

Salt Sensitivity: Challenging and Controversial Phenotype of Primary Hypertension

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Abstract Increases in life expectancy and cardiovascular adverse events in patients with hypertension highlight the need for new risk-reduction strategies to reduce the burden of degenerative diseases. Among the environmental factors, high salt consumption is currently considered the most important risk factor of hypertension. However, while high salt intake significantly raises blood pressure in some individuals, others do not show variation or even decrease their blood pressure. This heterogeneity is respectively classified as salt sensitivity and salt resistance. In this review, we propose salt sensitivity as a useful phenotype to unravel the mechanistic complexity of primary hypertension. The individual variability in blood pressure modification in response to salt intake changes derives from the combination of genetic and environmental determinants. This combination of random and non random determinants leads to the development of a personal index of sensitivity to salt. However, those genes involved in susceptibility to salt are still not completely identified, and the triggering mechanisms underlying the following development of hypertension still remain uncovered. One reason might be represented by the absence of a specific protocol, universally followed, for a standard definition of salt sensitivity. Another reason may be linked to the absence of common criteria for patient recruitment during clinical studies. Thus, the generation of a reliable approach for a proper recognition of this

personal index of sensitivity to salt, and through it the identification of novel therapeutic targets for primary hypertension, should be one of the aspirations for the scientific community.

Keywords Hypertension · Salt sensitivity · Endogenous ouabain · Renin-angiotensin-aldosterone system · Salt intake · Genetics · Candidate gene · GWAS · Personalized therapy

Introduction

Essential hypertension is one of the major public health challenges due to its high prevalence (in one third of the world's population) and the increased risk of adverse events, such as stroke, heart and kidney failure [1]. Essential hypertension is a complex disease, characterized by a large variety of determinants, such as genetic factors, race/ethnicity, age, body mass, and diet, as well as associated comorbidities (diabetes, chronic kidney diseases, etc.).

This interplay between environmental and genetic factors, both crucial determinants of hypertension, interacts and affects intermediary phenotypes which favor the increase in total vascular resistance and in cardiac output, consequently inducing hypertension [2]. Among all the intermediary phenotypes, the evaluation of salt sensitivity has been proposed to unravel the mechanistic complexity of primary hypertension [3–7].

Several studies have shown that primary hypertension is globally responsible for almost 50 % of the mortality rate [8]. However, these statistics mentioned above do not distinguish salt-sensitive from salt-resistant hypertension or they do not even include normotensive patients who are salt-sensitive [9••].

Salt sensitivity has been estimated in 51 % of hypertensive patients and in 26 % of normotensive subjects, posing a major public health problem in USA and in Westernized societies [7].

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Among the environmental factors, high salt consumption is currently considered the most important risk factor of hypertension [10, 11].

Nevertheless, the effect of dietary sodium (Na^+) on blood pressure (BP) varies according to the population's characteristics. Indeed, while high salt intake significantly raises BP in some individuals, others do not show variation or even decrease their BP. This heterogeneity is respectively classified as salt sensitivity and salt resistance [12, 13].

The distinction of these two phenotypes is relevant; salt sensitivity, even in absence of manifest hypertension, is per se a risk factor for cardiovascular morbidity and mortality [14, 15].

Previous family studies have documented a heritability estimated from moderate to high, generally ranging from 22 to 84 % [16, 17]. Furthermore, genetic studies have suggested that genetic mechanisms might play a pivotal role in salt-sensitive hypertension (SSH) [18].

This review combines most of the newest discoveries achieved in the field of salt sensitivity. Unfortunately, the definition of this individual susceptibility to salt still remains faint as well as the underlying mechanisms in either normotensive and hypertensive individuals continue to be unclear [13].

Despite the consistent lack of knowledge, we consider salt sensitivity as a powerful phenotype to explore the molecular and genetic mechanisms of primary hypertension. We highlighted all of the random and non-random determinants that should be better taken in consideration for the establishment of a "personal" salt risk profile. Our contention is that the future moving to the real clinical life will depend upon the ability to generate novel personalized therapeutic targets for a proper control of BP.

Mechanisms of Peripheral Sodium Handling in BP Regulation and Salt Sensitivity

The physiological mechanisms linking salt intake, total body Na^+ content, fluid balance, and BP have been already largely illustrated.

Since 1929, Cannon with his "steady-state theory" postulated that total-body Na^+ content is necessarily function of salt intake, identifying the kidney as unique source of Na^+ handling [19]. According to Cannon hypothesis, the whole amount of dietary salt and the amount of Na^+ eliminated through urination are directly correlated. The steady state between salt intake and urinary Na^+ excretion is achieved within a few days; whereas an unbalance between salt intake and renal excretion inevitably leads to fluid retention, and consequently to hypertension [19–22].

Later, Blaustein identified also the importance of the vascular tree in the BP modulation. Changes in Na^+ gradient augment calcium (Ca^{2+}) concentration, increasing vessel wall tension and peripheral resistance [3].

However, despite the importance of the vascular system, Guyton and collaborators demonstrated that the kidney plays a pivotal role in the control of total body Na^+ and in the regulation of body fluid homeostasis due to the modulation of pressure natriuresis (PNat) relationship (Fig. 1) [23]. PNat results from the relationship between BP and urinary salt output. While BP rises (graphically represented by the right shift of PNat curve), the urinary output becomes greater, leading to the fluid loss, until the BP falls back to the exact equilibrium point. Conversely, when BP falls below the equilibrium point (left shift of the curve), the fluid intake becomes greater than the output; this will increase the total body fluid volume, leading to the BP rise up to the equilibrium point (Fig. 1) [23]. Prolonged tendency to high Na^+ assumption leads to the kidney reabsorption of the additional Na^+ , resulting in fluid volume expansion and in the consequent hypertension [24, 25]. Salt-sensitive (SS) individuals in response to a high salt intake display a blunt PNat curve, characterized by a right shift along BP axis (Fig. 2) [24, 25].

The kidney ability to reabsorb salt is given by exchangers, transporters, and ion channels located within the nephrons [26]. Crucial determinants of the electrolytes body balance are several hormones, such as angiotensin II, aldosterone, atrial peptides, nitric oxide, and circulating endogenous ouabain (EO) [27, 28].

The principal site for the regulation and determination of Na^+ balance is the Na^+/K^+ -ATPase (Na^+ pump), ancestral enzyme regulated by two independent and complementary systems: EO and renin-angiotensin-aldosterone system (RAAS). Together, EO and RAAS play a pivotal role in the understanding of SSH. EO seems to increase the vascular tone and

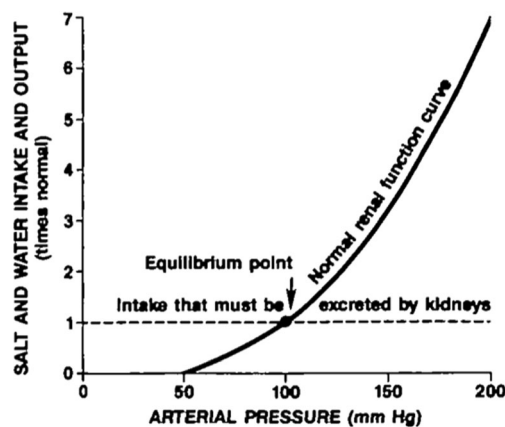


Fig. 1 Pressure-natriuresis curve (PNat). Illustration of the equilibrium point between arterial pressure and salt/water output. Whether blood pressure rises, the urinary salt and water output increases, causing right shift of the curve. There is fluid loss until the blood pressure returns to the equilibrium point. Conversely, if the arterial pressure falls below the equilibrium point, the curve is left oriented. The urinary output is suppressed until the blood pressure returns to the equilibrium point (from Guyton A, Hypertension 1990)

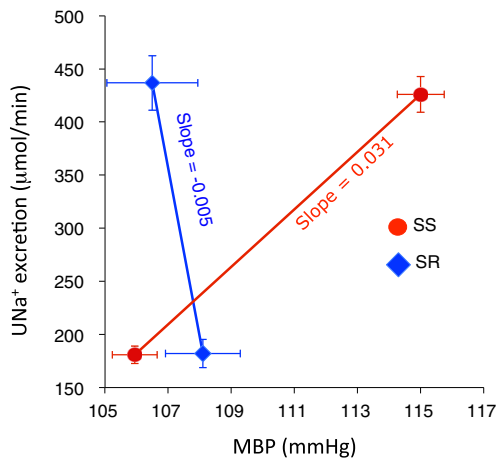


Fig. 2 Pressure Natriuresis relationship (PNat) in salt sensitive (SS) and salt resistant (SR) hypertensive patients evaluated during acute salt load. The slope of the curve is established according to the relationship between urinary Na⁺ excretion (UNa⁺) and the mean blood pressure (MBP). PNat is significantly different between SS and SR individuals: SS displays a blunted curve right shifted. On the opposite, SR curve is steeper and left shifted

contractility, whereas RAAS supports and maintains the renotubular Na⁺ retention [29•].

RAAS-Derived Homeostatic Response is Influenced by Salt Intake

RAAS is one of the major homeostatic systems, which controls body fluid volume, electrolyte balance, BP, and neuronal and endocrine functions related to cardiovascular control. RAAS is currently considered as a key factor of essential hypertension; indeed, its effector blockers are commonly adopted in clinical practice with successful BP control.

RAAS exerts its action through the effector molecule angiotensin II which binds specific membrane-bound receptors located in multiple tissues, including the vasculature [30, 31]. Angiotensin II activating aldosterone results in a homeostatic response that decreases the renal perfusion and vice versa increases the renal tubular Na⁺ reabsorption and potassium (K⁺) excretion [32]. In high-salt diets, for a good BP control, angiotensin II is physiologically suppressed.

Forty to fifty percent of essential hypertensive individuals do not exhibit the expected suppression of angiotensin II predicted by changes in Na⁺ intake [33]. This persistent RAAS activity [24, 25] has been attributed in part to genetics.

Giner et al. [34] and Poch et al. [35] have assessed a link between genetic polymorphisms of RAAS and SSH. Lack of plasma renin suppression has been found in SS hypertensive individuals in response to high sodium intake [34, 35].

Endogenous Ouabain Activity Changes According to Salt Assumption

EO is an adrenocortical cardiac glycoside whose circulating level is influenced by the body Na⁺ balance.

Na⁺ depletion raises circulating EO [29•], whereas long-term high-salt diet, much more than plasma aldosterone, suppresses circulating EO level in normotensive and non SS individuals. However, independently of Na⁺ intake, plasma EO [36] is inappropriately elevated in 45 % of patients with essential hypertension and correlates with BP [37].

Recently, Lanzani and collaborators have raised EO, as a functional marker, to predict the beneficial and adverse effects of low-salt diets [4••].

Furthermore, the elevated level of EO is related to cardiac [38] and renal [39] damage.

Manunta and collaborators have demonstrated that, even in absence of established hypertension, a high-salt assumption raises EO in SS individuals [25]. Moreover, on high-salt intake the hypertensiogenic action of EO has been found primarily related to the influence exerted on the kidneys and vascular system.

Ouabain administration in rabbit aortic strips augments the vessel tone [3]. On high-salt intake, the elevated circulating level of EO present in hypertensive patients enhances the activity of vascular α_2 isoform of the Na⁺ pump and activates the vascular sodium-calcium exchanger (NCX 1.3) [40–42]. The activation of NCX 1.3 enhances the vascular contractile activity due to the increase in intracellular Na⁺ and in extracellular Ca²⁺ [41–43] leading to essential hypertension [40].

On the other side, on high salt assumption EO binding of the renal α_1 isoform of the Na⁺ pump triggers the activation of tyrosine kinase c-Src. The activation of c-Src increases the renal tubular Na reabsorption and also promotes the transcription of several growth-related genes [44], further leading to hypertension and cardiovascular remodeling [45].

Central Nervous System Modulates Na⁺ Transport and BP

Osborn in 2005 postulated the “set point” hypothesis [46] which says that BP response to dietary salt changes depends not only on renal homeostatic regulatory function, but it is also regulated by central nervous system (CNS) [46].

The brain has been currently considered as a possible site of salt sensing. Several regulators of Na⁺ transport, such as aldosterone, mineralocorticoid receptors (MRs), EO, and Na⁺ pump play a central role in CNS synaptic plasticity. Those modulators seem to play a major role in the regulation of the Na⁺ content both in cerebrospinal fluid (CSF) and in the brain [26, 47].

Na⁺ content in CSF and plasma is physiologically equal, whereas SS rats exhibit increased CSF Na⁺ content [48]. A

high-salt diet does not affect the CSF Na^+ level of salt resistant (SR) rats [49, 50]. In other words, it is now evident that on high-salt intake, SS rats significantly increase their CSF and their brain parenchyma Na^+ content.

All the above data suggest that SS rats have less buffering capacity when compared to SR [48].

Huang and collaborators observed that in high-salt diet, SS rats increase their CSF Na^+ content before they became hypertensive [49], demonstrating that Na^+ entry across the brain barriers does not occur secondarily to the hypertension status [51]. All of these studies taken together suggest that salt sensitivity causes functional changes in the mechanisms regulating Na^+ transport across the blood–brain-barrier. However, the results obtained in humans are controversial; in high-salt diet (16–18 g/day for 7 days), salt-sensitive and non-salt-sensitive hypertensive subjects similarly increased their CSF Na^+ content [52].

The Nobel prize winner Paul Greengard [53] and Frans Leenen [51] highlighted the role of two distinct neuromodulatory activities, the brain-derived aldosterone and the aldosterone-EO pathways, respectively, activated in consequence of an acute and chronic increase in CSF Na^+ content. According to their proposals, acutely, the sympatho-excitatory and pressor responses observed in SS rats primarily occur due to an angiotensinergic sympatho-excitatory pathway [54, 55]; whereas a chronic increase in CSF Na^+ content appears to be dependent on a centrally released aldosterone-EO neuromodulatory activity [51, 53].

Angiotensinergic Sympatho-Excitatory Pathway

Aldosterone acting in the CNS may be either circulation derived [56, 57] or locally synthesized *de novo* directly from cholesterol or by conversion of circulating steroid precursors [58]. Whereas centrally synthesized aldosterone is physiologically relevant, is still a topic of debate [59]. However, there are convincing data that several enzymes involved in steroidogenesis are centrally released [59–62]. Central infusion of aldosterone in normotensive rats increases hypothalamic EO content, leading to hypertension [63, 64]. Even a small increase in CSF Na^+ content is able to produce sympatho-excitation [63].

Acute increases in CSF Na^+ content primarily excite Na^+ -sensitive nuclei, inducing activation of neuronal activity via angiotensin II release. Angiotensin II through the local AT_1 -receptor stimulation excites subpopulations of neurons thereby increasing sympathetic activity, BP, and heart rate [65].

However, how and where in the hypothalamus an increase in Na^+ concentration also increases the aldosterone production has not been addressed yet. Several experiments performed in animal models suggest that centrally located MRs, activated by aldosterone, are the principal mediators of the salt-induced hypertension, whereas aldosterone acts as the main agonist [66, 67].

Aldosterone-EO Neuromodulatory Activity

CNS has been already implicated in the control of EO [68, 69]. High level of EO has been detected both in the hypothalamus and pituitary gland [70–72].

The intra-cerebroventricular administration of NaCl causes hypertension in mice, due to an “ouabain-like substance,” present in the brain [73]. Furthermore, administration of ouabain in nuclei [54, 74, 75] causes dose-related increases in sympathetic nerve activity, BP, and heart rate.

All of these results suggest that the pressure pathway might be dependent in part on the brain released EO activity.

This centrally produced EO has been shown to exert its action via the high affinity binding of the α_2 -isoform of the Na^+ pump [76]. Indeed, both in vivo and in vitro experiments showed that central blockade of the ouabain high affinity binding of Na^+ pump suppresses the sympatho-excitatory and pressor responses of EO, and prevents the establishment of hypertension [73, 77]. Taking together all these data indicate that centrally released EO also plays a major role in the SSH [78–82].

Since the sympatho-excitation induced by the brain released EO is absent in transgenic rats without a functional brain renin–angiotensin system [83], and since it can be also prevented by the blockade of AT_1 -receptors [54, 55, 84], angiotensin II appears to be the principal actor of the EO mediated pressor response. Indeed, sympatho-excitatory and pressor responses to an increase in CSF Na^+ content are primarily mediated via angiotensinergic sympatho-excitatory pathways [54]. Nevertheless, during chronic increases in CSF Na^+ concentration the effectiveness of this pathway appears to become dependent on activation of slow synaptic transmission, sustained by the aldosterone-ouabain neuromodulation [53]. Centrally released EO, inhibiting the Na^+ pump and increasing the intracellular Ca^{2+} , lowers the membrane potential, slowing down the synaptic transmission [53]. As a result, more excitatory impulses reach the threshold and the neurons rate of firing increases. In other words, according to what P. Greengard proposed, it seems that chronic increase in CSF salt content activates a slow synaptic transmission to control the effectiveness of fast-synaptic transmission regulated by aldosterone, further contributing to the status of SSH [53].

Other Districts Involved in Sodium Handling

Currently, independently on CNS and periphery, other Na^+ homeostatic regulatory processes have been proposed. Two other systems emerged as involved in body Na^+ handling: being respectively the (neuro-)endocrine rhythmical release and the inflammatory response activated by tissue Na^+ storage.

High-Salt Intake in the Long-Term Induces an Endocrine Homeostatic Response

Rakova et al. analyzed Na^+ metabolism in response to ultra-long-term controlled and constant salt intake, administered to the participants of a space flight simulation [85].

“Mars 500” showed that total body Na^+ is regulated by a rhythmic endocrine homeostatic mechanism [85]. In contrast to the previous theories, the healthy spacemen exhibited regular fluctuations of total body Na^+ with monthly periodicity. Moreover, their steady state was not achieved within a few hours, and 24-h Na^+ excretion rarely matched the daily one. Even though salt intake was constant for weeks and months, there was considerable day-to-day variability in 24-h Na^+ excretion, accompanied by fluctuations of aldosterone, cortisol, and cortisone which peaked with a periodicity of about 1 week [85].

On the opposite, no changes in BP, body weight, and in the extracellular water excretion were evidenced according to fluctuations of total body Na^+ content [85].

Thus, it was shown that rhythmical accumulation and release of total body Na^+ content occurs independently of daily salt intake; it seems regulated by neuro-endocrine rhythmical pattern instead. However, whether this pattern is disrupted on salt sensitivity should be further established.

Sodium Deposition in Skin and Skeletal Muscle Influences BP

Recently, Na-MRI measurements of Na^+ content in humans suggested that relevant amounts of Na^+ accumulates in muscle and skin [86]. Na^+ deposition seems to disrupt the internal environment composition of tissues. Those amounts of Na^+ increase with age, seem to be more pronounced in men than in women, and increase in patients with essential hypertension [87].

Moreover, hypertensive patients treated with spironolactone showed reduction in their Na^+ storages [87], causally linking Na^+ deposition to primary hypertension.

Increases in Na^+ concentration in cell culture medium cause interstitial osmotic stress and boost adaptive or innate immune cell reaction [88, 89].

Thus, in high-salt diet, the hypertonicity established and maintained by the tissue Na^+ storages may triggers either the activation of adaptive or innate immune systems, leading to an inflammatory response which favors electrolyte concentration and mobilization [90].

Furthermore, Kopp et al. found that large amounts of Na^+ are stored in the skin interstitium, without concomitant water retention [87]. The absence of water retention evoked what Cannon proposed that skin is a sponge-like structure which stores and releases Na^+ independently of renal blood purification [20]. Recent evidence highlighted the role of the skin in the homeostatic regulatory processes, suggesting also a role in the BP regulation [91]. The amount of deposited Na^+ is

actively pumped from the keratinocyte to the cutaneous skin interstitium through a kidney-like countercurrent system. There is an electrolyte concentration gradient, which favors Na^+ mobilization, enhancing total body Na^+ content and increasing BP [92, 93].

Additionally, an emerging concept is taking place in the scientific field: macrophages may also actively regulate and modify the skin electrolyte composition [94, 95]. According to what was proposed by Machnick and collaborators, it also seems that macrophage-derived vascular epithelial growth factor C (VEGF-C), as a clearance factor, may be cause of lymphatic mobilization of Na^+ stored in the skin [94, 95].

Despite all the findings mentioned above, whether Na^+ deposition in tissues is cause of salt sensitivity, or salt sensitivity favors Na^+ deposition in tissues still remains controversial. Novel studies need to be performed to assess whether Na^+ storage contributes to cardiovascular morbidity and mortality, or whether it can be modified by lifestyle changes or medication. Moreover, the use of MRI in clinical practice to measure the tissues Na^+ content appears difficult and costly; and still, the 24-h urinary Na^+ excretion (UNaV) represents the primary metric universally adopted to estimate the dietary salt intake [96].

Environmental Factors: Crucial Determinants of Salt Sensitivity

Among the environmental factors, high salt consumption is currently considered the principal risk factor of hypertension [10, 11]. Moreover, a persistent high salt intake was associated to a higher rate of death for coronary disease and stroke [97].

Salt sensitivity in normotensive and hypertensive subjects has been either associated with increased cardiovascular events and reduced survival rate [14]. SS individuals display higher prevalence of left ventricular hypertrophy than SR [98], and hypertensive patients sensitive to salt are more prone to fatal and non fatal cardiovascular adverse events [99].

In 2010, among 1.6 million deaths for cardiovascular reasons, 1 of 10 deaths was attributed to high sodium consumption (mean level 3.95 g/day) [100].

Mozaffarian and collaborators, in their meta-analysis showed a linear dose-response relationship between reduction in salt intake and BP [100]. The effects of dietary salt on BP resulted more consistent among older subjects than among younger, among blacks than among whites, and among hypertensives than among normotensives [100]. The age-related differences observed and the larger effects found in hypertensive individuals seem to be consistent with the reduction in the vascular compliance and in the renal filtration, whereas the racial difference depends on changes in renal Na handling [101, 102].

Despite the evidence that Na^+ assumption lowers than 2.3 g per day either increase or decrease the incidence of

cardiovascular events [96], the guidelines commonly used do not consider the unsafe lower limit of sodium intake.

Furthermore, other groups have demonstrated the presence of paradoxical hypertension even whether the diet is salt-depleted [103], making the recommendation of a precise target of daily salt consumption further controversial.

O'Donnell and collaborators recently analyzed the correlation between the estimated urinary Na^+ and K^+ excretion with a composite outcome, represented by mortality and cardiovascular adverse events [104•]. Contrary to Mozaffarian et al., they found a nonlinear correlation between salt consumption and BP. According to the large International Prospective Urban Rural Epidemiological (PURE) Study, the lowest risk of the composite outcome was estimated for Na^+ excretion ranged between 3.00 and 6.00 g per day (Fig. 3), whereas a highest risk resulted for an estimated Na^+ excretion higher than 7.00 g and lower than 3.00 g per day (Fig. 3) [104•]. A daily K^+ excretion higher than 1.5 g resulted linked to a lower risk of cardiovascular events [104•]. Thus, higher and lower concentration of urinary Na^+ associates to an increased risk of

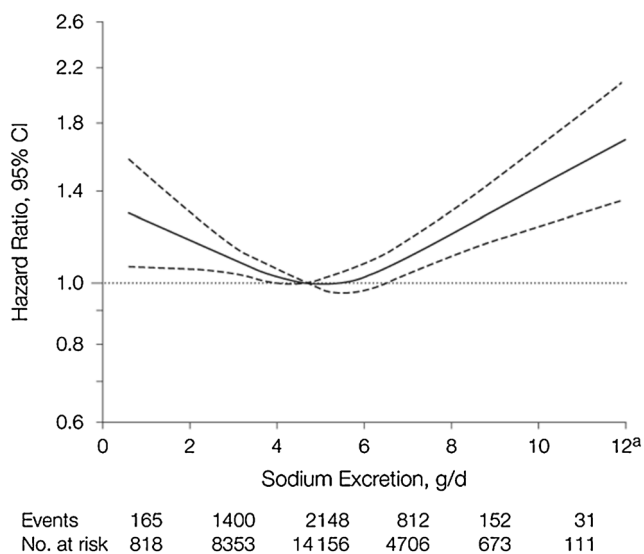


Fig. 3 Extended 24-h urinary excretion of sodium and composite of cardiovascular death, stroke, myocardial infarction, and hospitalization for congestive heart failure. Spline plot model adjusted for age, sex, race/ethnicity (white vs. nonwhite); prior history of stroke or myocardial infarction; creatinine, body mass index; comorbid vascular risk factors (hypertension, diabetes mellitus, atrial fibrillation, smoking, low- and high-density lipoprotein); treatment allocation (ramipril, telmisartan, neither, or both); treatment with statins, b-blockers, diuretic therapy, calcium antagonist, and antithrombotic therapy; fruit and vegetable consumption, level of exercise. Dashed lines indicate 95 % confidential intervals (CIs). Events and numbers at risk are shown between values on *x-axis* because they indicate the numeric range between these values. The Spline curve is truncated at 12 g per day. The association between estimated sodium excretion and CV events results J-shaped. Compared with baseline sodium excretion of 4 to 5.99 g per day, higher baseline sodium excretion (>7 g/day) and lower sodium excretion (<2 g/day) associates with an increased risk of the composite outcome (from O'Donnell MJ, JAMA 2011)

cardiovascular adverse events and mortality. This association is graphically represented in a J-shape curve [105–107] which is more pronounced among hypertensive participants, whereas it was less deep after adjustment for BP [104•], confirming that the adverse effects of high Na^+ assumption are somehow mediated by the effects of Na^+ intake on BP [108, 109]. There is indeed evidence that salt intake lowers than 2.5–3.00 g activates the RAAS and increase the EO circulating levels, leading to hypertension [110, 111].

In humans, a J-shape relationship was found between Na^+ balance and plasma EO [5]. This observation raises EO as a possible biomarker and also as a functional effector of cardiovascular outcomes [29•]. Elevated circulating level of EO has been associated with several adverse outcomes, such as cardiac enlargement, cardiac and renal failure, and a variety of other terminal disorders [27]. However, despite new remarkable discoveries, still many progresses have to be made in order to clarify the nonlinear effects of salt intake on cardiovascular disease and death [112].

Several epidemiological studies have been further performed to assess whether non-pharmacological interventions, such as lifestyle modifications, may also be considered in order to reduce BP and the related adverse events in patients with already established hypertension. The International Cooperative Study on the Relation of Sodium and Potassium to BP study (INTERSALT) demonstrates that wrong habits, such as habitual high Na^+ intake, low K^+ intake, reduced physical activity, elevated Body Mass Index (BMI), high alcohol intake, might play a critical role in increasing the prevalence of hypertension [113]. All of these habits seem to accelerate the progression of prehypertension to hypertension, as well as to increase the long-term risk of adverse events [114, 115, 116••, 117]. However, the establishment and implementation of worldwide healthier lifestyle is challenging. One barrier is represented by the problematic affordability of a diet rich in fruit and vegetables and low in Na^+ .

According to what was discussed during the World Health Organization meeting in 2007, interventions to reduce the population-wide salt intake have repeatedly proved to be highly cost-effective, hence, the urgency to implement policies in order to tackle the reduction of dietary salt intake [118].

Other vehicles as alternatives to salt should be therefore explored, and the currently recommended level of salt needs has to be further revised [118].

In addition, motivation would be expected in subjects with prehypertension, who do not have to face the alternative of taking medications [119].

Certainly, the future challenge is developing and implementing effective public health strategies, which lead to sustained lifestyle modification. Food industries should be encouraged to harmonize the salt content of

their products according to the lowest threshold and to avoid unnecessary variations in salt content of the same food product commercialized in different countries [118].

Controversial Tools for Salt Sensitivity Diagnosis

Several epidemiological and interventional studies have demonstrated a clear relationship between salt intake and hypertension [10, 11].

Salt sensitivity is manifested as a BP modification in response to an acute or chronic salt intake change and it can be defined as the tendency of BP to fall during salt reduction and rise during salt repletion [12, 13].

Even if the clinical importance of salt sensitivity is well recognized, a specific protocol for a univocal definition does not exist. Furthermore, an accurate diagnosis remains problematic, mainly due to the phenotype complexity.

There is a large consensus within the scientific community on the recognition of dietary manipulation as the standard reference procedure for evaluating the status of high susceptibility to salt. However, a long diet cycle requires at least 1-week period respectively for low and high salt intake, with additional compliance difficulties for many patients. Moreover, the formulation of standardized meals is costly.

In order to overcome these obstacles, a shorter methodology was proposed consisting of a rapid intravenous (i.v.) sodium load and depletion known as “Weinberger test” [120]. Grim et al., developed a rapid protocol in which BP was measured after i.v. infusion of 2 L of saline (0.9 %) and after volume depletion obtained through furosemide administration [120].

Even if comparison studies between dietary manipulation and Weinberger test are scarce, in an attempt to show that the effect of both maneuvers are similar, various studies took advantage of the Weinberger test to evaluate salt sensitivity.

Moreover, Weinberger et al. initially reported a significant correlation with dietary manipulation.

However, in the end, not all subjects responded to both maneuvers in a similar qualitative fashion. The discrepancy was more frequent among subjects having salt-resistant response to the rapid protocol [121].

Galletti et al. highlighted a good correlation between the degree of dietary salt restriction and BP in the subgroup of patients identified as SS by the rapid test. Their progressive reduction in BP after dietary decrement in salt content was consistent, while a negative, although not significant, association was found in the SR subgroup [122]. However, it was difficult to obtain valid conclusions from Galletti’s study, due to the small subset of patients undergone both protocols.

Later, de la Sierra et al. indicated that none of the comparisons showed good combinations of sensitivity and specificity between dietary manipulation and rapid protocol [123•].

Due to the number of variable results obtained, recently Weinberger’s protocol has been revisited and readapted in the Acute Saline Load Test. This novel test proposed can be administrated in regimen of day hospital, it is low cost, extremely rapid and it does not depend on the individual compliance [124].

The Acute Saline Load Test consists in the i.v. infusion of 2 l of saline solution (NaCl 0.9 %) within 2 h. The distinction between SS and SR phenotypes derives by the determination of the P_{NaT} curve (Fig. 2). The slope of that curve is established by the relationship between UNaV (collected at the beginning, at the end and 2 h after the test) and the mean blood pressure (MBP) observed under basal conditions and every 30 min during the test (Fig. 2),

The individuals having a blunt slope of that curve are SS, whereas those showing a steeper slope are evaluated as SR (Fig. 2).

Manunta et al. interpreted the volume repletion obtained by furosemide as just a reflection of the individual responsiveness to furosemide, rather than a faster reproduction of the effect obtained on BP thanks to the low-salt diet [124].

Therefore, despite the social impact of the disease and despite several useful methods proposed, salt sensitivity identification and diagnosis are still dramatically variable. Furthermore, a specific protocol universally adopted in clinical practice for a univocal definition of the disease is still missing.

Importance of Early Diagnosis of Salt Sensitivity

Clinical studies have shown that 30 to 60 % of essential hypertensive patients are salt sensitive [125–127]. Interestingly, normotensive SS individuals and hypertensive patients display similar mortality rate, whereas normotensive SR individuals exhibit improved survival [14]. Manunta and collaborators proposed the selection of Naïve Hypertensive Patients (NHP), defined as never treated individuals with newly discovered hypertension or with high-normal BP (systolic BP ranged between 120 and 140 mmHg and diastolic BP from 80 to 90 mmHg) (Fig. 4) [25]. According to the authors, NHP selection permits to verify the consistency of new molecular mechanisms detected by genetics and it further allows to unmask the essential hypertension triggering mechanisms as well as the study of the natural history and development of the disease [25]. Moreover, the selection of never pharmacologically treated individuals avoids the confounders of previous medications [128•]. Citterio et al. demonstrated that a single month of pharmacological washout is not enough to remove the treatment effects. Withdrawal, per se, may trigger a variety of alternative pressor mechanisms (e.g., increasing RAAS activity after removal of their inhibitors) which

Fig. 4 Progression of essential hypertension. The disease develops in 3 phases. In the latest phases (stages 2 and 3), both the severe vascular constriction and the consequent vascular auto-regulation and remodelling trigger superimposed pressure mechanisms and cause permanent organ damage. Selection of never treated individuals with newly discovered hypertension or high-normal blood pressure values (NHP) allows the investigators to unmask the natural history of hypertension

Phase	Symptoms	SBP mmHg	DBP
Hypertensive emergency (stage 3)	Symptoms (Angry face emoji)	≥ 180/120	Signs of irreversible organ damage
Hypertension (stage 3)	Symptoms (Frowning face emoji)	≥ 180/120	Progressive signs of organ dysfunction
Hypertension (stage 2)	Symptoms (Sad face emoji)	≥ 160/100	Lifestyle changes coupled with pharmacological treatment are mandatory
Hypertension (stage 1)	No symptoms (Neutral face emoji)	≥ 140/90	Lifestyle changes coupled with pharmacological treatment are mandatory
Pre-hypertension or borderline blood pressure	No symptoms (Happy face emoji)	> 120/80	Lifestyle changes are mandatory
Healthy blood pressure values	No symptoms (Happy face emoji)	≤ 120/80	

Naïve Hypertensive Patients

superimpose the triggering one, maintaining the initial status of hypertension [128•].

Nevertheless, the importance of an early diagnosis is not universally approved, and univocal criteria of patient selection do not currently exist. Literature still reveals extremely variable opinions and several studies are performed on individuals with more severe hypertension and already pharmacologically treated.

Genetic Variants: Crucial Determinants of Salt Sensitivity

Epidemiological studies have shown that heritability is crucial to determine hypertension [129–131].

Even if the pathophysiological mechanisms of salt sensitivity are still unknown, there is currently strong evidence that

genetic mechanisms underlie variation in BP response to salt intake changes [132–134].

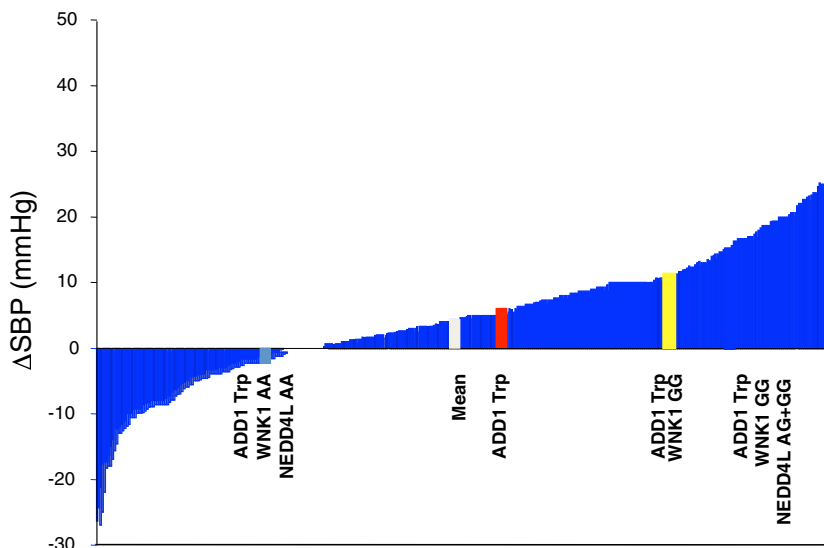
Moreover, several groups demonstrated that salt sensitivity and the related worst outcomes derive from the interaction between genetics and environmental factors [16–18].

Genetic mechanisms seem to play a pivotal role in the establishment of a personal index of sensitivity to salt variations (Fig. 5).

Despite a variety of genome-wide association studies (GWAS), those specific genes involved in hypertension development still remained elusive and those candidate genes identified by GWAS are not involved in salt sensitivity [135].

Thus, clinicians urge the identification of common allelic variants of those genes specifically candidates in salt sensitivity. Firstly, this will permit the establishment of a personal index of salt sensitivity based on the genetic background. Secondly, it will allow the selection of novel therapeutic

Fig. 5 During an Acute Salt Load test Systolic Blood Pressure variation (Δ SBP) of 350 individuals ranged from -28 to +49 mmHg. This variability is influenced by patients' genotype. In ADD1 460Trp background, individuals carrying both WNK1 GG and NEDD4L G variants show a greater increase of SBP during the test. On the contrary, individuals carrying WNK1 AA and NEDD4L AA variants decrease their SBP during the test (modified from Manunta P, Hypertension 2008)



targets as powerful forces to support the fight against primary hypertension.

Among all the genes described in literature as linked to SSH, we only selected those strongly implicated in the control of EO and aldosterone-dependent pressor pathways.

Alpha-Adducin (ADD1)

Adducin is a polymeric cytoskeleton protein involved in the internalization and recycling of Na⁺ pump. Several studies performed in Milan Hypertensive rats have shown that a functional point mutation in *ADD1* is cause of SSH. Furthermore, Milan Hypertensive rats mutated for *ADD1* exhibited increased tubular Na⁺ reabsorption [136–138]. In humans, two case-control studies have demonstrated that a missense mutation in *ADD1* gene (G460W) associates to hypertension. Furthermore, those patients carrying the mutation exhibited better responsiveness to thiazide treatment [126, 139, 140].

During “Weinberger test,” humans bearing *ADD1* 460W variants (G/W or W/W), if compared to the control (G/G) carriers, displayed a blunt slope of the P_{Na}t curve and increased tubular Na⁺ reabsorption, eventually resulting salt sensitive [138].

ADD1-WNK1-NEDD4L Pathway

WNK1 and NEDD4L are two regulator genes of luminal Na⁺ transport along distal nephrons.

Recent studies performed in humans have shown that common variants in both genes WNK1 (rs880054 and rs2301880) and NEDD4L (rs4149601) associates to hypertension [141, 142]. However, those gene variants taken individually did not show any significant effects in renal Na⁺ handling and in BP response, whereas significant effects were found when variants in *ADD1*, *WNK1*, and *NEDD4L* manifest in combination [124]. During Acute Saline Load Test, those individuals carrying *ADD1* Trp/*WNK1* GG/*NEDD4L* G variants showed increased slope of the P_{Na}t (Fig. 5) curve, eventually resulting salt sensitive, and displaying greater responsiveness to thiazide treatment [124].

Lanosterol Synthase (LSS)

In humans, *LSS* gene encodes for lanosterol synthase [143, 144], an oxidosqualene cyclase enzyme which converts (S)-2,3-oxidosqualene to lanosterol [145]. Lanosterol is a key four-ringed intermediate in cholesterol biosynthesis [146, 147].

Recent works have indicated *LSS* transcription in a considerable number of nephron sites [148]. Moreover, human adrenocortical H295R cells transfected with *LSS* rs2254524 642Leu exhibited higher *LSS* activity and increased ouabain levels underlying that, besides its already known functions, *LSS* is also involved in EO synthesis [149].

Recently, Lanzani et al. evaluated the role of *LSS* in modulating the EO response to the stress of a low-salt diet [4••]. In their study, during low-salt diet those never treated, recently discovered, essential hypertensive patients with *LSS* AA genotype showed greater decline in both systolic BP and diastolic BP, whereas EO resulted unchanged. Furthermore, their P_{Na}t curve was less steep than among C allele carriers, indicating increased salt sensitivity of BP to salt variations [4••]. While the counterpart carrying *LSS* AC or CC genotypes resulted less BP responsive to salt deprivation and showed greater increase in EO [4••]. Thus, this study further supports firstly the view that adrenocortical function is abnormal in some essential hypertensive individuals, and secondly, that genetic susceptibility is determinant in the establishment of a variable response of BP to salt modifications.

PRKG1

PRKG1 gene encodes for a type I cGMP-dependent protein kinase, nitrovascular effector involved in the release of the muscular smooth muscle cells (VSMC) [150, 151]. Literature shows a striking association between variation in diastolic BP and three SNPs in *PRKG1* gene (rs1904694, rs7897633 and rs7905063). During Acute Saline Load Test, individuals carrying one of those variants increased their diastolic BP, resulting salt sensitive [152•].

Moreover, it was recently found an association between *PRKG1* gene variants and left ventricular (LV) function [153]. Echocardiography performed in individuals bearing one of the mentioned above *PRKG1* SNPs, when compared to heterozygotes or non-carriers, showed significantly higher systolic radial strain and LV hypertrophy [153].

Serum and Glucocorticoid Inducible Kinase 1 (SGK1)

SGK1 plays a central role in ENaC dependent Na transport in distal nephrons. It regulates the aldosterone-induced Na reabsorption [154]. Recently, genetic polymorphisms in *SGK1*, affecting RAAS activity, have been linked to salt sensitivity development in humans [155•]. Participants to the study followed 2 dietary phases: 7 days of high-salt diet (>200 mmol/day) and 7 days of low-salt diet (10 mmol/day). Two SNPs (rs2758151 and rs9402571) frequently present in hypertensive individuals, associated with systolic BP fluctuations in response to dietary salt changes [155•].

SLC24A3 and SLC8A1

SLC24A3 and *SLC8A1* encode for NCKX3 and NCX1, respectively, two plasma membrane Na⁺/Ca²⁺ exchangers. Both genes are important regulators of intracellular Ca²⁺ homeostasis and are involved in the peripheral control of vascular resistance [156, 157].

Recently, one SNP in SLC24A3 (rs3790261) and one in SLC8A1 (rs434082), only when simultaneously present, were functionally related to variation in systolic BP. During Acute Saline Load a combined analysis showed an epistatic interaction of both SNPs with P_{Na}. During Acute Salt load, NHP simultaneously carrying the above mentioned gene variants, resulted SS [152•]. These results suggest that genes regulating contractility of the VSMC can evoke fluctuations in BP. In response to various signaling events, those genes act either increasing the contractile elements intracellular free Ca²⁺ concentration or the sensitivity to Ca²⁺ [152•].

UMOD

UMOD encodes for Uromodulin, the most abundant urinary protein, produced and secreted by the thick ascending limb (TAL) of Henle's Loop [158, 159]. It was demonstrated that some gene variants in UMOD are determinant of the individual susceptibility to hypertension [159, 160].

Furthermore, urinary excretion of Uromodulin augments in consequence of high salt intake [161, 162]. Recently, Uromodulin has been assessed as an important modulator of Na⁺ handling in TAL.

Humans bearing UMOD rs13333226 variant resulted prone to hypertension and showed lower Na⁺ urinary excretion [160].

Recently, Trudu et al. showed that during Acute Saline Load NHP carrying the gene variant rs4293393 increase their Uromodulin production and display a higher baseline diastolic BP, resulting SS [163•].

LSS and UMOD

Recently, Gatti et al. identified a genetic interaction between LSS, and UMOD [164]. This new pathway was relevant for BP response during both acute and chronic salt modification [164]. NHP carrying UMOD AA/LSS AA variants showed a right shift of P_{Na} curve, resulting more prone to salt sensitivity. Furthermore, on low-salt diet, those patients homozygous for the A alleles of both LSS and UMOD gene variants displayed a greater decline in systolic BP than patients carrying other allele combinations [164].

In this pathway, UMOD seems to act affecting the renal tubular Na excretions, whereas LSS affects vasoconstrictor activity by modulating circulating EO levels. When present in combination both genes seem to be relevant for BP response to acute and chronic salt modification.

Conclusion and Perspectives

Salt sensitivity has been largely assessed as a useful phenotype to unravel the mechanistic complexity of primary hypertension. Recent evidences suggest that SSH may derive from

anomalies in either centrally released Aldosterone or EO synaptic regulating pathways, as well as from the peripheral activation of Aldosterone-MR-EO system [165•]. Furthermore, on a constantly high salt intake, EO raises the vascular contractility and consequently BP, enhancing the sympathetic nerve response [166, 167]. This constitutively active neuronal response increases the arterial Ca²⁺ signaling and blunts the nitric oxide production in the renal medulla and collecting ducts, leading to renal vasoconstriction and enhancing Na⁺ reabsorption [168].

However, salt sensitivity, as a reflection of the individual BP susceptibility to salt changes, underlies the presence of predisposing environmental factors coupled with the individual genetic predispositions. The combination of random and non random determinants (race/ethnicity, age, body mass and diet, etc.) leads to the development of a personal index of sensitivity to salt, and also suggests the susceptibility to hypertension.

Moreover, salt sensitivity clearly represents a negative prognostic indicator of cardiovascular adverse events, as well as an important index of organ damage.

Noteworthy, the number of hypertensive subjects as well as the rate of cardiovascular mortality is exponentially increasing.

Despite the scientific progress, genetic evidence related to salt sensitivity is still dramatically controversial and even a proper definition of this phenotype remains extremely faint.

One possible reason for this may be represented by the heterogeneous methods adopted.

Although literature reports encouraging results, the methodology adopted for evaluating the disease still remain extremely variable. Several protocols, such as Acute Saline Load Test, Weinberger Test, and chronic high and low sodium dietary cycles have been proposed.

There is currently large consensus within the scientific community on the use of dietary cycle as a reliable method to evaluate salt sensitivity. However, the formulation of standardized meals is costly, the test formulation requires 2 weeks, and the accuracy in results completely depends on patient compliance.

Conversely, Weinberger's test perhaps represents a valid alternative to dietary cycle, thanks to the shorter timing and to the fast responses. However, the test requires a few days of hospitalization, which equally imply high costs. Instead, Acute Salt Load is very fast, it can be administered in regimen of day hospital, it is low cost, and the diagnostic accuracy of the test is not completely influenced by the patient compliance.

However, none of those mentioned above techniques were persuasively described as more reliable than the others. Moreover, the genetic evidences acutely established during Weinberger test, as well as during Acute Salt Load, and chronically triggered by low- and high-salt diet cycle often do not overlap [123•].

Another important aspect to be considered is that normotensive SS and hypertensive individuals exhibit similar mortality rate, suggesting how crucial an early recognition of this phenotype is before it becomes disease. Nevertheless, unanimous criteria for patient recruitment during clinical studies still remain under discussion; this variability may further represent another possible reason for all the controversies reported in literature.

Selection of NHP avoids the interference of previous medications and the influence of all the superimposed pressor mechanisms established in the latest phase of hypertension. Therefore, NHP recruitment allows the investigator to unmask the still unclear mechanisms of primary hypertension.

In summary, a reliable approach for salt sensitivity recognition, beyond large genetic studies and the development of new therapeutic targets should represent one of the future goals to achieve for the scientific community.

Besides, the relevance of salt sensitivity as a phenotype with poor prognosis, the universal designation of a reliable test is mandatory for the identification of novel therapeutic targets of primary hypertension.

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Compliance with Ethical Standards

Conflict of Interest Drs. Iatrino, Manunta, and Zagato declare no conflicts of interest relevant to this manuscript.

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