The development of the spontaneously hypertensive rat by or simply an extreme of the gaussian distribution of BP.2

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humans, the trait is normally distributed; therefore, the distinc-
tion between SS and SR members of the population has been
made by choosing an arbitrary magnitude of the salt-induced change in BP to define the groups. Regardless of possible causation by abnormalities of sodium handling, the SS phenotype is not usually characterized by alterations in salt balance (eg, impaired natriuresis or expanded plasma volume) but rather by a hypertensive response to maintain it.

In an unselected population, SSBP is a continuous, normally distributed quantitative trait.1 As with any other trait with these characteristics, there is the issue of whether population members with the largest and smallest quantities of the trait represent the randomness of its distribution or are qualitatively different from the population at large. An example of this controversy is the old analyses of the unimodality versus bimodality of BP incidence or prevalence in humans that tried to determine whether hypertension is a distinct entity or simply an extreme of the gaussian distribution of BP.2

The development of the spontaneously hypertensive rat by Japanese investigators3 showing that the trait could be selected by inbreeding made it clear that hypertension had a genetic component. The gaussian distribution of population BP is probably the result of a random mixture of prohypertensive and antihypertensive genes and genetic variants in a heterogeneous population interacting with environmental factors (eg, diet), physiological characteristics (eg, aging), and clinical features (eg, renal function).

Analogously, there were indirect clues suggesting genetic determination of SSBP: The trait was reproducible when measured with different methods4 or when repeatedly measured over time5; concordance in characteristics associated with the SSBP phenotype (eg, magnitude of natriuresis and BP and plasma renin responses after salt depletion) was shown in non-
twin6 and twin7 siblings; and other demographic, clinical, and biochemical characteristics clustered with the SS phenotype. However, it was not until the development of inbred strains of rodents (described below) homogeneous for SSBP or salt resistance of BP that definitive proof of a genetic component was provided.

Research on SSBP in humans is more complex than that in dichotomized SS and SR rodent strains. The reason is that methodological issues such as random error in BP measurements and physiological issues such as the multiple sources for BP variability may confound the assessment of the BP responses to salt loading or salt depletion. In addition, as shown in Figure 1,4 SSBP of humans is a continuous, normally

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distributed trait. Therefore, defining an individual as SS or SR depends on the selection of arbitrarily chosen cutoffs for the magnitude of the BP changes. Environmental factors substantially affect whatever the genetic component may be for SSBP in humans. Figure 2 shows the major effect of aging in increasing the prevalence of SSBP in both normotensive and hypertensive subjects. An important and encouraging observation is that the multiple phenotypic characteristics described in pure SS strains of rodents reproduce those observed in humans, indicating that the phenotypic cluster of SSBP can be brought out in humans by the current techniques used in carefully controlled research. Additionally, despite the unquestionable influence of environmental factors in the determination of SSBP in humans, estimates of its heritability have been as high as 74% in blacks and 50% in Chinese subjects, both higher than those for hypertension.

An important issue is the clinical significance of the SSBP phenotype. There was increasing understanding that it represents an abnormality. The reasons were that it contradicts the basic physiological tenet that salt balance can be maintained by natriuretic and antinatriuretic systems independently of BP, it occurs less frequently than salt resistance in normal subjects, and is associated with several forms of human and experimental hypertension. Finally, definitive proof that SSBP is an abnormal phenotype was provided by 2 long-term studies in normotensive and hypertensive subjects that demonstrated that it is a risk factor for cardiovascular mortality and morbidity.

Studies of salt sensitivity in rats were pioneered by Dahl, inspired by ecological and epidemiological studies of the association of salt intake with human hypertension. Dahl et al selected Sprague-Dawley rats with the highest BP response to a high-salt diet (facilitated by triiodothyronine administration) and mated them with equally responsive siblings. After few generations, an SS strain with a consistent hypertensive response to a high-salt diet was created. Contrary to common belief, this was not a pure inbred strain because its descendants were also outbred with other SS Sprague-Dawley rats as a result of breeding problems and small litters. Additionally, Sprague-Dawley rats without a hypertensive response to a high-salt diet were inbred to produce the SR strain. From the creation of these strains and the demonstration that a donor SS kidney transplanted into an SR rat conferred SSBP to the recipient (and vice versa), a major role for a renal abnormality was hypothesized as the factor determining the phenotype. Later, Rapp and Dene developed the fully inbred DS/Jr and DR/Jr strains that have subsequently been used by most researchers. The original SS and subsequent DS/Jr rats developed fulminant hypertension when exposed to a high-salt (8%) diet and died by the age of 8 weeks. They had a plethora of vascular lesions, renal fibrosis, and cardiac hypertrophy. Several investigators reported a variety of physiological abnormalities contributing to hypertension in DS/Jr rats, among them differences in cellular ion transport and concentration, enhanced sympathetic activity and blunted baroreflexes, reduced renal medullary blood flow, disturbed balance between vasoconstrictors and vasodilators with a special role for nitric oxide (NO), enhanced oxidative stress, and activated Rac1 GTPase mineralocorticoid receptor interaction.

In 1972, Ben-Ishay et al established a new model of experimental SS hypertension by inbreeding regular Sabra rats (Wistar substrain from Hebrew University) that were high-BP responders to deoxycorticosterone acetate (DOCA)-salt administration and designated them Sabra hypertensive (SBH). Nonresponders were also inbred and designated Sabra normotensive (SBN). Again, numerous physiological differences between SBH and SBN were found, including...
alterations in baroreflex function, sympathetic nervous system activity, adrenocorticotrophic hormone, vasopressin, metabolism of water and cations, ouabain-like activity, and NO expression and function. SBNs were resistant not only to DOCA-salt but also to renal artery clipping. Because of partial outbreeding and a lack of quality controls, Yagil et al. attempted to purify the colony and to re-establish its genetic and phenotypic homogeneity. These substrains, called SBH/y and SBN/y, had no spontaneous hypertension, and their SSBP was independent of gonadal hormones and aging. Although selected for inbreeding on the basis of their response to DOCA-salt, they were shown to be equally sensitive to 8% salt diet, making them comparable to the DS/Jr strain. SBH/y and SBN/y exhibited differences in oxidative stress, epithelial sodium channel, and leukocyte priming through tumor necrosis factor activity, among many other abnormalities.

Other rat models originally inbred for hypertension were also found to have a component of genetically determined salt sensitivity, for instance, the stroke-prone spontaneously hypertensive rat (SHRSP). In these animals, salt loading exacerbated hypertension and target organ damage. Lyon hypertensive rats, inbred for spontaneous hypertension not as severe as that of SHRSP, were also found to be SS. The Milan strains developed by Bianchi et al., inbred for spontaneous hypertension and normotension, differed by Na\(^+\) retention in the Milan hypertensive strain during the 3 weeks after weaning, during which time they sustained a 40–nmol Hg increase in BP. SSBP was also demonstrated by a significant antihypertensive response to diuretics. Kidney cross-transplantation experiments in this strain produced results analogous to those observed in Dahl S and R rats. Increased renal Na\(^+\)-K\(^+\)-ATPase activity was related to specific \(\alpha\)-adducin mutations that reduce Na\(^+\)-K\(^+\)-ATPase endocytosis, leading to increased tubular Na\(^+\) reabsorption. Activity and expression of other transporters (Na\(^+\)K\(^+\)2Cl\(^-\) and aquaporins) were also increased, as were the activities of endogenous ouabain-like compounds and the sympathetic nervous system.

Inbreeding of rabbits for high or low baroreflex heart rate responsiveness by Weinstock and Borosh produced a strain with genetically impaired baroreflex function. These rabbits develop hypertension when fed a high-salt diet that is associated with absent rise in renal plasma flow, impaired natriuresis, and increased proximal tubular Na\(^+\) reabsorption, all of which are dependent on sympathetic function as shown by reversibility with renal denervation.

The effect of varying salt consumption in chimpanzees was an overall increase in BP with high-salt intakes and an overall reduction with low intakes. Interestingly, increases in sodium intake from a magnitude consumed by primitive human societies (35 mmol/d) to levels that would be considered a relatively low-salt diet now in the Western world (120 mmol/d) were enough to increase the average population BP. Conversely, decreasing salt intake from that observed in many populations worldwide (248 mmol/d) to one below the current average in the United States (126 mmol/d) was enough to decrease the average population BP. However, from the confidence intervals of the BP changes, it is clear that some animals exhibited large whereas others showed minimal changes in BP with the dietary interventions. No specific attempt at classifying these chimpanzees as SS or SR was made in this work, but these responses suggest that the situation is akin to that in humans, in whom the phenotypes are defined on the basis of arbitrary cutoffs for the changes in BP. Although results in chimpanzees would intuitively be perceived as more applicable to humans than those in rodents, changes in BP in chimpanzees were observed over the period of several months, which limits extrapolation of these studies to humans, in whom physiological studies for classification of individuals into SS and SR groups were usually carried out over a period of days to a few weeks.

Figure 2. Mean arterial pressure decreases in response to salt depletion in normotensive (open bars) and hypertensive (hatched bars) individuals at different age decades. Asterisks indicate statistical significance for the larger blood pressure responses of hypertensive than normotensive subjects in some decades. The progressively larger bars from left to right indicate that the influence of aging increased these responses in both normotensive and hypertensive individuals. Reprinted from Weinberger and Fineberg. Copyright © 1991, American Heart Association, Inc.
The fact that rat strains inbred for SSBP may develop spontaneous hypertension (eg, Dahl SS and DS/Jr) whereas others inbred for hypertension are also SS (eg, SHRSP, Lyon hypertensive, and Milan hypertensive rats) makes it clear that the overlap and complexity of both phenotypes interfere with the identification of their causative mechanisms. An example is the presence of abnormal baroreflex in SS rat strains (DS/Jr, Milan hypertensive, SBH, SHRSP rats), deemed secondary to hypertension, whereas in the rabbits of Weinstock and Borosh, selected for a genetic primary abnormality of the baroreflex, SS hypertension is brought about as a secondary phenomenon.

Nonetheless, the dichotomization into SS and SR strains of rats has facilitated research in the area, taking into consideration the additional difficulty of conducting research in humans, in whom SSBP is a continuous trait without an obvious threshold and with a strong influence of age and baseline BP level.30

Physiology of SSBP
Research into the possible physiological mechanisms determining SSBP has been driven mostly by a conceptual framework derived from the work of Guyton and coworkers. The major tenet of such framework is that 1 or many mechanisms that normally regulate the adaptation of the cardiovascular system to a salt load must be impaired in SSBP. This somehow leads to the need for the whole animal to raise BP to excrete the salt load via pressure natriuresis. The result is that an SS animal or human being will be able to maintain a normal salt balance at the expense of developing hypertension, the main feature of SSBP. Obviously, the putative defect can involve a variety of mechanisms. Activation of a natriuretic system required to excrete a salt load (eg, natriuretic peptides, renal eicosanoids) may be impaired, or conversely, lack of physiological suppression of an antinatriuretic system in response to a salt load (eg, mineralocorticoid or renal transport activity) might be the culprit. The concept that “clamping” (ie, lack of regulation) of these systems leads to conservation of salt balance (Na balance) somehow requires an elevation in BP for maintenance of normal Na transport activity) might be the culprit. The concept that “clamping” (ie, lack of regulation) of these systems leads to conservation of salt balance (Na balance) somehow requires an elevation in BP for maintenance of normal Na balance at the expense of developing hypertension (Figure 3).32 A brief summary of abnormalities described in natriuretic and antinatriuretic systems in SS humans by many other investigators follows.

1. The renin-angiotensin-aldosterone system: The group of MacGregor (Parfrey et al35) showed that SSBP (assessed by the fall in mean arterial pressure [MAP] per unit urine sodium excretion after dietary salt reduction) increased in prevalence from normotensive to mildly hypertensive to severely hypertensive subjects and was inversely related to the plasma renin response to salt depletion. They suggested that a blunted renin response to salt depletion might be responsible for the decrease in BP in SS subjects, as supported by the observation that saralasin decreased BP in subjects with preserved but not in those with blunted renin responses to salt depletion.36 Years later, the same group showed that diminished renin, angiotensin II, and aldosterone responses to salt depletion were more prominent in blacks than in whites37 and in hypertensive whites than in normotensive whites.38 In both cases, these observations accounted for the concomitant greater BP decrease during salt depletion in blacks than in whites and in hypertensive compared with normotensive whites. In a study in which SSBP was assessed with acute and chronic protocols in the same subjects, Weinberger et al4 also showed that the renin response during acute salt depletion correlated negatively with the subsequent depressor responses to a low-salt diet. Similar to diminished stimulation of renin by salt depletion, SS subjects also had blunted suppression of renin in response to a salt load in some studies.39 This may be related to their excessive BP response to salt loading because preventing the decrease in angiotensin II or aldosterone that normally follows salt loading (eg, by infusion of these substances to maintain presalt levels) prevents the expected ensuing natriuresis.40 This bidirectional dampening of renin responses to changes in salt intake in SS has been confirmed by other groups,41–43 establishing that a blunted renin-angiotensin system is a phenotypic characteristic of SSBP. This is consistent with the conceptual framework of clamped pressor systems proposed by Guyton.
The research group of Williams and Hollenberg characterized a group of subjects who did not have increased aldosterone in response to either salt deprivation or angiotensin infusion and did not have increased renal blood flow in response to salt (nonmodulators). They showed that such subjects were likely to exhibit SS hypertension, although not all nonmodulators were SS (Rydstedt et al).55 In addition, whereas salt deprivation increases endogenous angiotensin II levels and decreases sensitivity to exogenous angiotensin II (via receptor occupancy or downregulation) in normal subjects, in normotensive and hypertensive SS subjects, sensitivity to exogenous angiotensin II is maintained or even increased after salt depletion.55

2. The endothelin system: Normally, urinary endothelin exhibits a circadian rhythm and correlates negatively with BP in normal and hypertensive subjects but positively with Na+ excretion during a salt load. SS hypertensives have diminished levels of urinary endothelin, which may contribute to their impaired natriuresis in response to a salt load.46

3. NO and oxidative stress: In SS hypertensives, a salt load increases free isoprostanes47 and paradoxically decreases excretion of NO metabolites, which normally increase in response to salt loading. This suggests that in such subjects, NO is diverted to the scavenging of salt-induced free radicals or that salt-stimulated production of an endogenous inhibitor of NO may play a role in SS hypertension. A similar situation may occur in patients with type 2 diabetes mellitus who have microalbuminuria. They are more SS than those without microalbuminuria and exhibit lower urinary excretion of NO.48 The latter is increased by valsartan. Therefore, reduction of NO scavenging by oxidative stress is a plausible explanation. In addition to NO scavenging, defects in NO production may be present in SS subjects. For example, SS blacks sustain greater BP reduction and smaller increases in renal blood flow when given intravenous l-arginine compared with SR or normotensive controls. This putative NO deficit may be responsible for their endothelial dysfunction, which in turn may contribute to SS hypertension by impeding vasodilation after a salt load.49

4. The sympathetic nervous system: Several investigators showed that in an SS strain of SHR, the pressor response to salt was associated with increased levels of plasma and urine catecholamines50 and renal nerve activity,51 whereas alterations in hypothalamic norepinephrine turnover with decreased norepinephrine content suggested diminished central sympathetic inhibition of peripheral sympathetic outflow.52,53 The interpretation was that the sympathetic nervous system was involved in the genetically determined pressor response to salt. However, other genetic SS strains have provided opposite findings. For example, renal nerves did not play a role in the SS hypertension of Dahl-S rats,54 and the hypothalamic levels of norepinephrine during a salt load were increased in this strain.55

In SS hypertensive subjects, plasma catecholamines do not decrease in response to a salt load as they do in normotensive or SR hypertensive subjects, likely contributing to the hypertensive response to salt.56–60 In contrast, plasma catecholamine responses to salt depletion are exaggerated in SS compared with SR hypertensive subjects, perhaps reflecting sympathetic stimulation by the decrease in BP.47,61 Renal natriuretic dopamine fails to be normally stimulated whereas renal norepinephrine fails to be normally inhibited during a salt load in SS hypertensive subjects, leading to an altered urine ratio between these catechols that may be related to impaired sodium excretion.62 The pressor response to exogenous norepinephrine is greater in SS than SR hypertensive subjects, whether on low- or high-salt intake,63,64 suggesting that increased vascular reactivity to catecholamines contributes to the maintenance of SS hypertension. All these observations suggest that sympathetic hyperactivity plays a role in human SS hypertension.

Further indirect support comes from patients who undergo cardiac transplantation who develop SS hypertension on cardiac denervation. Although other factors may contribute (immunosuppressant drugs, corticosteroids, renal dysfunction, etc.), this observation suggests that normal sympathetic innervation of the heart plays a role in the hemodynamic adaptation to dietary salt loading. Finally, normotensive subjects with a behavioral phenotype characterized by low anxiety scores but increased self-deception and increased autonomic responses to mental stress have associated SS hypertension, suggesting that increased autonomic reactivity (in contrast to reduced sympathetic tone as above) may also affect salt handling or its effect on arterial pressure.65

5. Atrial natriuretic peptides (ANPs): The SS substrain of SHR fails to respond to acute volume expansion or dietary salt supplementation with an increase in ANP compared with SR SHR or Wistar-Kyoto controls.66 An infusion of ANP that increases plasma levels to that of the controls prevents the SS component of the hypertension in this strain.67 These rodents have increased levels of ANP in the anterior hypothalamus, where they inhibit norepinephrine release, facilitating sympathetic outflow to the periphery,68 whereas in sympathetic ganglia, where ANP normally inhibits neural transmission, they have reduced levels in response to salt loading.69 Interpretation of these data in SS SHR was that genetically determined abnormalities in natriuretic peptide responses to changes in salt balance may be involved in the pathogenesis of SS hypertension. However, in genetic Dahl rat hypertension, some investigators have found no difference in plasma ANP or in its response to salt loading between the SS and the SR strains,69 whereas others have shown, contrary to the observations in SHR, that the SS strain has increased ANP levels compared with SR controls.70 This increase occurs once hypertension is established and severe71 and responds differently to the antihypertensive effect of agents that increase or decrease cardiac volume overload.72 Therefore, it seems that in Dahl rats there is a compensatory, not a pathogenic role, for ANP in SS hypertension.

An analogous situation characterizes research results on the role of ANP in human SS hypertension. Observations supporting a pathogenic role for ANP
include paradoxical decreases in plasma ANP of SS black hypertensive subjects in response to a high-salt (250 mEq/d) diet; blunted ANP responses to acute volume expansion by saline infusion in SS hypertensive subjects, particularly when preceded by a 5-day high-salt diet (200–220 mEq/d); and prediction of SSBP in the Framingham Offspring Cohort by lower levels of circulating N-terminal ANP. In contrast, other investigators have failed to detect differences in the ANP responses to high-salt intake between SS and SR subjects, and some actually detected significantly higher levels of ANP in SS than SR, whether on high- (220 mEq/d) or low- (20 mEq/d) salt diets.

Finally, opposite to the observations on N-terminal ANP in the Framingham cohort, increased levels of plasma pro-ANP have been shown to be predictors of SSBP in normal volunteers and prehypertensive subjects. This controversial information in humans can be reconciled only if ANP plays a pathogenic role in the BP response to salt in some SS subjects, whereas it is stimulated as a compensatory response to hypertension in others, analogous to the observations in different genetic SS strains of rats.

6. CYP450-derived metabolites of arachidonic acid: Two major products of this pathway, the vasoconstrictor 20-hydroxyeicosatetraenoic acid (20-HETE; produced by omega hydroxylases) and the vasodilator epoxyeicosatrienoic acids (EETs; produced by epoxygenases), are natriuretic agents in different parts of the renal tubule acting on different transporters. In Dahl-SS rats, a deficit in the synthesis of renal medullary 20-HETE has been shown to participate in SSBP. SS hypertensive subjects do not have reduced urinary excretion of 20-HETE but exhibit a loss of its normal relationship with Na+ excretion, an abnormality that resembles that of young SS Sprague-Dawley rats. In contrast, SS hypertensive patients have significantly lower plasma and urine EETs during low-salt intake compared with SR hypertensive patients. Fenoﬁbrate, an inducer of the cytochromes that produce 20-HETE and vasodilatory EETs in experimental animals, did not increase 20-HETE in these subjects but prevented inhibition of plasma and urine EETs by high salt in SS subjects. This resulted in a reduction of BP, heart rate, and renal vasoconstriction in SS subjects during high-salt intake, effects of fenofibrate not observed in SR hypertensive subjects. Plasma renin activity during a low-salt diet did not differ between SS and SR subjects in this study. Therefore, the known stimulatory effect of angiotensin II on the expression of soluble epoxide hydrolase (the enzyme that degrades EETs into inactive metabolites) cannot be responsible for the differences between SS and SR unless there are differences in other mechanisms of enhanced degradation of EETs by angiotensin-stimulated soluble epoxide hydrolase.

7. Hyperinsulinemia: Normotensive and hypertensive SS subjects are more insulin resistant than their SR counterparts, independently of BP. This is associated with hyperinsulinemia. Whether the stimulatory effect of insulin on tubular sodium re-absorption, sympathetic activity, or vascular remodeling contributes to the development of SS hypertension is not definitely known. However, insulin levels accentuate the effect of risk alleles of CYP4A11 in determining SSBP in humans.

Guyton’s conceptual framework also requires a mechanism by which a subtle degree of salt retention (usually not detected in balance studies) is translated into a pressor response. Hypotheses about total body autoregulation have been suggested over the years. For example, a longitudinal hemodynamic follow-up of patients with early essential hypertension documented the conversion of a high-cardiac-output state (early hypertension) into one of long-term normalization of cardiac output with increases in total peripheral resistance (late established hypertension). Although conceivably this could also take place over a shorter term, this “autoregulation” has not been observed in rats over 1 week of salt loading or in humans during a 7-day dietary protocol or over 24 hours using the Indiana University protocol for assessment of SSBP. In the human studies, both SS and SR subjects sustain equal increases in cardiac output in response to salt, but the characteristic abnormality of SS is an increase in total peripheral resistance.

The mechanism by which a defect in salt handling may lead to acute changes in vascular smooth muscle tone is not easily conceived within the traditional framework of sodium being distributed into intracellular, extracellular, and intra-vascular compartments. In addition, Guyton’s hypothesis is based on the premise that all mechanisms of salt handling are geared toward maintaining sodium excretion parallel to sodium intake (ie, achieving constant salt balance). Two major recent observations question this conventional knowledge. First, sodium can be stored in hyperosmolar concentrations (or at least without iso-osmolar water) in certain tissues such as skin and muscle, with a behavior different from that in the extracellular space. Second, in the longest-ever metabolic balance study in humans to date, salt excretion was subject to a circaseptan rhythm, completely independent of a constant salt intake. It is theoretically conceivable that alterations in compartmental storage or rhythmicity of sodium excretion may be factors involved in the pathophysiology of SSBP.

Genetics and SS Phenotype in Rodents

This section contains a review of information on putative genetic factors producing the SS phenotype in rodents to illustrate the complexity of the topic, which is relevant to the development of targeted therapies for the phenotype in humans. It is to be noted that the experimental protocols used in these studies (magnitudes of salt loading and salt depletion, route of administration, duration of the intervention, age of the animals, and concomitant interventions such as reduction of renal mass and use of diverse steroid hormones) varied widely. MEDLINE searches were conducted, crossing the terms defining the phenotype (salt and hypertension, salt sensitivity of BP) with those of the rodent strains of interest (inbred rat strains, Dahl rats, Lyon rats, Milan rats, Sabra rats) and those of gene research methodologies (consomic strains, congenic
strains, knockout mice, transgenic mice, interfering RNAs, etc. Of the >550 articles retrieved by these searches, 157 were chosen for review, excluding those dealing with hypertension in general (not SSBP) or those with inferred gene roles from physiological studies (not direct genetic studies).

The development of the techniques of linkage analysis and identification of quantitative trait loci for phenotypic features stimulated the search for genetic causes underlying SSBP in rats. Study of the role and gene composition of identified quantitative trait loci followed, through the creation of consomic or congenic strains in which putative causative or protective chromosomes or chromosome regions were introgressed from a control to an SS strain or vice versa. With the Dahl-SS strain used as the genetic background, concosomic strains harboring chromosome 9 from the Dahl-SR strain\(^95\); chromosomes 13,\(^{96} 12,^{97} 16,^{98} 1, 5, 7, 8, 13, \) and 18 from the Brown Norway\(^99\); or chromosome 10 from the Lewis\(^100,101\) normotensive strains showed protection from SS hypertension. Analogous results were observed by introgressing chromosome 2 of the Wistar-Kyoto strain into SS SHRSP\(^102\); chromosome 13 of the Brown Norway strain into the Lyon\(^103\) hypertensive strain, chromosome 1 (adducin-a gene) from the Milan normotensive to the hypertensive strain\(^104\), or chromosomes 15 and 20 of the Brown Norway into the Fawn Hooded rat with salt-induced hypertension\(^105\). Many of these observations detected sexual dimorphism in the chromosomal effects, and some characterized the mechanism for the antihypertensive effect of protective introgressed chromosomal segments (eg, the segment of chromosome 2 of the Brown Norway strain that improves SS hypertension in Dahl SS reduces inflammation and shifts the balance between CD4\(^+\) and regulatory T cells\(^106\)).

Interpretation of findings obtained with consomic strains is not straightforward. For example, the sum of the decreases in BPs for the nearly 30 loci identified in the autosomal chromosomes of the Dahl-SS rat is an order of magnitude greater than the total difference between the BP of the Dahl-SS and the control strain, demonstrating that epistasis is a major confounder\(^107\). Furthermore, hypertension suppressor quantitative trait loci of normotensive strains may be major modulators of the effect of hypertensive quantitative trait loci, to the point of almost abolishing the BP effect of their introgression into the normotensive strains\(^108\).

A role for narrower DNA segments was investigated by the creation of congenic strains with introgressed overlapping regions of these chromosomes. A combination of these experiments and the study of differential expression of genes between the congenic and parental strains, detection of non-synonymous single nucleotide polymorphisms in the regions of interest, and transcriptomic analysis has revealed a number of possible candidate genes\(^96,97,100,101,109,110\) the majority of which have no previously known cardiovascular regulatory function. However, even with high-resolution substitution mapping, there are usually additional variants flanking a substituted candidate gene, requiring genetic engineering to sort out the significance of the finding\(^111\). Furthermore, chromosomal regions without coding variants or with variants in intrinsic segments that may have possible regulatory functions have also been associated with hypertension, but the mechanism of the association remains obscure\(^112-114\).

Results of this approach make it clear that the SS phenotype of rats is determined by multiple different genes or by the combination of their effects into a polygenic form of inheritance. An additional contribution of this methodology has been the understanding that components of the SS phenotype (eg, insulin resistance\(^115\)) or the magnitude of its cardiac\(^98,116\) and renin\(^99,117-119\) target organ damage may be determined by genetic influences distinct from those that control the hypertensive response to salt.

With the advent of rapid and inexpensive DNA sequencing techniques and an explosion in the generation of knockout and transgenic models in mice, research has shifted to exploring possible roles for individual genes in SS hypertension. Studies have focused on genes that encode proteins in traditional cardiovascular regulatory (pressor and depressor) systems or renal transporters and their regulatory proteins. A summary of findings with possible relevance for understanding or treating the phenotype in humans is provided in the Table. In addition to the gene or gene product, the species or strain, and the genetic manipulation studied, the Table provides a very abbreviated summary of the findings with the references to access their full description.

Interpretation of the results of these studies, however, is complex. For example, genes that cause extremely rare forms of human monogenic hypertension (eg, epithelial sodium channel, WNKS, mineralocorticoid receptor, \(11\beta\)-hydroxysteroid dehydrogenase) cannot possibly explain the high frequency of salt sensitivity in humans. However, mechanistic information in the Table is pertinent because a possible role for minor variants with small effects, distinct from the disease-causing mutations, cannot be excluded, analogous to the significant effects on population BP exerted by genes that cause human monogenic hypotension\(^251\). Additionally, spontaneous overexpression of genes and overactivity of their products in the Dahl S strain are candidates for SSBP, but the evidence is strong only when these genes affect SSBP in other rat strains or another species or when there is a signal for a role in humans in large population studies. Moreover, knockout of genes that regulate natriuresis or antinatriuresis in mice and produce or prevent SSBP demonstrates that the affected system is involved in the regulation of BP and salt excretion but not necessarily in the causation of SSBP. Finally, the knockout of some genes (eg, renal angiotensin-converting enzyme or inflammatory cytokines) prevents SSBP in models with preceding renal damage (angiotensin II infusion or \(l-N^\circ\)-nitroarginine methyl ester administration), not by themselves. Transgenic mice perhaps provide the strongest evidence when introduction of the putative causative or protective gene into their genomes confers SSBP or SR phenotypes. Examples in the Table include production of SSBP by transfection with genes that regulate \(Na^+\) excretion in different portions of the nephron, including ARAP1, uromodulin, and GRK4.

In summary, most genetic abnormalities detected to date in SS hypertensive rodents relate one way or another to the regulation of natriuresis. However, novel pathways, including regulation of regional blood flows, cutaneous sodium storage, and innate immunity, are starting to emerge\(^70,155,156\) in part as the result of research enabled by the modern techniques of genetic investigation.
Table. Summary of Research on Genetic Observations Linked to SSBP in Experimental Animals

<table>
<thead>
<tr>
<th>Gene or Gene Product</th>
<th>Strain or Species</th>
<th>Genetic Observation or Intervention</th>
<th>Phenotypic Observation and/or Mechanism</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Renin-angiotensin system</td>
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<tr>
<td>Renin</td>
<td>Dahl, Milan, Lyon, Sprague-Dawley rats</td>
<td>Gene variants</td>
<td>Although gene variants are present in Dahl-SS vs SR, they are not found in Milan rats, do not relate to phenotype in Lyon rats, and are not more common in the Dahl than in the Sprague-Dawley strain.</td>
<td>120–123</td>
</tr>
<tr>
<td>Collectrin</td>
<td>WKY</td>
<td>siRNA knockdown</td>
<td>This homolog of ACE2 is a candidate for SSBP because it is involved in paradoxical conservation of proximal tubule Na⁺ reabsorption during a salt load (via membrane localization of AQP2, αENaC, and H⁺-ATPase). Na⁺ increases its promoter activity, mRNA, and protein. Its knockdown blunts promoter upregulation in response to salt.</td>
<td>124</td>
</tr>
<tr>
<td>Collectrin</td>
<td>Mice</td>
<td>Knockout</td>
<td>In addition to impaired natriuresis, knockout of collectrin results in SS hypertension with impaired endothelium-dependent vasorelaxation related to uncoupling of NO synthase and increased oxidative stress. Tempol attenuates SS in the knockout, confirming a role for vascular collectrin in SS hypertension.</td>
<td>125</td>
</tr>
<tr>
<td>Renal ACE</td>
<td>Mice</td>
<td>Expression in nonrenal tissues only</td>
<td>Lack of renal ACE prevents SSBP produced by renal injury (L-NAME or AngII infusion). It reduces renal AngII accumulation and the activity of Na⁺-transporters (NKCC2, NCC, ENaC, and pendrin) and the kinases SPAK and OSR1. It blunts AngII-induced antinatriuresis and hypertension.</td>
<td>126–128</td>
</tr>
<tr>
<td>Aminopeptidase N</td>
<td>Dahl-SR</td>
<td>Upregulated in Dahl-SR given salt</td>
<td>This enzyme generates proximal tubule natriuretic angiotensin IV (an inhibitor of ouabain-sensitive Na⁺-K⁺-ATPase, which reduces blood pressure in the rat). It plays a role in adaptation to high salt. Its transcript/protein abundance and its activity are greater in Dahl-SS than in SR, related to an SNP in the 5-flanking region that maps to a QTL described in Dahl-SS–Lewis cross. Upregulation in Dahl-SS probably confers salt resistance.</td>
<td>129</td>
</tr>
<tr>
<td>AT2 receptor</td>
<td>Mice</td>
<td>Knockout</td>
<td>Knockout exhibits rightward shift of pressure natriuresis (ie, loss of protective effect) involving effects on AT1 receptor and renal eicosanoids.</td>
<td>130</td>
</tr>
<tr>
<td>ARAP1</td>
<td>Mice</td>
<td>Transgenic</td>
<td>Overexpression increases AT1 receptor recycling to the proximal tubule membrane and Na⁺ reabsorption, leading to SS hypertension.</td>
<td>131</td>
</tr>
<tr>
<td>Mas receptor</td>
<td>Mice</td>
<td>Knockout</td>
<td>Knockout shows paradoxical natriuresis and protection from SS hypertension.</td>
<td>132</td>
</tr>
<tr>
<td>AngII-AVP dual receptor</td>
<td>Dahl rats</td>
<td>Mutations in Dahl-SS</td>
<td>Mutations in Dahl-SS increase binding to both agonists and receptor signaling; they cosegregate with BP in the F2 cross of Dahl-SS and SR.</td>
<td>133</td>
</tr>
<tr>
<td>Prorenin receptor</td>
<td>Mice</td>
<td>Neuron-specific knockout</td>
<td>In wild-type mice, ICV prorenin increases brain AngII formation and BP. These responses are abolished in the neuron-specific knockout. DOCA-salt fails to increase BP and sympathetic stimulation in these animals, with diminished expression of AngII. Hence, nonproteolytic activation of prorenin by binding to the prorenin receptor mediates AngII formation in the brain and DOCA-salt hypertension.</td>
<td>134</td>
</tr>
</tbody>
</table>

Sympathetic nervous system

| β2AR | Mice | Knockout | Na⁺ increases renal sympathetic activity and activation of β2AR decreases transcription of the WNK4 gene via effects on promoter acetylation. Inhibited WNK4 increases phosphorylated NCC, with increased Na⁺ reabsorption. Participation of this pathway in SSBP is supported by the abnormal excessive NE turnover during Na⁺ load in Dahl-SS rats (with inhibited WNK4 and increased phosphorylated NCC) and by the protection from SS hypertension conferred to the knockout β2AR mice or by transfecting WNK4 siRNA to the distal convoluted tubule. | 135 |
| WNK4 | Mice | Transfected siRNA to distal convoluted tubule | Salt infusion | |
| WNK4 | Dahl-SS and DOCA-salt | | | |
| Gαi2 | Sprague Dawley | ICV inhibitory oligonucleotide | Oligonucleotide produces Na⁺ retention, hypertensive, and increased plasma NE; indicating a protective role of SNS inhibitory G proteins against salt-induced hypertension. | 136 |
| Gαi2 | Dahl, Brown Norway, and congenic | ICV inhibitory oligonucleotide | Salt increases PVN Gαi2 and suppresses NE in the SR strains (Dahl-SR, Brown Norway, and a consomic) but not in Dahl-SS. The scrambled Gαi2 oligonucleotide produces renal nerve-dependent SS hypertension in Dahl-SR. Analogous to above, a PVN Gαi2 pathway mediates sympathoinhibitory responses to maintain salt resistance. | 137 |
| TRPV1 | Rat | Intrathecal shRNA | Silencing this channel produces SS hypertension with increased vascular tyrosine hydroxylase, indicating that it protects against Na⁺ loading by suppressing sympathetic tone. | 138 |

(Continued)
### Endothelin system

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<tr>
<th>Gene or Gene Product</th>
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<th>Genetic Observation or Intervention</th>
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<tbody>
<tr>
<td>ET-B receptor</td>
<td>Rat</td>
<td>ET-B deficient, rescued homozygous mutant</td>
<td>Rats develop severe SS hypertension, not corrected by ET-A receptor blocker but by amiloride instead, indicating a role for the ET-B receptor in natriuresis mediated by inhibition of ENaC.</td>
<td>139</td>
</tr>
<tr>
<td>ET-B receptor</td>
<td>Mice</td>
<td>Collecting duct–specific knockout</td>
<td>Knockout loses the ability to reduce the open probability of ENaC in response to salt, normally mediated by ET-1 stimulation of ET-B; hence, ENaC hyperactivity follows, with increased Na+ reabsorption and SS hypertension.</td>
<td>140</td>
</tr>
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### Prostaglandins

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<tbody>
<tr>
<td>PGE synthase-1</td>
<td>Mice</td>
<td>Knockout</td>
<td>Knockout loses normal PGE2 antagonism of aldosterone action (ie, aldosterone escape), with consequent Na+-induced hypertension.</td>
<td>141</td>
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### Kinins

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</thead>
<tbody>
<tr>
<td>BK-B2 receptor</td>
<td>Mice</td>
<td>Knockout</td>
<td>Knockout loses normal stimulation of prostaglandins and NO by BK, with loss of their compensatory effects on natriuresis and renal vasodilation in response to salt.</td>
<td>142</td>
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### Natriuretic peptides

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<tr>
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<tbody>
<tr>
<td>Corin</td>
<td>Knockout mice</td>
<td>Transgenic expression in heart</td>
<td>Transgenic variant gene leads to defective processing of prepronatriuretic peptides with diminished natriuresis, SS hypertension, and left ventricular hypertrophy.</td>
<td>143</td>
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</table>

### NO

<table>
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<tr>
<th>Gene or Gene Product</th>
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<th>Genetic Observation or Intervention</th>
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<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>iNOS2</td>
<td>Dahl rats</td>
<td>Mutation in Dahl-SS</td>
<td>Mutation produces a defect in NO synthesis at the protein (not mRNA) level in response to salt; this SS hypertension is prevented by arginine.</td>
<td>144</td>
</tr>
<tr>
<td>NOS1</td>
<td>Mice</td>
<td>Collecting duct–specific knockout</td>
<td>Mice have decreased production of nitrite and urinary Nox excretion; on high dietary salt, they have reduced urine volume, natriuresis, and Nox, with SS hypertension.</td>
<td>145</td>
</tr>
<tr>
<td>Caveolin-1</td>
<td>Mice</td>
<td>Knockout</td>
<td>Caveolin-1 normally limits activation of eNOS. The knockou has increased expression and phosphorylation of eNOS in heart and vessels and decreased vascular contractility with enhanced endothelium-dependent dilation during high salt. These vascular protective effects from salt loading are confirmed by the development of a more severe form of L-NAME hypertension in the knockout compared with wild-type mice.</td>
<td>146</td>
</tr>
<tr>
<td>Lysine-specific demethylase-1</td>
<td>Mice</td>
<td>Heterozygous knockout</td>
<td>Knockout of this regulator of DNA methylation and gene transcription leads to SS hypertension as a result of increased vascular contractility and decreased relaxation via the NO-cGMP pathway owing to diminished expression of eNOS mRNA and protein in heart and aorta.</td>
<td>147</td>
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</table>

### Oxidative stress

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<tr>
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</thead>
<tbody>
<tr>
<td>NOX2</td>
<td>Mice</td>
<td>Knockout</td>
<td>Deficit of NOX enzymes by knockout or by silencing in PVN blunts the aldosterone/salt-induced increase in ROS and hypertension; this blunting is reduced by ganglionic blockers, indicating that brain NOXs contribute to SS via increased SNS activity.</td>
<td>148</td>
</tr>
<tr>
<td>NOX2 and NOX4</td>
<td>Mice</td>
<td>siRNAs to the PVN</td>
<td>This contributes to oxidative stress and SS hypertension in the strain; disruption of this subunit with zinc-finger nucleases attenuates SS hypertension, preserves medullary blood flow, maintains glomerular filtration rate, and reduces proteinuria despite a salt load.</td>
<td>149,150</td>
</tr>
<tr>
<td>p67(phox)</td>
<td>Dahl rats</td>
<td>Overexpressed in renal medulla of Dahl-SS and zinc nuclease disruption</td>
<td>This contributes to oxidative stress and SS hypertension in the strain; disruption of this subunit with zinc-finger nucleases attenuates SS hypertension, preserves medullary blood flow, maintains glomerular filtration rate, and reduces proteinuria despite a salt load.</td>
<td>149,150</td>
</tr>
<tr>
<td>Proton channel HV1</td>
<td>Dahl rats</td>
<td>Overactivity in the medullary TAL of Dahl-SS</td>
<td>This Na+ sensor promotes ROS in the TAL of Dahl SS, its disruption by zinc finger nuclease deletion reduces ROS, Na+-induced hypertension, and renal injury.</td>
<td>151</td>
</tr>
<tr>
<td>Hemoxygenase-2</td>
<td>Mice</td>
<td>Knockout</td>
<td>This enzyme interacts with epoxygenases, increasing synthesis of EET. Knockout has hypertension and obesity, with insulin resistance, oxidative stress, and inflammation. A combined EET agonist/sEH inhibitor improves obesity, oxidative stress and hypertension.</td>
<td>152</td>
</tr>
</tbody>
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### Table. Continued

<table>
<thead>
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<tbody>
<tr>
<td><strong>Oxidative stress, continued</strong></td>
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<tr>
<td>PHD2</td>
<td>Dahl rats</td>
<td>Transfection of shRNA to renal medulla of Dahl-SS</td>
<td>Salt inhibits this enzyme, with consequent stimulation of HIF-1α, HO-1, COX-2, and natriuresis. In Dahl-SS, the effect of salt on PHD2 is impaired. Silencing of the gene counteracts the defect and improves SS hypertension.</td>
<td>153</td>
</tr>
<tr>
<td><strong>Angiogenesis factors</strong></td>
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<tr>
<td>HIF-1α</td>
<td>Mice</td>
<td>Keratinocyte-specific deletion</td>
<td>HIF-1α and HIF-2α regulate skin blood flow via opposing effects on skin macrophage NO. Reduced skin HIF-1α, with consequent decreases in NO and VEGF, leads to vasoconstriction and Na⁺-induced hypertension, demonstrating a role for regulation of skin blood flow in determining systemic vascular resistance.</td>
<td>154</td>
</tr>
<tr>
<td>TonEBP</td>
<td>Mice</td>
<td>Knockout in macrophages</td>
<td>Hypertonic salt storage in the skin stimulates TonEBP, which binds to the promoter of VEGF-C, increasing its production by macrophages. VEGF-C binds to VEGF-R3, with production of eNOS and lymph vessel proliferation, which facilitates extrusion of Na⁺. Impairing this mechanism by knockout of the transcription factor, by antagonism of the VEGF-R3 receptor, by trapping VEGF-C with soluble VEGF-R3, or by macrophage depletion leads to the development of SS hypertension, indicating a previously unknown role for the skin compartment in salt handling and BP regulation.</td>
<td>155,156</td>
</tr>
<tr>
<td>VEGF-R3</td>
<td>Mice</td>
<td>Antagonist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF-C</td>
<td>Mice</td>
<td>Soluble VEGF-R3 trapping or macrophage depletion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nr2f2</td>
<td>Dahl rats</td>
<td>Zinc-finger nuclease deletion in hinge region</td>
<td>A deletion in the hinge region of this transcription factor, involved in angiogenesis and heart development, reduces SS hypertension in Dahl-SS. Mechanism involves enhanced interaction of mutated Nr2f2 with another transcription factor (Fog2). Cardiac function and renal damage are improved in the mutant.</td>
<td>157</td>
</tr>
<tr>
<td><strong>Metabolic factors</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Insulin receptor</td>
<td>Mice</td>
<td>Targeted deletion in TAL and collecting duct</td>
<td>In this nephron segment, lack of insulin receptor (ie, lack of insulin-stimulated NOS1) leads to SS hypertension that is reversed by tempol.</td>
<td>158</td>
</tr>
<tr>
<td>Insulin receptor</td>
<td>Mice</td>
<td>Targeted deletion in collecting duct principal cells</td>
<td>In this nephron segment, insulin participates in Na⁺ conservation by increasing the expression of βENaC. Knockout of the receptor results in lowering of BP.</td>
<td>159</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>Mice</td>
<td>Knockout or KKAy (adiponectin-deficient) mice</td>
<td>In both models, adiponectin deficiency produces SS hypertension that can be improved with adenovirus delivery of adiponectin; BP is independent of insulin sensitivity and relates to reduced vascular eNOS and prostacyclin.</td>
<td>160</td>
</tr>
<tr>
<td><strong>Inflammation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rag1</td>
<td>Dahl rats</td>
<td>Zinc finger nuclease disruption</td>
<td>These rats lack T and B cells in blood, spleen, or kidney and are protected from SS hypertension.</td>
<td>161</td>
</tr>
<tr>
<td>Rag1</td>
<td>Mice</td>
<td>Knockout</td>
<td>As above, mice lacking lymphocytes do not develop SS hypertension; adoptive transfer of T cells, which must contain AT1 receptor and NADPH, restores the hypertension.</td>
<td>162</td>
</tr>
<tr>
<td>Interferon-γ and interleukin-17A</td>
<td>Mice</td>
<td>Knockouts</td>
<td>The knockouts for these cytokines fail to develop AngII antinatriuresis and have blunted BP responses to the peptide. Natriuresis despite AngI is attributable to decreased abundance of proximal tubule NHE3 and decreased phosphorylation of NKCC2 and NCC.</td>
<td>163</td>
</tr>
<tr>
<td>HSP70</td>
<td>Wistar rats; L-NAME hypertension</td>
<td>Peptide that induces immune tolerance to HSP70</td>
<td>T cells of hypertensive rats have overexpression of renal HSP70 with CD4 clonal expansion. HSP70 immune tolerance and adoptive transfer of T cells from tolerized rats protect against SS hypertension, whereas HSP70 gene delivery to the kidney of sensitized rats causes SS hypertension. Hence, autoimmunity to HSP70 triggers influx of T cells to the kidney and SS hypertension.</td>
<td>164</td>
</tr>
<tr>
<td>T cells</td>
<td>Sprague-Dawley rats; L-NAME hypertension</td>
<td>Mycophenolate mofetil</td>
<td>Transient minor renal damage by L-NAME predisposes to SS hypertension, which is associated with renal infiltration of T cells, segmental glomerulosclerosis, and thickening of afferent arterioles. All of these changes are prevented by the immunosuppressant mycophenolate mofetil, which abolishes CD5 T-cell infiltration of the kidney.</td>
<td>165</td>
</tr>
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Mineralocorticoids

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<thead>
<tr>
<th>Gene or Gene Product</th>
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<tbody>
<tr>
<td>MCR</td>
<td>Mice</td>
<td>Conditional knockout in vascular smooth muscle</td>
<td>Knockout of the MCR in vascular smooth muscle abolishes salt-induced hypertension by preventing salt-induced increases in arterial stiffness.</td>
<td>166</td>
</tr>
<tr>
<td>Rac1 GTPase</td>
<td>Dahl rats</td>
<td>Abnormal activation by salt in Dahl-SS</td>
<td>Despite reduced aldosterone, Dahl-SS have abnormal (ie, paradoxically increased) MCR translocation to the nucleus and signaling (renal SGK1); this is attributable to paradoxical increases in the small GTPase Rac1 in response to salt. A blocker of Rac1 activity prevents MCR activation, hypertension, and renal damage.</td>
<td>167</td>
</tr>
<tr>
<td>GiDrx</td>
<td>Arhgdia−/− mice</td>
<td>Knockout</td>
<td>The role of Rac1 is supported by knocking out 1 of its inhibitors in this strain of mice. Lack of inhibition of the dissociation of inactive GDP-bound Rac1 leads to Rac1 hyperactivity and SS hypertension.</td>
<td>167</td>
</tr>
<tr>
<td>SGK1</td>
<td>Mice</td>
<td>Knockout</td>
<td>Systemic lack of this MCR-stimulated kinase leads to hypotension and to resistance to SS hypertension produced by fat, fructose, or DOCA-salt.</td>
<td>168–171</td>
</tr>
<tr>
<td>APC</td>
<td>Mice</td>
<td>Random mutagenesis of APC</td>
<td>SGK1 is upregulated by β-catenin, which in turn is degraded by the adenomasus polyposis coli gene. Knockout of the latter leads to SGK1 overactivity, Na+ retention, increased plasma volume, and hypertension.</td>
<td>172</td>
</tr>
<tr>
<td>Striatin</td>
<td>Mice</td>
<td>Heterozygous knockout</td>
<td>Striatin is a downregulator of the pathway mediating genomic effects of aldosterone (MCR-SGK1-ENaC). These heterozygous knockout mice have increased renal expression of the components of this pathway and develop SS hypertension. They also have enhanced phenylephrine vasoconstriction and attenuated acetylcholine vasodilation with decreased eNOS expression, suggesting an additional salt-dependent role for striatin in regulation of vascular function in SS hypertension.</td>
<td>173</td>
</tr>
<tr>
<td>11-β Hydroxylase</td>
<td>Dahl rats</td>
<td>SNPs on chromosome 7</td>
<td>These protective SNPs of Dahl-SR cosegregate with decreased 18-OH-DOC; introgression of chromosome 7 of Dahl-SR into Dahl-SS reduces SSBP.</td>
<td>174, 175</td>
</tr>
<tr>
<td>11-β HSD isoform 1</td>
<td>Dahl rats</td>
<td>siRNA renal medullary knockout in Dahl-SS</td>
<td>Dahl-SS lack normal downregulation of this enzyme in response to salt. Knockdown in the renal medulla corrects this defect, decreasing formation of corticosterone (ie, stimulation of MCR) with attenuation of SS hypertension.</td>
<td>176–178</td>
</tr>
<tr>
<td>11-β HSD isoform 2</td>
<td>Mice</td>
<td>Heterozygous knockout</td>
<td>These mice develop SS hypertension with impaired Na+ excretion, hypokalemia, and cardiac hypertrophy; corticosterone is increased and aldosterone is suppressed during a salt load.</td>
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</tr>
<tr>
<td>Striatin</td>
<td>Mice</td>
<td>Zinc finger nuclease disruption</td>
<td>These rats develop SS hypertension with many features of the AME syndrome in humans (eg, reduced body size, polydipsia, polyuria, and chronic renal damage).</td>
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Renal transporters: Na⁺-K⁺-ATPase and regulatory molecules

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<tbody>
<tr>
<td>α-1 Isoform</td>
<td>Dahl rats</td>
<td>Spontaneous mutation</td>
<td>Mutation in Dahl-SS reported in some but not all laboratories; interaction with NKCC2 transporter in the F2 cross of Dahl-SS and -SR is supported by digenic cosegregation with BP. However, introgression of the α-1 isoform gene from the Milan normotensive rat into Dahl-SS lowers BP by mechanisms unrelated to Na⁺-K⁺-ATPase activity.</td>
<td>182–185</td>
</tr>
<tr>
<td>α-2 Isoform</td>
<td>Mice</td>
<td>Knock-in of ouabain resistant isoform in brain</td>
<td>The ouabain-binding site of this isoform is involved in the response to increased Na⁺ in the CNS. Knock-in of a ouabain-resistant form of the α-2 Na⁺-K⁺-ATPase chain abolishes the pressor effect of ICV Na⁺- and ICV ouabain.</td>
<td>186</td>
</tr>
<tr>
<td>α-Adducin</td>
<td>Milan rats</td>
<td>Spontaneous polymorphisms</td>
<td>Salt loading normally decreases Na⁺-K⁺-ATPase activity via dopamine-induced endocytosis of the pump. The α-adducin mutation of Milan hypertensive rats impairs the response to dopamine, blunting the interaction between the pump and adaptin proteins, leading to inappropriate Na⁺ reabsorption and hypertension. The effect of the α-adducin mutation is enhanced in rats carrying spontaneous mutations of the β- and γ-chain genes.</td>
<td>187, 188</td>
</tr>
</tbody>
</table>

(Continued)
This kinase, through intermediate steps, ultimately leads to dephosphorylation of the Na+-K+-ATPase α subunit, which increases its catalytic activity. Its expression is increased in the proximal tubule of Milan rats carrying the α-adducin mutation. The role of SIK1 was proven in vitro in proximal tubule cells transfected with the human α-adducin variant. These cells have increased SIK1 activity and Na+ transport mediated by elevated Na+-K+-ATPase; both alterations are abolished by dominant-negative (kinase-deficient) SIK1 mutants.

Renal transporters: Na+H− exchangers

Ouabain hypertension in rats is associated with Src-dependent, increased α1-Na+-K+-ATPase activity. Proximal tubule cells lacking NHE1 do not respond to ouabain, consistent with the depressor effect of NHE1 inhibitors in the whole animal. The effect of NHE1 on Na+K+-ATPase is mediated by an interaction between specific domains of the 2 proteins.

Renal transporters: NKCC2 and ROM-K

Alternative spliced isoforms of the channel are quantitatively different in Dahl-SS (A>F) vs Dahl-SR (F>A), probably as a result of intronic mutations (intron 3) upstream of alternatively spliced exons 4B, 4A, and 4F, which otherwise exhibit no changes in nucleotide sequence.

Uromodulin promoter

Variants of this promoter, which have been associated with SS hypertension in humans, produce activation of NKCC2 and SS hypertension when overexpressed in these transgenic mice.

ROM-K

Homozygous rats recapitulate the Bartter phenotype; heterozygous rats have reduced BP responses to salt and protection from renal damage.

Renal transporters: NCC and its regulatory molecules

Deficit of this protein in distal convoluted tubule leads to impaired phosphorylation of NCC with reduced BP.

WNK4 mutation

Transgenic mice harboring the human mutated gene reproduce the PHA II phenotype with increased expression and phosphorylation of NCC in the distal convoluted tubule.

SPAK

This kinase is an effector of WNK4 activity; its knockout results in a Gitelman phenotype and in the rescue of the PHA II phenotype when crossed with mice with a knocked-in causative WNK4 mutation.

WNK1

An intronic deletion analogous to that responsible for PHA II in humans leads to overexpression of long WNK1 in the distal convoluted tubule, with stimulation of NCC activity and reproduction of the PHA II phenotype. WNK1 activation of NCC is mediated by SPAK and independent of but somewhat inhibited by WNK4.

WNK1

Consistent with the above, disruption of the expression of WNK1 by gene trapping techniques produces low BP in the heterozygous knockouts.
### Renal transporters: NCC and its regulatory molecules, continued

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</thead>
<tbody>
<tr>
<td>Kidney-specific (alternatively spliced, without catalytic domain) WNK1</td>
<td>Mice</td>
<td>Overexpression in kidney</td>
<td>This kidney-specific WNK1 antagonizes full-length WNK1 kinase with reduced expression and phosphorylation of NCC and NKCC2. Its overexpression in mice leads to kidney Na+ wasting and lower BP.</td>
<td>206</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Targeted deletion of initiation exon 4</td>
<td>Conversely, knockout mice have increased expression and phosphorylation of NCC and NKCC2 and develop SS hypertension, recapitulating PHA II.</td>
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### Renal transporters: Na+ bicarbonate cotransporter

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</tr>
</thead>
<tbody>
<tr>
<td>SLC4A5</td>
<td>Mice</td>
<td>Knockout</td>
<td>Deletion of this transporter causes paradoxical Na+ retention and hypertension that are abolished by amiloride but not hydrochlorothiazide. Knockouts had increased membrane αENaC protein in kidney cortex extracts and upregulation of electrogenic ENaC tubular activity measured by micropuncture. Therefore, knockout of SLC4A5 induces hypertension by disinhibition of ENaC Na+ reabsorption.</td>
<td>207</td>
</tr>
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</table>

### Renal transporters: ENaC and its regulatory molecules

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<td>Rats</td>
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### Renal dopaminergic natriuretic system

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Renal dopaminergic natriuretic system, continued

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<td>Cyp4a14 monoxygenase</td>
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<td>Cyp2c44 epoxygenase</td>
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<td>Knockout of this epoxygenase produces a low-estrogen, blunted gonadotrophin SS hypertension in females, which is normalized by estrogen replacement; there is no reduction in renal EETs because of compensatory synthesis by Cyp2j9; mechanisms of hypertension include increased proximal tubule Na+ reabsorption and afferent arteriolar reactivity to AngII and ET-1.</td>
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(Continued)
Evidence for a genetic basis of salt sensitivity has come from heritability estimates in family studies. Miller et al examined the change in BP between random-sodium and low-sodium diets among white US families and found a higher correlation in monozygotic pairs compared with sibling pairs: 0.72 for systolic BP (SBP), 0.62 for diastolic BP (DBP), and 0.68 for MAP in monozygotic twins compared with 0.50, 0.33, and 0.36, respectively, for siblings. Svetkey et al used an established inpatient protocol to examine the change in BP between intravenous sodium loading and furosemide-induced volume depletion in black US families and found evidence of heritability, although effects of variable family sizes contributed to variation in estimates. Additional evidence was provided by the description of an association of salt sensitivity with a rise in the frequency of such variants. Because hypotension is a greater threat to survival before reproductive age, it has been assumed that negative selection would be strongest against a BP-lowering variation.

Mendelian syndromes of hypotension and hypertension in which families segregate mutations of strong effect have been exploited by genome-wide linkage studies to define key contributors to BP variation. Mendelian hypertension results from mutations that lead to renal salt retention and mendelian hypotension results from mutations that lead to renal salt loss. Common variants contributing to salt sensitivity have been sought with association methods. Although many such variants have been identified by genome-wide association studies that contribute to BP variation in the general population of multiple ancestries, they have required very large sample sizes to achieve sufficient statistical significance to detect modest effects after adjustment for multiple testing.

Table. Continued

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<tbody>
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<td>CYP450 enzymes, eicosanoids and soluble epoxide hydrolase, continued</td>
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<tr>
<td>sEH</td>
<td>Mice</td>
<td>Targeted disruption</td>
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<td>sEH</td>
<td>Mice</td>
<td>Targeted disruption</td>
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ACE indicates angiotensin-converting enzymes; AME, syndrome of apparent mineralocorticoid excess; AngII, angiotensin II; APC, adenomatous polyposis coli; AQP2, aquaporin 2; ARAP1, angiotensin II type 1 receptor–associated protein 1; AT1, angiotensin receptor type 1; AT2, angiotensin receptor type 2; AVP, arginine vasopressin; β2AR, β2 adrenergic receptor; BK, bradykinin; BP, blood pressure; cGMP, cyclic guanosine monophosphate; CNS, central nervous system; COX-2, cyclooxygenase-2; DHET, dihydroxyeicosatrienoic acid; DOCA, deoxycorticosterone; EET, epoxycosatrienoic acid; Elijovich et al |
such sample sizes have been unavailable in individuals phenotyped for SSBP; therefore, no statistically significant associations have yet been found and confirmed that meet genome-wide significance thresholds ($P < 5 \times 10^{-8}$).

Many candidate gene studies have therefore focused on pathways suspected of playing a role in salt sensitivity. Candidate genes in the renin-angiotensin-aldosterone system, adrenergic system, NO, renal salt handling, vascular smooth muscle calcium handling, regulators of renal transporters, natriuretic peptides, arachidonic acid metabolites, and kallikrein system have all been examined. However, multiple methodological challenges have plagued the field.

The studies are limited by relatively modest sample sizes; a small number of study samples make up the majority of the association studies. Individual studies lack adequately powered replication samples that have assessed the same phenotype. Most studies have used statistical thresholds that do not account for the multitude of genes and variants examined within individual articles and across articles for an individual study sample. Some studies that claim replication fail to show replication of a specific genetic variant across multiple studies but rather report putative association of an independent variant at the same genetic locus. Ultimately, for the discovery of common genetic variants that influence salt sensitivity, larger sample sizes, meta-analysis of individual variants across multiple study samples, and application of statistical thresholds appropriate to the total number of hypotheses tested will be needed. Given the lack of bona fide associations in adequately powered human genetic studies of rare or common variants at the population level, human genetic validation of mechanisms suggested in rodent models is currently not possible.

**Environmental and Demographic Factors and SSBP**

The BP response to a change in salt (sodium chloride) intake is not uniform. Different types of study designs have been used to identify subgroups of the population whose BP response to salt is greater (or lesser) than other subgroup responses. Studies include small, brief challenge studies, feeding trials, and meta-analyses of trials. Factors that might influence the BP response to salt include sex, age, adiposity, race-ethnicity, and clinical conditions (hypertension, diabetes mellitus, and chronic kidney disease). For several factors, evidence is insufficient to make strong conclusions because individual studies were not designed to test the effects of salt reduction simultaneously in a comparator group. For example, a few trials have tested the effects of salt reduction in patients with diabetes mellitus but none tested the effects concurrently in patients without diabetes mellitus. Additionally, most meta-analyses that aggregate published data across studies rather than analyzing individual-level data are poorly suited to identify subgroups that are SS because of potential residual confounding. In contrast, in some studies, the effects of salt were examined in both sexes, in blacks (versus whites), across the age span in adults, and over a broad range of BPs. Simultaneously testing of the effects of salt reduction in a broad population was carried out in the large Dietary Approaches to Stop Hypertension (DASH)–Sodium feeding trial. This permitted comparison in subgroups, as shown in Figure 4.

In summary, a strong and consistent body of evidence has documented that on average blacks compared with whites have a greater BP response to a change in salt intake and that this finding is independent of baseline BP level. Likewise, individuals with hypertension have a greater BP response to a change in salt than individuals without hypertension, and older individuals have a greater BP response than younger adults. The effects of salt reduction depend on concurrent diet. The effects of salt on BP are greater in the setting of a low-potassium intake and in the setting of poor-quality diet compared with the DASH diet.

A less consistent body of evidence suggests that women might be more SS than men and that overweight individuals are more SS than normal-weight individuals. The effects of salt reduction in Asians and in individuals with diabetes mellitus or chronic kidney disease have been tested in few
studies, in which salt reduction lowered BP. However, the absence of comparator groups precludes strong statements about whether these groups are more SS than corresponding groups without the factor.

**Prognostic Significance of SSBP**

Investigation of SSBP began as an outgrowth of general research into the mechanisms controlling the circulation and the function of the kidneys. Once it became apparent that the BP response to changes in salt intake might vary from very large to almost nonexistent in experimental animals, research began focusing on this intriguing observation. The inbred SS strains of rodents that were generated for this research exhibited a specific pattern of target organ damage and increased mortality. However, these experiments were conducted in animals that had developed established hypertension. Therefore, it was not possible to tease out the relative roles of SSBP and an elevated BP itself in determining organ damage or mortality.

Interestingly, target organ damage of SS hypertension in humans resembles that of SS rats, with a predominance of left ventricular hypertrophy, renal damage, and stroke. In 1997, Morimoto and colleagues showed that SSBP may be a cardiovascular risk factor above and beyond the detrimental prognosis conferred by hypertension. They presented the outcomes, over an average follow-up of 7.3 years, of 62 SS and 94 SR hypertensive patients. Patients were classified into these groups by use of a dietary protocol (12–15 g NaCl per day for 1 week and 1–3 g NaCl per day another week) and were deemed SS if they had an increase of >10% in MAP from the low-salt to the high-salt week. By the end of follow-up, SS subjects had more than double the rate of nonfatal and fatal cardiovascular events compared with those who were SR (4.3 versus 2.0 per 100 patient-years). Multivariate regression confirmed that BP, smoking, and SSBP were independent predictors of cardiovascular risk. This study had a relatively small population and derived its conclusions from statistical analyses of the data because all participants were hypertensive.

More definitive proof of an independent role for SSBP as a cardiovascular risk factor was provided by Weinberger et al. These investigators conducted long-term follow-up, up to 27 years, in a large cohort of subjects (278 with and 430 without hypertension) who had been classified as SS or SR at baseline using the acute inpatient protocol of intravenous saline loading and diet plus diuretic-induced salt depletion. They showed that SSBP was associated with an increased mortality risk ratio of 1.73 (95% confidence interval, 1.02–2.94). Survival was lowest in SS hypertensive subjects and highest in SR normotensive subjects, as expected. The novel observation was that the survival curves of SR hypertensive subjects and SS normotensive subjects were not significantly different, indicating that SSBP in normotensive subjects carried a prognosis similar to that of hypertension in SR individuals.

Some have argued that conclusions about the role of salt in determining outcomes cannot be drawn from the above study because actual data on sodium intake over the years were not available in their population. This criticism appears irrelevant because the study was about the effect of a phenotype, not about an effect of salt intake. Others have argued that some of the outcomes may have been attributable to the development of hypertension over time in SS normotensive subjects because it is well known that SSBP predicts the onset of hypertension over the ensuing decades. It is not conceivable that future development of hypertension may be a factor determining prognosis, but the fact remains that hypertension was not present at the time the prognostic phenotype was detected in otherwise normal subjects. Furthermore, the SS phenotype has clinical characteristics that may determine its prognosis independently of BP. For example, SSBP is one of the features that develop in otherwise healthy adults who had low birth weight, a well-known predictor for cardiovascular disease in adult life. In addition, SS subjects are more insulin resistant than their counterparts, whether normotensive or hypertensive, and they exhibit reduced nocturnal dipping of BP, which is in general associated with worse cardiovascular prognosis. Finally, despite the limitations discussed below about use of data from ambulatory BP monitoring (ABPM) as surrogates for the prediction of SSBP, a large study of outcomes using this methodology that included 2064 subjects with 20 years of follow-up also showed that subjects with predicted SSBP had an increased risk of mortality, particularly men.

A summary of the observations above is that SSBP has ceased to be exclusively an object of physiological inquiry but has become instead an issue of clinical importance because the phenotype carries prognostic implications potentially as strong as those of traditional cardiovascular risk factors. Conceivably, unraveling of its causative mechanisms, via genetic or physiological studies, may allow treatment of the phenotype with drugs that target its causation, not necessarily antihypertensive agents. A preliminary example of such a strategy has been provided by the demonstration of an antihypertensive effect of fenofibrate in SS hypertension. This is probably mediated by stimulation of CYP450 enzymes that improve the abnormalities in arachidonic acid metabolites observed in human SS hypertension.

**Measurement of SSBP in Humans**

**History of Methods Used**

The initial description of SSBP was made in 1978 by Kawasaki et al. They measured BP supine every 4 hours for the entire study in 19 “idiopathic” hypertensive patients in a clinical research center and compared MAP on the sixth day of each dietary regimen: 1 week each of normal (109 mmol/d) followed by a low (9 mmol/d) and then high (249 mmol/d) intake of sodium. BP decreased significantly in all subjects after the low-sodium diet compared with baseline and increased in all but 1 of the subjects with the high-salt diet. Those researchers arbitrarily divided the 19 subjects into an SS group in whom the increase in MAP was ≥10% and a non-SS group in whom the increase was <10%. There were no differences in BP among the subjects between the beginning and end of the study (ie, between normal- and high-sodium intake). Thus, the changes in BP were observed only after the low-sodium intake when all subjects exhibited a decrease, but there was an arbitrarily defined difference in the magnitude of this decrease.
between the SS and non-SS groups. The SS subjects retained more sodium than the non-SS subjects during the high-sodium diet, as assessed by 24-hour urinary sodium excretion.

The Kawasaki study design points out a potentially confounding physiological issue, that is, the sequence of sodium intake, which has plagued several subsequent studies. A decrease in dietary sodium intake or a reduction in extracellular fluid volume normally activates the renin-angiotensin-aldosterone system, but the degree of activation of this system is quite variable among individuals and known to be blunted in SS subjects. Therefore, starting with a low-salt intake with variable degrees of renin-angiotensin-aldosterone system activation may influence the effect of subsequent high-salt intake on sodium and fluid reabsorption, whereas starting with a high-salt intake with uniform suppression of the renin-angiotensin-aldosterone system may confer more uniformity to the subsequent response to low-salt intake. Evidence to support this contention was provided in the protocol of rapid extracellular volume expansion with intravenous fluids described below. The 578 subjects in this study exhibited virtually complete suppression of plasma renin activity and plasma aldosterone concentration after salt loading regardless of BP (normotension versus hypertension) or salt sensitivity (SS versus SR) status.1

Not all experiments of dietary sodium loading produced changes in BP. In 1976, Kirkendall and colleagues69 studied 8 normotensive prisoners after a month each of diets containing 10, 210, and 410 mmol/d sodium without observing a change in BP. However, the BP measurements made in that study were not as frequent or meticulous as those in the Kawasaki study. The importance of these 2 initial studies is that they show that the sequence of dietary manipulation (ie, whether the BP changes are based on an increase or a decrease in dietary sodium intake) and the number and circumstances (position, times of day, etc) of BP measurements are critical, including those obtained during the subjects’ normal ambient diet. Verification of the dietary intake, the duration of the interventions, and the ingestion of other nutrients are also important factors to consider.

Another careful clinical research center study of 8 normotensive, young male subjects was conducted at the end of 7 days of 10-mmol/d sodium intake followed by 3 days each of 300-, 800-, and 1500-mmol/d sodium intake. When sodium intake is reduced below ambient levels, a longer period of time is required to achieve balance than when it is increased. Daily collection of 24-hour urine with measurements of sodium, potassium, and creatinine excretion served to verify compliance with the dietary regimen. MAP was significantly increased with the 800- and 1500-mmol/d intakes compared with values at the end of the 10- and 300-mmol/d periods.70

In an extension of this study, 14 additional normotensive men, 7 black and 7 white, were studied at 6 levels of dietary sodium intake (10, 300, 600, 800, 1200, and 1500 mmol/d), the first for 7 days and the others for 3 days each. Although BP increased in all subjects as sodium intake increased, there were differences in magnitude and threshold of BP responses between races. White subjects exhibited a significant increase in BP between the 10- and 1200-mmol/d intakes, whereas blacks had a significant increase between the 10- and 800-mmol/d intakes and again at 1500 mmol/d. Significant 24-hour kaliuresis was observed at levels of sodium intake ≥300 mmol/d. No difference in cumulative sodium retention could be demonstrated between blacks and whites, but blacks retained more potassium on the low-sodium diet than whites and lost less potassium during high-sodium intake. When potassium losses were prevented by replacing the urinary losses at the higher levels of sodium intake, the magnitude of BP increase at the higher levels of sodium intake was significantly attenuated, indicating an interaction between potassium balance and SSBP.71

Sullivan and colleagues372 studied 27 normal subjects and 19 borderline hypertensive subjects after a 10-mmol/d sodium intake for 5 days, at the end of which urinary sodium excretion was ≈20 mmol/24 h. Subjects then received 200- and/or 400-mmol/d sodium diets. BP responses were varied, but in general, both normal and hypertensive subjects had a decrease in MAP and DBP after the low-sodium period. A small number of normotensive subjects had an increase in BP with sodium repletion, whereas the majority of hypertensive subjects did so.

A large number of subsequent studies have used a variety of dietary approaches in many different populations with variable confirmation of intake by urine measurements. These have ranged from high-sodium intake being represented by ambient diets or nominal levels of 100, 200, or 300 mmol/d or as much as 20 g and a low intake ranging from 9 to 20, 30, or 50 mmol/d to 5 g/d in >75 published studies. Moreover, the duration of each period and the sequence were highly variable.

A different approach to the assessment of sodium and volume sensitivity of BP has used rapid extracellular volume expansion with intravenous fluids and volume and sodium depletion induced by diuretic administration. The prototype of this approach was the development of an inpatient protocol giving an intravenous infusion of 2 L normal saline over a 4-hour period in the morning, originally intended for the identification of nonsuppressible aldosterone production.373 A prepared diet provided a total of 335 mmol sodium on this volume-expansion day. On the following day, sodium and volume depletion was induced by a diet of 10 mmol sodium and 40 mg oral furosemide given at 10 AM, 2 PM, and 6 PM to assess the response of plasma renin activity to this maneuver. Subjects were housed for the duration of the study in a clinical research center where all measurements were obtained and all food was prepared and eaten. BP was measured 3 times in the sitting position at 8 AM and noon on admission, and again after salt loading and salt depletion. Plasma renin activity, plasma aldosterone, serum electrolytes, serum creatinine, and 24-hour urinary sodium, potassium, creatinine, and catecholamine excretion were measured daily.74 This protocol was initially conducted in >100 normal subjects to establish normative data and in >200 hypertensive subjects for identification of secondary forms of hypertension and other research studies. More than 1000 subjects have been studied with this protocol at the original institution and many other sites around the world. Some investigators have attempted to shorten this rapid approach to a single day of intravenous saline infusion followed by short-acting diuretic administration,375 but an attempted validation of this procedure failed to show concordance with the traditional one.376
Results of this protocol were used to establish a definition of salt sensitivity and resistance of BP based on MAP calculated from sphygmomanometric readings in triplicate, obtained by trained and certified nurses at the end of the saline infusion and again after 2 hours of ambulation on the morning after the low-sodium diet and diuretic administration. This report included observations in 378 normal volunteers and 198 hypertensive subjects in whom secondary forms of hypertension had been excluded by the previous study. The distribution of BP responses at the 2 observations points was gaussian in both groups, but the curves were significantly different. From these data, an arbitrary classification of subjects was made into salt (and volume) sensitive (decrease in MAP \( \geq 10 \) mm Hg), resistant (<5–mm Hg decrease or an increase in MAP), or indeterminate (decrease of 5– to 9–mm Hg MAP). The use of an indeterminate category provided greater reliability for the comparisons between subjects in the SS and SR groups. This protocol generated the largest database using this approach and has identified many characteristics associated with salt sensitivity and resistance of BP. Notably, in this study, as in the index dietary study, the major change in BP was seen after sodium and volume depletion rather than after saline infusion. Although small patient series studied with a dietary approach identified subjects who sustained depressor responses to a salt load in this acute intravenous protocol, none of the 576 subjects exhibited a decrease in BP after the saline load. They sustained an average increase in MAP of <5 mm Hg, which was not different between the normotensive and hypertensive subjects, perhaps reflecting the fact that individuals with an activated renin-angiotensin system were not selected for these studies. Hypertensive subjects were significantly more SS (51%) than the normotensive ones (26%), and 33.3% of the former were SR versus 58% of the latter. In both groups, 16% fell in the indeterminate category. In both populations, SS subjects were significantly older and had significantly lower levels of plasma renin activity. SSBP was more frequent among black hypertensive subjects than among white hypertensive subjects, but the prevalence of SSBP showed no such racial difference in the normotensive group.

Much longer dietary interventions have also been attempted. In a study by Miller et al., 16 normotensive parent pairs were provided dietary instruction to reduce sodium intake to 60-mmol sodium excretion over a period of 12 weeks, during which multiple measures of sodium excretion and BP were obtained. Average SBP showed a significant decrease of 3 to 5 mm Hg, with comparable reductions in DBP. Individual BP responses displayed a wide continuous range, with 23% showing no decrease. Responses were correlated with changes in sodium excretion and were larger in those with higher initial BP. In a later analysis of 74 subjects, Weinberger et al. reported a gaussian distribution of change in MAP after the low-sodium diet. Arbitrary cut points for sodium sensitivity were defined as a decrease of at least 3 mm Hg in MAP after the sodium intervention; sodium resistance was defined as an increase of at least 3 mm Hg. In further analyses, Miller et al. found that BP response to dietary salt manipulation was correlated with age and was higher among those >40 years of age.

Large population-based sodium reduction trials with dietary approaches over longer time periods have also been carried out. The effects of sodium reduction on BP were consistently greater among those with hypertension than in normotensive subjects. Several lifestyle research interventions examined subgroup effects and the relation of change in BP to change in urinary Na⁺ excretion. Investigators of the DASH-Sodium and other trials found that the BP response to sodium reduction varies by group characteristics, including age, race, and sex, as well as hypertensive status. These group differences have also been found in physiological studies of BP responses. A small body of research identified other factors such as body weight, insulin resistance and diabetes mellitus, and increased sympathetic nervous system activity as factors influencing salt sensitivity or resistance of BP. Such observations require further confirmation.

Reproducibility of the BP Response to Sodium

Several investigators have examined the reproducibility of the BP response to the sodium challenge. When the inpatient protocol was repeated in the same subjects within the year, the correlation between changes in MAP was 0.56, suggesting modest reproducibility. Four of 28 subjects changed their status from SS to SR or vice versa. Sharma et al. also found a significant correlation (r=0.60) in repeat studies. Zoccali et al. however, reported a lower degree of reproducibility, with correlations of 0.13 to 0.15, using a similar protocol, although details of their methodology are scarce. Efforts to use simpler and shorter protocols have failed validation.

Four subsequent studies have performed to assess the congruence of BP responses to the rapid intravenous saline loading plus diuretic-induced sodium and volume depletion protocol with slower dietary sodium intake approaches. These comparisons were consistently significant. Thus, although limited in number, the studies that have examined the reproducibility of techniques for the assessment of salt sensitivity and resistance and the congruence between 2 different approaches to these measurements have been largely significant despite the well-known variability of BP over time in numerous studies.

Investigators of the DASH-Sodium study looked at the reproducibility of patterns of response to dietary interventions over time among participants. This feeding study used a crossover design with high, median, and low levels of sodium intake within groups randomized to the DASH diet versus a control diet. Each sodium level was maintained for 30 days. Obarzanek et al. examined the individual BP response to changes in the sodium diet within the control arm of the study while keeping weight and other nutrients constant. BPs were measured multiple times to reduce measurement error and to assess the reproducibility of the response to sodium. BP change in response to lower sodium exhibited gaussian distribution, supporting previous observations that sodium sensitivity lies on a continuum. There was also wide variability in BP changes during periods of stable salt intake, and this variability exhibited normal distribution. Lower-sodium intake shifted the BP distribution to lower levels without affecting its variability. The correlation of changes in BP response to similar changes in sodium intake, directly observed from contrasts.
between the 3 randomly ordered sodium conditions, was 0.27. When sodium sensitivity was defined as a BP response above or below the median, only 57% of participants had a consistent dichotomous response. This suggests that this definition of salt sensitivity, under the conditions of the DASH study, is affected by random variation in BP.

Investigators from the GenSalt Study evaluated reproducibility of the BP response over a long-term interval (4–5 years) among 487 participants in northern China. The same protocol, including 1 week of a low-salt (51 mEq) followed by 1 week of a high-salt diet (308 mEq), was used in both assessments. Strict compliance to the dietary intervention was maintained. BP was measured 3 times over 3 days during baseline and after each diet. The correlations of changes in SBP in response to the low- and high-sodium diets were both 0.37. Although these \( r \) values are not large, the persistence of the relationship suggests that the underlying BP response may be reproducible over long periods of time. The inherent variability in BP measurements, however, makes it challenging to identify and classify individuals who have a heightened response to salt.

A few recent studies have used ABPM to reduce the inherent variability in BP measurements. Although attractive, this approach is probably easier to apply during inpatient rapid protocols than during prolonged outpatient dietary ones. Furthermore, some controversy has arisen about the period of BP readings from these monitors that should be used for assessment of the BP response to sodium manipulation.

**BP Variability and Assessment of SSBP**

BP measurement has long been known to be extremely variable over time. Sphygomanometer-based values vary according to the number of measurements, the number of visits or days of measurement, and the month of measurement, resulting in substantial within-person variability. The predictive value of BP measurements for the individual’s true underlying average increases as the number of measurement and visits increases, and multiple measures are needed to accurately estimate the underlying BP. For clinical purposes, the Eighth Joint National Committee followed the recommendation of the Seventh Joint National Committee, that is, 2 to 3 measurements taken while seated after 5 minutes of rest. The Seventh Joint National Committee also recommended that readings be taken over at least 2 office visits to minimize measurement variability. Canadian guidelines recommend at least 3 visits to classify individual patients with borderline levels of hypertension. Finally, it is well recognized that arm shape, cuff size selection, and operator training affect these measurements. Therefore, training of personnel is crucial, and recommendations and guidelines have been issued in this regard.

Oscillometric devices are replacing mercury sphygmomanometers as standard devices for clinical measurement of BP and generally provide consistent results. Advantages of these devices over conventional ones include elimination of observer bias and averaging of multiple measures over time. Use of these devices does not eliminate the inherent variability of BP. Additionally, questions about the actual accuracy of some devices have been raised. Within-person variability of conventional clinic, automated office, and home BP measurements decreases substantially with increases in the number of readings or visits, reaching a plateau after 4 to 6 measurements, although this may vary with the characteristics of the studied population. ABPM has additional advantages: It is fully automated, assesses nocturnal variation, and averages longer periods of readings. Because it is usually used for 24 hours, it does not solve the issue of BP variability over weeks or months. In the case of automated BP monitors, methods have been proposed to adjust for the drift in readings over time and the alerting responses to the presence of the operator. Newer devices include capabilities such as dual arm readings, timed repeat measurements that do not require the presence of the operator in the room, and electronic downloading of the data for subsequent analysis.

Because assessment of SSBP depends on at least 2 readings, that is, before and after manipulation of salt intake, the variability of this change in BP builds in the variability (ie, random error) of both readings. If the period between them is short, much of the variability of the BP change may be attributable to within-person variability as opposed to change in salt balance. Therefore, reduction of the variability of pre-intervention and post-intervention BP by the use of multiple measurements will tend to produce a more accurate representation of the effect of the change in salt balance. Nonetheless, even with multiple measurements, substantial variation in BP persists, making classification of individuals into SS and SR problematic outside of carefully controlled research settings.

**Measurement of Sodium Intake/Excretion and Assessment of SSBP**

Assessment of sodium intake through diet recall is subject to bias, underreporting, and day-to-day and other random variation. Therefore, measurement of urinary sodium excretion remains the preferred means of estimating sodium intake in individuals. Estimation of urinary sodium excretion from multiple, high-quality 24-hour urine collections has been considered the gold standard. Several investigators have tried to use spot urine collections to obviate the difficulties and inconvenience involved in 24-hour collections. Although sodium excretion is known to have diurnal variation, with lower levels overnight, the Kawasaki et al equation has been used to calibrate these spot urines to 24-hour values. This equation was developed in a Japanese sample and may not be generalizable to other populations. In addition, the diurnal pattern of urinary sodium excretion is closely related to the time of sodium ingestion, typically during the day. More recently, other equations have been developed to estimate 24-hour excretion of sodium from spot samples, for example, Tanaka et al in the Japanese INTERSALT (International Cooperative Study on Salt, Other Factors, and Blood Pressure) population, Mage et al for NHANES (National Health and Nutrition Examination Survey) samples, and most recently, Brown et al in INTERSALT. Rather than simply extrapolating the timed collection and volume to a 24-hour period, these equations estimate sodium on the basis of the ratio of sodium to creatinine concentration. Additionally, INTERSALT estimates were derived from a regression analysis using the sodium, potassium, and creatinine from a spot measurement, along...
with age, region, and body mass index. Researchers who examined the agreement between the 24-hour specimen and 4 timed voids collected throughout the day concluded that in a US population, the Western INTERSALT equations exhibited less bias than the other 3 equations.417 In the Prospective Urban Rural Epidemiology (PURE) study, which used samples from 11 countries, including many Asians, the Kawasaki equation instead was the best fit.418 Most investigators418–420 found that although corrected spot urines may be sufficient to estimate mean population sodium excretion levels, there was substantial disagreement between the measures for individuals. Hence, spot urines, which exhibit increased variability compared with actual 24-hour measurements, should be considered inadequate for the assessment of sodium excretion in research studies dealing with SSBP.

It must be pointed out that 24-hour urine collections, although considered the gold standard, also have inherent variability. In addition to potential problems with undercollection or inconsistent timing, such samples exhibit substantial day-to-day variability. For example, the correlation of 24-hour sodium excretion obtained with samples of 2 visits spaced by ≈2 weeks was only 0.36.421 Coefficients of variation over 4 days to day were estimated as 19% to 23%,412 and individual coefficients of variation over multiple years of observation averaged 15% to 20%.422 More recently, it has been shown that under conditions of extremely controlled constant sodium intake (simulation of spaceflight lasting 520 days), salt excretion was nonetheless subject to a circaseptan rhythm, the nature of which is not yet understood.423 Multiple 24-hour urine samples collected over several weeks would theoretically be a better estimate of salt intake by reducing within-person variability. However, they are obviously impractical in shorter-term studies assessing salt sensitivity. Another means of improving the studies of SSBP is to use a rigidly controlled dietary intake, which would be an alternative to relying on 24-hour urine measurements. Prepared meals for dietary protocols and absolute control of metabolic diets in patients admitted to a clinical research center are possible approaches to achieve this.

**Surrogate Markers of SSBP in Humans**

Current methods for determining SSBP are labor intensive and therefore costly; thus, they are rarely if ever undertaken outside the clinical research arena. Two areas of research seeking easily obtainable surrogate markers for SSBP have developed recently, one based on analysis of BPs and heart rates from ambulatory monitors and the other based on excretion of proximal tubular cells or renal exosomes.

A group of Italian researchers hypothesized that characteristics in a 24-hour ABPM would reflect SSBP in individuals on habitual salt intake.423 Other investigators had measured beat-by-beat BP and pulse rate variability in 34 essential hypertensive subjects studied during 1 week of low- and high-salt diets and determined by sophisticated spectral analysis methods that SSBP was associated with lesser baroreflex sensitivity and higher pulse interval power.424 In other words, pulse rate and SSBP increased in parallel in their SS patients but were unaffected by salt intake in SR individuals. These observations were consistent with many others in which subjects with the metabolic syndrome, who are insulin resistant and SS, have increased sympathetic nervous system activity and heart rates.39,425–427 Another characteristic of many groups of hypertensive patients (eg, secondary hypertension, obstructive sleep apnea, chronic renal insufficiency) is a diminished sleep-associated reduction in BP (nondipping of BP), which is known to have detrimental prognostic implications. Subjects with features of the SS hypertensive phenotype such as the metabolic syndrome also have a high prevalence of nondipping.428 In general, salt blunts63 whereas salt deprivation enhances429,430 nocturnal dipping, and a reduced dipping has been described in association with SSBP.365,431,432 It is conceivable that this association may contribute to the increased morbidity of the SS phenotype. It followed that analogous to the need for the pressure natriuresis response in general, SS subjects may depend on higher sleep BPs to excrete a salt load.

Therefore, Castiglioni et al423 studied both 24-hour pulse rates >70 bpm and the nocturnal dipping of mean arterial BP as surrogate markers for the risk of SSBP in 46 hypertensive patients on a habitual salt diet. Subjects underwent a formal dietary protocol for the assessment of SSBP. The analysis divided patients into those with 2 (high risk), 1 (intermediate risk), or no (low risk) hypothesized surrogates. There was fair agreement between the high SSBP risk and the actual SSBP measured (ie, the surrogate markers had fair sensitivity). However, they found that about one quarter of the predicted low SSBP risk group was actually SS (low specificity for the observation or substantial misclassification of the lower-risk group). Subsequently, they suggested that the prediction could be improved by using a combination of the nocturnal dipping of MAP and pulse pressure, parameters that are weakly correlated and may provide different information derived from the magnitude and pulsatile components of BP. With this new method, misclassification of the low-risk group was reduced to 5%, and receiver-operating curve analysis showed a sensitivity and specificity for the high-risk group of 74% and 78%, respectively.433

Bursztyn and Ben-Dov367 used the SSBP surrogate markers to label a large cohort (n=2064) of untreated patients who had been referred for ABPM and had 20 years of follow-up. They assessed the impact of this classification on mortality. About 27% were categorized as low SSBP risk, 62% as intermediate risk, and 13% as high risk with the methodology of Castiglioni and coworkers.423 Women were more likely to fall into the last group (16% versus 10% men), consistent with conventional knowledge about SSBP. The high-risk group was characterized by older age, higher body mass index, and higher prevalence of diabetes mellitus, which are clinical characteristics usually associated with the SS phenotype.5,39,427,434,435 In Cox proportional hazards model, with the use of dipping of SBP rather than MAP and adjustment for all the phenotypic variables above and for 24-hour SBP and DBP, high-risk status predicted an increased risk of mortality (hazard ratio, 1.59; P<0.03) in the whole population. However, the effect was attributable solely to observations in men (hazard ratio, 2.40; 95% confidence interval, 1.36–4.26), whereas in women, the effect was marginal (hazard ratio, 1.17; 95% confidence interval, 0.63–2.17). Limitations of the study included
the unknown salt intake of the subjects (average habitual for middle-aged Israelis is 8.5 g/d), selection bias (referred population), lack of adjustment for risk factors unknown for the cohort (eg, smoking status and cholesterol levels), and unknown morbidity and causes of mortality.

Reproducibility of the results of Castiglioni et al is also an issue. Some have been able to reproduce their results in women with a history of preeclampsia, whereas others were unable to do so in a population classified as SS or SR with the acute inpatient protocol of Indiana University that was composed of hypertensive and normotensive SS subjects of diverse ethnic background, with a significant proportion of blacks.

Another promising line of research is emerging from the study of proximal tubular cells or renal exosomes shed in the urine. In the former, the ability to recruit dopamine-1 receptors to the cell membrane in response to increased intracellular sodium by monensin (a sodium ionophore) and the intracellular calcium response to cell exposure to angiotensin II appear to be impaired in SS individuals. The negative continuous relationship between these 2 parameters and the magnitude of SSBP supports the view that impairment of tubular sodium transport by these 2 pathways may be involved in SSBP. Hence, assessing the activity of these pathways may allow personalized identification of SS and SR phenotypes. Importantly, characterization of cell pathway activity took place a long time after phenotyping, indicating independence of the marker from the state of salt intake of the subject. In addition, subjects with decreased BP in response to salt (designated inverse SS) had the highest activity of these pathways, suggesting that the marker could serve to identify patients who might be harmed by a reduction in salt intake. In urine exosomes, the same group reported differential expression of 45 renally produced (not systemic) microRNAs among SS, SR, and inverse SS subjects. Some of these microRNAs are known to participate in the regulation of signaling pathways involved in hypertension (eg, peroxisome proliferator-activated receptor-γ, epidermal growth factor receptor, transforming growth factor-β1, phosphatase and tensin homolog/phosphoinositide 3-kinase). Therefore, microRNA profiling of individual patients may serve as a marker of their SSBP phenotype. Both observations were made in small number of patients (10–12) and require confirmation in larger number of individuals.

At this stage of the research, it can be stated that surrogate markers for SSBP from ABPM may correctly identify some but not all subjects for their actual SSBP phenotype and that those from urine renal tubular cells and exosomes need to be replicated and validated. In terms of research into mechanisms of SSBP in humans (an important goal if therapeutic interventions were to derive from understanding mechanisms), classification of subjects will remain the domain of the research laboratory, with the dietary protocols or acute inpatient protocols discussed in other sections of this publication.

Implications of SSBP for the Clinical Management of Individual Subjects
The reproducibility of SSBP tests separated by weeks or years supports the conclusion that underlying differences in sodium sensitivity exist among individuals. As reviewed above, correlation coefficients for BP responses to changes in sodium were as high as 0.56 for the acute inpatient protocol repeated within a year and significant but as low as 0.27 to 0.37 in the dietary DASH-Sodium and GenSalt studies. Comparisons of the intravenous sodium challenge protocol with an ambulatory dietary sodium protocol also demonstrated significant correlations of 0.40 and 0.56. These observations strengthen the interpretation that there is an inherent individual BP response to dietary sodium. Heritability estimates for BP response to dietary sodium of 0.2 to 0.3 are in the same range as the R² values that correspond to these correlation coefficients.

Translating this knowledge on SSBP to clinical practice is practically impossible because the traditional protocol requires a 3-day hospital stay and attempted briefer protocols in the clinic failed validation. Additionally, even the accepted protocols used in research with reproducibility correlation coefficients of 0.56 explain only 31% of the variability among individual responses. The ambulatory dietary sodium tests show lower R² values (8% for DASH-Sodium and 10% for GenSalt). Therefore, the moderate degree of biological reproducibility, the substantial BP variability unrelated to sodium, and the complexity of the testing conspire to render current methods impractical and ineffective for identifying the true magnitude of SSBP in an individual and for eventual applicability to clinical practice.

Conceivably, a clinical assumption about SSBP could be made from assessing other phenotypic characteristics related to it. However, the definition of some of these phenotypes is even more complex than that of SSBP itself. For example, the so-called nonmodulators, who are commonly but not uniformly SS, are defined by measurement of renal blood flow and aldosterone responses to angiotensin II, a very involved research protocol. Phenotypes easier to define such as low-renin essential hypertension or lack of renin response to salt deprivation or upright posture are common in groups of SS subjects but hardly useful for prediction in an individual patient.

Ideally, a biomarker (DNA, blood, or urine) that identifies patients in whom reduced sodium intake would lead to a clinically significant reduction in BP would help clinicians target therapy. Discovery of such a marker is complicated by the fact that it should emerge from studies conducted with current research protocols that use arbitrary BP cutoffs to define the phenotype. However, once uncovered, these putative markers could be further validated by their association with the multiple characteristics of the phenotype and with its prognostic implications. Reclassifying subjects from their dichotomization according to BP responses to salt into groups characterized by the presence or absence of the marker should theoretically strengthen these associations. Conceivably, subjects with the positive marker would be strongly encouraged to achieve low-sodium intake, whereas those without it would predominantly resort to other therapeutic modalities. The latter might be important because diminished salt intake, when not useful for BP reduction, may produce detrimental metabolic or cardiovascular effects and excessive hypertension in the elderly. Furthermore, along the lines advocated in the current era of precision medicine, a biomarker...
might help guide pharmacological therapy because certain antihypertensive drugs have been shown to reduce SSBP in some patient subgroups. Examples include valsartan for SS subjects with type II diabetes mellitus and microalbuminuria, fenofibrate for SS essential hypertensive subjects, and amiloride for blacks with volume-dependent, resistant essential hypertension.

In terms of dietary advice, it could be argued that such a marker would not be useful unless it could identify individuals beyond an SS BP cut point that defines a clinically actionable level. Such a cut point would be necessarily arbitrary because BP responses to reduced salt intake are normally distributed; that is, they are not an “all or none” phenomenon. Furthermore, the relationship between BP and cardiovascular risk is continuous; hence, virtually any magnitude of reduction of BP by diminished salt intake could conceivably produce a health benefit.

No definitive marker for SS BP has been identified yet. The surrogates discussed above, derived from 24-hour ABPM, have not been uniformly successful, and those from the study of urine proximal tubular cells and exosomes are too preliminary. In the absence of a marker, demographic and clinical phenotypes associated with SS BP can be used to guide dietary advice. Sodium sensitivity is more common in those with hypertension, in blacks compared with other ethnic groups, in women, in people >45 years of age, and in those with the metabolic syndrome. A large proportion of the US adult population falls into these categories, and clinicians can use this information, albeit imperfect, to target groups (rather than individuals) that may benefit from more intensive reduction in salt intake. A population approach to the reduction of salt intake is advocated by the US Dietary Guidelines Advisory Committee, joint American College of Cardiology/American Heart Association lifestyle guidelines, and recent Institute of Medicine reports, although the maximum amount of sodium that should be consumed daily has been the topic of recent scientific debate.

Regardless of the considerations above, discovery of a marker may be more relevant for practice in the future, unrelated to dietary advice. Available evidence shows that SS BP is a risk factor for cardiovascular morbidity independent of BP. Presumably, once the mechanisms of this phenotype are unraveled and therapies devised, treatment of SS BP may need to be addressed as a risk factor in itself, above and beyond treatment of BP, in the hypertensive population and perhaps even in normotensive SS individuals.

**Implications of SS BP for Public Health**

Recommendations for an optimal salt intake in the population have been issued mostly on the basis of epidemiological study results. There has been no major controversy about the presence of a direct relationship between salt intake and BP, particularly at high levels of BP. In contrast, there is persistent controversy about the effects of salt intake or those of diminished salt consumption on cardiovascular outcomes and mortality. Some investigators have reported a putative increase in detrimental outcomes at lower-than-average salt intakes, resulting in a J-shaped curve for the relationship between salt intake and cardiovascular disease. Others have provided possible pathophysiological mechanisms for potential harmful effects of reduced salt intake, including impaired hemodynamics in the elderly, activation of the renin-angiotensin system, and precipitation of insulin resistance.

The controversy continues because the designs of the epidemiological studies supporting worse cardiovascular morbidity and mortality with diminished salt intake have been plagued by methodological weaknesses, including residual confounding, reverse causation, error in the estimation of sodium intake from dietary recall or from measurement of salt excretion in random samples, and lack of repeated measurements of salt excretion over time. Meta-analyses dealing with the same issue are not devoid of the problems of heterogeneity in the included cohorts, to the point that a 2013 Institute of Medicine committee report stated that “lack of consistency among studies in the methods used for defining sodium intakes at both high and low ends of the range of typical intakes among various population groups precluded deriving a numerical definition for high and low salt intakes” in its findings and conclusions.

For decades, most Americans, regardless of age or sex, have had daily sodium intake levels that exceed recommendations, and public health approaches have been recommended to help reduce sodium intake in the United States. These strategies include setting mandatory national standards for the sodium content of foods because the primary source of sodium in the food supply is commercially processed foods.

The highest daily sodium intake level that is likely to pose no risk of adverse health effects to most individuals in the general population is characterized as the tolerable upper level. The tolerable upper level for sodium is set at 2300 mg/d, and SS BP is one factor that may affect the tolerable upper level. However, despite the heterogeneity in BP response to sodium intake, a convincing body of evidence suggests that reductions in population-level sodium intake should result in a favorable shift in the population distribution of BP levels.

On theoretical grounds, it is conceivable that reducing salt intake would be more important for SS than for SR individuals, not only because of the differential effect on BP of the 2 groups but also perhaps because of additional differential effects on other cardiovascular risk factors. Although important on an individual level, the applicability of SSBP to public health approaches to sodium reduction is currently limited. Limitations include a lack of uniformity across research studies in defining SSBP, the lack of an established practical method of assessment, and the fact that the prevalence of SSBP is heavily influenced by the definition used.

To improve the utility of SSBP in informing the public health efforts for sodium reduction, there should be better definition, measurement, and understanding of the correlates and consequences of SSBP. Development of an easily obtained, inexpensive marker for SSBP could allow the assessment of potential differential effects of reduced sodium intake on cardiovascular morbidity in SS versus SR individuals. This possible confounder has never been evaluated because of the complexity and cost of the current labor-intensive protocols required for characterization of the phenotype.
Knowledge Gaps

From the review of the major topics in the previous sections, it is obvious that there are significant gaps in our current knowledge about SSBP and unresolved methodological issues in the techniques and research designs used for its measurement in humans. Areas identified in this review include the following:

1. The increasing number of genes and mechanisms described as involved in determining the SS phenotype in experimental animal models strongly suggests that it may ultimately be caused by diverse clusters or networks of gene variants. Application of bioinformatic techniques may be required to identify such networks to test the hypothesis that the SSBP phenotype is inherited as a polygenic trait.

2. Investigation of the genetics of hypertension in humans will require genome-wide association studies in which the number of participants phenotyped for SSBP is large enough to confer power for detection of associations at genome-wide significant thresholds and to account for the number of genes or gene variants examined. Such studies could be modeled on the American Heart Association cardiovascular genome-phenome study, provided that all participants are phenotyped for SSBP.

3. Such genetic studies, and physiological studies, would be facilitated by the discovery of an easily measurable biomarker for the SSBP phenotype because current methodologies for phenotyping humans are laborious, cumbersome, and costly, constituting a major barrier to the conduct of large studies. Lack of a marker also has implications for clinicians because for a diagnostic presumption of SSBP in individual patients, they will have to continue relying on the demographic and biochemical characteristics (eg, age, race, plasma renin activity) that relate to the SSBP phenotype (which are not highly specific phenotypic surrogates) or use the response to diuretics or low-salt diet post hoc.

4. Continued research on hemodynamic characteristics that are surrogates of SSBP such as those conducted with ABPM techniques may conceivably provide a biomarker, particularly with incorporation of predictive variables into multivariate models that achieve high sensitivity and specificity compared with direct measurement of SSBP with currently accepted techniques. Additionally, an easily obtained biomarker from urine samples might be developed from continued research on properties of urine renal tubular cells or exosomes.

5. Emerging novel knowledge about the storage of sodium in tissue compartments and the study of possible differences in such storage between SS and SR animals or humans, coupled with the ability to use magnetic resonance imaging techniques to measure such storage, may also lead to the development of a radiological marker for the SSBP phenotype.

6. Investigations exploring the role of diverse biochemical substances (hormones, vasoactive agents, regulators of sodium metabolism, etc) in humans should be geared to the exploration of multivariate model prediction of the phenotype.

Methodological Recommendations for the Measurement of SSBP in Humans

There is no evidence base to determine best research practices in terms of measurement of SSBP in humans. Therefore, the following recommendations are made on a consensus basis.

1. Regardless of the design used, assessment of SSBP in humans requires large changes in sodium and volume balance. Clinical scientists working with the inpatient acute protocol of salt loading and salt deprivation should adhere to the traditionally used manipulations of salt intake, 460 mmol for the day of salt loading (300 mmol in the saline infusion and 160-mmol diet) and 10-mmol diet plus furosemide (three 40 mg doses over 8 hours) the following day. Clinical scientists working with outpatient dietary protocols must take into consideration that ambient intake varies widely within and between populations. Therefore, it seems reasonable to suggest that the high-salt phase of the protocols should have a daily intake of at least 250 mmol for about a week, followed by an equal period of low-salt intake of ≤50 mmol Na+, with SSBP assessed by the change in BP from the end of the high-salt period to the end of the low-salt period.

2. An ideal experimental design requires absolute control of the intake (parenteral or metabolic kitchen diets) and careful 24-hour urinary sodium excretion measurements. That is, achievement of the intended high- and low-salt phases of the experiment must be confirmed by measurement, not estimated from the intervention or from recall because both are subject to error. Furthermore, other components of the diet such as its overall composition and potassium content must be controlled because they modify the effects of sodium on BP.

3. Multiple, careful BP measurements reduce the inherent variability of BP and thus provide a more accurate estimate of the effect of changes of salt balance on BP. Options include the use of multiple standardized clinic measurements or ABPM. However, when multiple BP is obtained during the acute inpatient protocol, there is no gold standard or consensus about which parameter (24-hour average versus the average of shorter subsets after salt loading and salt depletion) should be used to assess the effect of salt on BP. Research using this methodology should investigate this issue further.
## Writing Group Disclosures

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<td>Richard A. Dart</td>
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<td>Christopher H. Newton-Cheh</td>
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<td>Frank M. Sacks</td>
<td>Harvard School of Public Health</td>
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*Significant.

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<th>Other</th>
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<td>Robert Carey</td>
<td>University of Virginia Health System</td>
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References


47. Laffer CL, Boltermann RJ, Romero JC, Elijojchv et al. Salt Sensitivity of Blood Pressure. e33


Salt Sensitivity of Blood Pressure

Elijio et al


White PC, Dupont J, New MI, Leiberman E, Hochberg Z, Rössler A. A mutation in CYP11B1 (Arg-448→Gly) associated with steroid 11


Elijovich et al  Salt Sensitivity of Blood Pressure e43


335. Dahlberg J, Nilsson LO, von Woson F, Melander O. Polymorphism in NEDD4L is associated with increased salt sensitivity, reduced levels of P-renin and increased levels of Nt-proANP. PLoS One. 2007;2:e432. doi: 10.1371/journal.pone.0000432.


348. Kirkendall AM, Connor WE, Abboud F, Rastogi SP, Anderson TA, Fry M. The effect of dietary sodium chloride on blood pressure, body fluids,
393. Gueorguiev CM, Grim CE. A curriculum for the training and certification of blood pressure measurement for health care providers. Can J Cardiol. 1995;11(suppl H):H38–42H.


Key Words: AHA Scientific Statements ◼ biomedical research ◼ blood pressure determination ◼ genetic research ◼ physiopathology ◼ salt-sensitivity hypertension ◼ sodium
Salt Sensitivity of Blood Pressure: A Scientific Statement From the American Heart Association
on behalf of the American Heart Association Professional and Public Education Committee of the Council on Hypertension; Council on Functional Genomics and Translational Biology; and Stroke Council

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In the article by Elijovich et al, “Salt Sensitivity of Blood Pressure: A Scientific Statement From the American Heart Association,” which published online July 21, 2016, and appears in the September 2016 issue of the journal (Hypertension. 2016;68:e7–e46. DOI: 10.1161/HYP.0000000000000047), a correction was needed.

On page e7, in the byline, the fellowship designation “FAHA” has been added for Dr. Richard A. Dart.

This correction has been made to the current online version of the article, which is available at http://hyper.ahajournals.org/content/68/3/e7.full.