at virtually all loci, is quite different from the genetic architecture of humans. The various genetic rat strains can be used as models of slowly-developing primary hypertension (48, 67, 68), juvenile hypertension (69, 70), salt-induced hypertension (51), and hypertension associated with end-organ damage (56)~~various genetic rat strains can be used as models of slowly developing essential~~

~~hypertension, juvenile hypertension, salt-induced hypertension, and hypertension-associated with end-organ damage~~. However, understanding the genetic composition of the hypertensive strain and the choice of an appropriate control strain are important (66). A direct comparison of two strains of commonly-used DSS rats demonstrated over 1.3 million different base pairs between the strains, and a significant difference in BP when the rats were fed a high salt diet (45). Genetic differences between control strains must also be considered. Kurtz and Morris (48) demonstrated that the Wistar Kyoto (WKY) rat, a common genetic control for SHR, had profound differences in phenotypes (growth rate and BP) when obtained from different commercial suppliers. Moreover, all "control" strains are necessarily limited in the absence of a complete understanding of the genetic differences between the control and hypertensive strains. These examples emphasize the importance of careful identification of and consistent use of strains when performing experiments. Equally important are environmental influences on phenotypes. In inbred DSS rats, a simple substitution of the sodium-independent components of the diet could profoundly alter the salt-sensitive hypertension and renal damage phenotype (71).

In summary, genetic rat models have demonstrated great utility and provided exceptional insight into the genetics and pathophysiology of hypertension. A researcher interested in employing such models should carefully consider the disease traits of the different strains and appropriate genetic controls, and pay careful attention to controlling the environment.

Transgenic models of hypertension

The construction of high density comparative genetic maps between mouse, rat and human (72) reinforces the similarities (as well as differences) between these species. The millennium genome project for hypertension (73) stated as its aim the identification of hypertension-susceptibility genes and pathways by a systemic multiple candidate gene approach. Candidate genes identified as possible contributors by GWAS screening can be interrogated in animal models using transgenesis - the stable introduction of modifications into the genome.

Gene addition, resulting in over-expression of a given gene of interest, was classically achieved by microinjection of DNA into the single-cell embryo and monitoring its subsequent expression and phenotypic effect in the mouse (74, 75), and subsequently in rats and other species. Alternatively, the using of bacterial artificial chromosomes (BACs), which incorporate 100s of kilobases of DNA, human chromagranin variants have been analyzed in mouse models, for their ability to reduce the risk of hypertension (76, 77). Gene targeting, resulting in loss of

gene function, was also achieved initially in the mouse following the development of embryonic stem cells (ESC). The gene-targeting construct is introduced by transfection or electroporation into ESC, and those cells that have been correctly targeted by homologous recombination, are selected and injected into blastocysts. The resultant chimaeras are then bred and their progeny screened for incorporation of the genetic modification.

ESC technology led to the supremacy of the mouse for generating new animal modeling of human disease over the following 25 years. Many developments, improvements and refinements in gene targeting during this time allowed the researcher to introduce precise and ever more ingenious genetic changes (Table A). Gene expression cassettes may be targeted to safe havens such as the ROSA 26 locus (78, 79). Fluorescent reporters, expressed in place of the gene of interest, but under its endogenous promoter, highlight the tissue- or developmental-specific pattern of expression. In some cases knockout of a gene may prove to be embryonic lethal, because it contributes to a critical stage in development. By flanking key exons of the gene with loxP sites and crossing the transgenic animal with animals that express the enzyme Cre Recombinase in a tissue- or developmental-specific manner, the gene can be knocked out later in development. Alternatively, transgene expression may be driven by an inducible promoter, so that its expression is under the control of the researcher.

Prior to 2008 gene knockout in the rat or rabbit relied upon random mutagenesis using N-ethyl-N-nitosourea, transposon-based systems such as Sleeping beauty, or spermatagonial stem cell targeting. Following identification of the signaling pathways that control self-renewal and differentiation of ESC, it was discovered that incorporation of certain inhibitors in rat ESC growth medium was sufficient to maintain them in the self-renewal, pluripotent state (80, 81), and a variety of species are now targetable through the ESC route. Hence, all the techniques developed in mice are potentially applicable to other species, like APOE knockout in rabbit (82).

More recently, generation of transgenic animals has been transformed by the introduction of gene editing technologies using sequence-specific nucleases. The first of these, Zinc Finger nuclease (ZFN) targeting, requires two ZFNs to bind upstream and downstream of the target site, eventuating in a double strand break at the target site. The cell repairs the break, but it often results in introduction of small deletions or insertions. If the target site is within an exon, then such changes may lead to missense or nonsense mutations, effectively knocking out the target gene (83). Alternatively, a single strand oligo or plasmid can be added to direct a desired

sequence alteration or insertion at the target site. Geurts et al (65, 84, 85) used ZFNs (and subsequently CRISPr Cas9) to systematically knockout a number of GWAS candidate cardiovascular disease-related genes (86) through the PhysGen Knockout program.

Transcription activator-like effector nucleases (TALENs) are a second class of nucleases, which also bind up- and down-stream of the target site and require the close proximity of FokI monomers to introduce a double strand break. Unlike ZFNs, TALENs targeting a specific sequence can be readily constructed from libraries of domains, each of which recognizes one nucleotide, making them widely accessible and relatively cheap. Recently, TALENs were used to generate two Pde1a null mouse lines, which revealed a role for phosphodiesterase 1 in BP regulation in addition to renal pathogenesis (87).

The third and most readily available nuclease class is the clustered regularly interspaced short palindromic repeat (CRISPr/Cas9) system derived from the bacterial immune system of Streptococcus pyogenes. Though the CRISPr/Cas9 system is susceptible to off-target events, it has proved its worth in generating knockout models in a wide range of species. Of note is the recent CRISPR/Cas9 targeted knockout and subsequent knock-in of a 19bp indel polymorphism in a rat long non-coding RNA (*Rffl-inc1*) (88). Recent advances in CRISPR technology include generation of Cas9 derivatives with increased specificity (89, 90) and altered PAM recognition sequences (91) and development of other CRISPRs from alternative bacterial sources (92). The versatility of nucleases extends to the generation of large-scale deletions (93) and insertions (94), which allows for the humanization of animal models.

Gene reduction or knockout has also been achieved using siRNAs. To prevent rapid degradation in vivo, miRNA mimics or anti-miR oligonucleotides require chemical modifications such as locked nucleic acid (LNA) modifications. LNA-modified anti-miRs proved to be effective in targeting miR-29b, which affects collagen gene expression in the renal medulla in DSS rats (95). Genetic modification can be achieved using recombinant lentiviral or adeno-associated viral-mediated delivery. Adverse reactions to the delivery vehicle need to be addressed, in addition to target specificity and cellular uptake (96).

Cell ablation is a transgenic technique with numerous potential applications. Classically, diphtheria toxin A was used to achieve cell ablation (97). More recently, introduction of the nitro-reductase gene, together with pharmacological treatment with a pro-drug, Metronidazole or KillerRed, a far-red fluorescent protein, which is phototoxic to the cell on exposure to

appropriate laser light, have been used (98). Optogenetic cell ablation can be tightly controlled both spatially and temporally, allowing single cells to be ablated (99).

The application of transgenesis to hypertension research

The utilities of a transgenic model for investing the role of a specific gene in hypertension might include: 1) elucidating its basic function; 2) mechanistic understanding of its involvement in a particular pathway; 3) determining its contribution to ~~essential~~primary hypertension; and 4) faithfully modeling the etiology of human hypertension, with a view toward identifying new therapeutic targets and drug treatments.

Many candidate genes for human hypertension identified by GWAS have been individually found to make only small contributions to BP (<1mm Hg), suggesting that there are no common gene variants with major effects to promote ~~essential~~primary hypertension (100-102). Though considerable basic knowledge regarding the complexities of homeostatic BP control has been gleaned from these studies, the lack of clinically relevant therapeutic targets emerging from this work is disappointing. Recently, a SNP in the third intron of the PHACTR1 gene, identified through GWAS studies, has been shown to enhance endothelin-1 expression in the vasculature (103). This is an example of a common non-coding variant contributing to vascular disease and hypertension. Similarly, Ji et al (104) found that rare gene variants in 3 genes, Na^+-K^+-$2Cl^-$ cotransporter [NKCC]2, ROMK and NCC, causing rare Mendelian syndromes with recessive inheritance, are surprisingly common, with 1 in 64 subjects in the Framingham Heart Study cohort carrying such a mutation (104). Since such rare variants cannot be detected by GWAS, this suggests that whole genome sequencing may be a more fruitful source of rare gene variants or epigenetic modifications that potentially contribute to individual propensity for hypertension. Relevant transgenic models might then be designed to confirm the effect of such variants on BP, and provide a valuable test-bed for therapeutic development. While the clinical relevance of a particular model is an important consideration, advancing understanding of basic mechanisms of hypertension is also of value and not incompatible with long-term translational goals.

With the abundance of gene targeting tools particularly for the mouse, substantial basic understanding of rare Mendelian disorders associated with altered BP and renal sodium handling has been gained. A mouse model of Liddle's syndrome (2) containing a gain-of-function stop codon in □-ENaC (reflecting the human mutation) exhibits normal BP unless placed on a high salt diet, when mice develop hypertension and hypokalemia. The hypervolemia suggested by

increased sodium reabsorption and low aldosterone on normal salt diet, together with salt-sensitivity, replicates the human syndrome. The basic mechanisms of positive modulators of ENaC trafficking or expression, such as Sgk1, and Af17 and negative modifiers such as Nedd4-2 have all been revealed with respective animal knockout models.

Mice lacking NKCC2, which models Bartter syndrome, die from dehydration prior to weaning because of uncompensated polyuria (105). Bartter syndrome can also be modeled by knockout of ROMK (106) with animals exhibiting hypotension. NCC knockout, despite recapitulating features of Gitelman's syndrome such as hypocalciuria, causes no reduction in BP unless the animals are put on a low salt diet (107). Renal knockout of Nedd4-2 (108) leads to salt-sensitive hypertension with increased NCC phosphorylation. NCC is also activated in Gordon's syndrome through mutations in WNK4 (109), leading to hypertension and hyperkalemia.

Rare conditions such as the syndrome of apparent mineralocorticoid excess, where hydroxysteroid dehydrogenase (Hsd)11b2 deficiency allows the mineralocorticoid receptor to be activated illicitly by glucocorticoids, have been modeled in both mouse (110-112) and rat (83) models. The global mouse knockout (112) replicates the human syndrome with hypertension and hypokalemia, and heterozygous animals exhibit salt-sensitivity (110). Recently, kidney-specific knockout of mouse Hsd11b2 was found to be sufficient to cause salt-sensitive hypertension attributed to ENaC and NCC activation (111).

Renin knockout in both mouse (113, 114) and rat (115), results in significant hypotension. Knockout of the duplicated renin gene, Ren2, has no effect on BP in the mouse (114), but its over-expression in the SD rat leads to extreme hypertension (116). Subsequent crossing of the transgene onto inbred strains of rat that are susceptible (F344) or resistant (Lewis) to malignant hypertension, allowed identification of angiotensin converting enzyme as a modifier of end organ damage (117). The model has been improved further by placing expression of the mouse renin gene under an inducible (Cyp1a1) promoter (118). The timing and severity of hypertension in the Cyp1a1(Ren2) transgenic rat is thus under the control of the researcher.

The action of AngII in the proximal tubule was determined in Ang receptor (AT1a)-deficient mice. Kidney expression of AT1a is necessary for AngII dependent hypertension, and specific removal (119, 120) or overexpression (120) in proximal tubules causes hypo- or hypertension, respectively, on normal salt diet. Meanwhile, animals lacking all three Ang II receptors reveal

that AngII controls BP by acting solely through these receptors (121). Humanization of both rats and mice with human renin and Ang genes (122, 123) has been useful for establishing species specificity of the RAAS, but more importantly such transgenics are useful for investigating the function of different haplotypes and species-specific RAAS inhibition.

Conditional knockouts have been used to investigate the roles of endothelin-1 (ET1) and its receptor, ETA. Specific knockout of ET-1 in the collecting duct causes hypertension and sodium retention (124), while knockout of the ETA receptor in collecting duct prevents receptor antagonist related fluid retention (125).

Gene knockdown is a potential therapeutic strategy. When adenovirus was used to direct anti-miR against the AT_{1a} receptor to the paraventricular nucleus of SHR, hypertension was attenuated (126). Likewise, siRNA against Nox2 or Nox4 has been used to attenuate BP in the aldosterone-salt mouse model (127). Transgenic technology has been used to ascertain the causative genes in multiple models of ~~essential~~primary and salt-sensitive hypertension. For example, knocking out either NADPH oxidase 4 (Nox4) or the subunit, $p67^{phox}$ on the Dahl salt-sensitive background significantly ameliorates both salt-sensitivity and albuminuria (128, 129). An extensive list of transgenic models that exhibit salt-sensitive hypertension is given in a recent AHA review (130).

The size of the mouse can sometimes preclude its use in studies where substantial or multiple sampling is required. Though surgical instrumentation is routine (e.g. telemetric devices), there may be time constraints where, for example, battery life is limiting. Historically, the rat has been the model of choice in the pharmaceutical industry, so there is a wealth of physiological knowledge associated with this species (131). With development of protocols for ESC isolation and gene editing, rats, rabbits, and larger animals are now more likely to be considered as useful genetic model systems. With the rapidly expanding range of tissue and spatio-temporal controls that can be placed on the gene(s) of interest, and the possibility for multiple targeting or humanization, animal models can now be designed to answer ever more complex questions relating to human disease.

There are caveats to the use of transgenic modeling, and these must be considered on a case–by-case basis. Vector sequences may cause anomalous expression of a reporter, as reported in the vasculature of fli1:EGFP zebrafish (132). The pharmacological profile of the mouse and the rat may differ from that of the human, exemplified by the TRPA1 channel (133). Homologues to

human genes may be absent in animal models, or vice-versa. Finally, there may also be species-specific differences in genetic mechanisms of disease progression or end-organ damage (134).

The optically clear zebrafish has been very successful in informing basic understanding of organ development or function (135, 136). The advantage of ex-vivo development, together with optical clarity, facilitates the use of unparalleled optical and imaging techniques such as SPIM, optogenetics and optical ablation techniques. Whilst a relatively new species in cardiovascular science, the use of Zebrafish for studies of heart development and function and the cardio-renal axis combined with its facile genetic tractability and utility for high throughput screening of drugs and small molecules position it as a likely important species in future translational studies.

In summary, transgenic technology affords a great deal of scope for generation of informative animal models, from identification of gene function to humanization. SNPs, suspected of being disease-related, can be investigated *in vivo* and potential therapeutic strategies tested. Also, with the latest developments in stem cell technology and genome editing, the choice of species is no longer limited to small rodents.

Large animal genetic models

Large animal models of spontaneous hypertension have been recognized and developed over the past 50 years. Spontaneous (or primary) elevation of BP has been reported in a variety of large animal species including chickens, turkeys (137), rabbits (138), swine (139), dogs (140) and nonhuman primates (141). In the avian species, elevated BP likely does not represent true "hypertension", since BP differences are characteristic of comparisons with other species rather than intra-species variation. Other large animal models show intra-species variation in BP by individuals, indicative of hypertension and similar to that observed in humans. Those species all exhibit clear genetic correlates among individuals with strong degrees of heritability. Similar to the SHR, selective breeding of New Zealand and Dutch white rabbits generated one of the earliest large animal models of hypertension (138). Hypertensive animals tended to exhibit varying degrees of renal pathologies, possibly reflecting cause and/or a consequence of the hypertension.

There are large animal models of spontaneous hypertension in dogs and swine exhibiting significant elevation in BP that is spontaneous and may be expanded by selective breeding. Interestingly, both dog (140, 142) and pig (139) models of spontaneous hypertension also have LVH, one of the most common cardiovascular complications of human hypertension portending

significant risk for morbidity and mortality. The canine model was derived from diagnosed ~~essential~~ primary hypertension in a female Siberian husky selectively bred over a 5-year period to normotensive males. The offspring produced a colony of hypertensive animals with dominant heritability. Similar to the rabbit model, subsets of Guizhou mini-pigs and Sichuan domestic pigs exhibit renal disease with mild renal fibrosis, which may be partly responsible for hypertension (139). In this spontaneous model of systemic hypertension, bilateral renal denervation normalized BP (143). The swine model also demonstrates spontaneous hypertension, due to a neurogenic mechanism potentially related to increased renal sympathetic outflow. Yet, unlike non-human primate models, mini-pigs do not typically have elevated heart rates.

A nonhuman primate model of systemic hypertension was first reported in a small number of individual African Green Monkeys or vervets (144). These findings have recently been extensively expanded through phenotyping of nearly 400 animals by forearm plethysmography (141). In this model, the mechanism is likely to be neurological in origin based upon the direct correlation between BP to elevated heart rate. Although RAAS components were unaltered, these monkeys developed significant renal pathology in glomeruli and small vessels, resembling humans with ~~essential~~primary hypertension. Since the vervet genome has now been fully sequenced and a basic annotation published (145), defining the genomic mechanisms underlying the development of spontaneous hypertension may be possible. Accordingly, this nonhuman primate model may have substantial utility for translational research on the genetic basis of human ~~essential~~primary hypertension.

PLATFORMS OF EXPERIMENTAL HYPERTENSION: INDUCED

Renovascular

The pioneering work by Goldblatt (37) and later Page (146) in the 1930s planted the seed for the development of surgically induced models of hypertension and opened a new chapter for hypertension research. Their work positioned the kidney and renal arteries on the complex map of the pathophysiology of hypertension and disclosed the unique relationship between the kidney and BP control that also propelled the development of various therapeutic interventions. Surgical induction of hypertension by reducing blood flow in the suprarenal aorta or main renal arteries with and without removal of renal mass, inducing compression of the renal parenchyma, subtotal nephrectomy, or sinoaortic baroreceptor denervation, are applicable to both small and large animal models with similar outcomes, underscoring pathophysiological mechanisms of

hypertension that likely are conserved across species.

A common pathophysiological feature shared by models of renovascular hypertension is the driver of hypertension and target organ damage: a reduction of blood flow to the kidneys resulting in decreased perfusion pressure activating the RAAS, leading to vasoconstriction, and salt/water retention, with systemic hypertension developing in a matter of days. Systemic hypertension induces progressive endothelial dysfunction, stretch, and organ damage, whereas chronic reduction of blood flow to the kidneys leads to tissue ischemia and subsequent release of hypoxia-activated factors and oxidative stress that activate inflammation. These processes in turn induce microvascular remodeling, fibrosis, and loss of renal function, which likely play a dual role by both maintaining hypertension and promoting target organ injury.

Alternative approaches to surgically-induced hypertension, although less widely used, include direct damage of the renal parenchyma through compression (147), renal micro-embolization (148), and ureteral obstruction (149, 150), which often also activate the RAAS and elicit inflammation, renal fibrosis, and subsequent loss of renal function. A potential limitation of these approaches is a difficulty in controlling the degree of renal damage, and thus the development and severity of hypertension is less predictable. Another potential limitation is clinical relevance, since hypertension caused by parenchymal compression is infrequent in humans. Nevertheless, hypertension can be observed in humans with renal masses, such as subcapsular hematomas, tumors. Parenchymal compression has also been implicated in development of hypertension associated with obesity (151), thus potentially conferring significance for these surgical models of hypertension.

Summary: Recommendations for using animal models of renovascular hypertension

Although reduction of blood flow to the kidneys or induction of parenchymal injury represent less than 10% of the cases of clinical hypertension, target organ injury develops in a similar pattern to ~~essential~~primary hypertension. Yet, models of secondary hypertension might be more suitable for assessing target organ injury and developing therapeutic interventions than for understanding the pathogenesis of human ~~essential~~primary hypertension. Furthermore, the prominent involvement of the RAAS in many of these models may distinguish secondary hypertension from some forms of low-renin hypertension.

Large animal models of renovascular hypertension may have several advantages beyond their

anatomical, physiological, and pathophysiological similarities to humans. For example, they may be more amenable than rodents for studies in chronic stable hypertension, allowing longitudinal quantification of relevant hemodynamic and biochemical parameters that characterize the progression of hypertension, target organ injury, and response to therapeutic interventions in a translational fashion (152). On the other hand, targeted interventions to resolve the vascular occlusion (153-157) are now feasible in both small and large animal models. Thus, both types of models might be used to tease out the driving force initiating hypertension, studying the potential for reversibility of target organ injury, and identifying mechanisms of organ damage that may be independent from hypertension.

The ability to induce renovascular hypertension in genetically modified rodents offers unique opportunities for in-depth elucidation of pathophysiological mechanisms, opening the possibility for a more comprehensive understanding of the consequences of hypertension. Finally, both large and small animal models of renovascular hypertension offer the possibility of imposing other comorbidities, such as metabolic derangements, and determining their contributions to target organ damage (40, 158, 159) thereby realistically mimicking the clinical population of elderly hypertensive patients in which multiple cardiovascular risk factors often coexist.

Angiotensin II-Dependent Hypertension

The RAAS plays a fundamental role in normal sodium and water homeostasis. Accordingly, one of the most widely used pre-clinical models of hypertension, particularly in rodents, is chronic subcutaneous infusion of Ang II. The utility of this model accrues in part from resembling some forms of human hypertension. The RAAS is broadly activated in human ~~essential~~primary hypertension, and the level of BP elevation achieved with commonly used doses of Ang II in mice is on par with that seen in uncontrolled, Stage II hypertension. After 4 weeks of chronic Ang II infusion in susceptible rodent strains, target organ damage is quite similar to that seen in human patients with sustained elevations in BP, including cardiac hypertrophy, vascular remodeling, and chronic kidney disease (160-163). Nevertheless, because the renal vasoconstriction attributable to Ang II can induce ischemia, particularly at higher doses, chronic Ang II infusion more closely models the renal injury that accrues from chronic renal ischemia in human hypertension rather than from barotrauma (164).

The Ang II infusion model was employed in early dog studies that characterized the functions of the RAAS (165, 166), but was adapted for chronic subcutaneous infusion in rodents.

Among rodents, rats develop target organ injury in response to Ang II more readily than mice and can more easily be surgically implemented with BP monitoring devices. On the other hand, mice breed more quickly, have a rapid onset of hypertension, and are amenable to gene targeting even within selected cell lineages. Among mouse strains, C57LB/6 mice are more resistant to renal injury and are not salt-sensitive, making this strain ideal for investigating RAAS-dependent hypertension in the absence of these 2 conditions (167, 168). By contrast, 129SVE mice are salt-sensitive, more susceptible to kidney injury, and manifest greater levels of BP elevation at similar AngII doses relative to C57BL/6(169). Mice from the FVB strain develop marked injury in several compartments within the kidney during Ang II infusion, particularly when combined with unilateral nephrectomy (170). Thus, the choices of species and strain are key considerations when designing experiments employing chronic Ang II infusion.

In mouse studies, several doses of Ang II have been employed to induce and analyze hypertension of different severities. The "slow pressor" dose of 400ng/kg/min Ang II may mimic the gradual onset of hypertension in humans with ~~essential~~primary hypertension (171). The intermediate dose of 490-500 ng/kg/min is the most widely used in recent years (161). A higher dose of 1000ng/kg/min Ang II was used to dissect the functions of AT_1 receptors in distinct tissue pools during hypertension, and in conjunction with other modifications can provoke measurable renal damage in susceptible strains (162). Even higher doses are employed, albeit less commonly, to provoke cardiac fibrosis (172). Notably, these doses far exceed Ang II levels observed naturally in hypertensive humans.

The duration of infusion can be adjusted based on the cardiovascular control center studied. Acute BP effects of Ang II can be appreciated within seconds to minutes (173), whereas with chronic subcutaneous infusions of ≥ 500 ng/kg/min Ang II, increases in BP emerge within the first 24 hours (160). Vascular remodeling due to Ang II is evident within 2 weeks (161), and cardiac hypertrophy at 2-4 weeks (160, 174). Renal injury is reproducible at 4 weeks on susceptible strains (162, 175), but far more robust after 8 weeks (170). Thus, investigators should titrate the Ang II dose and study duration based the experimental question.

The current state-of-the-art methodology for measuring BPs during chronic Ang II infusion is radiotelemetry (176). Using the older method of tail-cuff plethysmography the level of vasoconstriction with higher dose Ang II is so profound (164, 168), that extrapolating BP from blood flow velocity through the tail circulation may be misleading. Radiotelemetry also permits

accurate measurement of heart rate and arrhythmias.

A few modifications to murine Ang II infusion can enhance its applicability to study injury and salt sensitivity. Unilateral nephrectomy, high salt diet, and/or an extended infusion period (8 weeks) can yield more robust renal damage in mice (162, 170). As Ang II promotes sodium retention, a low salt diet can be added to chronic Ang II to understand the extent to which the BP elevation seen during Ang II accrues from its capacity to promote sodium retention (163).

Summary and Future Considerations. While an aggressive pharmacological challenge to induce hypertension has obvious limitations, in several circumstances chronic Ang II infusion offers distinct advantages. First, it is the most direct approach for investigating the in vivo actions of angiotensin receptors and their downstream signaling cascades. Second, pairing chronic subcutaneous Ang II infusions with acute IV infusions allows a comprehensive profile of a protein's vascular and renal functions during RAAS activation. Third, the model is reproducible across species. Finally, chronic Ang II infusion engages all the cardiovascular control centers in pathogenesis, allowing in vivo assessment of interactions between sympathetic and immune activation, systemic and renal vasoconstriction, and renal sodium transport. Thus, chronic Ang II infusion remains a useful tool to dissect coordinated contributions of multiple cardiovascular control centers in hypertension.

Mineralocorticoid-Salt hypertension

Administration of mineralocorticoids together with a high salt diet can induce hypertension in both large and small animals. In animals ~~given~~ a high salt-intake, administration of deoxycorticosterone ~~(DOC)~~, usually in the form of ~~deoxycorticosterone acetate (~~DOCA~~)~~, has been the most widely used approach for inducing mineralocorticoid-salt hypertension. Deoxycorticosterone ~~DOC~~ appears to have both glucocorticoid and mineralocorticoid properties, but its tendency to cause sodium retention appears central to the DOCA-salt model. Its mineralocorticoid potency, however, is less than that of aldosterone itself. While elevated levels of deoxycorticosterone ~~DOC~~ can contribute to some rare human forms of hypertension, the most common human form of mineralocorticoid-dependent hypertension involves hyperaldosteronism. Thus, the development of animal models of hyperaldosteronism is a subject of considerable scientific interest (177). Recently, for example, a mouse model of aldosterone-salt hypertension was developed by transgenically expressing the human gene for aldosterone synthase under control of the human promoter for the gene encoding 11β hydroxylase (178).

Early studies showed that the effects of mineralocorticoids like DOCA were greatly enhanced in animals 'sensitized' to its actions by a high salt intake (typically 0.6-1% NaCl in drinking water) and often by uninephrectomy (179). While initial studies emphasized the need for extremely high DOCA doses, lower doses reproducibly induce hypertension; doses typically currently range from 20-50 mg/kg (in rats) (180). Notably, using saline drinking solution without access to free water imposes a non-physiological stress on the animals that may affect the results; further, the models often become hypokalemic, and some investigators have therefore supplemented potassium. Although rats are most commonly used for this model, it has been employed successfully using many different species, including mice, dogs, sheep, and pigs. The model manifests low plasma renin activity (and low circulating AngII), so it bears some similarity to a common form of primary human hypertension, low renin hypertension.

The DOCA-salt model appears to have volume-dependent, vascular, and neurogenic components. Mineralocorticoids, dietary salt loading, and uninephrectomy would each be expected to promote increases in extracellular fluid volume, and it is thus not surprising that salt and water balances can be positive, especially early in the course of mineralocorticoid-salt administration. Both plasma and extracellular fluid (ECF) volume are expanded (181), and these effects may appear to be sustained (182). However, the extent to which a high salt diet causes greater sodium retention and volume expansion in animals treated with deoxycorticosterone~~DOC~~ or aldosterone than in non-treated salt-loaded controls remains controversial (183). While the classical model of mineralocorticoid-salt hypertension typically involves expansion of blood volume and ECF volume, hypertension may occur even when prominent volume expansion does not occur, as in the setting of a normal salt diet (182). It should be noted that administration of mineralocorticoids to animals given a low salt diet usually induces little or no effect on BP.

Shortly after DOCA-salt hypertension was described, it was recognized to have a substantial neurogenic component. DOCA-salt animals typically manifest sympathetic hyperactivity, perhaps resulting in part from increased plasma osmolality, which regulates lumbar sympathetic nerve activity. DOCA has been postulated to sensitize the brain to salt and osmolality (184).

Sympathetic hyperactivity increases vasoconstriction in both arterioles and in the venous circulation. The importance of these effects has been supported by reports that lesions in the area postrema, anterolateral third ventricle, and paraventricular nucleus of the hypothalamus attenuate the hypertension (185).

Although DOCA-salt hypertension is characterized by suppressed plasma renin activity, Ang II concentrations in the brain may actually increase, likely contributing to sympathetic activation and salt and water retention through effects on brain AT1 receptors (186). There is also evidence for a role of the prorenin receptor in these processes, as its blockade reduces BP in DOCA-salt animals (187). In contrast, intrarenal RAAS does not appear to play a major role. The early studies of Selye and others indicated that DOCA-salt animals exhibited substantial systemic inflammation (188). More recently, interest in the immune contribution to hypertension generally has reemerged. Harrison and colleagues have shown that T cells are important for the full effects of DOCA-salt to increase BP and to enhance superoxide production, clearly implicating a role for T cells in the model (161).

In summary, the combination of mineralocorticoid treatment and ~~a~~ high ~~-~~ salt intake, and sometimes with uninephrectomy, provides a reliable animal model that can develop severe hypertension with some features of human low-renin hypertension. Studies of such models might provide insight into the salutary effects of mineralocorticoid receptor blockade in the setting of resistant hypertension, even in patients without frank hyperaldosteronism. Thus, the mineralocorticoid-salt models may have broad applicability to those hypertensive patients in whom controlling BP presents a challenge. Such models could also have value for understanding why cardiovascular risk may be higher in humans with primary aldosteronism than in individuals with similar BP levels and hypertension of unknown etiology,

Renoprival Hypertension

The prevalence of hypertension among patients with kidney disease highlights the relevance of renoprival models to human hypertension, as more than 90% of patients with end-stage kidney disease are afflicted with hypertension. Pre-clinical renoprival models correspond precisely to the phenotype in the nephrology clinic that garners concern – a hypertensive patient who has developed chronic kidney disease. Another salient feature of renoprival models is the impairment in salt excretion that accrues from reduced nephron mass, allowing one to study the salt-sensitivity that afflicts roughly half of human hypertensive patients.

——Remnant kidney models were developed in rats to explore the adaptive effects of renal mass reduction on the remaining nephrons (189). Accordingly, use of this model revealed the detrimental consequences of glomerular hyper-filtration, and the benefits of lowering glomerular pressure via inhibition of the RAAS. Extrapolation from these rat models provided some of the rationale for use of RAS blockers in patients with kidney disease and hypertension. Infarction of 2/3 of one rat kidney leads to BP elevation, whereas 2/3 nephrectomy together with total contralateral nephrectomy, the modern "subtotal nephrectomy" model, yields both hypertension and glomerulosclerosis. In rats, 2/3 nephrectomy is typically performed on the left kidney and achieved via ligation of the posterior branch and the inferior segment of the renal artery (190). Adaptation to mice, typically as a "3/4" nephrectomy (1 nephrectomy plus resection of half the contralateral kidney), is technically challenging but has been executed with ligation of renal artery segments as in rats (191) or with direct excision of half a kidney using electrocautery or glue to achieve hemostasis (192). Hypertension develops universally in remnant kidney rats. By contrast, subtotal nephrectomy in mice induces hypertension in the 129SVE salt-sensitive strain but not in the C57BL/6 strain that is relatively salt-resistant (191, 193, 194). Hypertension and kidney disease progress more slowly and less severely in this model than in more aggressive pharmacological hypertension models, with studies reporting several months of data in rats and mice (193, 194). In rats, tailcuff plethysmography may be sufficient to detect a BP rise, but given the variability of responses in mice, radiotelemetry is recommended for BP monitoring in mice.

Summary and Future Considerations. In the future, subtotal nephrectomy will likely remain a useful model of hypertension relevant to patients with advanced and progressive CKD. The salt sensitivity in the remnant kidney model seen with rats or 129SVE mice enhances its relevance to human subpopulations, such as African Americans, who are prone to salt sensitivity. Abnormalities in cardiac morphology and function such as LVH and altered diastolic relaxation seen with subtotal nephrectomy will provide a timely model of cardiorenal syndrome (190, 192). The glomerulosclerosis seen particularly in the rat model mimics the secondary focal segmental glomerulosclerosis that develops after several years of uncontrolled hypertension. The gradual increase in BP in this model emulates the BP trajectory seen in human ~~essential~~primary hypertension more smoothly than abrupt induction of hypertension via pharmacologic perturbations. Nevertheless, hybrid models of renal mass reduction are now being used to enhance injury and/or shorten the time required for readouts. For example, when vigorous

RAAS activation is needed, unilateral nephrectomy combined with Ang II infusion yields robust glomerular and tubular damage after 8 weeks, or after 4 weeks when a high salt diet is administered (162, 170). Alternatively, Ang II infusion combined with true subtotal nephrectomy in salt-resistant mouse strains like C57BL/6 can overcome resistance to hypertension in these strains (194). Thus, at institutions with skilled surgical personnel, the remnant kidney model alone or in combination with other approaches will continue to yield data relevant to human patients with hypertension and CKD.

Nitric oxide system

Nitric oxide (NO) is catalysed by endothelial nitric oxide synthase (eNOS) and its local release occurs on a continual basis thereby modulating effects of local and systemic vasoconstrictors and fine-tuning of BP and organ blood flow. Based on the premise that NO inhibition would lead to predominance of vasoconstrictors and consequent increase in BP, researchers began to explore the possibility that NOS inhibition would cause hypertension in animal models. In 1990, Gardiner et al showed that acute treatment of Brattelboro rats with NG-monomethyl-L-arginine (L-NMMA), a methyl derivative of arginine and NOS inhibitor, caused an increase in BP (195). Ribeiro followed up on these studies and showed that chronic treatment of Wistar rats with the NO inhibitor, Nω-nitro-L-arginine (L-NAME), a nitro derivative of L-arginine, increased systolic BP by over 60 mmHg (196). L-NAME-treated rats also show renal vasoconstriction and hypoperfusion (195-197). As the disease progresses, it is characterized by renal dysfunction, renal hypertensive microangiopathy, cardiac, vascular and renal fibrosis and features of malignant hypertension. These studies defined a new model of hypertension induced by chronic NO inhibition and provided the hypertension community with a robust experimental model of severe/malignant hypertension with evidence of target organ damage.

The mechanisms underlying L-NAME-induced hypertension seem to involve processes beyond inhibition of endotheliumal-derived NO, because infusion of L-arginine, which activates eNOS to produce NO, does not completely reverse hypertension. Chronic inhibition of NO likely has impact on BP regulatory systems beyond direct effects on vasodilation and vascular tone. In particular, persistent inhibition of NO biosynthesis with L-NAME is associated with profound vasoconstriction, activation of the sympathetic nervous system and the RAAS, oxidative stress, kidney damage and structural alterations of the vascular wall. Since RAAS inhibitors fail to completely ameliorate L-NAME-induced hypertension, other humoral factors, such as

endothelin-1, have also been implicated (197).

As with all experimental models of human disease there are strengths and limitations that need to be considered for the chronic NO inhibition model of hypertension. The strengths of this pharmacological model of hypertension include the relatively simple technical approach, the reproducibility of the model, the development of systemic hypertension, the robust nature of severe hypertension, evidence of target organ damage, and reversibility of hypertension with L-arginine and various commonly used antihypertensive drugs (198). However, the mechanisms underlying L-NAME likely involve processes beyond NOS inhibition, and more importantly, the pathophysiological role of decreased NO biosynthesis in human hypertension remains unclear. Moreover, the BP increase in NO deficiency hypertension occurs rapidly, usually within hours of L-NAME or L-NMMA infusion, while the development of hypertension in humans occurs slowly and becomes established after many years. Hence, while the L-NAME/L-NMMA rat is an excellent model of hypertension-induced target organ damage mimicking many of the complications observed in human hypertension, it is probably not an appropriate model for ~~essential~~primary hypertension, which has a slow onset that develops over the lifetime, and caution needs to be considered when extrapolating findings from this model to the clinic (199). On the other hand, superimposing the L-NAME model onto conventional diabetic mouse strains accelerates kidney injury, recapitulating many features of human diabetic nephropathy (200, 201).

Summary and future directions: What we have learned from the chronic NOS inhibition model is that NO is a critical factor involved in the physiological regulation of cardiovascular function and homeostasis and is a key regulatory molecule involved in multiple functions, including vascular tone, renal function, salt-volume homeostasis and renin secretion. As such, it is a potentially attractive therapeutic target. While organic nitrates have been used to treat angina since the 1800s, these compounds have a short half-life with little benefit in chronic hypertension. Hence, there is now growing interest in the antihypertensive effects of inorganic nitrates in the diet, and in the development of modulators of NO that are stable and that will deliver NO in a tissue-and site-specific manner. Animal models may be useful in understanding the therapeutic potential of NO modulation in human hypertension.

Several additional forms of hypertension that may develop in animal models are less common and have not been described here. These include models of hypertension induced by infection,

heavy metals, stress (e.g., air jet, psychosocial, cold-induced, etc.), medications and herbal supplements (acetaminophen, NSAIDs, licorice, etc.), hypothyroidism, hyperparathyroidism, and others, which have been described elsewhere.

OBESITY

Overweight and obesity contributes to up to 75% of the risk for ~~essential~~primary hypertension, and to most cases of treatment-resistant hypertension. While the physiological and molecular mechanisms of obesity-related hypertension remain unclear, sodium retention, RAAS activation, increased sympathetic activity, leptin resistance, and endothelial dysfunction have been implicated. Elucidating the mechanisms responsible for obesity-related hypertension may allow development of novel strategies for treating these patients.

Overall, animal models that exhibit concomitant obesity and hypertension can be divided into 2 distinct categories: models of obesity that spontaneously develop hypertension, and models of hypertension with superimposed obesity. Despite their different etiologies, both types of models allow exploring the role of obesity in the development and progression of hypertension. While models of primary obesity permit evaluating the mechanisms by which obesity predisposes to hypertension, models of hypertension enable studying how obesity alters its course.

Several models of experimental obesity spontaneously develop hypertension as they gain body weight. Genetically induced obesity models that develop hypertension include the obese Zucker rat (OZR), ZSF1 rat, Wistar Fatty rat (WFR), and ob/ob mouse, which result from mutations that interfere with leptin signaling and transduction. These models are the most commonly used and offer the advantage of minimizing the impact of confounding factors, allowing mechanistic studies to investigate the role of specific genes in obesity-induced hypertension. Genetically induced obesity-hypertension can be also achieved in large animal models. For example, DNA transposition of D374Y gain-of-function cDNA of chimp proprotein convertase subtilisin/kexin type-9 is associated with elevated BP (202). However, coexisting obesity and hypertension result from the combination of genetic and environmental epigenetic factors. Thus, genetically induced obesity models may not fully address this need.

Diets rich in saturated fats and refined carbohydrates induce weight gain and changes in body composition and adipose tissue cellularity both in rodents (203, 204) and several large animals, including dogs (205), rabbits (206), and pigs (207). The rat might be the optimal small animal

model for diet-induced hypertension, because of its size and the propensity to develop hypertension faster than mice. High-carbohydrate diets, alone or in combination with high-fat diets, have been shown to induce obesity, hypertension, hyperlipidemia, and glucose intolerance in several rat strains, including SD and Wistar rats within just 3 weeks (208, 209). However, C57BL/6 mice fed a high-fat/high-carbohydrate diet supplemented with NaCl may need as much as 3 months to develop hypertension (203). Unlike rodents, development of hypertension is relatively similar among large animals of obesity ranging from 5 weeks in the dog (210) to 12-16 in the pig (207). Although a mixture of chow and added fat may be sufficient to develop obesity-hypertension, purified-ingredient diets are the preferred choice, due to their low batch-to batch variability and lack of plant-derived phytochemicals, which may alter disease progression (211). Purified diets contain high levels of carbohydrates, which are indispensable to achieve several features of metabolic syndrome, and particularly hypertension. Pigs fed a high-fat diet alone are characterized by dyslipidemia and vascular dysfunction, but do not increase BP levels (212). Contrarily, feeding domestic pigs with a diet containing high levels of carbohydrates, fat, and cholesterol over 16 weeks induces spontaneous hypertension (207). Although high-fructose diets seems to be more effective in inducing metabolic syndrome compared to high-sucrose diets, diet-induced increase in BP levels is comparable (213).

Lastly, chemical agents (e.g., monosodium glutamate) primarily used to induce obesity may result in spontaneous hypertension (214). Yet, the association between obesity and hypertension in these models is often weak.

In addition to models of primary obesity, models of concurrent, independently achieved hypertension are critical to answer the fundamental question of whether obesity exacerbates existing hypertension. For example, fat loading in DSS rats aggravated BP and salt-induced renal damage, preceded by body weight gain, visceral fat accumulation, and insulin resistance (215). Similarly, studies in Ossabaw pigs with coexisting obesity and renal artery stenosis demonstrated that obesity amplifies both renal and cardiac injury, yet BP levels remained unaltered (216, 217). These observations suggest that the primary mechanism implicated in the development of hypertension is an important determinant of the effect of obesity. While obesity exacerbates salt sensitivity of BP, the effect of hypertension may be dissociated from obesity in surgically induced hypertensive models.

Alternatively, the impact of obesity on hypertension could be studied by genetic or dietary

manipulations. In the spontaneously hypertensive obese (Koletsky) rat, hypertension and obesity are induced by independent genetic mutations (218). Interestingly, BP levels are comparable to rats with salt-sensitive hypertension, suggesting that obesity induced by this mutation does not exacerbate genetically induced hypertension. Contrarily, DOCA-salt treatment of obese Zucker rats increases BP as fast as with 4 days (219). Therefore, these models are suitable to explore if obesity increases the sensitivity of animals to experimentally induced hypertension. Presumably, the involvement of the RAAS, oxidative stress, or other mechanisms, may dictate the sensitivity of the models to coexisting obesity and hypertension.

The choice between small or large animal models of obesity-hypertension should consider several elements ~~(see section)~~. Specifically, large animal models of diet-induced obesity may require a long period of time to develop spontaneous hypertension, increasing experimental costs. In addition, body weight, length, and fat percentage may reach significant proportions, limiting animal mobility and increasing the risk for infections and surgical complications.

SEX DIFFERENCES AND AGING IN HYPERTENSIVE SMALL ANIMAL MODELS

Profound sex differences are an important feature of hypertension and cardiovascular diseases in humans. Similarly, sex differences in BP have been noted in most animals, including rodents, dogs, chickens, and rabbits (220, 221), but the mechanisms responsible for the sex differences in hypertension or BP control have mainly been studied in rodents (Table B). Almost without exception, young adult male rats have higher BP than females regardless of whether they are normotensive (SD) or hypertensive (SHR, DSS), just as in humans. In normotensive SD rats, mean BP is lower in females than in males (222), possibly due to lower proximal sodium reabsorption and greater ability to excrete sodium (223). There are also greater pressor responses in males with DOCA-salt hypertension (224) and DSS rats (225). In both mice and rats, normotensive males also develop higher BP in response to a “slow pressor” dose of Ang II (226, 227). The mechanisms responsible for the elevated BP or response to Ang II vary with the strain and genetics of the rodents. The kidney and perhaps the sex steroid milieu might be responsible for the sex differences in the Ang-II mediated hypertension. However, in general, radiotelemetry monitoring shows that the estrus cycle of the female does not affect daily BP markedly.

Aging in humans is associated with an increase in BP. In hypertensive rat models, BP increases in females as they stop estrous cycling. In SHR, BP is lower in females prior to stopping estrous cycling, and then increases progressively such that by 16 months of age, BP in

females is similar to or higher than males (228). In DSS rats on a low salt diet, aging in both males and females is also associated with increases in BP (229). In many hypertensive rat models hypertension is mediated by different mechanisms for males and females. Contributions of hormones, sympathetic and renal nervous systems, immune system, metabolic syndrome effects, and oxidative stress, which are different between males and females, must be taken into account. Thus, sex differences in BP control in animals are as complicated as in humans.

Role of Sex steroids: The presence or absence of sex steroids is an important mediator of hypertension in many of the spontaneously hypertensive rodent models. Castration of male SHR, DSS, and mREN2 models causes a reduction in BP, and ovariectomy increases BP in DS rats, on a high or low salt diet (229), and in mREN2 females (224). In contrast, ovariectomy of SHR has no effect on their BP (225). Exogenous androgens given chronically increase BP in female SD rats, a model of polycystic ovary syndrome (230). The mechanisms by which sex steroids can impact BP include stimulation of renal angiotensinogen synthesis by androgens, and estrogen-mediated stimulation of the vasodilator arm of the RAAS or decreased AT1 receptor synthesis. Estradiol also increases expression of endothelial nitric oxide synthase.

Sex and aging-related differences in mechanisms responsible for hypertension in young SHR

In young male and female SHRs the mechanisms responsible for hypertension are different. For example, in young adult male SHR, the hypertension is mediated by androgens since castration reduces BP to similar levels as in females, and androgen supplementation restores the hypertension (231), whereas ovariectomy has no effect in females, suggesting that estrogens have no effect on BP in females. Removal of the renal nerves or adrenergic blockade normalizes BP in both male and female SHR (232). Central melanocortin-4 receptor (MC4R) antagonism also reduces BP in young male SHR (233), but not young females (234), suggesting that sympathetic activation in males may be due to MC4R activation. Blockade of the RAAS with ACEI (231, 235) normalizes BP in both young male and female SHR.

~~In young adult male and female SHR, removal of the renal nerves or adrenergic blockade normalizes BP (222), but the mechanism responsible is different for the sexes. Central blockade of the melanocortin-4 receptor (MC4R) reduces BP in males, (223), but not females. However, with aging, BP falls with renal denervation or adrenergic blockade, but both male and female remain hypertensive (223), implicating multiple mechanisms in the hypertension in aging SHR.~~

The role of oxidative stress in mediating hypertension in SHR is also sex-dependent, although females have similar or higher levels of oxidative stress markers. Overall, antioxidants prevent development of hypertension in both sexes of SHR (236, 237), but once established, maintenance of hypertension is independent of oxidative stress in females, but not males. The NO system may also be more activated in young females than males SHR (238). Interestingly, tetrahydrobiopterin supplementation reduces BP in male SHR, but this is due to a concomitant reduction in androgen levels rather than protecting against eNOS uncoupling (239). Inhibition of 20-HETE synthesis, either by non-specific arachidonic acid metabolism inhibitor or a specific ω-hydroxylase inhibitor, reduces BP in male SHR (240), but not young females (230). Blockade of the RAAS reduces BP to similar levels in both male and female SHR when they are young (231), but becomes less efficacious with aging in females (235). In addition, the endothelin and the 20-HETE eicosanoid systems may contribute to hypertension in aging females.

Sex differences in inflammation and the immune system also contribute to the hypertension (Table B). Both male and female SHR have a depressor response to the lymphocyte inhibitor, mycophenolate mofetil, but the greater response was in females (241). Females have more circulating CD3+, CD4+, and pro-inflammatory CD3+CD4+RORγ+ Th17 cells, whereas males have more immune-suppressive CD3+CD4+Foxp3+ T regulatory cells. In the kidney, females had greater numbers of CD8+ and regulatory T cells infiltration, whereas males had greater CD4+ and Th17 cell infiltration. The role of T cells in mediating hypertension is emerging. For example, females show greater pressor response to Ang II after male-to-female T cell transfer, and males become less responsive after female to male T cell transfer. Future studies will be necessary to determine the role that T cells play in mediating ~~essential~~primary hypertension in humans, in order to refine studies in animal models.

Aging and sex differences in mechanisms responsible for hypertension

As noted above, with aging and cessation of estrous cycling, BP increases in female SHR to levels similar to or higher than in males (228), despite the lack of effect of ovariectomy on BP in young females (231). The mechanisms responsible for hypertension are also different by sex in aging SHR. For example, while BP falls with renal denervation or adrenergic blockade, both aging males and females remain hypertensive (234), which does not occur in young animals, suggesting that other components contribute to the hypertension in aging SHR. Similarly, blockade of the RAAS reduces BP but becomes less efficacious with aging in females (235), but

normalizes BP in aging males as in young males. While the endothelin system plays no role in mediating hypertension in young male or female SHR, with aging ET_A receptor antagonism modestly reduces BP in females, but not males (230). Blockade of 20-HETE synthesis fails to reduce BP in young female SHR, whereas 20-HETE synthesis inhibition reduces BP in old females (242), suggesting that unlike in young females, 20-HETE contributes to regulation of BP in old female SHR.

In DSS rats, BP in females does increase with ovariectomy and decreases with estradiol supplements (229). Interestingly, by 12 months of age, estradiol-supplemented ovariectomized DSS rats, ovariectomized DSS rats, and intact DSS rats have similar BP (229), suggesting that with aging changes occur in the estrogen receptors or intracellular signaling pathways that make the estradiol no longer able to attenuate the BP.

Other comparisons for BP regulation in male and female DSS rats are not as comprehensive as in SHR and are thus not discussed further.

Other mechanisms underlying sex differences in hypertension

Sex differences in BP have been reported in most models of diet-induced obesity. Male C57Bl/6 mice on a 16-week high fat diet gained less weight than females, but developed hypertension (243). Male Obese Zucker rats have higher BP than females (244). The mechanisms responsible for the sex differences in BP in these rodent models remain unclear.

In large animal models, many studies have been performed in dogs, although the sex has not been often considered. Ang II given intra-cerebroventricularly to dogs caused a pressor and dipsogenic effect in male dogs but not females (245). Male rabbits have higher BPs than females, but neither are salt sensitive (221).

In summary, many rodent models show sex differences in the BP levels, and hypertensive mechanisms responsible for these are different. As shown in Table B, in general, in female animal models, the mechanisms responsible for hypertension are more multifaceted than in males, and these mechanisms tend to increase in number and significance with aging. Therefore, investigators should be cognizant of and look for sex differences in BP, recognize that there may be sex differences in the mechanisms responsible for BP control, and recognize that there may be age-related differences in the mechanisms responsible for BP control as well.

END-ORGAN DAMAGE

The impact of hypertension on human health occurs through damage to critical target organ systems including the brain, heart, kidneys and vasculature. Investigations on target organ damage in many established rodent models of hypertension have revealed insights into mechanisms of BP-induced tissue injury. However, with the growing importance of co-morbidities and aging in hypertension and its complications, studying aged animals and animals with induced co-morbidities, such as hyperglycemia, hyperlipidemia and obesity, would further increase the translational relevance of animal models of human hypertension.

Vasculature: Vascular dysfunction and remodelling

In hypertension, endothelial dysfunction is associated with ~~may precede overt evidence of~~ target organ damage, and has been demonstrated in peripheral, coronary, cerebral, renal and conduit vessels in experimental and clinical hypertension. Molecular processes causing endothelial dysfunction include decreased endothelial NO production, increased bioavailability of reactive oxygen species, increased ET-1 and Ang II production, and immune mechanisms. Hypercontractility also contributes to high vascular tone in hypertension. Processes underlying this involve increased vascular smooth muscle cell contraction and reduced relaxation, mediated in large part through changes in intracellular calcium concentration and activation of RhoA-Rho kinase pathways.

In addition to functional alterations, small and large arteries exhibit structural changes in hypertension, characterized by remodeling, fibrosis, and inflammation, processes that are amplified with aging in SHR (246). Resistance artery narrowing and large artery stiffening are not only target organ effects of high blood pressure, but these vascular changes contribute to the development of hypertension by increasing peripheral resistance and compromising arterial compliance.

Structural remodeling of the vascular wall leads to reduced lumen diameter and thickening of the vascular media. Remodeling of small arteries may be the first manifestation of target organ damage in hypertension. In clinical studies, 100% of patients with stage 1 hypertension have small vessel remodeling whereas only 60% have endothelial dysfunction and 45% have left ventricular hypertrophy (247). The concept of 'vascular remodeling' was first suggested in 1989 when pial arterioles from SHRSP were found to have significant structural alterations (248). Arterial remodeling is characteristically associated with increased wall thickness, of which two types are described: inward or outward, depending on whether the lumen diameter is reduced or increased

respectively. Remodeling is further classified into eutrophic, hypotrophic or hypertrophic depending on whether there is no change, reduced or increased vascular material (vascular smooth muscle cells, extracellular matrix) respectively (249, 250). Eutrophic inward remodeling is usually found in SHR and Ang II-induced hypertension, whereas hypertrophic remodeling is found in renovascular hypertension, salt-sensitive hypertension, aldosterone-induced hypertension and other forms of secondary hypertension **(Table D).** Different types of remodeling may occur in different vascular beds, for example in SHR, small resistance arteries exhibit eutrophic inward remodeling whereas conduit arteries undergo hypertrophic remodeling. Associated with structural alterations are mechanical changes that promote arterial stiffness, decreased elasticity and reduced distensibility, which further contribute to vascular target organ damage.

~~In addition to functional alterations, vessels exhibit structural changes in hypertension, characterized by remodelling, fibrosis, and inflammation, processes that are amplified with aging in SHR (246). Structural alterations of the vascular wall lead to reduced lumen diameter and thickening of the vascular media. Associated with structural remodelling are mechanical changes that promote arterial stiffness and reduced distensibility.~~

The most commonly used approach~~es~~ to directly assess ~~in experimental models of hypertension~~ vascular function (endothelial function, vasoconstrictor and vasodilator properties), along with structure and mechanical properties, ~~are~~ is by myography (wire and pressure) ~~myography~~. Wire myography allows *ex vivo* measurement of transverse isometric tension in an arterial segment in response to different factors, and assessing biochemical and molecular pathways and passive properties of the vessel (251). Pressure myography is used to assess small vessel function and structure under near physiological conditions of pressure and flow by digitally tracking diameter and flow in real time (252). Parameters studied ~~assessed~~ by pressure myography include media structure, wall stress, strain and myogenic tone, and allows evaluation of responses to increases in pressure, flow and pharmacological stimuli. The system can be linked to imaging equipment~~systems~~, for example to assess calcium transients. These approaches have been used extensively in various rodent models of hypertension, including L-NAME-rats, SHR, SHRSP, salt-sensitive rats, renovascular models, Ang II-induced hypertension and transgenic mice, and have contributed enormously to elucidating the vascular phenotype as a cause and target of hypertension.

In addition to impaired endothelial function, hyper-reactivity and structural remodeling,

hypertension-induced vascular damage may involve rarefaction, which is characterized by a decrease in microvascular density. Rarefaction may be functional (vasoconstriction) or structural, and may contribute to ≈25% of peripheral resistance in experimental hypertension (249).

Heart: Cardiac Fibrosis, Hypertrophy, and Hypertensive Heart disease.

Cardiac injury is a major consequence of persistent, uncontrolled hypertension. Elevated BP culminates in myocardial strain resulting in LVH, an independent risk factor for cardiovascular mortality. Disruption in cardiac architecture with LVH is associated with aberrant electrical conduction leading to atrial or ventricular arrhythmias and sudden death. When the heart can no longer sustain normal function in the face of elevated afterload, persistent hypertension leads to diastolic and ultimately systolic heart failure. Accordingly, hypertension is a leading cause of congestive heart failure in humans.

In many rodent models of hypertension, approximating human Stage 2 hypertension leads to LVH within 2-4 weeks~~BP elevation leads to LVH~~, measured by augmented heart-to-body-weight or heart-to-tibia-length ratios. ~~Indeed, t~~The level of BP measured by radiotelemetry correlates with the extent of cardiac hypertrophy (160), making heart weight a possible surrogate for hypertension where direct measurements are unavailable. Rodent echocardiography allows direct assessment of changes in cardiac filling patterns and left ventricular wall thickness. These detailed measurements allow discrimination between signaling pathways that favor physiological versus pathogenic cardiac hypertrophy.

After one month, hypertensive cardiac injury in rodents is marked at the histologic level ~~At the histologic level, hypertensive cardiac injury in rodents is marked~~ by myocyte damage, mild perivascular fibrosis, and sparse mononuclear cell infiltrates (160), which nonetheless modulate cardiac injury during hypertension. At the molecular level, cardiac hypertrophy is characterized by recapitulation of fetal gene expression in experimental hypertension (253). Severe hypertension provides a model of cardiac fibrosis, which can be quantitated by immunohistochemistry and molecular signatures of cardiac scar formation. Scarring disrupts electrical conduction in the heart with consequent discrete dysrhythmias that can be captured and quantified with current radiotelemetry monitoring systems (254).

Kidney: Renal Fibrosis and Hypertensive Kidney Failure

Hypertension-induced renal damage (HIRD) comprises at least three patterns: benign

nephrosclerosis, malignant nephrosclerosis, and hypertension-accelerated kidney disease (255). Benign nephrosclerosis is characterized by arteriosclerosis, interstitial fibrosis, and global glomerulosclerosis. The individual risk of end-stage kidney disease from benign nephrosclerosis is surprisingly small, but the net effect of benign hypertension is significant, because hypertension itself is so common. In contrast, malignant hypertension, which itself is uncommon, typically leads to kidney damage, often associated with fibrinoid necrosis and thrombosis of small vessels and glomeruli. HIRD most commonly occurs in the setting of underlying kidney disease, in which hypertension accelerates progression, for instance of diabetic kidney disease.

The three subtypes of HIRD have been replicated in animal models. Rodent models like SHR develop kidney damage very slowly. This appears to reflect preserved renal vascular autoregulation, with normal pressure-induced afferent vasoconstriction, preventing high arterial pressure from being transmitted to the glomerular capillaries (256). This model resembles benign hypertension in humans, in which the risk for hypertensive nephrosclerosis is low, and damage is restricted primarily to pre-capillary blood vessels and interstitium. In contrast, when arterial pressure rises above a critical threshold, for example in stroke-prone SHR rats exposed to high salt intake, renal damage develops rapidly, with lesions characteristic of malignant hypertension (257). In this case, the pressure is above the autoregulatory range, and is therefore transmitted directly to the glomerulus. This causes proteinuria and rapidly progressive renal dysfunction, resulting from glomerular damage. These characteristic features of human malignant hypertension can also be observed in *Ren2*-transgenic rats.

In the setting of underlying renal disease, the relationship between arterial pressure and kidney damage shifts, and BPs that do not normally lead to progressive damage do so. Mechanisms involved are controversial and depend on the models employed. Many studies use $5/6^{th}$ nephrectomy (See under “Renoprival Hypertension”). Kidney damage has been suggested to result from resulting dilation of the afferent arteriole, with efferent vasoconstriction, which together increase glomerular capillary pressure, independent of changes in arterial pressure. In contrast, a surgical approach to reduce renal mass without generating hypertension showed that systemic hypertension is required for renal damage (258). Mouse strains vary in their susceptibility to kidney damage. Rodent models of diabetic kidney disease, for example that induced by streptozotocin on a 129SvE background, have been used to demonstrate the impact of

superimposed hypertension on baseline kidney damage (259).

Brain: Hypertensive Cerebral Damage

Hypertension is a major risk factor for cerebrovascular diseases, such as stroke (ischemic and hemorrhagic) and vascular dementia, but also for neurodegenerative diseases, including Alzheimer's disease (260). Hypertension has damaging effects on cerebral blood vessels, which have been implicated in its harmful effects on the brain. Lacking energy reserves, the brain is highly susceptible to alterations in blood supply, and hypertension can promote both acute and chronic ischemic brain injury (261). As in systemic arteries, hypertension accelerates atherosclerosis and induces stiffening, remodeling and hypertrophy in cerebral arteries. Distinctive alterations, similar to those observed in the kidney (lipohyalinosis), are observed in penetrating arterioles of the brainstem and basal ganglia. Functionally, hypertension alters myogenic tone and cerebrovascular autoregulation, induces endothelial dysfunction, impairs the ability of neural activity to increase cerebral blood flow (neurovascular coupling), and damages the blood-brain barrier. These structural and functional alterations promote vascular occlusions, leading to acute ischemic brain injury, and chronic vascular insufficiency causing white matter damage. A major consequence of the hypertensive white matter damage is cognitive impairment. Indeed, hypertension is the major cause of cognitive impairment on vascular bases, the most common cause of dementia after Alzheimer's disease (260). Executive dysfunction and psychomotor slowing are the typical cognitive deficits, but memory impairment, more characteristic of Alzheimer's disease, can also occur in more advanced cases (260). In addition, hypertension induces rupture of cerebral microvessels causing intracerebral hemorrhage, typically in the basal ganglia, or bursting of aneurysmal dilatation in arteries at the base of the brain resulting in subarachnoid hemorrhage.

Several animal models of hypertension have been used to investigate the effects of hypertension on the brain (Table C). Although these models do not fully recapitulate the harmful effects of hypertension, they have provided valuable knowledge on the potential mechanisms underlying the susceptibility of the brain to hypertension. Most of the models used for cerebrovascular research have been in rodents, although there have been studies in larger animals, mainly pigs and monkeys. As noted earlier, models based on administration of pharmacological agents have the advantage that the cause hypertension is known, and can be induced in a defined time frame and in transgenic animals, allowing studying early mechanisms

of disease at the molecular level, as well as cognitive dysfunction. A disadvantage is that that the hypertension is limited in time (usually weeks) and does not mimic the long-lasting impact on the brain of the human disease. Nevertheless, these models have been some of the most commonly used.

Genetic models based on intercrossing and selecting for the hypertensive phenotype, e.g., SHR-SP, BPH2 mice, exhibit life-long hypertension and provide insight on the effects of hypertension on the brain, including cognitive dysfunction, over the life course. However, the precise cause of the hypertension remains unknown, raising the possibility that the cerebrovascular alterations are not attributable to hypertension, but to unrelated genetic factors. For example, the increased susceptibility to ischemic brain injury in SHR and SP-SHR could be related, in part, to an inherited vulnerability of neurons to excitotoxicity (262, 263). Some transgenic models have life-long hypertension with a known cause, e.g., mice overexpressing human angiotensinogen and renin (R+/A+) or lacking eNOS, and have been very useful to investigate the role of specific pathways and mediators in the effects of life-long hypertension on the brain.

Some hypertension models that produce brain lesions (infarcts, hemorrhages, or white matter lesions) usually require the combination of pharmacological, dietary, genetic and/or surgical manipulations to enhance the effects of hypertension on the brain (Table C). While mimicking the neuropathological impact of hypertension, the time when lesions develop cannot be predicted and the location of the lesions is highly variable.

Of particular interest are models in larger animals, such as pig and monkeys, in which brain size, gray-white matter ratio, vascular topology, cognitive testing, and cardiovascular function have greater translational relevance (264). Monkeys made hypertensive by aortic coarctation exhibit white matter lesions and microinfarcts and, like the pig model, lend themselves to more detailed assessment of cognitive endpoints (Table C). However, these models are expensive, not well suited to high throughput investigations, and less amenable to genetic manipulations.

In summary, while investigations on target organ damage in animal models of human hypertension have focused mainly on vessels, the heart, and the kidney, there is a paucity of information on the brain effects in these models. This is particularly evident in renovascular hypertension and low-renin hypertension. Considering the devastating impact of hypertension on the brain and its vessels, and its pathogenic role in wide variety of brain diseases, there is a

strong rationale for expanding application of state-of-the-art cerebrovascular and neurovascular investigative tools to delve deeper into the mechanisms through which hypertension promotes neurovascular and neurodegenerative diseases.

APPLICATIONS OF GENETICS AND SYSTEMS BIOLOGY TO ANIMAL MODELS

Great insight has been gained from the genetic study of human hypertension. Studies of monogenic forms of hypertension have revealed the molecular basis of several related syndromes. More recently, GWAS analyses uncovered common variants of modest effect as well as low-frequency variants that contribute to BP variation in patients. These studies provide important insight into human disease, which can be complemented by animal studies, often in models exhibiting phenotypic characteristics observed in human hypertension, which can provide mechanistic biological insight into gene function and underlying cardiovascular risk.

Identification of Quantitative Trait Loci Influencing BP Traits*:*

Of the different inbred species used for genetic studies of hypertension, the rat has been widely utilized for the identification of QTL using linkage analysis approaches. This has been driven by the large number of rat genetic models of hypertension, the relatively low cost of rat experimentation, and ease and accessibility of techniques for assessing cardiovascular phenotypes in rats. The functional validation of QTL has been enabled by generation of congenic or consomic strains, in which defined segments of DNA from one strain are introgressed onto the genetic background of a second strain using a genetic marker-assisted breeding strategy. With this approach, phenotypic differences detected between the parental and congenic strains can indicate that a gene or genes within a particular substituted region of genomic DNA have an influence on the functional trait of interest. The subsequent identification of genes within these QTL has been difficult and depends upon complementary approaches including transcriptomic analyses, gene sequencing, and gene editing (45, 63). Development of modern sequencing techniques and sequencing of the full rat genome provided further opportunities to fully define and design experiments to elucidate key sequence differences of candidate genes within a QTL.

Epigenetics

Rodent models have served as excellent platforms to validate the impact of deletion or overexpression of individual genes associated with hypertensive traits in GWAS or other linkage studies. In addition to their value for genetic and genomic studies, these models have also

contributed to recognition of the influence of environmental factors on disease phenotypes, including hypertension, fueling the study of epigenetics. Epigenetics refers to effects of environmental factors that induce changes in an organism due to modifications in gene expression rather than a direct alteration of DNA sequence. These modifications commonly occur through DNA methylation, post-translational histone modifications, and noncoding RNAs. Of these factors, DNA methylation has been most studied. For example, elevated methylation of the promoter region of 11-beta HSD2 has been correlated with reduced activity of the enzyme and hypertension in patients (265). Similarly, the promoter of the NKCC-1 is hypomethylated in the aorta and heart of SHR compared to the WKY rat, correlating with increased NKCC-1 activity in those tissues and more severe hypertension (266). The use of animal models to assess epigenetic regulation of gene expression in hypertension promises to be a productive area of focus in the future.

Non-coding RNAs

RNAs that do not code for proteins can influence disease pathogenesis by regulating the effectiveness of gene expression through modulation of messenger RNA (mRNA) levels and repression of mRNA translation. Various forms of non-coding RNAs, such as microRNAs, long non-coding RNAs, and circular RNAs, have been proposed to play a role in regulation of BP and risk for hypertension, and the risk for target organ damage associated with hypertension (88, 267, 268). While controversy surrounds the appropriate criteria for identifying non-coding RNAs of functional significance, there is growing interest in studying them in animal models of hypertension, and of hypertension related cardiovascular disorders, from both a mechanistic and therapeutic perspective (88, 267-269). Furthermore, non-coding RNAs may be involved in the mechanisms of kidney injury in some forms of hypertension (270)

Microbiome

Recent studies in humans and animal models have highlighted the powerful impact of the microbiome on a range of disorders. Likewise, the gut microbiota can have a profound influence on BP regulation. In animal models of hypertension, recent observations suggest that there are differences in the microbiota between DSS and Dahl R rats (271) and between SHR and WKY (272). The mechanisms of action whereby the gut microbiota influence BP are not fully elucidated, but the release or stimulation of trimethylamine N-oxide, short chain fatty acids, or other factors can influence cardiovascular phenotypes (273, 274). In addition, recent studies have

identified specific changes in the gut microbiome influencing blood pressure effects of high salt diet (275). As this field matures, it is likely that the influence of the microbiome on complex phenotypes such as hypertension will receive wider recognition. Animal modeling will likely be very useful in unraveling these actions.

Systems Biology*:*

The integrated scientific approach to interrogate and understand the contribution of individual biological components including genes, transcripts, proteins, metabolites, epigenetic modifications, the microbiome, and environmental modifiers, along with the integrated function of these components to a cell, tissue, organ, organ system, or organism is termed Systems Biology. Systems Biology approaches utilize high throughput analytical and bioinformatic tools to understand the entire system rather than any single individual aspect. There is no individual 'Systems Biology' approach, nor is there agreement on the precise definition of this approach (276, 277), but Systems Biology approaches, integrating quantitative measurement of biological variables obtained in animal models, mathematical modeling, reconstruction, and theory (277) show great promise for understanding complex multifactorial human diseases like hypertension.

In summary, studies at the level of genetics, epigenetics, the microbiome, and systems biology show great promise for hypertension research. At present, h~~H~~owever, due to substantial gaps in understanding pathogenesis and genetic determinants of human hypertension, opportunities to incorporate relevant causal pathways or genetic variants into animal modeling efforts have been limited. For the same reasons, it has not been possible to utilize molecular profiling to verify authenticity of existing animal models. Yet, data emerging from agnostic systems biology studies using genetics, genomics, proteomics and analysis of the microbiome show promise for advancing basic understanding of human hypertension. These approaches should also allow better validation of animal models based upon identification and recapitulation of specific "omic" signatures derived from human hypertension.

SUMMARY: RELEVANCE OF MODELS TO HUMAN HYPERTENSION: CONCORDANCES AND GAPS.

Animal models of human disorders have proved to be immensely useful in translational research in a number of fields, including hypertension. These models allow incisive approaches

not possible in clinical studies for understanding pathophysiology, genetic mechanisms, identification of new disease markers and potential therapeutic targets. As we have highlighted in this paper, insights derived from animal models of hypertension have contributed significantly to understanding this highly prevalent human disorder. Here we summarize a few key points that are especially relevant for those working in the field.

Recent high profile publications have decried the poor reproducibility of published studies using animal models for pre-clinical assessment of therapeutic agents in human disorders from cancer (278) to neurological disease (279). Various factors have been implicated to explain these inconsistencies, including: deploying insufficient numbers of experimental animals, inadequate power calculations and statistical analyses, assessment of outcomes by individuals who are not blinded to experimental groups, and failure to pursue independent replication of critical experiments. Accordingly, these remediable methodological problems must be taken into account in designing any study using animal models of hypertension (4-8).

Another key issue is consideration of how closely the experimental model truly captures what is observed in humans (see "construct validity" above). In this regard, one limitation to developing animal models of human hypertension is that the pathogenesis of the human disease is not well understood, and the primary cause of elevated BP is not apparent in the vast majority of affected individuals. On the other hand, the cardinal feature of human hypertension, elevated BP, can be modeled relatively easily in animals by activating systems known to be involved in human hypertension, including the renin-angiotensin system and sympathetic nervous system, or inhibition of protective factors, such as nitric oxide. Yet, these models all suffer from their limited duration of hypertension compared to humans. The obscure pathogenesis of human hypertension also complicates interpretation and relevance of existing animal models with spontaneous hypertension. Nonetheless, most of these models respond to anti-hypertensive therapies used in humans and develop similar long-term complications, suggesting overlaps in pathogenesis. Recent progress in understanding genetic mechanisms of primary hypertension in humans should provide opportunities for generating more reliable models, and for analyzing the veracity of existing models using systems biology approaches.

While elevated BP is the key diagnostic feature of human hypertension, its morbidity and mortality result from complications in the brain, heart, kidney and vasculature. The contribution of elevated BP to these complications has been well established in clinical trials showing that BP

lowering reduces complications. However, understanding the molecular mechanisms of these complications is an unmet need, where animal models should continue to add value through identifying pathways and/or markers associated with increased risk for complications, understanding mechanisms for known risk factors such as APOl1, and for potentially identifying therapies that can protect against complications, above and beyond blood pressure control.

Another key feature of human hypertension is its frequent association with chronic co-morbidities such as obesity, diabetes, heart and kidney disease, which can influence the disease characteristics and outcomes. Likewise, sex and ethnicity also have major impact in human hypertension. These factors are not typically incorporated into most animal models of hypertension, but should be to improve concordance with the human condition.

The choice of a suitable model, such as small versus large animal, spontaneous versus induced hypertension, etc., will depend on a number of factors including the specific experimental question and an investigator's available resources and expertise. No individual model will recapitulate all features of human hypertension, and all have advantages and shortcomings, which we have highlighted in this manuscript and its accompanying tables. These factors obviously must be taken into account in the design and interpretation of experiments, and the most powerful insights will often be derived from studies carried out in multiple, complementary models.

CONCLUSIONS

Hypertension is the most common chronic disease in the world, and increased understanding of the pathogenesis, prevention, and treatment of hypertension and its comorbidities is imperative. Animal models of hypertension have been, and will likely remain, very useful in provide insights into the pathogenesis and novel treatment options of hypertension. Clearly, investigators need to make informed choices as to the appropriate animal model for specific application, and the experiments need to be carefully designed, executed, and interpreted. In this Statement we summarize a few key points that are especially relevant for those working in the field, and may aid in propelling it forward.

Acknowledgements

Partly supported by NIH grant numbers DK100081 (LOL), DK104273 (LOL), R37-NS089323-

04 (CI), R01-NS095441 (CI), R01-NS100447 (CI).

Table A. ***Examples of transgenic techniques related to hypertensive models***

Transgenic technique	**Species**	**Examples**
Gene over-expression	M M/R	Mendelian models of hypertension Salt-sensitive hypertension (120, 130)
Inducible expression	R	Inducible hypertension & end organ damage (118)
BAC incorporation	M	Reduced risk of hypertension (76, 77)
Global gene knockout/	M M/R M	Mendelian models of hypertension (105-109) Salt-sensitive hypertension (110-112, 128-130) Pulmonary hypertension (280) Renin hypotension (113-115)
Gene knock-in	M/R	Renin (119); RAS humanization (122, 123)
Targeted gene knockout	M	Kidney specific Hsd11b2 knockout (111)
Safe haven targeting	R	ROSA 26 (93, 94)
Conditional knockout	M	Pulmonary hypertension (124, 125)
Rat / rabbit ES cells	R Rb	TALEN targeting (281) ApoE knockout (82)
Zinc finger nucleases	R	Salt-sensitive hypertension (86)
TALENs	M/R	Pde1a(87)
CRISPR-Cas9	R	(88); Humanisation & ROSA-26 (93, 94)
Gene knock-down/ Anti-miRs / siRNA	M/R	AT1A receptor (126); Reduced hypertension in aldosterone/salt treated mice (127); Reduced hypertension in SHR (95).

~~Small animal models~~ ~~Large animal models~~

Anatomical, physiological, hemodynamic properties	Distant from humans	Closer to humans
Developmental pathophysiology	Distant from humans	Closer to humans
Tissue availability	Low	High
Costs	Low	High
Genetic modification	Readily available	Possible
Reproductive cycle	Short	Long
Characterization	Extensive	Adequate
Availability of specific antibodies	High	Low

Table B. Sex Differences in Blood Pressure and Response to Treatment in Animal models

	Males		**Females**	
Strain/model	**Young adult**	**Aging**	**Young adult**	**Aging (post cycling)**
SHR				
No treatment	M > F	M ≤ F	F < M	F ≥ M
ET_AR antagonism	No effect	No effect	No effect	Modest fall
Enalapril/losartan	Normalize	Normalize	Normalize	Decrease
20-HETE		Yes	No effect	Decrease F > M
Gonadectomy	Castrated < M	Castrated < M	No effect. OVX = F	No effect. OVX= F
Adrenergic block/ renal denervation	Decrease M>F	Decrease	Decrease	Decrease
MC4R antagonist	Decrease	Decrease	No effect	No effect
Nitric oxide	NOx/NOS1/NOS 3 activities M < F	E2 increase eNOS synthesis		
Antioxidants	Decrease	Decrease	If start pre-puberty	No effect
T cells			Tregs F > M	
Pregnancy			Falls last trimester	
Dahl S				
Low NaCl	M > F	Rise with aging	F < M	Rise with aging
+ High NaCl diet	M > F			
Gonadectomy	Castrated < M	Castrated < M	OVX > F	Ovx=F rise with age
Pregnancy			No fall last trimester	
mREN2				
No treatment	M > F		F < M	
Gonadectomy	Castrated < M		OVX > F	OVX = F
Normotensive strains				
No treatment (rats)	M > F	M < F	F < M	F ≥ M by 18 mos
+ DOCA-salt	M > F			
+ Ang II				
+ ACEI	M > F (mice)		F > M (rats)	
- ACEI	M > F (mice/rats)			
+ACEI + high NaCl	M = salt sensitive		F =not salt sensitive	

T cells	M > F		F < M	
Pregnancy			Decrease late	
+ RUPP			Increase late	
+ L-NAME			Increase	
Offspring effect RUPP dams	M > F	Normalizes	F < M	Increase F > M
Obesity (mice)	M > F		No effect	
Rabbits	M > F		F < M	
Dogs + Ang II	Increase M > F		Increase F < M	
Chickens	M > F		F < M	

SHR, Spontaneously hypertensive rats; M, male; F, female; OVX, ovariectomized female; ET_AR, endothelin-A receptor; MC4R, melanocortin 4 receptor; DOCA, deoxycorticosterone acetate; Ang II, angiotensin II; ACEI, angiotensin I converting enzyme; NOS, nitric oxide synthase; RUPP, reduced uterine perfusion pressure; L-NAME, nitro-L-arginine methyl ester.

Table C. Cerebrovascular pathologies in selected animal models of hypertension

Hypertension model	Species	Neurovascular pathology	References
AngII infusion (2-4 weeks)	Mouse Rat	• Hypertrophy and remodeling • Neurovascular dysfunction • Increased BBB permeability • Inflammation • Oxidative stress • Cognitive deficits • Brain amyloid□□eta accumulation	(282-286)
Chronic AngII+Acute AngII+LNAME	Mouse	• Micro-hemorrhages • Increased BBB permeability • Inflammation • Cognitive deficits	(287, 288)
Ren and/or Agt overexpression	Mouse Rat	• Hypertrophy and remodeling (R+/A+ mouse) • Increased stiffness (Renin rat) • Endothelial dysfunction • Cognitive deficits (R+/A+ mouse) • Larger infarcts (after MCAO)	(289-293)
Ren/Agt overexpression +LNAME +High salt	Mouse	• Micro-hemorrhages • Inflammation • Oxidative stress	(294)
ET1 overexpression in endothelial cells+MCAO	Mouse	• Larger infarcts • Increased BBB permeability • Cognitive deficits	(295, 296)
eNOS deficiency LNAME hypertension	Mouse Rat	• Hypertrophy • Endothelial dysfunction • Larger infarcts (after MCAO)	(297-300)
Chronic intermittent hypoxia/obstructive sleep apnea	Mouse Rat	• Neurovascular dysfunction • Oxidative stress • Larger infarcts (after MCAO) only with more severe cyclic hypoxia ($6\%O_2$)	(301-305)
BPH2	Mouse	• Hypertrophy • Neurovascular dysfunction • Increased BBB permeability • Cognitive deficits	(283, 290, 306)
SHR-SP+Western diet	Rat	• Infarcts and hemorrhages • Neurovascular dysfunction • Increased BBB permeability • Retinopathy	(307-309)
SHR+MCAO	Rat	• Larger infarcts	(310, 311)

SHR-SP+Carotid occlusion	Rat	• White matter lesions • Increased BBB permeability • Inflammation • Cognitive deficits	(312)
Dahl Rat+High salt	Rat	• Oxidative stress • Reduced BBB marker proteins • Increased BBB permeability • Loss of myogenic tone • Infarcts and hemorrhages	(15, 313, 314)
DOCA salt	Mouse Rat	• Hypertrophy • Neurovascular dysfunction • Inflammation • Oxidative stress • Cognitive deficits • No change in infarct size or larger infarcts (after MCAO)	(286, 315-318)
• DOCA salt+elastase • AngII (2 weeks)+ elastase • Carotid & renal artery ligation+AngII (2 weeks) +elastase	Mouse	• Cerebral aneurysms formation and subarachnoid hemorrhage	(316, 319, 320)
Renovascular hypertension	Rat	• Infarcts and hemorrhages • Larger lesions (after MCAO)	(321, 322)
Aortic coarctation	Mouse	• Hypertrophy • Endothelial dysfunction • Oxidative stress • Brain amyloid□□eta accumulation • Cognitive impairment	(323, 324)
	Yucatan pig	• Vascular stiffening • Cognitive deficits	(325, 326)
	Cynomolgus or Rhesus monkey	• White and gray matter microinfarcts (<1mm) • Cognitive deficits	(327)
Aortic coarctation+ High fat diet±aging	Cynomolgus or Rhesus monkey	• Worse cognitive deficits	(328, 329)

AngII: angiotensin-II; BBB: blood-brain barrier; BPH2: blood pressure high-2; DOCA: deoxycorticosterone acetate; LNAME: Nitro-L-arginine-metylester; MCAO: middle cerebral artery occlusion; Ren/Agt: renin angiotensinogen; SHR-SP: stroke-prone spontaneously hypertensive rats; elastase injected in the basal cistern

Table D. Vascular target organ damage in different experimental models of hypertension

Hypertension model	Vascular target organ damage	Reference
Genetic rat models		
SHR	Inward eutrophic remodeling (resistance artery)	(330-335)
	Hypertrophic remodeling (conduit artery)	
	Rarefaction	
	Endothelium-dependent dysfunction	
	Impaired endothelium-independent vasorelaxation (conduit arteries)	
SHRSP	Hypertrophic remodeling Fibrosis Vascular hypercontractility Endothelial dysfunction Increased myogenic tone	(336, 337)
GHR	Hypotrophic outward remodeling (basilar artery)	(338)
Salt-sensitive hypertension		
DSS	Hypertrophic remodeling Endothelial dysfunction Impaired myogenic response (cerebral artery)	(51, 339-341)
DOCA-salt	Hypertrophic remodeling Endothelial dysfunction Vascular inflammation	(333, 342)
Renovascular		
One-clip Goldblatt	Inward eutrophic remodeling (resistance artery) Hypertrophic remodeling (conductance artery)	(343)
2K-1C	Hypertrophic remodeling (aorta but not mesenteric artery)	(344)
NO-dependent models		
L-NAME	Outward hypotrophic remodeling Aortic stiffness Increased pulse wave velocity Impaired endothelium-independent vasorelaxation Cerebral artery remodeling	(345-348)
SHR/L-NAME	Aortic stiffness Reduced distensibility Excessive fibrosis	(349)
Ang II-dependent models		

Ren-2-transgenic rats	Aortic endothelial dysfunction Decreased aortic contraction Fibrosis Endothelial dysfunction	(350)
dTGR	Medial hypertrophy Intimal thickening Fibrinoid necrosis	(351)
Ang II-infused (400 ng/kg/min; Slow pressor)	Inward eutrophic remodeling Endothelial dysfunction Vascular hypercontractility Low-grade vascular inflammation	(160-163) (352-354)
Ang II-infused (>1000 ng/kg/min)	Aortic medial thickening Outward aortic remodeling Vascular inflammation Vascular hyper-reactivity Increased vascular tone Increased aortic stiffness Endothelial dysfunction	(355-358)
Large mammals		
AGM	Renal vascular remodeling Vascular hypertrophy	(141, 359)
Hypertensive obese pig	Cardiac microvascular remodeling	(360)
Fat-fed mini-pig	Endothelial dysfunction Vascular inflammation Hypertrophic remodeling	(361)

SHR, spontaneously hypertensive rat; DSS, Dahl salt-sensitive; SHRSP, stroke-prone SHR; GHR, genetically hypertensive rat; DOCA, deoxycorticosterone; NO, nitric oxide; L-NAME, N^{ω}-nitro- L-arginine methyl ester; Ang II, angiotensin II; Ren, renin; agm, African green monkey; 2K-1C, two-kidney, one-clip; dTGR, human renin-angiotensinogen double transgenic rat.

References

1. Yang SS, Morimoto T, Rai T, Chiga M, Sohara E, Ohno M, Uchida K, Lin SH, Moriguchi T, Shibuya H, Kondo Y, Sasaki S, Uchida S: Molecular Pathogenesis of Pseudohypoaldosteronism Type II: Generation and Analysis of a Wnk4(D561A/+) Knockin Mouse Model. Cell Metab. 5: 331-44, 2007.
2. Pradervand S, Wang Q, Burnier M, Beermann F, Horisberger JD, Hummler E, Rossier BC: A mouse model for Liddle's syndrome. J Am Soc Nephrol 10: 2527-33, 1999.
3. Galis ZS, Thrasher T, Reid DM, Stanley DV, Oh YS: Investing in high blood pressure research: a national institutes of health perspective. Hypertension 61: 757-61, 2013.
4. Reproducibility Issues in Research with Animals and Animal Models: Workshop in Brief. 2015, Washington, DC: The National Academies Press. 8.
5. Sjoberg EA: Logical fallacies in animal model research. Behav Brain Funct 13: 3, 2017.
6. Zeiss CJ, Johnson LK: Bridging the Gap between Reproducibility and Translation: Data Resources and Approaches. ILAR J: 1-3, 2017.
7. Avey MT, Moher D, Sullivan KJ, Fergusson D, Griffin G, Grimshaw JM, Hutton B, Lalu MM, Macleod M, Marshall J, Mei SH, Rudnicki M, Stewart DJ, Turgeon AF, McIntyre L: The Devil Is in the Details: Incomplete Reporting in Preclinical Animal Research. PLoS One 11: e0166733, 2016.
8. Reichlin TS, Vogt L, Wurbel H: The Researchers' View of Scientific Rigor-Survey on the Conduct and Reporting of In Vivo Research. PLoS One 11: e0165999, 2016.
9. McGonigle P, Ruggeri B: Animal models of human disease: challenges in enabling translation. Biochem Pharmacol 87: 162-71, 2014.
10. Pinto YM, Paul M, Ganten D: Lessons from rat models of hypertension: from Goldblatt to genetic engineering. Cardiovasc Res 39: 77-88, 1998.
11. Pfeffer JM, Pfeffer MA, Fishbein MC, Frohlich ED: Cardiac function and morphology with aging in the spontaneously hypertensive rat. Am J Physiol 237: H461-8, 1979.
12. Bing OH, Conrad CH, Boluyt MO, Robinson KG, Brooks WW: Studies of prevention, treatment and mechanisms of heart failure in the aging spontaneously hypertensive rat. Heart Fail Rev 7: 71-88, 2002.
13. Hultstrom M: Development of structural kidney damage in spontaneously hypertensive rats. J Hypertens 30: 1087-91, 2012.
14. Bailey EL, Smith C, Sudlow CL, Wardlaw JM: Is the spontaneously hypertensive stroke prone rat a pertinent model of sub cortical ischemic stroke? A systematic review. Int J Stroke 6: 434-44, 2011.
15. Werber AH, Baumbach GL, Wagner DV, Mark AL, Heistad DD: Factors that influence stroke in Dahl salt-sensitive rats. Hypertension 7: 59-64, 1985.
16. Doi R, Masuyama T, Yamamoto K, Doi Y, Mano T, Sakata Y, Ono K, Kuzuya T, Hirota S, Koyama T, Miwa T, Hori M: Development of different phenotypes of hypertensive heart failure: systolic versus diastolic failure in Dahl salt-sensitive rats. J Hypertens 18: 111-20, 2000.
17. Wilde E, Aubdool AA, Thakore P, Baldissera L, Jr., Alawi KM, Keeble J, Nandi M, Brain SD: Tail-Cuff Technique and Its Influence on Central Blood Pressure in the Mouse. J Am Heart Assoc 6, 2017.
18. Li Y, Wei FF, Wang S, Cheng YB, Wang JG: Cardiovascular risks associated with diastolic blood pressure and isolated diastolic hypertension. Curr Hypertens Rep 16: 489, 2014.
19. Kurtz TW, Griffin KA, Bidani AK, Davisson RL, Hall JE, Subcommittee of P, Public Education of the American Heart A: Recommendations for blood pressure measurement in

humans and experimental animals. Part 2: Blood pressure measurement in experimental animals: a statement for professionals from the subcommittee of professional and public education of the American Heart Association council on high blood pressure research. Hypertension 45: 299-310, 2005.
20. Sim JJ, Bhandari SK, Shi J, Liu IL, Calhoun DA, McGlynn EA, Kalantar-Zadeh K, Jacobsen SJ: Characteristics of resistant hypertension in a large, ethnically diverse hypertension population of an integrated health system. Mayo Clin Proc 88: 1099-107, 2013.
21. Mancia G, Dell'Oro R, Trevano FQ, Grassi G: Novel Agents in Development, in *Clinical Pharmacology and Therapeutics of Hypertension*, McGinnis GT, Editor. 2008, Elsevier: Amsterdam. p. 391-412.
22. Zandberg P: Animal models in experimental hypertension: relevance to drug testing and discovery, in *Pharmacology of Antihypertensive Drugs*, Van Zwieten PA, Editor. 1984, Elsevier: Amsterdam. p. 6-45.
23. Greek CR, Greek JS: Sacred cows and golden geese : the human cost of experiments on animals. 2000, New York: Continuum. 256 p.
24. Ondetti MA: From peptides to peptidases: a chronicle of drug discovery. Annu Rev Pharmacol Toxicol 34: 1-16, 1994.
25. Pfeffer MA: Janice M. Pfeffer Memorial Lecture. J Card Fail 8: S248-52, 2002.
26. Wood JM, Maibaum J, Rahuel J, Grütter MG, Cohen N-C, Rasetti V, Rüger H, Göschke R, Stutz S, Fuhrer W, Schilling W, Rigollier P, Yamaguchi Y, Cumin F, Baum H-P, Schnell CR, Herold P, Mah R, Jensen C, O'Brien E, Stanton A, Bedigian MP: Structure-based design of aliskiren, a novel orally effective renin inhibitor. Biochemical and Biophysical Research Communications 308: 698-705, 2003.
27. Wood JM, Schnell CR, Cumin F, Menard J, Webb RL: Aliskiren, a novel, orally effective renin inhibitor, lowers blood pressure in marmosets and spontaneously hypertensive rats. J Hypertens 23: 417-26, 2005.
28. Jensen C, Herold P, Brunner HR: Aliskiren: the first renin inhibitor for clinical treatment. Nat Rev Drug Discov 7: 399-410, 2008.
29. Tsui BM, Kraitchman DL: Recent advances in small-animal cardiovascular imaging. J Nucl Med 50: 667-70, 2009.
30. Zambraski EJ, Ciccone CD, Izzo JL, Jr.: The role of the sympathetic nervous system in 2-kidney DOCA-hypertensive Yucatan miniature swine. Clin Exp Hypertens A 8: 411-24, 1986.
31. Ciccone CD, Zambraski EJ: Effects of acute renal denervation on kidney function in deoxycorticosterone acetate-hypertensive swine. Hypertension 8: 925-31, 1986.
32. Olmsted F, Page IH: Hemodynamic Aspects of Prolonged Infusion of Angiotensin into Unanesthetized Dogs. Circ Res 16: 140-9, 1965.
33. McCubbin JW, DeMoura RS, Page IH, Olmsted F: Arterial hypertension elicited by subpressor amounts of angiotensin. Science 149: 1394-5, 1965.
34. Hofstaetter JG, Blouin S, Friehs I, Klaushofer K, Roschger P: No effect of short-term hypertension on bone matrix mineralization in a surgical animal model in immature rabbits. Clin Exp Hypertens 34: 107-12, 2012.
35. Fossum TW, Baltzer WI, Miller MW, Aguirre M, Whitlock D, Solter P, Makarski LA, McDonald MM, An MY, Humphrey JD: A novel aortic coarctation model for studying hypertension in the pig. J Invest Surg 16: 35-44, 2003.
36. Cody RJ, Jr., Rodger RF, Hartley LH, Burton J, Herd JA: Acute hypertension in a nonhuman primate: humoral and hemodynamic mechanisms. Hypertension 4: 219-25, 1982.

37. Goldblatt H, Lynch J, Hanzal RF, Summerville WW: Studies on Experimental Hypertension : I. The Production of Persistent Elevation of Systolic Blood Pressure by Means of Renal Ischemia. J Exp Med 59: 347-79, 1934.
38. Panek RL, Ryan MJ, Weishaar RE, Taylor DG, Jr.: Development of a high renin model of hypertension in the cynomolgus monkey. Clin Exp Hypertens A 13: 1395-414, 1991.
39. Lerman LO, Schwartz RS, Grande JP, Sheedy PF, Romero JC: Noninvasive evaluation of a novel swine model of renal artery stenosis. J Am Soc Nephrol 10: 1455-65, 1999.
40. Chade AR, Rodriguez-Porcel M, Grande JP, Krier JD, Lerman A, Romero JC, Napoli C, Lerman LO: Distinct renal injury in early atherosclerosis and renovascular disease. Circulation 106: 1165-1171, 2002.
41. Rhoads MK, Goleva SB, Beierwaltes WH, Osborn JL: Renal Vascular and Glomerular Pathologies Associated with Spontaneous Hypertension in the Nonhuman Primate Chlorocebus aethiops sabaeus. Am J Physiol Regul Integr Comp Physiol: ajpregu 00026 2017, 2017.
42. Rat Genome Database (http://rgd.mcw.edu/rgdweb/search/strains.html?term=hypertensive+rats&obj=strain). Accessed on 10-18-2017.
43. Festing, MFW: Index of Major Rat Strains (http://www.informatics.jax.org/inbred_strains/rat/STRAINS.shtml). Accessed on 10-18-2017.
44. Doggrell SA, Brown L: Rat models of hypertension, cardiac hypertrophy and failure. Cardiovasc Res 39: 89-105, 1998.
45. Padmanabhan S, Joe B: Towards Precision Medicine for Hypertension: A Review of Genomic, Epigenomic, and Microbiomic Effects on Blood Pressure in Experimental Rat Models and Humans. Physiol Rev 97: 1469-1528, 2017.
46. Rapp JP: Genetic analysis of inherited hypertension in the rat. Physiol Rev 80: 135-72, 2000.
47. Okamoto K, Aoki K: Development of a strain of spontaneously hypertensive rats. Jpn Circ J 27: 282-93, 1963.
48. Kurtz TW, Morris RC, Jr.: Biological variability in Wistar-Kyoto rats. Implications for research with the spontaneously hypertensive rat. Hypertension 10: 127-31, 1987.
49. Okamoto K, Yamoci Y, Nagaoka A: Establishment of the stroke-prone spontaneously hypertensive rat (SHR). Circ Res 34 (Suppl I): I-143-I153, 1974.
50. Nabika T, Ohara H, Kato N, Isomura M: The stroke-prone spontaneously hypertensive rat: still a useful model for post-GWAS genetic studies? Hypertens Res 35: 477-84, 2012.
51. Joe B: Dr Lewis Kitchener Dahl, the Dahl rats, and the "inconvenient truth" about the genetics of hypertension. Hypertension 65: 963-9, 2015.
52. Tschopp TB, Baumgartner HR: Defective platelet adhesion and aggregation on subendothelium exposed in vivo or in vitro to flowing blood of fawn-hooded rats and storage pool disease. Thromb Haemost 38: 620-9, 1977.
53. Provoost A, De Keijzer M: The fawn-hooded rat: a model for chronic renal failure. In: Gretz N, Strauch M, eds. Experimental and genetic rat models of chronic renal failure. Basel: Karger: 100-114, 1993.
54. Bianchi G, Fox U, Imbasciati E: The development of a new strain of spontaneously hypertensive rats. Life Sci 14: 339-47, 1974.
55. Bianchi G, Fox U, Di Francesco GF, Giovanetti AM, Pagetti D: Blood pressure changes produced by kidney cross-transplantation between spontaneously hypertensive rats and normotensive rats. Clin Sci Mol Med 47: 435-48, 1974.

56. Ge Y, Fan F, Didion SP, Roman RJ: Impaired myogenic response of the afferent arteriole contributes to the increased susceptibility to renal disease in Milan normotensive rats. Physiol Rep 5, 2017.
57. Dupont J, Dupont JC, Froment A, Milon H, Vincent M: Selection of three strains of rats with spontaneously different levels of blood pressure. Biomedicine 19: 36-41, 1973.
58. Vincent M, Dupont J, Sassard J: Plasma renin activity as a function of age in two new strains of spontaneously hypertensive and normotensive rats. Clin Sci Mol Med 50: 103-7, 1976.
59. Ma MCJ, Pettus JM, Jakoubek JA, Traxler MG, Clark KC, Mennie AK, Kwitek AE: Contribution of independent and pleiotropic genetic effects in the metabolic syndrome in a hypertensive rat. PLoS One 12: e0182650, 2017.
60. Ben-Ishay D, Zamir N, Feurstein G, Kobrin I, Le Quan-Bui KH, Devynck MA: Distinguishing traits in the Sabra hypertension-prone (SBH) and hypertension-resistant (SBN) rats. Clin Exp Hypertens 3: 737-47, 1981.
61. Ben-Ishay D, Mekler J, Saliternick-Vardi R: Sabra hypertension-prone and hypertension-resistant rats. Hypertension 9 [Suppl I]: I-24-I-26.
62. Yagil C, Katni G, Rubattu S, Stolpe C, Kreutz R, Lindpaintner K, Ganten D, Ben-Ishay D, Yagil Y: Development, genotype and phenotype of a new colony of the Sabra hypertension prone (SBH/y) and resistant (SBN/y) rat model of salt sensitivity and resistance. J Hypertens 14: 1175-82, 1996.
63. Cowley AW, Jr.: The genetic dissection of essential hypertension. Nat Rev Genet 7: 829-40, 2006.
64. Stoll M, Jacob HJ: Genetic rat models of hypertension: relationship to human hypertension. Curr Hypertens Rep 3: 157-64, 2001.
65. Geurts AM, Cost GJ, Freyvert Y, Zeitler B, Miller JC, Choi VM, Jenkins SS, Wood A, Cui X, Meng X, Vincent A, Lam S, Michalkiewicz M, Schilling R, Foeckler J, Kalloway S, Weiler H, Menoret S, Anegon I, Davis GD, Zhang L, Rebar EJ, Gregory PD, Urnov FD, Jacob HJ, Buelow R: Knockout rats via embryo microinjection of zinc-finger nucleases. Science 325: 433, 2009.
66. Rudemiller NP, Mattson DL: Candidate genes for hypertension: insights from the Dahl S rat. Am J Physiol Renal Physiol 309: F993-5, 2015.
67. Pravenec M, Kren V, Landa V, Mlejnek P, Musilova A, Silhavy J, Simakova M, Zidek V: Recent progress in the genetics of spontaneously hypertensive rats. Physiol Res 63 Suppl 1: S1-8, 2014.
68. Doris PA: Genetics of hypertension: an assessment of progress in the spontaneously hypertensive rat. Physiol Genomics 49: 601-617, 2017.
69. Bianchi G, Ferrari P, Barber B: The Milan hypertensive strain. in Handbook of HYpertension, ed de Jong W (Elsevier, Amsterdam. 328-349, 1984.
70. Cusi D, Alberghini E, Pati P, Tripodi G, Barlassina C, Colombo R, Cova T, Niutta E, Vezzoli G, Bianchi G: Pathogenetic mechanisms in essential hypertension. Analogies between a rat model and the human disease. Int J Cardiol 25 Suppl 1: S29-36, 1989.
71. Mattson DL, Meister CJ, Marcelle ML: Dietary protein source determines the degree of hypertension and renal disease in the Dahl salt-sensitive rat. Hypertension 45: 736-41, 2005.
72. Kwitek AE, Tonellato PJ, Chen D, Gullings-Handley J, Cheng YS, Twigger S, Scheetz TE, Casavant TL, Stoll M, Nobrega MA, Shiozawa M, Soares MB, Sheffield VC, Jacob HJ: Automated construction of high-density comparative maps between rat, human, and mouse. Genome Res 11: 1935-43, 2001.

73. Kohara K, Tabara Y, Nakura J, Imai Y, Ohkubo T, Hata A, Soma M, Nakayama T, Umemura S, Hirawa N, Ueshima H, Kita Y, Ogihara T, Katsuya T, Takahashi N, Tokunaga K, Miki T: Identification of hypertension-susceptibility genes and pathways by a systemic multiple candidate gene approach: the millennium genome project for hypertension. Hypertens Res 31: 203-12, 2008.
74. Gordon JW, Scangos GA, Plotkin DJ, Barbosa JA, Ruddle FH: Genetic transformation of mouse embryos by microinjection of purified DNA. Proc Natl Acad Sci U S A 77: 7380-4, 1980.
75. Palmiter RD, Brinster RL: Transgenic mice. Cell 41: 343-5, 1985.
76. Zhang K, Mir SA, Hightower CM, Miramontes-Gonzalez JP, Maihofer AX, Chen Y, Mahata SK, Nievergelt CM, Schork NJ, Freedman BI, Vaingankar SM, O'Connor DT: Molecular Mechanism for Hypertensive Renal Disease: Differential Regulation of Chromogranin A Expression at 3'-Untranslated Region Polymorphism C+87T by MicroRNA-107. J Am Soc Nephrol 26: 1816-25, 2015.
77. Mir SA, Zhang K, Milic M, Gu Y, Rieg T, Ziegler M, Vaingankar SM: Analysis and validation of traits associated with a single nucleotide polymorphism Gly364Ser in catestatin using humanized chromogranin A mouse models. J Hypertens 34: 68-78, 2016.
78. Hasegawa Y, Daitoku Y, Sekiguchi K, Tanimoto Y, Mizuno-Iijima S, Mizuno S, Kajiwara N, Ema M, Miwa Y, Mekada K, Yoshiki A, Takahashi S, Sugiyama F, Yagami K: Novel ROSA26 Cre-reporter knock-in C57BL/6N mice exhibiting green emission before and red emission after Cre-mediated recombination. Exp Anim 62: 295-304, 2013.
79. Yang D, Song J, Zhang J, Xu J, Zhu T, Wang Z, Lai L, Chen YE: Identification and characterization of rabbit ROSA26 for gene knock-in and stable reporter gene expression. Sci Rep 6: 25161, 2016.
80. Buehr M, Meek S, Blair K, Yang J, Ure J, Silva J, McLay R, Hall J, Ying QL, Smith A: Capture of authentic embryonic stem cells from rat blastocysts. Cell 135: 1287-98, 2008.
81. Li P, Tong C, Mehrian-Shai R, Jia L, Wu N, Yan Y, Maxson RE, Schulze EN, Song H, Hsieh CL, Pera MF, Ying QL: Germline competent embryonic stem cells derived from rat blastocysts. Cell 135: 1299-310, 2008.
82. Ji D, Zhao G, Songstad A, Cui X, Weinstein EJ: Efficient creation of an APOE knockout rabbit. Transgenic Res 24: 227-35, 2015.
83. Mullins LJ, Kenyon CJ, Bailey MA, Conway BR, Diaz ME, Mullins JJ: Mineralocorticoid Excess or Glucocorticoid Insufficiency: Renal and Metabolic Phenotypes in a Rat Hsd11b2 Knockout Model. Hypertension 66: 667-73, 2015.
84. Geurts AM, Moreno C: Zinc-finger nucleases: new strategies to target the rat genome. Clin Sci (Lond) 119: 303-11, 2010.
85. Jacob HJ, Lazar J, Dwinell MR, Moreno C, Geurts AM: Gene targeting in the rat: advances and opportunities. Trends Genet 26: 510-8, 2010.
86. Flister MJ, Tsaih SW, O'Meara CC, Endres B, Hoffman MJ, Geurts AM, Dwinell MR, Lazar J, Jacob HJ, Moreno C: Identifying multiple causative genes at a single GWAS locus. Genome Res 23: 1996-2002, 2013.
87. Wang X, Yamada S, LaRiviere WB, Ye H, Bakeberg JL, Irazabal MV, Chebib FT, van Deursen J, Harris PC, Sussman CR, Behfar A, Ward CJ, Torres VE: Generation and phenotypic characterization of Pde1a mutant mice. PLoS One 12: e0181087, 2017.
88. Cheng X, Waghulde H, Mell B, Morgan EE, Pruett-Miller SM, Joe B: Positional cloning of quantitative trait nucleotides for blood pressure and cardiac QT-interval by targeted CRISPR/Cas9 editing of a novel long non-coding RNA. PLoS Genet 13: e1006961, 2017.

89. Kleinstiver BP, Pattanayak V, Prew MS, Tsai SQ, Nguyen NT, Zheng Z, Joung JK: High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. Nature 529: 490-5, 2016.
90. Slaymaker IM, Gao L, Zetsche B, Scott DA, Yan WX, Zhang F: Rationally engineered Cas9 nucleases with improved specificity. Science 351: 84-8, 2016.
91. Kleinstiver BP, Prew MS, Tsai SQ, Nguyen NT, Topkar VV, Zheng Z, Joung JK: Broadening the targeting range of Staphylococcus aureus CRISPR-Cas9 by modifying PAM recognition. Nat Biotechnol 33: 1293-1298, 2015.
92. Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, Volz SE, Joung J, van der Oost J, Regev A, Koonin EV, Zhang F: Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell 163: 759-71, 2015.
93. Yoshimi K, Kaneko T, Voigt B, Mashimo T: Allele-specific genome editing and correction of disease-associated phenotypes in rats using the CRISPR-Cas platform. Nat Commun 5: 4240, 2014.
94. Yoshimi K, Kunihiro Y, Kaneko T, Nagahora H, Voigt B, Mashimo T: ssODN-mediated knock-in with CRISPR-Cas for large genomic regions in zygotes. Nat Commun 7: 10431, 2016.
95. Liu Y, Taylor NE, Lu L, Usa K, Cowley AW, Jr., Ferreri NR, Yeo NC, Liang M: Renal medullary microRNAs in Dahl salt-sensitive rats: miR-29b regulates several collagens and related genes. Hypertension 55: 974-82, 2010.
96. Kriegel AJ, Mladinov D, Liang M: Translational study of microRNAs and its application in kidney disease and hypertension research. Clin Sci (Lond) 122: 439-47, 2012.
97. Ivanova A, Signore M, Caro N, Greene ND, Copp AJ, Martinez-Barbera JP: In vivo genetic ablation by Cre-mediated expression of diphtheria toxin fragment A. Genesis 43: 129-35, 2005.
98. Teh C, Korzh V: In vivo optogenetics for light-induced oxidative stress in transgenic zebrafish expressing the KillerRed photosensitizer protein. Methods Mol Biol 1148: 229-38, 2014.
99. Buckley C, Carvalho MT, Young LK, Rider SA, McFadden C, Berlage C, Verdon RF, Taylor JM, Girkin JM, Mullins JJ: Precise spatio-temporal control of rapid optogenetic cell ablation with mem-KillerRed in Zebrafish. Sci Rep 7: 5096, 2017.
100. Wain LV, Verwoert GC, O'Reilly PF, Shi G, Johnson T, Johnson AD, Bochud M, Rice KM, Henneman P, Smith AV, Ehret GB, Amin N, Larson MG, Mooser V, Hadley D, Dorr M, Bis JC, Aspelund T, Esko T, Janssens AC, Zhao JH, Heath S, Laan M, Fu J, Pistis G, Luan J, Arora P, Lucas G, Pirastu N, Pichler I, Jackson AU, Webster RJ, Zhang F, Peden JF, Schmidt H, Tanaka T, Campbell H, Igl W, Milaneschi Y, Hottenga JJ, Vitart V, Chasman DI, Trompet S, Bragg-Gresham JL, Alizadeh BZ, Chambers JC, Guo X, Lehtimaki T, Kuhnel B, Lopez LM, Polasek O, Boban M, Nelson CP, Morrison AC, Pihur V, Ganesh SK, Hofman A, Kundu S, Mattace-Raso FU, Rivadeneira F, Sijbrands EJ, Uitterlinden AG, Hwang SJ, Vasan RS, Wang TJ, Bergmann S, Vollenweider P, Waeber G, Laitinen J, Pouta A, Zitting P, McArdle WL, Kroemer HK, Volker U, Volzke H, Glazer NL, Taylor KD, Harris TB, Alavere H, Haller T, Keis A, Tammesoo ML, Aulchenko Y, Barroso I, Khaw KT, Galan P, Hercberg S, Lathrop M, Eyheramendy S, Org E, Sober S, Lu X, Nolte IM, Penninx BW, Corre T, Masciullo C, Sala C, Groop L, Voight BF, Melander O, O'Donnell CJ, Salomaa V, d'Adamo AP, Fabretto A, Faletra F, Ulivi S, Del Greco F, Facheris M, Collins FS, Bergman RN, Beilby JP, Hung J, Musk AW, Mangino M, Shin SY, Soranzo N, Watkins H, Goel A, Hamsten A, Gider P, Loitfelder M, Zeginigg M, Hernandez D, Najjar SS, Navarro P, Wild SH, Corsi AM, Singleton A, de Geus EJ, Willemsen G, Parker AN, Rose LM, Buckley B, Stott D, Orru M, Uda M, LifeLines Cohort S,

van der Klauw MM, Zhang W, Li X, Scott J, Chen YD, Burke GL, Kahonen M, Viikari J, Doring A, Meitinger T, Davies G, Starr JM, Emilsson V, Plump A, Lindeman JH, Hoen PA, Konig IR, EchoGen c, Felix JF, Clarke R, Hopewell JC, Ongen H, Breteler M, Debette S, Destefano AL, Fornage M, AortaGen C, Mitchell GF, Group CCHFW, Smith NL, KidneyGen c, Holm H, Stefansson K, Thorleifsson G, Thorsteinsdottir U, consortium CK, Cardiogenics c, CardioGram, Samani NJ, Preuss M, Rudan I, Hayward C, Deary IJ, Wichmann HE, Raitakari OT, Palmas W, Kooner JS, Stolk RP, Jukema JW, Wright AF, Boomsma DI, Bandinelli S, Gyllensten UB, Wilson JF, Ferrucci L, Schmidt R, Farrall M, Spector TD, Palmer LJ, Tuomilehto J, Pfeufer A, Gasparini P, Siscovick D, Altshuler D, Loos RJ, Toniolo D, Snieder H, Gieger C, Meneton P, Wareham NJ, Oostra BA, Metspalu A, Launer L, Rettig R, Strachan DP, Beckmann JS, Witteman JC, Erdmann J, van Dijk KW, Boerwinkle E, Boehnke M, Ridker PM, Jarvelin MR, Chakravarti A, Abecasis GR, Gudnason V, Newton-Cheh C, Levy D, Munroe PB, Psaty BM, Caulfield MJ, Rao DC, Tobin MD, Elliott P, van Duijn CM: Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure. Nat Genet 43: 1005-11, 2011.

101. Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, Najjar SS, Zhao JH, Heath SC, Eyheramendy S, Papadakis K, Voight BF, Scott LJ, Zhang F, Farrall M, Tanaka T, Wallace C, Chambers JC, Khaw KT, Nilsson P, van der Harst P, Polidoro S, Grobbee DE, Onland-Moret NC, Bots ML, Wain LV, Elliott KS, Teumer A, Luan J, Lucas G, Kuusisto J, Burton PR, Hadley D, McArdle WL, Wellcome Trust Case Control C, Brown M, Dominiczak A, Newhouse SJ, Samani NJ, Webster J, Zeggini E, Beckmann JS, Bergmann S, Lim N, Song K, Vollenweider P, Waeber G, Waterworth DM, Yuan X, Groop L, Orho-Melander M, Allione A, Di Gregorio A, Guarrera S, Panico S, Ricceri F, Romanazzi V, Sacerdote C, Vineis P, Barroso I, Sandhu MS, Luben RN, Crawford GJ, Jousilahti P, Perola M, Boehnke M, Bonnycastle LL, Collins FS, Jackson AU, Mohlke KL, Stringham HM, Valle TT, Willer CJ, Bergman RN, Morken MA, Doring A, Gieger C, Illig T, Meitinger T, Org E, Pfeufer A, Wichmann HE, Kathiresan S, Marrugat J, O'Donnell CJ, Schwartz SM, Siscovick DS, Subirana I, Freimer NB, Hartikainen AL, McCarthy MI, O'Reilly PF, Peltonen L, Pouta A, de Jong PE, Snieder H, van Gilst WH, Clarke R, Goel A, Hamsten A, Peden JF, Seedorf U, Syvanen AC, Tognoni G, Lakatta EG, Sanna S, Scheet P, Schlessinger D, Scuteri A, Dorr M, Ernst F, Felix SB, Homuth G, Lorbeer R, Reffelmann T, Rettig R, Volker U, Galan P, Gut IG, Hercberg S, Lathrop GM, Zelenika D, Deloukas P, Soranzo N, Williams FM, Zhai G, Salomaa V, Laakso M, Elosua R, Forouhi NG, Volzke H, Uiterwaal CS, van der Schouw YT, Numans ME, Matullo G, Navis G, Berglund G, Bingham SA, Kooner JS, Connell JM, Bandinelli S, Ferrucci L, Watkins H, Spector TD, Tuomilehto J, Altshuler D, Strachan DP, Laan M, Meneton P, Wareham NJ, Uda M, Jarvelin MR, Mooser V, Melander O, Loos RJ, Elliott P, Abecasis GR, Caulfield M, Munroe PB: Genome-wide association study identifies eight loci associated with blood pressure. Nat Genet 41: 666-76, 2009.

102. Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, Glazer NL, Morrison AC, Johnson AD, Aspelund T, Aulchenko Y, Lumley T, Kottgen A, Vasan RS, Rivadeneira F, Eiriksdottir G, Guo X, Arking DE, Mitchell GF, Mattace-Raso FU, Smith AV, Taylor K, Scharpf RB, Hwang SJ, Sijbrands EJ, Bis J, Harris TB, Ganesh SK, O'Donnell CJ, Hofman A, Rotter JI, Coresh J, Benjamin EJ, Uitterlinden AG, Heiss G, Fox CS, Witteman JC, Boerwinkle E, Wang TJ, Gudnason V, Larson MG, Chakravarti A, Psaty BM, van Duijn CM: Genome-wide association study of blood pressure and hypertension. Nat Genet 41: 677-87, 2009.

103. Gupta RM, Hadaya J, Trehan A, Zekavat SM, Roselli C, Klarin D, Emdin CA, Hilvering CRE, Bianchi V, Mueller C, Khera AV, Ryan RJH, Engreitz JM, Issner R, Shoresh N, Epstein CB, de Laat W, Brown JD, Schnabel RB, Bernstein BE, Kathiresan S: A Genetic Variant Associated with Five Vascular Diseases Is a Distal Regulator of Endothelin-1 Gene Expression. Cell 170: 522-533 e15, 2017.
104. Ji W, Foo JN, O'Roak BJ, Zhao H, Larson MG, Simon DB, Newton-Cheh C, State MW, Levy D, Lifton RP: Rare independent mutations in renal salt handling genes contribute to blood pressure variation. Nat Genet 40: 592-599, 2008.
105. Takahashi N, Chernavvsky DR, Gomez RA, Igarashi P, Gitelman HJ, Smithies O: Uncompensated polyuria in a mouse model of Bartter's syndrome. Proc Natl Acad Sci U S A 97: 5434-9, 2000.
106. Zhou X, Zhang Z, Shin MK, Horwitz SB, Levorse JM, Zhu L, Sharif-Rodriguez W, Streltsov DY, Dajee M, Hernandez M, Pan Y, Urosevic-Price O, Wang L, Forrest G, Szeto D, Zhu Y, Cui Y, Michael B, Balogh LA, Welling PA, Wade JB, Roy S, Sullivan KA: Heterozygous disruption of renal outer medullary potassium channel in rats is associated with reduced blood pressure. Hypertension 62: 288-94, 2013.
107. Schultheis PJ, Lorenz JN, Meneton P, Nieman ML, Riddle TM, Flagella M, Duffy JJ, Doetschman T, Miller ML, Shull GE: Phenotype resembling Gitelman's syndrome in mice lacking the apical Na+-Cl- cotransporter of the distal convoluted tubule. J Biol Chem 273: 29150-5, 1998.
108. Ronzaud C, Loffing-Cueni D, Hausel P, Debonneville A, Malsure SR, Fowler-Jaeger N, Boase NA, Perrier R, Maillard M, Yang B, Stokes JB, Koesters R, Kumar S, Hummler E, Loffing J, Staub O: Renal tubular NEDD4-2 deficiency causes NCC-mediated salt-dependent hypertension. J Clin Invest 123: 657-65, 2013.
109. Chowdhury JA, Liu CH, Zuber AM, O'Shaughnessy KM: An inducible transgenic mouse model for familial hypertension with hyperkalaemia (Gordon's syndrome or pseudohypoaldosteronism type II). Clin Sci (Lond) 124: 701-8, 2013.
110. Bailey MA, Craigie E, Livingstone DEW, Kotelevtsev YV, Al-Dujaili EAS, Kenyon CJ, Mullins JJ: Hsd11b2 haploinsufficiency in mice causes salt sensitivity of blood pressure. Hypertension 57: 515-520, 2011.
111. Ueda K, Nishimoto M, Hirohama D, Ayuzawa N, Kawarazaki W, Watanabe A, Shimosawa T, Loffing J, Zhang MZ, Marumo T, Fujita T: Renal Dysfunction Induced by Kidney-Specific Gene Deletion of Hsd11b2 as a Primary Cause of Salt-Dependent Hypertension. Hypertension 70: 111-118, 2017.
112. Kotelevtsev Y, Brown RW, Fleming S, Kenyon C, Edwards CR, Seckl JR, Mullins JJ: Hypertension in mice lacking 11beta-hydroxysteroid dehydrogenase type 2. J Clin Invest 103: 683-9, 1999.
113. Clark AF, Sharp MG, Morley SD, Fleming S, Peters J, Mullins JJ: Renin-1 is essential for normal renal juxtaglomerular cell granulation and macula densa morphology. J Biol Chem 272: 18185-90, 1997.
114. Sharp MG, Fettes D, Brooker G, Clark AF, Peters J, Fleming S, Mullins JJ: Targeted inactivation of the Ren-2 gene in mice. Hypertension 28: 1126-31, 1996.
115. Moreno C, Hoffman M, Stodola TJ, Didier DN, Lazar J, Geurts AM, North PE, Jacob HJ, Greene AS: Creation and characterization of a renin knockout rat. Hypertension 57: 614-9, 2011.
116. Mullins JJ, Peters J, Ganten D: Fulminant hypertension in transgenic rats harbouring the mouse Ren-2 gene. Nature 344: 541-4, 1990.

117. Liu X, Bellamy CO, Bailey MA, Mullins LJ, Dunbar DR, Kenyon CJ, Brooker G, Kantachuvesiri S, Maratou K, Ashek A, Clark AF, Fleming S, Mullins JJ: Angiotensin-converting enzyme is a modifier of hypertensive end organ damage. J Biol Chem 284: 15564-72, 2009.
118. Kantachuvesiri S, Fleming S, Peters J, Peters B, Brooker G, Lammie AG, McGrath I, Kotelevtsev Y, Mullins JJ: Controlled hypertension, a transgenic toggle switch reveals differential mechanisms underlying vascular disease. J Biol Chem 276: 36727-33, 2001.
119. Gurley SB, Riquier-Brison ADM, Schnermann J, Sparks MA, Allen AM, Haase VH, Snouwaert JN, Le TH, McDonough AA, Koller BH, Coffman TM: AT1A angiotensin receptors in the renal proximal tubule regulate blood pressure. Cell Metab 13: 469-475, 2011.
120. Li H, Weatherford ET, Davis DR, Keen HL, Grobe JL, Daugherty A, Cassis LA, Allen AM, Sigmund CD: Renal proximal tubule angiotensin AT1A receptors regulate blood pressure. Am J Physiol Regul Integr Comp Physiol 301: R1067-77, 2011.
121. Gembardt F, Heringer-Walther S, van Esch JH, Sterner-Kock A, van Veghel R, Le TH, Garrelds IM, Coffman TM, Danser AH, Schultheiss HP, Walther T: Cardiovascular phenotype of mice lacking all three subtypes of angiotensin II receptors. FASEB J 22: 3068-77, 2008.
122. Ganten D, Wagner J, Zeh K, Bader M, Michel JB, Paul M, Zimmermann F, Ruf P, Hilgenfeldt U, Ganten U, et al.: Species specificity of renin kinetics in transgenic rats harboring the human renin and angiotensinogen genes. Proc Natl Acad Sci U S A 89: 7806-10, 1992.
123. Fukamizu A, Sugimura K, Takimoto E, Sugiyama F, Seo MS, Takahashi S, Hatae T, Kajiwara N, Yagami K, Murakami K: Chimeric renin-angiotensin system demonstrates sustained increase in blood pressure of transgenic mice carrying both human renin and human angiotensinogen genes. J Biol Chem 268: 11617-21, 1993.
124. Ahn D, Ge Y, Stricklett PK, Gill P, Taylor D, Hughes AK, Yanagisawa M, Miller L, Nelson RD, Kohan DE: Collecting duct-specific knockout of endothelin-1 causes hypertension and sodium retention. J Clin Invest 114: 504-11, 2004.
125. Stuart D, Chapman M, Rees S, Woodward S, Kohan DE: Myocardial, smooth muscle, nephron, and collecting duct gene targeting reveals the organ sites of endothelin A receptor antagonist fluid retention. J Pharmacol Exp Ther 346: 182-9, 2013.
126. Fan ZD, Zhang L, Shi Z, Gan XB, Gao XY, Zhu GQ: Artificial microRNA interference targeting AT(1a) receptors in paraventricular nucleus attenuates hypertension in rats. Gene Ther 19: 810-7, 2012.
127. Xue B, Beltz TG, Johnson RF, Guo F, Hay M, Johnson AK: PVN adenovirus-siRNA injections silencing either NOX2 or NOX4 attenuate aldosterone/NaCl-induced hypertension in mice. Am J Physiol Heart Circ Physiol 302: H733-41, 2012.
128. Cowley AW, Jr., Yang C, Zheleznova NN, Staruschenko A, Kurth T, Rein L, Kumar V, Sadovnikov K, Dayton A, Hoffman M, Ryan RP, Skelton MM, Salehpour F, Ranji M, Geurts A: Evidence of the Importance of Nox4 in Production of Hypertension in Dahl Salt-Sensitive Rats. Hypertension 67: 440-50, 2016.
129. Feng D, Yang C, Geurts AM, Kurth T, Liang M, Lazar J, Mattson DL, O'Connor PM, Cowley AW, Jr.: Increased expression of NAD(P)H oxidase subunit p67(phox) in the renal medulla contributes to excess oxidative stress and salt-sensitive hypertension. Cell Metab 15: 201-8, 2012.
130. Elijovich F, Weinberger MH, Anderson CA, Appel LJ, Bursztyn M, Cook NR, Dart RA, Newton-Cheh CH, Sacks FM, Laffer CL, American Heart Association P, Public Education Committee of the Council on H, Council on Functional G, Translational B, Stroke C: Salt

Sensitivity of Blood Pressure: A Scientific Statement From the American Heart Association. Hypertension 68: e7-e46, 2016.
131. Aitman TJ, Critser JK, Cuppen E, Dominiczak A, Fernandez-Suarez XM, Flint J, Gauguier D, Geurts AM, Gould M, Harris PC, Holmdahl R, Hubner N, Izsvak Z, Jacob HJ, Kuramoto T, Kwitek AE, Marrone A, Mashimo T, Moreno C, Mullins J, Mullins L, Olsson T, Pravenec M, Riley L, Saar K, Serikawa T, Shull JD, Szpirer C, Twigger SN, Voigt B, Worley K: Progress and prospects in rat genetics: a community view. Nat Genet 40: 516-22, 2008.
132. Liu Z, Liu F: Cautious use of fli1a:EGFP transgenic zebrafish in vascular research. Biochem Biophys Res Commun 427: 223-6, 2012.
133. Bianchi BR, Zhang XF, Reilly RM, Kym PR, Yao BB, Chen J: Species comparison and pharmacological characterization of human, monkey, rat, and mouse TRPA1 channels. J Pharmacol Exp Ther 341: 360-8, 2012.
134. Conway BRR, J.; Bailey, M.A.; Dunbar, D.R.; Manning, J.R.; Bellamy, C.O.; Hughes, J.; Mullins, J.J.: Hyperglycemia and renin-dependent hypertension synergize to model diabetic nephropathy. J Am Soc Nephrol. 23: 405-411, 2012.
135. Kotb AM, Muller T, Xie J, Anand-Apte B, Endlich K, Endlich N: Simultaneous assessment of glomerular filtration and barrier function in live zebrafish. Am J Physiol Renal Physiol 307: F1427-34, 2014.
136. Huang J, McKee M, Huang HD, Xiang A, Davidson AJ, Lu HA: A zebrafish model of conditional targeted podocyte ablation and regeneration. Kidney Int 83: 1193-200, 2013.
137. Schlager G: Spontaneous Hypertension in Laboratory Animals. A review of the genetic implications. . J Hered 63: 35-38, 1972.
138. Alexander N, L.B. Hinshaw, D.R. Drury: Further observations on development of a colony of spontaneously hypertensive rabbits. Proc. Soc. Exptl. Med. 92: 249-253, 1956.
139. Li D, Wang Q, Zhang Y, Li D, Yang D, Wei S, Su L, Ye T, Zheng X, Peng K, Zhang L, Zhang Y, Yang Y, Ma S: A Novel Swine Model of Spontaneous Hypertension With Sympathetic Hyperactivity Responds Well to Renal Denervation. Am J Hypertens 29: 63-72, 2016.
140. Frederick E. Tippett GAP, George Eyster, Gary Blanchard,, Bell aT: Primary hypertension in a colony of dogs. Hypertension 9: 49-58, 1987.
141. Rhoads MK, Goleva SB, Beierwaltes WH, Osborn JL: Renal vascular and glomerular pathologies associated with spontaneous hypertension in the nonhuman primate Chlorocebus aethiops sabaeus. Am J Physiol Regul Integr Comp Physiol 313: R211-R218, 2017.
142. Littman MP RJ, Bovée KC: Spontaneous systemic hypertension in dogs: five cases (1981-1983). J Am Vet Med Assoc. 193: 486-94, 1988.
143. Osborn JaJF: Renal Nerves and Long-Term Control of Arterial Pressure. Compr Physiol 7: 263-320, 2017.
144. Martin S. PR, Goldwater R, Gutkowsa J, Hughes C, Hamet P. Ervin FR: The response of hypertensive and normotensive male vervets (Cercopithecus aethiops) to cold pressor stress, captopril administration and acute bolus of atrial natriuretic factor. Am J Hypertens 3: 27-32, 1990.
145. Harding J: Genomic tools for the use of nonhuman primates in translational research. ILAR J 58: 59-68, 2017.
146. Page IH: A Method for Producing Persistent Hypertension by Cellophane. Science 89: 273-4, 1939.
147. DeForrest JM, Scalese RJ, Oehl RS, Waldron TL, Mitch S, Brittain RJ, Free CA, Asaad M, Burkett D: Perinephritis hypertension in Macaca fascicularis (cynomolgus monkey): studies of

the renin-angiotensin-aldosterone axis and renal hemodynamic function. J Hypertens 7: 763-7, 1989.
148. Misra S, Gordon JD, Fu AA, Glockner JF, Chade AR, Mandrekar J, Lerman L, Mukhopadhyay D: The porcine remnant kidney model of chronic renal insufficiency. J Surg Res 135: 370-9, 2006.
149. Carlstrom M, Wahlin N, Skott O, Persson AE: Relief of chronic partial ureteral obstruction attenuates salt-sensitive hypertension in rats. Acta Physiol (Oxf) 189: 67-75, 2007.
150. Carlstrom M, Wahlin N, Sallstrom J, Skott O, Brown R, Persson AE: Hydronephrosis causes salt-sensitive hypertension in rats. J Hypertens 24: 1437-43, 2006.
151. Hall JE: Pathophysiology of obesity hypertension. Curr Hypertens Rep 2: 139-47, 2000.
152. Lerman LO, Chade AR, Sica V, Napoli C: Animal models of hypertension: an overview. J Lab Clin Med 146: 160-173, 2005.
153. Chade AR, Tullos N, Stewart NJ, Surles B: Endothelin-a receptor antagonism after renal angioplasty enhances renal recovery in renovascular disease. J Am Soc Nephrol 26: 1071-80, 2015.
154. Urbieta-Caceres VH, Zhu XY, Gibson ME, Favreau FD, Jordan K, Lerman A, Lerman LO: Reversal of experimental renovascular hypertension restores coronary microvascular function and architecture. Am J Hypertens 24: 458-65, 2011.
155. Chade AR, Kelsen S: Renal microvascular disease determines the responses to revascularization in experimental renovascular disease. Circ Cardiovasc Interv 3: 376-83, 2010.
156. Chelko SP, Schmiedt CW, Lewis TH, Lewis SJ, Robertson TP: A novel vascular clip design for the reliable induction of 2-kidney, 1-clip hypertension in the rat. J Appl Physiol (1985) 112: 362-6, 2012.
157. Huang WC, Tsai RY, Fang TC: Nitric oxide modulates the development and surgical reversal of renovascular hypertension in rats. J Hypertens 18: 601-13, 2000.
158. Hollander W, Prusty S, Kemper T, Rosene DL, Moss MB: The effects of hypertension on cerebral atherosclerosis in the cynomolgus monkey. Stroke 24: 1218-26; discussion 1226-7, 1993.
159. Xu CP, Glagov S, Zatina MA, Zarins CK: Hypertension sustains plaque progression despite reduction of hypercholesterolemia. Hypertension 18: 123-9, 1991.
160. Crowley SD, Gurley SB, Herrera MJ, Ruiz P, Griffiths R, Kumar AP, Kim HS, Smithies O, Le TH, Coffman TM: Angiotensin II causes hypertension and cardiac hypertrophy through its receptors in the kidney. Proc Natl Acad Sci U S A 103: 17985-90, 2006.
161. Guzik TJ, Hoch NE, Brown KA, McCann LA, Rahman A, Dikalov S, Goronzy J, Weyand C, Harrison DG: Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. J Exp Med 204: 2449-60, 2007.
162. Crowley SD, Frey CW, Gould SK, Griffiths R, Ruiz P, Burchette JL, Howell DN, Makhanova N, Yan M, Kim HS, Tharaux PL, Coffman TM: Stimulation of lymphocyte responses by angiotensin II promotes kidney injury in hypertension. Am J Physiol Renal Physiol 295: F515-24, 2008.
163. Crowley SD, Zhang J, Herrera M, Griffiths R, Ruiz P, Coffman TM: Role of AT1 receptor-mediated salt retention in angiotensin II-dependent hypertension. American Journal of Physiology - Renal Physiology 301: F1124-F1130, 2011.
164. Polichnowski AJ, Griffin KA, Picken MM, Licea-Vargas H, Long J, Williamson GA, Bidani AK: Hemodynamic basis for the limited renal injury in rats with angiotensin II-induced hypertension. Am J Physiol Renal Physiol 308: F252-60, 2015.

165. Olsen ME, Hall JE, Montani JP, Guyton AC, Langford HG, Cornell JE: Mechanisms of angiotensin II natriuresis and antinatriuresis. Am J Physiol 249: F299-307, 1985.
166. Cowley AW, Miller JP, Guyton AC: Open‐Loop Analysis of the Renin‐Angiotensin System in the Dog. Circulation Research 28: 568-581, 1971.
167. Hartner A, Cordasic N, Klanke B, Veelken R, Hilgers KF: Strain differences in the development of hypertension and glomerular lesions induced by deoxycorticosterone acetate salt in mice. Nephrol Dial Transplant 18: 1999-2004, 2003.
168. Sparks MA, Stegbauer J, Chen D, Gomez JA, Griffiths RC, Azad HA, Herrera M, Gurley SB, Coffman TM: Vascular Type 1A Angiotensin II Receptors Control BP by Regulating Renal Blood Flow and Urinary Sodium Excretion. J Am Soc Nephrol 26: 2953-62, 2015.
169. Gurley SB, Riquier-Brison AD, Schnermann J, Sparks MA, Allen AM, Haase VH, Snouwaert JN, Le TH, McDonough AA, Koller BH, Coffman TM: AT1A angiotensin receptors in the renal proximal tubule regulate blood pressure. Cell Metab 13: 469-75, 2011.
170. Lautrette A, Li S, Alili R, Sunnarborg SW, Burtin M, Lee DC, Friedlander G, Terzi F: Angiotensin II and EGF receptor cross-talk in chronic kidney diseases: a new therapeutic approach. Nat Med 11: 867-74, 2005.
171. Kawada N, Imai E, Karber A, Welch WJ, Wilcox CS: A mouse model of angiotensin II slow pressor response: role of oxidative stress. J Am Soc Nephrol 13: 2860-8, 2002.
172. Sopel MJ, Rosin NL, Lee TDG, Legare J-F: Myocardial fibrosis in response to Angiotensin II is preceded by the recruitment of mesenchymal progenitor cells. Lab Invest 91: 565-578, 2011.
173. 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 272: F515-20, 1997.
174. Zhang J, Rudemiller NP, Patel MB, Karlovich NS, Wu M, McDonough AA, Griffiths R, Sparks MA, Jeffs AD, Crowley SD: Interleukin-1 Receptor Activation Potentiates Salt Reabsorption in Angiotensin II-Induced Hypertension via the NKCC2 Co-transporter in the Nephron. Cell Metab 23: 360-8, 2016.
175. Zhang JD, Patel MB, Song YS, Griffiths R, Burchette J, Ruiz P, Sparks MA, Yan M, Howell DN, Gomez JA, Spurney RF, Coffman TM, Crowley SD: A novel role for type 1 angiotensin receptors on T lymphocytes to limit target organ damage in hypertension. Circ Res 110: 1604-17, 2012.
176. Butz GM, Davisson RL: Long-term telemetric measurement of cardiovascular parameters in awake mice: a physiological genomics tool. Physiol Genomics 5: 89-97, 2001.
177. Aragao-Santiago L, Gomez-Sanchez CE, Mulatero P, Spyroglou A, Reincke M, Williams TA: Mouse Models of Primary Aldosteronism: From Physiology to Pathophysiology. Endocrinology 158: 4129-4138, 2017.
178. Gu H, Ma Z, Wang J, Zhu T, Du N, Shatara A, Yi X, Kowala MC, Du Y: Salt-dependent Blood Pressure in Human Aldosterone Synthase-Transgenic Mice. Sci Rep 7: 492, 2017.
179. Selye H: Production of hypertension and hyalinosis by desoxocortisone. Br Med J 1: 203-6, 1950.
180. Basting T, Lazartigues E: DOCA-Salt Hypertension: an Update. Curr Hypertens Rep 19: 32, 2017.
181. Haack D, Mohring J, Mohring B, Petri M, Hackenthal E: Comparative study on development of corticosterone and DOCA hypertension in rats. Am J Physiol 233: F403-11, 1977.

182. Tajima Y, Ichikawa S, Sakamaki T, Matsuo H, Aizawa F, Kogure M, Yagi S, Murata K: Body fluid distribution in the maintenance of DOCA-salt hypertension in rats. Am J Physiol 244: H695-700, 1983.
183. Kurtz TW, Dominiczak AF, DiCarlo SE, Pravenec M, Morris RC, Jr.: Molecular-based mechanisms of Mendelian forms of salt-dependent hypertension: questioning the prevailing theory. Hypertension 65: 932-41, 2015.
184. O'Donaughy TL, Qi Y, Brooks VL: Central action of increased osmolality to support blood pressure in deoxycorticosterone acetate-salt rats. Hypertension 48: 658-63, 2006.
185. Fink GD, Pawloski CM, Blair ML, Mangiapane ML: The area postrema in deoxycorticosterone-salt hypertension in rats. Hypertension 9: III206-9, 1987.
186. Grobe JL, Buehrer BA, Hilzendeger AM, Liu X, Davis DR, Xu D, Sigmund CD: Angiotensinergic signaling in the brain mediates metabolic effects of deoxycorticosterone (DOCA)-salt in C57 mice. Hypertension 57: 600-7, 2011.
187. Danser AH: The Role of the (Pro)renin Receptor in Hypertensive Disease. Am J Hypertens 28: 1187-96, 2015.
188. Selye H, Hall CE, Rowley EM: Malignant Hypertension Produced by Treatment with Desoxycorticosterone Acetate and Sodium Chloride. Can Med Assoc J 49: 88-92, 1943.
189. Deen WM, Maddox DA, Robertson CR, Brenner BM: Dynamics of glomerular ultrafiltration in the rat. VII. Response to reduced renal mass. Am J Physiol 227: 556-62, 1974.
190. Zhang Y, Kompa AR: A practical guide to subtotal nephrectomy in the rat with subsequent methodology for assessing renal and cardiac function. Nephrology (Carlton) 19: 552-61, 2014.
191. Salzler HR, Griffiths R, Ruiz P, Chi L, Frey C, Marchuk DA, Rockman HA, Le TH: Hypertension and albuminuria in chronic kidney disease mapped to a mouse chromosome 11 locus. Kidney Int 72: 1226-32, 2007.
192. Kennedy DJ, Elkareh J, Shidyak A, Shapiro AP, Smaili S, Mutgi K, Gupta S, Tian J, Morgan E, Khouri S, Cooper CJ, Periyasamy SM, Xie Z, Malhotra D, Fedorova OV, Bagrov AY, Shapiro JI: Partial nephrectomy as a model for uremic cardiomyopathy in the mouse. Am J Physiol Renal Physiol 294: F450-4, 2008.
193. Kren S, Hostetter TH: The course of the remnant kidney model in mice. Kidney Int 56: 333-7, 1999.
194. Leelahavanichkul A, Yan Q, Hu X, Eisner C, Huang Y, Chen R, Mizel D, Zhou H, Wright EC, Kopp JB, Schnermann J, Yuen PS, Star RA: Angiotensin II overcomes strain-dependent resistance of rapid CKD progression in a new remnant kidney mouse model. Kidney Int 78: 1136-53, 2010.
195. Gardiner SM, Kemp PA, Bennett T, Palmer RM, Moncada S: Nitric oxide synthase inhibitors cause sustained, but reversible, hypertension and hindquarters vasoconstriction in Brattleboro rats. Eur J Pharmacol 213: 449-51, 1992.
196. Ribeiro MO, Antunes E, de Nucci G, Lovisolo SM, Zatz R: Chronic inhibition of nitric oxide synthesis. A new model of arterial hypertension. Hypertension 20: 298-303, 1992.
197. Qiu C, Engels K, Baylis C: Angiotensin II and alpha 1-adrenergic tone in chronic nitric oxide blockade-induced hypertension. Am J Physiol 266: R1470-6, 1994.
198. Jain M, Bhosale V, Tripathi D, Singh H, Pal N, Hanif K, Jagavelu K: Antihypertensive Drugs Aliskiren, Nebivolol, and Olmesartan Reduce Hypertension by Reducing Endothelial Microparticles and Regulating Angiogenesis. J Cardiovasc Pharmacol 70: 176-183, 2017.
199. Zatz R, Baylis C: Chronic nitric oxide inhibition model six years on. Hypertension 32: 958-64, 1998.

200. Mohan S, Reddick RL, Musi N, Horn DA, Yan B, Prihoda TJ, Natarajan M, Abboud-Werner SL: Diabetic eNOS knockout mice develop distinct macro- and microvascular complications. Lab Invest 88: 515-28, 2008.
201. Zhao HJ, Wang S, Cheng H, Zhang MZ, Takahashi T, Fogo AB, Breyer MD, Harris RC: Endothelial nitric oxide synthase deficiency produces accelerated nephropathy in diabetic mice. J Am Soc Nephrol 17: 2664-9, 2006.
202. Hedayat AF, Park KH, Kwon TG, Woollard JR, Jiang K, Carlson DF, Lerman A, Lerman LO: Peripheral vascular atherosclerosis in a novel PCSK9 gain-of-function mutant Ossabaw miniature pig model. Transl Res, 2017.
203. Yu Q, Larson DF, Slayback D, Lundeen TF, Baxter JH, Watson RR: Characterization of high-salt and high-fat diets on cardiac and vascular function in mice. Cardiovasc Toxicol 4: 37-46, 2004.
204. Dobrian AD, Davies MJ, Prewitt RL, Lauterio TJ: Development of hypertension in a rat model of diet-induced obesity. Hypertension 35: 1009-15, 2000.
205. Hariri N, Thibault L: High-fat diet-induced obesity in animal models. Nutr Res Rev 23: 270-99, 2010.
206. Carroll JF, Dwyer TM, Grady AW, Reinhart GA, Montani JP, Cockrell K, Meydrech EF, Mizelle HL: Hypertension, cardiac hypertrophy, and neurohumoral activity in a new animal model of obesity. Am J Physiol 271: H373-8, 1996.
207. Pawar AS, Zhu XY, Eirin A, Tang H, Jordan KL, Woollard JR, Lerman A, Lerman LO: Adipose tissue remodeling in a novel domestic porcine model of diet-induced obesity. Obesity (Silver Spring) 23: 399-407, 2015.
208. Wong SK, Chin KY, Suhaimi FH, Fairus A, Ima-Nirwana S: Animal models of metabolic syndrome: a review. Nutr Metab (Lond) 13: 65, 2016.
209. Thirunavukkarasu V, Anitha Nandhini AT, Anuradha CV: Lipoic acid attenuates hypertension and improves insulin sensitivity, kallikrein activity and nitrite levels in high fructose-fed rats. J Comp Physiol B 174: 587-92, 2004.
210. Rocchini AP, Moorehead C, Wentz E, Deremer S: Obesity-induced hypertension in the dog. Hypertension 9: III64-8, 1987.
211. Thigpen JE, Setchell KD, Saunders HE, Haseman JK, Grant MG, Forsythe DB: Selecting the appropriate rodent diet for endocrine disruptor research and testing studies. ILAR J 45: 401-16, 2004.
212. Chade AR, Mushin OP, Zhu X, Rodriguez-Porcel M, Grande JP, Textor SC, Lerman A, Lerman LO: Pathways of renal fibrosis and modulation of matrix turnover in experimental hypercholesterolemia. Hypertension 46: 772-9, 2005.
213. Oron-Herman M, Kamari Y, Grossman E, Yeger G, Peleg E, Shabtay Z, Shamiss A, Sharabi Y: Metabolic syndrome: comparison of the two commonly used animal models. Am J Hypertens 21: 1018-22, 2008.
214. Tschop M, Heiman ML: Rodent obesity models: an overview. Exp Clin Endocrinol Diabetes 109: 307-19, 2001.
215. Nagae A, Fujita M, Kawarazaki H, Matsui H, Ando K, Fujita T: Effect of high fat loading in Dahl salt-sensitive rats. Clin Exp Hypertens 31: 451-61, 2009.
216. Zhang X, Li ZL, Woollard JR, Eirin A, Ebrahimi B, Crane JA, Zhu XY, Pawar AS, Krier JD, Jordan KL, Tang H, Textor SC, Lerman A, Lerman LO: Obesity-metabolic derangement preserves hemodynamics but promotes intrarenal adiposity and macrophage infiltration in swine renovascular disease. Am J Physiol Renal Physiol 305: F265-76, 2013.

217. Li ZL, Ebrahimi B, Zhang X, Eirin A, Woollard JR, Tang H, Lerman A, Wang SM, Lerman LO: Obesity-metabolic derangement exacerbates cardiomyocyte loss distal to moderate coronary artery stenosis in pigs without affecting global cardiac function. Am J Physiol Heart Circ Physiol 306: H1087-101, 2014.
218. Ernsberger P, Ishizuka T, Liu S, Farrell CJ, Bedol D, Koletsky RJ, Friedman JE: Mechanisms of antihyperglycemic effects of moxonidine in the obese spontaneously hypertensive Koletsky rat (SHROB). J Pharmacol Exp Ther 288: 139-47, 1999.
219. Morrison RG, Carpenter AB, Moore SK, Mangiarua EI, Valentovic MA, Walker EM, Jr., Wehner PS, Rhoten WB, Touchon RC, McCumbee WD: Increased sensitivity of the obese Zucker rat to deoxycorticosterone-salt-induced hypertension. J Hypertens 20: 2247-55, 2002.
220. Sandberg K, Ji H: Sex differences in primary hypertension. Biol Sex Differ 3: 7, 2012.
221. Evans RG, Stevenson KM, Bergstrom G, Denton KM, Madden AC, Gribben RL, Weekes SR, Anderson WP: Sex differences in pressure diuresis/natriuresis in rabbits. Acta Physiol Scand 169: 309-16, 2000.
222. Sartori-Valinotti JC, Iliescu R, Yanes LL, Dorsett-Martin W, Reckelhoff JF: Sex differences in the pressor response to angiotensin II when the endogenous renin-angiotensin system is blocked. Hypertension 51: 1170-6, 2008.
223. Veiras LC, Girardi ACC, Curry J, Pei L, Ralph DL, Tran A, Castelo-Branco RC, Pastor-Soler N, Arranz CT, Yu ASL, McDonough AA: Sexual Dimorphic Pattern of Renal Transporters and Electrolyte Homeostasis. J Am Soc Nephrol 28: 3504-3517, 2017.
224. Cohen JA, Lindsey SH, Pirro NT, Brosnihan KB, Gallagher PE, Chappell MC: Influence of estrogen depletion and salt loading on renal angiotensinogen expression in the mRen(2).Lewis strain. Am J Physiol Renal Physiol 299: F35-42, 2010.
225. Brinson KN, Rafikova O, Sullivan JC: Female sex hormones protect against salt-sensitive hypertension but not essential hypertension. Am J Physiol Regul Integr Comp Physiol 307: R149-57, 2014.
226. Xue B, Pamidimukkala J, Hay M: Sex differences in the development of angiotensin II-induced hypertension in conscious mice. Am J Physiol Heart Circ Physiol 288: H2177-84, 2005.
227. Zimmerman MA, Baban B, Tipton AJ, O'Connor PM, Sullivan JC: Chronic ANG II infusion induces sex-specific increases in renal T cells in Sprague-Dawley rats. Am J Physiol Renal Physiol 308: F706-12, 2015.
228. Fortepiani LA, Zhang H, Racusen L, Roberts LJ, 2nd, Reckelhoff JF: Characterization of an animal model of postmenopausal hypertension in spontaneously hypertensive rats. Hypertension 41: 640-5, 2003.
229. Hinojosa-Laborde C, Lange DL, Haywood JR: Role of female sex hormones in the development and reversal of dahl hypertension. Hypertension 35: 484-9, 2000.
230. Yanes LL, Romero DG, Moulana M, Lima R, Davis DD, Zhang H, Lockhart R, Racusen LC, Reckelhoff JF: Cardiovascular-renal and metabolic characterization of a rat model of polycystic ovary syndrome. Gend Med 8: 103-15, 2011.
231. Reckelhoff JF, Zhang H, Srivastava K: Gender differences in development of hypertension in spontaneously hypertensive rats: role of the renin-angiotensin system. Hypertension 35: 480-3, 2000.
232. Foss JD, Fink GD, Osborn JW: Reversal of genetic salt-sensitive hypertension by targeted sympathetic ablation. Hypertension 61: 806-11, 2013.

233. da Silva AA, do Carmo JM, Kanyicska B, Dubinion J, Brandon E, Hall JE: Endogenous melanocortin system activity contributes to the elevated arterial pressure in spontaneously hypertensive rats. Hypertension 51: 884-90, 2008.
234. Maranon RO, Lima R, Mathbout M, do Carmo JM, Hall JE, Roman RJ, Reckelhoff JF: Postmenopausal hypertension: role of the sympathetic nervous system in an animal model. Am J Physiol Regul Integr Comp Physiol 306: R248-56, 2014.
235. Yanes LL, Romero DG, Iliescu R, Zhang H, Davis D, Reckelhoff JF: Postmenopausal hypertension: role of the Renin-Angiotensin system. Hypertension 56: 359-63, 2010.
236. Fortepiani LA, Reckelhoff JF: Role of oxidative stress in the sex differences in blood pressure in spontaneously hypertensive rats. J Hypertens 23: 801-5, 2005.
237. Sullivan JC, Sasser JM, Pollock JS: Sexual dimorphism in oxidant status in spontaneously hypertensive rats. Am J Physiol Regul Integr Comp Physiol 292: R764-8, 2007.
238. Sullivan JC, Pardieck JL, Hyndman KA, Pollock JS: Renal NOS activity, expression, and localization in male and female spontaneously hypertensive rats. Am J Physiol Regul Integr Comp Physiol 298: R61-9, 2010.
239. Fortepiani LA, Reckelhoff JF: Treatment with tetrahydrobiopterin reduces blood pressure in male SHR by reducing testosterone synthesis. Am J Physiol Regul Integr Comp Physiol 288: R733-6, 2005.
240. Su P, Kaushal KM, Kroetz DL: Inhibition of renal arachidonic acid omega-hydroxylase activity with ABT reduces blood pressure in the SHR. Am J Physiol 275: R426-38, 1998.
241. Tipton AJ, Baban B, Sullivan JC: Female spontaneously hypertensive rats have a compensatory increase in renal regulatory T cells in response to elevations in blood pressure. Hypertension 64: 557-64, 2014.
242. Yanes LL, Romero DG, Cucchiarelli VE, Fortepiani LA, Gomez-Sanchez CE, Santacruz F, Reckelhoff JF: Role of endothelin in mediating postmenopausal hypertension in a rat model. Am J Physiol Regul Integr Comp Physiol 288: R229-33, 2005.
243. Gupte M, Thatcher SE, Boustany-Kari CM, Shoemaker R, Yiannikouris F, Zhang X, Karounos M, Cassis LA: Angiotensin converting enzyme 2 contributes to sex differences in the development of obesity hypertension in C57BL/6 mice. Arterioscler Thromb Vasc Biol 32: 1392-9, 2012.
244. Riazi S, Madala-Halagappa VK, Dantas AP, Hu X, Ecelbarger CA: Sex differences in renal nitric oxide synthase, NAD(P)H oxidase, and blood pressure in obese Zucker rats. Gend Med 4: 214-29, 2007.
245. Doursout MF, Chelly JE, Wouters P, Lawrence C, Liang YY, Buckley JP: Effect of gender in centrally induced angiotensin II hypertension in dogs. Hypertension 15: I117-20, 1990.
246. Shi X, Bai Y, Ke Y, Chen R, Lin X, Chen L, Hong H: Ageing-related aorta remodelling and calcification occur earlier and progress more severely in rats with spontaneous hypertension. Histol Histopathol: 11971, 2018.
247. Park JB, Schiffrin EL: Small artery remodeling is the most prevalent (earliest?) form of target organ damage in mild essential hypertension. J Hypertens 19: 921-30, 2001.
248. Baumbach GL, Heistad DD: Remodeling of cerebral arterioles in chronic hypertension. Hypertension 13: 968-72, 1989.
249. Boudier HA: Arteriolar and capillary remodelling in hypertension. Drugs 58 Spec No 1: 37-40, 1999.
250. Mulvany MJ, Baumbach GL, Aalkjaer C, Heagerty AM, Korsgaard N, Schiffrin EL, Heistad DD: Vascular remodeling. Hypertension 28: 505-6, 1996.

251. del Campo L, Ferrer M: Wire Myography to Study Vascular Tone and Vascular Structure of Isolated Mouse Arteries. Methods Mol Biol 1339: 255-76, 2015.
252. Schjorring OL, Carlsson R, Simonsen U: Pressure Myography to Study the Function and Structure of Isolated Small Arteries. Methods Mol Biol 1339: 277-95, 2015.
253. Zhang Y, Carreras D, de Bold AJ: Discoordinate re-expression of cardiac fetal genes in N(omega)-nitro-L-arginine methyl ester (L-NAME) hypertension. Cardiovasc Res 57: 158-67, 2003.
254. Kvakan H, Kleinewietfeld M, Qadri F, Park JK, Fischer R, Schwarz I, Rahn HP, Plehm R, Wellner M, Elitok S, Gratze P, Dechend R, Luft FC, Muller DN: Regulatory T cells ameliorate angiotensin II-induced cardiac damage. Circulation 119: 2904-12, 2009.
255. Griffin KA: Hypertensive Kidney Injury and the Progression of Chronic Kidney Disease. Hypertension 70: 687-694, 2017.
256. Griffin KA, Churchill PC, Picken M, Webb RC, Kurtz TW, Bidani AK: Differential salt-sensitivity in the pathogenesis of renal damage in SHR and stroke prone SHR. Am J Hypertens 14: 311-20, 2001.
257. Griffin KA, Polichnowski A, Litbarg N, Picken M, Venkatachalam MA, Bidani AK: Critical blood pressure threshold dependence of hypertensive injury and repair in a malignant nephrosclerosis model. Hypertension 64: 801-7, 2014.
258. Griffin KA, Picken M, Bidani AK: Method of renal mass reduction is a critical modulator of subsequent hypertension and glomerular injury. J Am Soc Nephrol 4: 2023-31, 1994.
259. Gangadhariah MH, Luther JM, Garcia V, Paueksakon P, Zhang MZ, Hayward SW, Love HD, Falck JR, Manthati VL, Imig JD, Schwartzman ML, Zent R, Capdevila JH, Pozzi A: Hypertension is a major contributor to 20-hydroxyeicosatetraenoic acid-mediated kidney injury in diabetic nephropathy. J Am Soc Nephrol 26: 597-610, 2015.
260. Iadecola C: The pathobiology of vascular dementia. Neuron 80: 844-66, 2013.
261. Iadecola C: The Neurovascular Unit Coming of Age: A Journey through Neurovascular Coupling in Health and Disease. Neuron 96: 17-42, 2017.
262. Lecrux C, Nicole O, Chazalviel L, Catone C, Chuquet J, MacKenzie ET, Touzani O: Spontaneously hypertensive rats are highly vulnerable to AMPA-induced brain lesions. Stroke 38: 3007-3015, 2007. r14689
263. Jeffs B, Clark JS, Anderson NH, Gratton J, Brosnan MJ, Gauguier D, Reid JL, Macrae IM, Dominiczak AF: Sensitivity to cerebral ischaemic insult in a rat model of stroke is determined by a single genetic locus. Nature genetics 16: 364-367, 1997. r14697
264. Hainsworth AH, Allan SM, Boltze J, Cunningham C, Farris C, Head E, Ihara M, Isaacs JD, Kalaria RN, Oberstein SAMJL, Moss MB, Nitzsche B, Rosenberg GA, Rutten JW, Salkovic-Petrisic M, Troen AM: Translational models for vascular cognitive impairment: a review including larger species. BMC medicine 15: 1-12, 2017.
265. Friso S, Pizzolo F, Choi SW, Guarini P, Castagna A, Ravagnani V, Carletto A, Pattini P, Corrocher R, Olivieri O: Epigenetic control of 11 beta-hydroxysteroid dehydrogenase 2 gene promoter is related to human hypertension. Atherosclerosis 199: 323-7, 2008.
266. Lee HA, Baek I, Seok YM, Yang E, Cho HM, Lee DY, Hong SH, Kim IK: Promoter hypomethylation upregulates Na+-K+-2Cl- cotransporter 1 in spontaneously hypertensive rats. Biochem Biophys Res Commun 396: 252-7, 2010.
267. Cheng X, Joe B: Circular RNAs in rat models of cardiovascular and renal diseases. Physiol Genomics 49: 484-490, 2017.

268. Gopalakrishnan K, Kumarasamy S, Mell B, Joe B: Genome-wide identification of long noncoding RNAs in rat models of cardiovascular and renal disease. Hypertension 65: 200-10, 2015.
269. Shi L, Liao J, Liu B, Zeng F, Zhang L: Mechanisms and therapeutic potential of microRNAs in hypertension. Drug Discov Today 20: 1188-204, 2015.
270. Zhu X-Y, Ebrahimi B, Eirin A, Woollard JR, Tang H, Jordan KL, Ofori M, Saad A, Herrmann SMS, Dietz AB, Textor SC, Lerman A, Lerman LO: Renal Vein Levels of MicroRNA-26a Are Lower in the Poststenotic Kidney. J Am Soc Nephrol 26: 1378-1388, 2015.
271. Mell B, Jala VR, Mathew AV, Byun J, Waghulde H, Zhang Y, Haribabu B, Vijay-Kumar M, Pennathur S, Joe B: Evidence for a link between gut microbiota and hypertension in the Dahl rat. Physiol Genomics 47: 187-97, 2015.
272. Yang T, Santisteban MM, Rodriguez V, Li E, Ahmari N, Carvajal JM, Zadeh M, Gong M, Qi Y, Zubcevic J, Sahay B, Pepine CJ, Raizada MK, Mohamadzadeh M: Gut dysbiosis is linked to hypertension. Hypertension 65: 1331-40, 2015.
273. Jose PA, Raj D: Gut microbiota in hypertension. Curr Opin Nephrol Hypertens 24: 403-9, 2015.
274. Pevsner-Fischer M, Blacher E, Tatirovsky E, Ben-Dov IZ, Elinav E: The gut microbiome and hypertension. Curr Opin Nephrol Hypertens 26: 1-8, 2017.
275. Wilck N, Matus MG, Kearney SM, Olesen SW, Forslund K, Bartolomaeus H, Haase S, Mahler A, Balogh A, Marko L, Vvedenskaya O, Kleiner FH, Tsvetkov D, Klug L, Costea PI, Sunagawa S, Maier L, Rakova N, Schatz V, Neubert P, Fratzer C, Krannich A, Gollasch M, Grohme DA, Corte-Real BF, Gerlach RG, Basic M, Typas A, Wu C, Titze JM, Jantsch J, Boschmann M, Dechend R, Kleinewietfeld M, Kempa S, Bork P, Linker RA, Alm EJ, Muller DN: Salt-responsive gut commensal modulates TH17 axis and disease. Nature 551: 585-589, 2017.
276. Wanjek C: Systems biology as defined by NIH: an intellextual resource for integrative biology. The NIH Catalyst Volume 19, (https://irp.nih.gov/catalyst/v19i6/systems-biology-as-defined-by-nih).2011; accessed November 1, 2017.
277. Kirschner MW: The meaning of systems biology. Cell 121: 503-4, 2005.
278. Begley CG, Ellis LM: Drug development: Raise standards for preclinical cancer research. Nature 483: 531-3, 2012.
279. Perrin S: Preclinical research: Make mouse studies work. Nature 507: 423-5, 2014.
280. Gomez-Arroyo J, Voelkel NF, Bogaard HJ, Taraseviciene-Stewart L: Usefulness of a mouse model of reversible pulmonary arterial hypertension: be cautious, choose carefully. Am J Respir Crit Care Med 185: 1326; author reply 1326-7, 2012.
281. Ponce de Leon V, Merillat AM, Tesson L, Anegon I, Hummler E: Generation of TALEN-mediated GRdim knock-in rats by homologous recombination. PLoS One 9: e88146, 2014.
282. Toth P, Tucsek Z, Sosnowska D, Gautam T, Mitschelen M, Tarantini S, Deak F, Koller A, Sonntag WE, Csiszar A, Ungvari Z: Age-related autoregulatory dysfunction and cerebromicrovascular injury in mice with angiotensin II-induced hypertension. J Cereb Blood Flow Metab 33: 1732-42, 2013.
283. Faraco G, Sugiyama Y, Lane D, Garcia-Bonilla L, Chang H, Santisteban MM, Racchumi G, Murphy M, Van Rooijen N, Anrather J, Iadecola C: Perivascular macrophages mediate the neurovascular and cognitive dysfunction associated with hypertension. J Clin Invest 126: 4674-4689, 2016.

284. Capone C, Faraco G, Peterson JR, Coleman C, Anrather J, Milner TA, Pickel VM, Davisson RL, Iadecola C: Central cardiovascular circuits contribute to the neurovascular dysfunction in angiotensin II hypertension. J Neurosci 32: 4878-86, 2012.
285. Duchemin S, Belanger E, Wu R, Ferland G, Girouard H: Chronic perfusion of angiotensin II causes cognitive dysfunctions and anxiety in mice. Physiol Behav 109: 63-8, 2013.
286. Faraco G, Park L, Zhou P, Luo W, Paul SM, Anrather J, Iadecola C: Hypertension enhances Abeta-induced neurovascular dysfunction, promotes beta-secretase activity, and leads to amyloidogenic processing of APP. J Cereb Blood Flow Metab 36: 241-52, 2016.
287. Wakisaka Y, Chu Y, Miller JD, Rosenberg GA, Heistad DD: Spontaneous intracerebral hemorrhage during acute and chronic hypertension in mice. J Cereb Blood Flow Metab 30: 56-69, 2010.
288. Meissner A, Minnerup J, Soria G, Planas AM: Structural and functional brain alterations in a murine model of Angiotensin II-induced hypertension. J Neurochem 140: 509-521, 2017.
289. Dunn WR, Gardiner SM: Differential alteration in vascular structure of resistance arteries isolated from the cerebral and mesenteric vascular beds of transgenic [(mRen-2)27], hypertensive rats. Hypertension 29: 1140-7, 1997.
290. Baumbach GL, Sigmund CD, Faraci FM: Cerebral arteriolar structure in mice overexpressing human renin and angiotensinogen. Hypertension 41: 50-55, 2003.
291. Inaba S, Iwai M, Tomono Y, Senba I, Furuno M, Kanno H, Okayama H, Mogi M, Higaki J, Horiuchi M: Exaggeration of focal cerebral ischemia in transgenic mice carrying human Renin and human angiotensinogen genes. Stroke 40: 597-603, 2009.
292. Inaba S, Iwai M, Furuno M, Tomono Y, Kanno H, Senba I, Okayama H, Mogi M, Higaki J, Horiuchi M: Continuous activation of renin-angiotensin system impairs cognitive function in renin/angiotensinogen transgenic mice. Hypertension 53: 356-62, 2009.
293. Faraci FM, Lamping KG, Modrick ML, Ryan MJ, Sigmund CD, Didion SP: Cerebral vascular effects of angiotensin II: new insights from genetic models. J Cereb Blood Flow Metab 26: 449-455, 2006.
294. Iida S, Baumbach GL, Lavoie JL, Faraci FM, Sigmund CD, Heistad DD: Spontaneous stroke in a genetic model of hypertension in mice. Stroke 36: 1253-8, 2005.
295. Leung JW, Ho MC, Lo AC, Chung SS, Chung SK: Endothelial cell-specific over-expression of endothelin-1 leads to more severe cerebral damage following transient middle cerebral artery occlusion. J Cardiovasc Pharmacol 44 Suppl 1: S293-300, 2004.
296. Zhang X, Yeung PK, McAlonan GM, Chung SS, Chung SK: Transgenic mice over-expressing endothelial endothelin-1 show cognitive deficit with blood-brain barrier breakdown after transient ischemia with long-term reperfusion. Neurobiol Learn Mem 101: 46-54, 2013.
297. Baumbach GL, Sigmund CD, Faraci FM: Structure of cerebral arterioles in mice deficient in expression of the gene for endothelial nitric oxide synthase. Circulation research 95: 822-829, 2004.
298. Faraci FM, Sigmund CD, Shesely EG, Maeda N, Heistad DD: Responses of carotid artery in mice deficient in expression of the gene for endothelial NO synthase. The American journal of physiology 274: H564-70, 1998.
299. Girouard H, Park L, Anrather J, Zhou P, Iadecola C: Cerebrovascular nitrosative stress mediates neurovascular and endothelial dysfunction induced by angiotensin II. Arterioscler Thromb Vasc Biol 27: 303-309, 2007.

300. Huang Z, Huang PL, Ma J, Meng W, Ayata C, Fishman MC, Moskowitz MA: Enlarged infarcts in endothelial nitric oxide synthase knockout mice are attenuated by nitro-L-arginine. Journal of Cerebral Blood Flow & Metabolism 16: 981-987, 1996.
301. Capone C, Faraco G, Coleman C, Young CN, Pickel VM, Anrather J, Davisson RL, Iadecola C: Endothelin 1-dependent neurovascular dysfunction in chronic intermittent hypoxia. Hypertension 60: 106-113, 2012.
302. Jackman KA, Zhou P, Faraco G, Peixoto PM, Coleman C, Voss HU, Pickel V, Manfredi G, Iadecola C: Dichotomous effects of chronic intermittent hypoxia on focal cerebral ischemic injury. Stroke 45: 1460-1467, 2014.
303. Durgan DJ, Crossland RF, Lloyd EE, Phillips SC, Bryan RM: Increased cerebrovascular sensitivity to endothelin-1 in a rat model of obstructive sleep apnea: a role for endothelin receptor B. J Cereb Blood Flow Metab 35: 402-411, 2015.
304. Crossland RF, Durgan DJ, Lloyd EE, Phillips SC, Reddy AK, Marrelli SP, Bryan RM: A new rodent model for obstructive sleep apnea: effects on ATP-mediated dilations in cerebral arteries. American journal of physiology Regulatory, integrative and comparative physiology 305: R334-42, 2013.
305. Phillips SA, Olson EB, Morgan BJ, Lombard JH: Chronic intermittent hypoxia impairs endothelium-dependent dilation in rat cerebral and skeletal muscle resistance arteries. 286: H388-93, 2004.
306. Hartman RE, Kamper JE, Goyal R, Stewart JM, Longo LD: Motor and cognitive deficits in mice bred to have low or high blood pressure. Physiol Behav 105: 1092-7, 2012.
307. Takahashi S, Hamai Y, Okamoto K: Fluorescein fundus angiography of stroke-prone SHR. Jpn Heart J 19: 646-7, 1978.
308. Takahashi S, Hamai Y, Okamoto K: On the findings of stroke-prone SHR. Jpn Heart J 19: 645, 1978.
309. Tamaki K, Sadoshima S, Baumbach GL, Iadecola C, Reis DJ, Heistad DD: Evidence that disruption of the blood-brain barrier precedes reduction in cerebral blood flow in hypertensive encephalopathy. Hypertension 6: I75-81, 1984.
310. Spratt NJ, Fernandez J, Chen M, Rewell S, Cox S, van Raay L, Hogan L, Howells DW: Modification of the method of thread manufacture improves stroke induction rate and reduces mortality after thread-occlusion of the middle cerebral artery in young or aged rats. Journal of Neuroscience Methods 155: 285-290, 2006.
311. Barone FC, Price WJ, White RF, Willette RN, Feuerstein GZ: Genetic hypertension and increased susceptibility to cerebral ischemia. Neuroscience & Biobehavioral Reviews 16: 219-233, 1992.
312. Jalal FY, Yang Y, Thompson J, Lopez AC, Rosenberg GA: Myelin loss associated with neuroinflammation in hypertensive rats. Stroke 43: 1115-22, 2012.
313. Kalani A, Pushpakumar SB, Vacek JC, Tyagi SC, Tyagi N: Inhibition of MMP-9 attenuates hypertensive cerebrovascular dysfunction in Dahl salt-sensitive rats. Molecular and cellular biochemistry 413: 25-35, 2016.
314. Smeda JS, Payne GW: Alterations in autoregulatory and myogenic function in the cerebrovasculature of Dahl salt-sensitive rats. Stroke 34: 1484-1490, 2003.
315. Rodrigues SF, Granger DN: Cerebral microvascular inflammation in DOCA salt-induced hypertension: role of angiotensin II and mitochondrial superoxide. J Cereb Blood Flow Metab 32: 368-375, 2012.

316. Tada Y, Wada K, Shimada K, Makino H, Liang EI, Murakami S, Kudo M, Kitazato KT, Nagahiro S, Hashimoto T: Roles of hypertension in the rupture of intracranial aneurysms. Stroke 45: 579-86, 2014.
317. Jabaris SSL, Sumathy H, Girish R, Narayanan S, Sugumar M, Saravana Babu C, Thanikachalam S, Thanikachalam M: Phosphodiesterase-4 inhibitors ameliorates cognitive deficits in deoxycorticosterone acetate induced hypertensive rats via cAMP/CREB signaling system. Brain research 1622: 279-291, 2015.
318. Dorrance AM, Rupp NC, Nogueira EF: Mineralocorticoid receptor activation causes cerebral vessel remodeling and exacerbates the damage caused by cerebral ischemia. Hypertension 47: 590-595, 2006.
319. Nuki Y, Tsou T-L, Kurihara C, Kanematsu M, Kanematsu Y, Hashimoto T: Elastase-induced intracranial aneurysms in hypertensive mice. Hypertension 54: 1337-1344, 2009.
320. Hosaka K, Downes DP, Nowicki KW, Hoh BL: Modified murine intracranial aneurysm model: aneurysm formation and rupture by elastase and hypertension. Journal of neurointerventional surgery 6: 474-479, 2014.
321. Zeng J, Zhang Y, Mo J, Su Z, Huang R: Two-kidney, two clip renovascular hypertensive rats can be used as stroke-prone rats. Stroke 29: 1708-13- discussion 1713-4, 1998.
322. Ménard B, Chazalviel L, Roussel S, Bernaudin M, Touzani O: Two-kidney one-clip is a pertinent approach to integrate arterial hypertension in animal models of stroke: Serial magnetic resonance imaging studies of brain lesions before and during cerebral ischemia. Journal of Cerebral Blood Flow & Metabolism 7: 0271678X1771581-12, 2017. r14687
323. Chan S-L, Baumbach GL: Nox2 deficiency prevents hypertension-induced vascular dysfunction and hypertrophy in cerebral arterioles. International journal of hypertension 2013: 793630, 2013.
324. Carnevale D, Mascio G, D'Andrea I, Fardella V, Bell RD, Branchi I, Pallante F, Zlokovic B, Yan SS, Lembo G: Hypertension induces brain β-amyloid accumulation, cognitive impairment, and memory deterioration through activation of receptor for advanced glycation end products in brain vasculature. Hypertension 60: 188-197, 2012.
325. Hu JJ, Fossum TW, Miller MW, Xu H, Liu JC, Humphrey JD: Biomechanics of the Porcine Basilar Artery in Hypertension. Annals of Biomedical Engineering 35: 19-29, 2006.
326. Olver TD, Klakotskaia D, Ferguson BS, Hiemstra JA, Schachtman TR, Laughlin MH, Emter CA: Carotid Artery Vascular Mechanics Serve as Biomarkers of Cognitive Dysfunction in Aortic ‐ Banded Miniature Swine That Can Be Treated With an Exercise Intervention. Journal of the American Heart Association 5: e003248-12, 2016.
327. Kemper T, Moss MB, Hollander W, Prusty S: Microinfarction as a result of hypertension in a primate model of cerebrovascular disease. Acta Neuropathol 98: 295-303, 1999.
328. Prusty S, Kemper T, Moss MB, Hollander W: Occurrence of stroke in a nonhuman primate model of cerebrovascular disease. Stroke 19: 84-90, 1988.
329. Moss MB, Jonak E: Cerebrovascular disease and dementia: a primate model of hypertension and cognition. Alzheimers Dement 3: S6-15, 2007.
330. Schiffrin EL: Vascular remodeling in hypertension: mechanisms and treatment. Hypertension 59: 367-74, 2012.
331. Sharifi AM, Schiffrin EL: Apoptosis in vasculature of spontaneously hypertensive rats: effect of an angiotensin converting enzyme inhibitor and a calcium channel antagonist. Am J Hypertens 11: 1108-16, 1998.

332. Geng J, Zhao Z, Yang L, Zhang M, Liu X: Protein Kinase D was involved in vascular remodeling in spontaneously hypertensive rats. Clin Exp Hypertens: 1-8, 2018.
333. Rizzoni D, Muiesan ML, Porteri E, De Ciuceis C, Boari GE, Salvetti M, Paini A, Rosei EA: Vascular remodeling, macro- and microvessels: therapeutic implications. Blood Press 18: 242-6, 2009.
334. Jiang J, Zheng JP, Li Y, Gan Z, Jiang Y, Huang D, Li H, Liu Z, Ke Y: Differential contribution of endothelium-derived relaxing factors to vascular reactivity in conduit and resistance arteries from normotensive and hypertensive rats. Clin Exp Hypertens 38: 393-8, 2016.
335. Deng LY, Schiffrin EL: Effects of endothelin-1 and vasopressin on resistance arteries of spontaneously hypertensive rats. Am J Hypertens 5: 817-22, 1992.
336. Harvey AP, Montezano AC, Hood KY, Lopes RA, Rios F, Ceravolo G, Graham D, Touyz RM: Vascular dysfunction and fibrosis in stroke-prone spontaneously hypertensive rats: The aldosterone-mineralocorticoid receptor-Nox1 axis. Life Sci 179: 110-119, 2017.
337. Pires PW, Jackson WF, Dorrance AM: Regulation of myogenic tone and structure of parenchymal arterioles by hypertension and the mineralocorticoid receptor. Am J Physiol Heart Circ Physiol 309: H127-36, 2015.
338. Ledingham JM, Laverty R: Basilar artery remodelling in the genetically hypertensive rat: effects of nitric oxide synthase inhibition and treatment with valsartan and enalapril. Clin Exp Pharmacol Physiol 27: 642-6, 2000.
339. Rizzoni D, Porteri E, Castellano M, Bettoni G, Muiesan ML, Muiesan P, Giulini SM, Agabiti-Rosei E: Vascular hypertrophy and remodeling in secondary hypertension. Hypertension 28: 785-90, 1996.
340. d'Uscio LV, Barton M, Shaw S, Moreau P, Luscher TF: Structure and function of small arteries in salt-induced hypertension: effects of chronic endothelin-subtype-A-receptor blockade. Hypertension 30: 905-11, 1997.
341. Fan F, Geurts AM, Murphy SR, Pabbidi MR, Jacob HJ, Roman RJ: Impaired myogenic response and autoregulation of cerebral blood flow is rescued in CYP4A1 transgenic Dahl salt-sensitive rat. Am J Physiol Regul Integr Comp Physiol 308: R379-90, 2015.
342. Sharifi AM, Schiffrin EL: Apoptosis in aorta of deoxycorticosterone acetate-salt hypertensive rats: effect of endothelin receptor antagonism. J Hypertens 15: 1441-8, 1997.
343. Li JS, Knafo L, Turgeon A, Garcia R, Schiffrin EL: Effect of endothelin antagonism on blood pressure and vascular structure in renovascular hypertensive rats. Am J Physiol 271: H88-93, 1996.
344. Paulis L, Becker ST, Lucht K, Schwengel K, Slavic S, Kaschina E, Thone-Reineke C, Dahlof B, Baulmann J, Unger T, Steckelings UM: Direct angiotensin II type 2 receptor stimulation in Nomega-nitro-L-arginine-methyl ester-induced hypertension: the effect on pulse wave velocity and aortic remodeling. Hypertension 59: 485-92, 2012.
345. Ferreira-Melo SE, Yugar-Toledo JC, Coelho OR, De Luca IM, Tanus-Santos JE, Hyslop S, Irigoyen MC, Moreno H, Jr.: Sildenafil reduces cardiovascular remodeling associated with hypertensive cardiomyopathy in NOS inhibitor-treated rats. Eur J Pharmacol 542: 141-7, 2006.
346. Hsieh NK, Wang JY, Liu JC, Lee WH, Chen HI: Structural changes in cerebral arteries following nitric oxide deprivation: a comparison between normotensive and hypertensive rats. Thromb Haemost 92: 162-70, 2004.

347. Pistea A, Bakker EN, Spaan JA, Hardeman MR, van Rooijen N, VanBavel E: Small artery remodeling and erythrocyte deformability in L-NAME-induced hypertension: role of transglutaminases. J Vasc Res 45: 10-8, 2008.
348. Isabelle M, Simonet S, Ragonnet C, Sansilvestri-Morel P, Clavreul N, Vayssettes-Courchay C, Verbeuren TJ: Chronic reduction of nitric oxide level in adult spontaneously hypertensive rats induces aortic stiffness similar to old spontaneously hypertensive rats. J Vasc Res 49: 309-18, 2012.
349. Arnet UA, Novosel D, Barton M, Noll G, Ganten D, Luscher TF: Endothelial dysfunction in the aorta of transgenic rats harboring the mouse Ren-2 gene. Endothelium 6: 175-84, 1999.
350. Tochitani T, Mori M, Matsuda K, Kouchi M, Fujii Y, Matsumoto I: Histopathological characteristics of renal changes in human renin-angiotensinogen double transgenic rats. J Toxicol Pathol 29: 125-9, 2016.
351. Montezano AC, Nguyen Dinh Cat A, Rios FJ, Touyz RM: Angiotensin II and vascular injury. Curr Hypertens Rep 16: 431, 2014.
352. Antunes TT, Callera GE, He Y, Yogi A, Ryazanov AG, Ryazanova LV, Zhai A, Stewart DJ, Shrier A, Touyz RM: Transient Receptor Potential Melastatin 7 Cation Channel Kinase: New Player in Angiotensin II-Induced Hypertension. Hypertension 67: 763-73, 2016.
353. Schiffrin EL: Mechanisms of remodelling of small arteries, antihypertensive therapy and the immune system in hypertension. Clin Invest Med 38: E394-402, 2015.
354. Zhang L, Wu JH, Huang TQ, Neplioue v I, Brian L, Zhang Z, Wertman V, Rudemiller NP, McMahon TJ, Shenoy SK, Miller FJ, Crowley SD, Freedman NJ, Stiber JA: Drebrin Regulates Angiotensin II-Induced Aortic Remodeling. Cardiovasc Res, 2018.
355. Avendano MS, Martinez-Revelles S, Aguado A, Simoes MR, Gonzalez-Amor M, Palacios R, Guillem-Llobat P, Vassallo DV, Vila L, Garcia-Puig J, Beltran LM, Alonso MJ, Cachofeiro MV, Salaices M, Briones AM: Role of COX-2-derived PGE2 on vascular stiffness and function in hypertension. Br J Pharmacol 173: 1541-55, 2016.
356. Avendano MS, Garcia-Redondo AB, Zalba G, Gonzalez-Amor M, Aguado A, Martinez-Revelles S, Beltran LM, Camacho M, Cachofeiro V, Alonso MJ, Salaices M, Briones AM: mPGES-1 (Microsomal Prostaglandin E Synthase-1) Mediates Vascular Dysfunction in Hypertension Through Oxidative Stress. Hypertension, 2018.
357. Leloup AJA, De Moudt S, Van Hove CE, Dugaucquier L, Vermeulen Z, Segers VFM, De Keulenaer GW, Fransen P: Short-Term Angiotensin II Treatment Affects Large Artery Biomechanics and Function in the Absence of Small Artery Alterations in Mice. Front Physiol 9: 582, 2018.
358. Brouwers-Ceiler DL, Nelissen-Vrancken HJ, Smits JF, De Mey JG: The influence of angiotensin II-induced increase in aortic wall mass on compliance in rats in vivo. Cardiovasc Res 33: 478-84, 1997.
359. Zhou X, Wang J, Fa Y, Ye H: Signature microRNA expression profile is associated with spontaneous hypertension in African green monkey. Clin Exp Hypertens: 1-5, 2018.
360. Zhang X, Li ZL, Eirin A, Ebrahimi B, Pawar AS, Zhu XY, Lerman A, Lerman LO: Cardiac metabolic alterations in hypertensive obese pigs. Hypertension 66: 430-6, 2015.
361. Yongming P, Zhaowei C, Yichao M, Keyan Z, Liang C, Fangming C, Xiaoping X, Quanxin M, Minli C: Involvement of peroxisome proliferator-activated receptors in cardiac and vascular remodeling in a novel minipig model of insulin resistance and atherosclerosis induced by consumption of a high-fat/cholesterol diet. Cardiovasc Diabetol 14: 6, 2015.