Salt, Kidney and Hypertension: Why and What to Learn from Genetic Analyses?

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Key Words
Salt · Hypertension · Juxtaglomerular apparatus · Macula densa · Civilization · Genetic polymorphism · Angiotensin · Angiotensinogen · Liddle syndrome

Abstract
The relation between salt intake and high blood pressure has been widely recognized, but its exact mechanisms and the reason of such a relation are not clearly understood. In this review, I discuss the sequence of factors relevant to our understanding of the pathophysiology of 'essential' hypertension. I will first consider the relation between two major determinants of systemic blood pressure, i.e. extracellular fluid volume (ECFV) and the renin-angiotensin-aldosterone system, and then the renal sensing mechanism of changes in ECFV, the historical background of salt intake in our culture, and finally suggest explanations of results of genetic analyses of hypertension. The discussion is aimed at furthering our understanding of how and why hypertension develops in response to present day high salt intakes. In addition, data are presented for the implementation of a practical health care policy to effectively reduce the incidence of hypertension.

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Introduction

Hypertension is a very common medical condition and is the major contributing factor in the development of vascular complications in many adults with diabetes mellitus, atherosclerosis, obesity and chronic renal disease, and a common disease entity in both developed and developing countries. The causal relation between salt intake and high blood pressure is widely recognized, but the exact pathophysiological mechanisms and the reason for the relation are less clear. Moreover, recent advances in analytical techniques have uncovered several genetic abnormalities at the genome level, which may be causally related to hypertension. In this brief review, I address the issues on the relation between salt intake and hypertension and describe future perspectives on genetic analyses and prevention of hypertension.

Two Major Determinants of Systemic Blood Pressure

It is vital to maintain a minimal systemic blood pressure to ensure the adequate supply of oxygen to every cell of the body. This has become critically important through millions of years of vertebrate evolution since the emer-

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gence of amphibians around 300 million years ago. In aquatic life prior to the amphibians (e.g. teleosts), a mechanism to maintain high systemic blood pressure was not needed because of the relatively low gravitational force (one sixth of that of terrestrial life). Indeed, the blood pressure of a fish or teleost is in the range of 10–15 mg Hg. In land-dwelling vertebrates, a mechanism is necessary to maintain a relatively high blood pressure to deliver sufficient quantities of oxygenated blood via the blood stream to the organs positioned higher than the heart. The two major determinants for maintaining systemic blood pressure are the extracellular fluid volume (ECFV) and the renin-angiotensin-aldosterone system (RAAS). There are also modifiers of this ECFV-RAAS axis which are of lesser physiological significance for maintaining systemic blood pressure.

ECFV, the Primary Variable

In a practical sense, almost all NaCl is present in the ECF and NaCl is the main determinant of ECF tonicity. Therefore, ECFV is determined by the NaCl content of the body, and thus varies with and reflects salt intake. It stands to reason that ECFV is a variable which will be affected primarily by salt intake and may vary from one day to another, from one individual to another and from one culture to another. ECFV will change with salt intake to maintain its 'normal' value. The kidney plays a central role in this homeostatic system by adjusting NaCl excretion into the urine as a function of change in salt intake. Indeed, when salt intake is reduced to zero, urinary NaCl excretion can decrease to zero within a few days and ECFV is maintained, but at a slightly reduced level. In response to excess salt intake, NaCl excretion will increase accordingly within a few days to match the intake, maintaining the homeostasis, but with a slightly elevated ECFV. This system is one of the most fundamental homeostatic systems in the body.

The RAAS, the Secondary Variable

While serving as a critical ECFV regulator via aldosterone by acting on the aldosterone receptors of the distal nephron Na channel, the RAAS plays a central role in blood pressure maintenance via the actions of angiotensin II (AII) on vascular smooth muscle cells. Renin concentration in the blood stream is the key rate-limiting step or reaction of RAAS. The blood renin concentration is almost entirely dependent upon its secretion from the juxtaglomerular cells, which are vascular smooth muscle cells at the end of the afferent arteriole, just at the entrance to the glomerulus. Renin secretion is stimulated in response to a fall in ECFV, leading to activation of the RAAS system and maintenance of blood pressure; at the same time, an increase in aldosterone production and secretion from glomerulosa cells of the adrenal glands via AII enhances Na reabsorption at the distal nephron, thereby conserving NaCl to maintain ECFV. It is critically important to recognize that the RAAS is the secondary variable which responds appropriately to a change in ECFV, the primary variable in this homeostatic system.

Coupling of ECFV and RAAS: The Central Role of the Juxtaglomerular Apparatus

How is a change in ECFV transmitted to the RAAS? The kidney is the central organ for this coupling of the ECFV-RAAS axis [1]. In the kidney, it is the juxtaglomerular apparatus (JGA) which plays this role [2–4]. Renin is produced and secreted from the juxtaglomerular cells, and it is thought that renin secretion is primarily regulated by renal perfusion pressure, adrenergic stimulation, AII and the macula densa mechanism. It is widely accepted that the major signals for renin secretion triggered in response to a change in ECFV are both renal perfusion pressure and macula densa mechanisms. Indeed, when ECFV decreases in response to a low salt intake or other circumstances, such as bleeding, diarrhea or vomiting, renin secretion is increased via these two mechanisms. Thus blood pressure is held constant despite a reduction in ECFV. However, a quantitative comparison of the effects of renin secretion by a change in perfusion pressure and by a macula densa mechanism has remained inconclusive in part due to technical limitations.

Recent studies utilizing single isolated JGAs with glomerular afferent arteriole and/or with the macula densa portion of the thick ascending limb of Henle perfused in vitro provided interesting results [5–8]. As shown in figure 1b, renin release changes little, if at all, in response to changes in perfusion pressure ranging from 15 to 100 mm Hg. By contrast, a reduction in macula densa Cl concentration or Cl delivery markedly stimulates renin release [6, 7]. These results strongly suggest that it is the macula densa mechanism which is primarily responsible for renin release in response to a reduction in ECFV. Under this condition macula densa Cl delivery will decrease even though systemic and renal perfusion pressure may remain

stable through autoregulation of the renal afferent arteriole. In physiological settings, one can imagine little change in systemic blood pressure when the salt intake is reduced from 100 to 50 mEq/day, yet urine salt excretion is effectively reduced to match the intake and renin secretion is appropriately stimulated to activate the RAAS.

The highly sensitive and effective coupling of the ECFV-RAAS axis at the JGA is again consistent with the notion that the JGA has emerged in amphibia at the transition of vertebrates from aquatic to terrestrial life where blood pressure must be maintained at a higher level at 1.0 g gravity in an environment where salt intake must be small or very limited.

Salt Intake of Mammals in the Natural Terrestrial Environment

If one considers salt intake in a natural environment, one will easily realize that it must be very low [9]. Foodstuffs available in such an environment will be primarily animal bodies, vegetables and fruits, all of which contain little salt except for that contained in the ECF of animal tissues. Thus, considering animal behavior, one can estimate that salt intake will be 1–2 g/day/60 kg body weight (BW) for carnivores and less than 0.5 g/day/kg BW for herbivores [10]. Salt intake is particularly critical for herbivores as they are subject to constant salt depletion with a maximally stimulated RAAS. Indeed, urine aldosterone secretion is very high in herbivores living in wild, natural environments and marked hyperplasia of the zona glomerulosa is present [9]. Also, they have a behavior known as 'salt craving' [9]. How about humans? It has been estimated that the salt intake of Paleolithic human beings was less than 1.5 g/day [10].

Human Civilization, Salt Intake and Essential Hypertension

Even today, salt intake is very low in tribes living in wild, natural environments, such as the Zulu of Africa, Papua New Guineans and the Yanomamo Indians in the Amazon. Their blood pressure does not significantly rise as they age, and essential hypertension was not encountered [9]. Indeed, salt has been a precious commodity not available in abundance in the past. In fact, one can find many stories on how important salt was: such examples are 'Via salaria' and 'salary or salarium', 'give a piece of rock salt' of the Romans, the long and harsh salt caravans
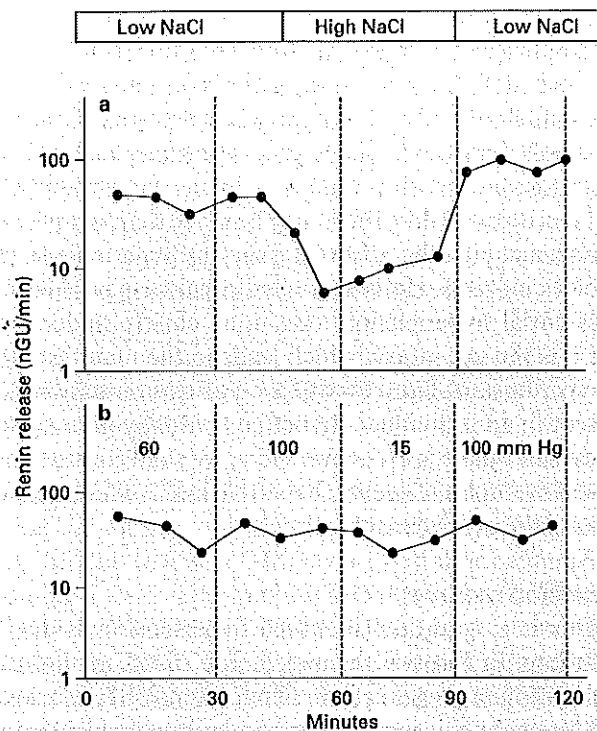

Fig. 1. Comparative effect on renin secretion of macula densa chloride delivery (a) renal and afferent arteriolar pressure (b). Preparations of single isolated JGAs were cannulated and either its afferent arteriole or the thick ascending limb of Henle was perfused in vitro. Renin release was expressed as nanoGoldblatt units/min/single JGA. It is clear that renin secretion changed dramatically, rapidly, and reversibly in response to a change in macula densa chloride delivery in which perfusate NaCl concentrations were 141/122 mEq/l for 'High NaCl' and 26/7 mEq/l for 'Low NaCl', respectively [7]. By contrast, renin release changed little, if at all, in response to changes in arteriolar perfusion pressure between 15 and 100 mm Hg [8]. The data suggest that the macula densa chloride delivery is a more sensitive and powerful regulator than arteriolar perfusion pressure of renin release. Reproduced with modifications from ref. 7 and 8.

of Ethiopian mountain tribes, the 'Salt March' of Mahatma Gandhi protesting against a 'salt tax' imposed by the British and a palace-like salt factory built by Louis XIV. The importance of salt as a precious commodity can also be found in many cities named after salt in modern European civilization and elsewhere: for example, 'Salzburg' was named after the source of its wealth, its nearby salt mine. In modern days, when higher salt intakes became more common among a majority of people we witness the emergence of essential hypertension, a civilization disease.

Information from Genome Analyses

It is thought that genetic and environmental factors each play defining roles in the genesis of many disorders. The magnitude of each component may vary from one disease to another: in single-gene disorders, such as retinoblastoma, a specific mutation in the target gene will lead to a disease while there may be more than one genetic derangement in other disorders, such as hypertension and diabetes mellitus. However, when considering the nature of essential hypertension, one must clearly understand that it is the salt intake, which leads to the manifestation of hypertension. Thus, even if such genetic mutations are present in an individual, 'hypertension' may not develop unless salt intake is excessive. Here, we shall consider two typical examples of genetic hypertension in which genetic abnormalities are clearly defined.

Liddle Syndrome

This is a syndrome in which hypertension is due to mutations in the Na channel in the distal nephron; it represents a monogenic form of hypertension (see below). The Na channel is the target of the action of aldosterone. Reported mutations are found either in the beta or in the gamma subunit of the channel with a gain in function or enhanced Na reabsorption. Thus, the disease resembles primary aldosteronism with expanded ECFV and suppressed RAAS.

Angiotensinogen Polymorphism

There are two genetic types of angiotensinogen, depending upon the 235th amino acid, which may be either threonine (T) or methionine (M), with two corresponding genes, T and M. The ratio of T235 and M235 differs among countries and races (see below). It is generally recognized that 235T is associated more frequently with essential hypertension and indeed the circulating angiotensinogen level is higher in genotype TT than TM and is lowest in MM. It is known that renin is the pacesetter of RAAS, but a higher substrate or angiotensinogen level will provide a proportionally higher AII generation at any renin secretion. Indeed, some forms of hypertension are due to elevated angiotensinogen. With these backgrounds, it may be expected that TT may cause higher blood pressure than MM.

Genetic Mutation through Human Evolution

Through millions of years of human evolution, genetic mutations occurred spontaneously and randomly and at any site. Some genome sites may be more susceptible to mutation than others. Only 3–5% of a given genome may be of functional significance, with exons coding for mRNAs occupying approximately 1% of the entire genome. The consequence of mutation could be: (a) of no biological and physiological relevance; (b) deleterious so that the mutation may be fatal or cannot be transferred to subsequent generations, or (c) advantageous so that the mutation will be transferred with a higher probability to subsequent generations. However, whether a mutation is advantageous or not may depend upon the environment.

Now consider the two types of hypertension mentioned above, Liddle syndrome and angiotensinogen T235M polymorphism. Liddle syndrome is a monogenic form of hypertension due to a gain of function of the distal Na channels simulating primary aldosteronism [12]. There are several patients and families with Liddle syndrome, but why do we not recognize patients with a loss of function of the Na channel causing distal Na loss? Such a patient is similar to a baby with congenital aldosterone deficiency who dies within a few weeks after birth due to dehydration, hyperkalemia and metabolic acidosis unless the disorder is discovered early enough to be treated properly. Thus, in a natural environment such mutations are fatal and cannot be transferred to the next generation. By contrast, the mutations seen in Liddle syndrome will provide an advantage over 'normals' in a natural environment of very limited salt availability and are more likely to be transferred to subsequent generations. However, over the last centuries, modern civilization has provided easier and abundant daily supply of salt, a life style now disadvantageous to those who carry these mutations, manifesting a condition known as 'hypertension'. As reviewed above, the salt intake of humans through thousands of years must have been less than about 1.5 g/day and this is what our genes have adapted to for survival [10]. Currently, salt intake may range around 10–20 g/day, obviously an excessive quantity compared to how our genetic machinery has been programmed [10, 11].

How about angiotensinogen 235T and M mutations? 235T is associated with higher angiotensinogen or renin substrate in the circulation and provides higher AII at any given renin secretion. 235T is more often associated with subjects with 'essential hypertension' in our modern civilization with a higher salt intake [13–15]. In a natural environment where salt intake is limited, 235T is more advan-

tageous than 235M and thus has been conserved through generations. But the truth may be more complex than this. In fact, 235T is the form almost exclusively present in a natural environment. Analyses of angiotensinogen in apes have demonstrated that all apes analyzed show only T [13]. In a natural environment where salt intake is very limited, 235T will confer a slightly higher blood pressure than the low normal pressure associated with low salt intake. This will be of advantage for strength and increased probability of mating and conservation of 235T in the ape society. By contrast, the 235M will be a weaker phenotype to compete in such a society of low salt intake. This lessens the chances of transmitting this genotype to subsequent generations. Thus it will not be conserved. Because apes are, in general, herbivores, salt intake will be very low. It would be interesting to examine the frequency of 235T and 235M in carnivores with some, though limited, salt intake in order to gain insights into the genetic adaptation and the biological significance of RAAS in evolution.

Analyses of T235M mutations in different human racial subgroups provide interesting results [13–15]. 235T is seen in over 90% of Africans whereas in African Americans it is 80–90%; in Caucasians in Europe and the USA it is around 40–50% and in the Japanese it is about 80%. The data suggest that (1) this site is hypervariable and mutates with high frequency, (2) the frequency of this mutation may provide some clues to the way of life in a given modern society. The data on Africans and African Americans who have lived in the New World for some 100–200 years and who are primarily inbred suggest the rate of mutation of this specific site. The lower frequency of 235T in Caucasians is consistent with the way of life in modern Europe. Parenthetically, it is of interest to note that there is always a mutation of A/G at the sixth base of the 5' upstream of exon 1 of the angiotensinogen gene coupled with the 235T/M mutation. The mechanism and the reason for this coupling will be of great interest in order to understand the tertiary structure of the gene and the factors affecting the genetic mutation.

These two types of genetic abnormalities underlying these forms of 'essential hypertension' indicate that the environment the organisms live in will determine the selection of any genetic mutations. The mutations with an advantageous phenotype will be transmitted to subsequent generations. As to blood pressure regulation, the mutation that favors either salt conservation or higher RAAS will be advantageous in natural environments, thus it is conserved. However, the changes from a low to a higher salt intake due to modern habits over the past few

centuries have suddenly turned these advantageous mutations conserved over generations into a disadvantage that results in a condition called 'essential hypertension'. There are two types of essential hypertension, i.e. 'salt sensitive' and 'salt insensitive or non-salt sensitive'. As should be clear by now, all essential hypertension is in principle 'salt sensitive' or 'salt-dependent' and so-called 'salt-sensitive' and 'non-salt-sensitive' simply indicates the distinct pathophysiology of the maintenance of elevated blood pressure in established hypertension.

Salt Consumption and the Prevalence of Hypertension

The relation between salt intake in one culture, civilization, or society and the prevalence of hypertension is very difficult to assess. Moreover, the public campaign to promote a lower salt intake is also difficult to implement effectively. The reasons for such difficulties are multiple: one of them is the true prevalence of hypertension and the impact of a higher salt intake. It is generally stated that the daily salt intake should be in the range of 5–8 g/day. Can this be achieved by educating the public? Even physicians and hypertension specialists may not know the level of salt intake of a patient and the society in which he or she lives. Thus, it is difficult to specifically teach the patient and his/her family to deal with this problem and comply with a salt intake of 5–8 g/day.

The data from Japan are of interest in this respect, because they are fairly homogeneous. The annual data of salt intake in Japan by region show that the average salt intake has been gradually increasing from 11 to 13 g/day over the last decade. However, two regions are of extreme interest in their distinction. In the Tohoku region, the average salt intake has always exceeded that of the rest of Japan by about 1 g/day; in the Kinki region, it has always been lower than in the rest of Japan by about 1 g/day (fig. 2). This difference has been widely but vaguely known and is suggested in the less salty dishes in Kansai and the salty cooking in Tohoku. Available data substantiate this general perception. Does this difference reflect the prevalence of hypertension?

The data on the sales of several drugs, including antihypertensive agents, are highly informative. Figure 3 shows the percent shares of the 'economy index' and the sales of antihypertensive drugs, antipeptic ulcer drugs and antibiotics. The percent shares of these 4 items are comparable in all regions except for Tohoku and Kinki, where the percent share of antihypertensive drugs shows distinct
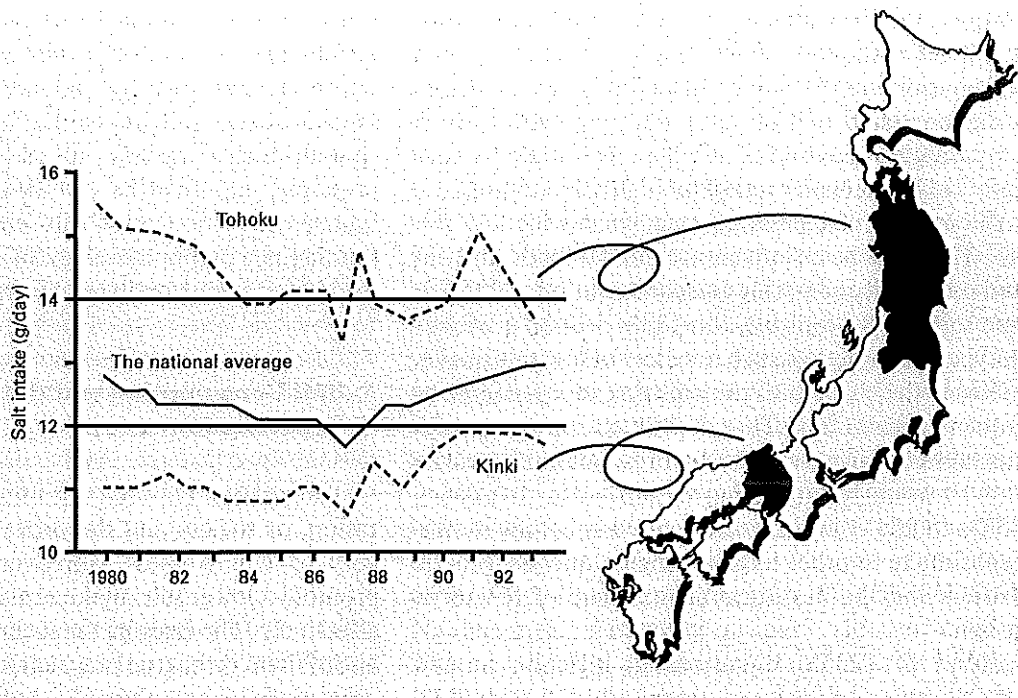
Fig. 2. Average daily salt intake in the different regions of Japan. Note that daily salt intake has been about 1 g/day higher in the Tohoku region and about 1 g/day lower in the Kinki region than in the other regions of Japan, i.e. not markedly different from the national average. Data are based on the reports of the Ministry of Health and Welfare of Japan.

Fig. 3. The percent share of the economy index (EI), and of 3 major pharmaceutical products, i.e. antihypertensives (HBP), antipeptic ulcer H_2 antagonists (GI) and antibiotics, by two regions of Japan. Note that the percent market shares of these 4 categories of indices did not differ considerably except for a disproportionately higher share of antihypertensives in Tohoku, a disproportionately lower share of antihypertensives in Kinki. The data strongly suggest that 1 g/day higher or 1 g/day lower salt intakes or a 10% difference may be associated with a 20–30% higher or lower prevalence of hypertension. Data were gathered from the wholesale distributors of pharmaceutical drugs.
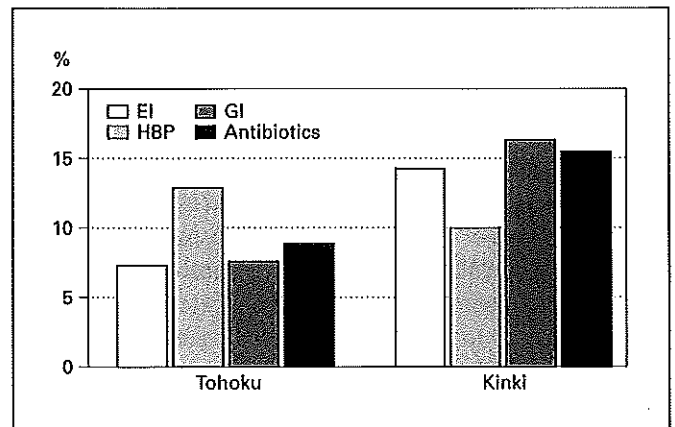
differences. Thus, in the Tohoku region, percent shares of the latter items are in the range of 8–9%, probably representative of the population in this region, but the percent share of antihypertensive drugs is around 14%. By contrast, in the Kinki region, the percent shares of the latter

items are in the range of 14–16%, representative of the population in this area, the share of antihypertensive drugs is about 10%. These data clearly show that a mere difference of 1 g/day difference in the average daily salt intake translates into differences of 20–30% in the inci-

dence of hypertension among the population. The data also suggest that, whatever the major sources of salt in any given civilization or society (in Japan they will be largely soy sauce, miso, tsukemono and salt), to recommend and campaign for a 10–20% cut in the sources of salt in daily meals is more practical and more easily understood by the public than simply emphasizing the importance of a 5–8 g/day salt intake without any specific directions. This practical and easy-to-understand approach will effectively reduce the incidence of hypertension by 20–30%, with a major impact on health economy and disease prevention. It is also of critical importance that the entire society be involved since the taste for high-salt meals develops through many years from childhood. Thus the entire family and society have to be brought up in the lower, as a start 10% less, salt dietary habits and culture.

Future Perspectives

Advances in analytical methodologies at the genetic and molecular levels have made it possible to gain more and more information invaluable to our understanding of the genetic and molecular mechanisms of diseases. However, how we utilize the technologies is dependent on our public wisdom. If we are truly concerned about the prevention and eradication of hypertension, it will be a wise public policy to implement and spend more money on effective educational programs on reducing salt intake as mentioned above, by redirecting some of the research money spent on genetic analyses of hypertension.

Recent DNA chip technologies have made significant inroads to simplifying the detection of single nucleotide mutations which may be associated with specific disease conditions. However, it should be noted that (1) in each individual about 50 mutations in the entire genome occur spontaneously from the parent and (2) through many gen-erations and among the 6 billion humans on earth, single nucleotide differences are present in 5–10 million sites per genome or 3 billion nucleotides, or stated in another way, 0.2–0.3% of the nucleotides are different among individuals [16, 17]. Thus, it should be clear to anyone that it will be an extremely difficult task to relate one nucleotide difference of a single nucleotide polymorphism over this sizable 0.2–0.3% single nucleotide difference or 'basal noise' to a specific disease. Alternatively, to identify genetic mutations responsible for such a common and obviously polygenic disease state as hypertension, one must identify, secure and analyze subpopulations of at least 1,000–2,000 patients, with hypertension of clearly defined pathophysiology. This task by itself is very difficult to accomplish. Moreover, any genetic analysis of hypertension should be interpreted keeping in mind the underlying concept of evolution and adaptation to low salt intake in a wild natural environment over thousands of years, that resulted in a 'maladaptation' to the high salt intake over a few hundred years.

Scientists should think over and over again what they wish to know, why they wish to do research, how a specific disease such as hypertension develops through human evolution and civilization. They should strive to gain a better understanding of genetic mutations and the evolution of life. Without such insights and wisdom, considerable amounts of research money provided by the public will be wasted, as research investments consume personnel, animals, reagents, equipment, and quite commonly result in redundant and meaningless publications. We, as conscientious scientists, must offer advice to the government and the public without selfish motives on the research priorities for the prevention and treatment of such common diseases as hypertension. The limited resources should be properly and wisely distributed between genetic research, effective prevention strategies and public education and health promotion.

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