Pathogenesis of Higher Blood Pressure and Worse Renal Function in Salt-Sensitive Hypertension

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Keywords

Salt-sensitive hypertension · Ambulatory blood pressure · Growth factor · Renal damage · PI3K-AKT signaling pathway

Abstract

Background: The underlying pathogenesis of patients with salt-sensitive hypertension expressing higher blood pressure and severer renal damage remains uncertain. **Methods:** We recruited 329 subjects, 131 in salt-sensitive (SS) group, 148 in nonsalt-sensitive (NSS) group, and 50 healthy people in normal group and tested their renal function, 24-h ambulatory blood pressure, and growth factor series. **Results:** The SS group showed worse renal function with lower estimated glomerular filtration rate and higher urinary microalbumin, α -microglobulin, urinary protein Cr ratio, and urinary immunoglobulin. Most indicators in 24-h ambulatory blood pressure of the SS group were significantly enhanced than the NSS group, indicating their higher blood pressure. The significantly elevated growth factors in the SS group were AR, BMP-5, EG-VEGF, GH, HGF, IGFBP-2, IGFBP-3, IGFBP-6, MCSFR, NT-4, PDGF-AA, SCF, SCFR, VEGFR2, VEGFR3, and VEGF-D,

compared to other 2 groups or one of them. PI3K-AKT pathway was activated in the SS group. **Conclusions:** Differences in growth factors and pathways may account for the manifestations of the SS group. Activated PI3K-AKT pathway with higher IGFBP-3 and GH can lead to renal damage. Higher MCSFR in the SS group indicates that high blood pressure and severe kidney damage may be associated with the activation of the immune system. EG-VEGF, VEGFR2, VEGFR3, and VEGF-D can also explain the elevated blood pressure due to the dilated lymphatic system which drains excess sodium and water back into circulation. The SS group presented higher AR and HGF which may worsen renal function by regulating cell proliferation and tumor formation. However, due to the potential low awareness rate of hypertension at the very beginning, we cannot ensure the exact occurrence order of blood pressure, renal damage, and salt sensitivity. Therefore, further studies which can track data from the onset of hypertension are needed.

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Introduction

Ranked third among the mortality rates of cardiovascular and cerebrovascular diseases in China (271.8/100,000), hypertension is becoming an increasingly important public health problem, with its prevalence among Chinese residents over 18 years old was 25.2% in 2015 [1]. Every individual in a same mammalian population responses differently in blood pressure to salt intake, which was first found by Dahl in salt-sensitive (SS) rats [2]. The relationship between salt and blood pressure in humans was first proposed by Luft and Kawasaki who also defined the concept of SS hypertension [3, 4]. In the main ethnic group, population with SS hypertension make up over half of the total hypertensive population [5] and undergo higher morbidity and mortality of cardiovascular diseases. An estimated rate of the morbidity of cardiovascular diseases in SS hypertensive patients is 4.7%, comparing to 2% in nonsalt-sensitive (NSS) hypertensive patients [6]. Previous research studies have shown abundant evidences that SS hypertension was associated with more serious organ damage than NSS hypertension. So it is obvious that SS hypertension, as a specific type of hypertension, can induce distinct clinical characteristics in patients which cover special inducing factors (salt), high incidence of complications, and severe organ damage. In recent years, researchers have explored the possible mechanisms of hypertension and renal damage in SS patients from the perspective of immune system including aspects about macrophages, inflammatory cytokines, and so on [7]. In our study, we recruited 131 patients in the SS group, 148 patients in the NSS group, and 50 people in the normal group. With their 24-h ambulatory blood pressure monitored and indicators related to kidney damage measured, we found that the SS group showed higher blood pressure and more serious renal damage. In order to explore the potential biological mechanisms, we utilized protein array to test the growth factor series, the main factors regulating tissue growth and proliferation, and imported the differential proteins to Kyoto Encyclopedia of Genes and Genomes (KEGG) database to find out the activated signaling pathways.

Ambulatory blood pressure monitoring which can present the fluctuation of blood pressure acts as a practical and cost-effective way for diagnosis, treatment, prognosis, and research of hypertension and is considered to be an ideal method to diagnose hypertension, estimate outcomes of therapy, and diagnose impairment of target organs [8]. Protein array is a high-throughput analysis

technology which is deemed to serve as a convenient and reliable tool in analyzing protein expression profiles and allows for simultaneous investigations of thousands of targets in a single experiment. With certain known protein molecules immobilized on a solid chemical-processed surface, the target proteins that can bind specifically to the molecules will be captured [9].

KEGG is a comprehensive knowledge base for assisting biological interpretations of large-scale molecular datasets. As a collection of manually drawn pathway maps, it contains numerous networks of molecular interactions and reactions. Enrichment analysis is utilized to integrate the pathways of all input proteins and show the shared pathways legibly and distinctly [10].

Methods

Study Population

We recruited 279 hypertensive patients who were hospitalized in the department of cardiology during a period from June 2015 to June 2016 and 50 gender- and age-matched healthy people who experienced medical examination in the same hospital, Chinese Academy of traditional Chinese Medicine Affiliated Guang'anmen Hospital. We distributed these people into 3 groups, 131 patients in the SS group, 148 patients in the NSS group, and 50 healthy people in the normal group.

All the recruited 279 patients had a clear diagnosis of hypertension according to the European Guidelines for Hypertension (2013), which states hypertension is defined as systolic blood pressure ≥ 140 mm Hg and/or diastolic pressure ≥ 90 mm Hg without taking any antihypertensive drugs [11]. The 131 patients with SS hypertension were determined by adopting the modified Sullivan acute oral saline loading method.

Exclusion criteria included patients with secondary hypertension, cerebral hemorrhage, acute cerebral infarction, acute myocardial infarction, severe heart failure, severe liver damage, acute renal damage, chronic kidney failure (stage CKD3-5), mental disorders, malignant tumors, tuberculosis, or other infectious diseases and after severe trauma or major operations. The study protocol was approved by Ethics Committee of Guang'anmen Hospital, Chinese Academy of traditional Chinese Medicine, Beijing, China (2018-039-SQ). All study subjects provided informed consent before enrollment.

Ambulatory Blood Pressure Monitoring

Each recruited patient underwent a 24-h ambulatory blood pressure monitoring by wearing a ambulatory blood pressure detector (ABPM 6100; US.Welch Allyn, Inc.). The detection started at 8:00 a.m. on the first day and completed at 8:00 a.m. on the second day. The interval between each measurement was set as every half hour from 8:00 a.m. to 9:00 p.m. and every hour from 9:00 p.m. to 8:00 a.m. After the process, we withdrew the detectors and imported data to Welch Allyn CardioPerfect analysis software and got comprehensive data of their blood pressure.

Table 1. Clinical characteristics of recruited subjects (median $\pm$ IQ range)

	SS group, <i>n</i>	NSS group, <i>n</i>	Normal group, <i>n</i>
Cases	131	148	50
Age, years	77 $\pm$ 17	73.5 $\pm$ 17	70.5 $\pm$ 10
Male	49	61	23
Female	82	87	27
Course of disease, months	15 $\pm$ 20 ^a	10 $\pm$ 14 ^b	0 $\pm$ 0
BMI, kg/m ²	26 $\pm$ 6 ^c	25 $\pm$ 0	24 $\pm$ 3 ^b
Abdominal perimeter, cm	98 $\pm$ 10 ^c	98 $\pm$ 8	96 $\pm$ 4 ^b
Neck circumference, cm	38 $\pm$ 3 ^c	38 $\pm$ 4 ^c	36 $\pm$ 3 ^{a,b}

When Adj. *p* is smaller than 0.05, the difference is significant. Course of disease: Adj. *p* between the SS and NSS group is 0.000; BMI: Adj. *p* between the SS and normal group is 0.001; abdominal perimeter: Adj. *p* between the SS group and normal Group is 0.007; neck circumference: Adj. *p* between the SS group and normal group is 0.000, Adj. *p* between the NSS group and normal group is 0.000. Adj. *p*, adjusted *p*; SS, salt-sensitive; NSS, nonsalt-sensitive. ^a Adj. *p* < 0.05 compared with the NSS group. ^b Adj. *p* < 0.05 compared with the SS group. ^c Adj. *p* < 0.05 compared with the normal group.

Renal Function and Urinary Microprotein

The morning fasting venous blood of all participants were obtained to measure serum Cr which is an important indicator of renal function and essential for calculating estimated glomerular filtration rate (eGFR). The collected samples of morning clean urine were tested to determine levels of urinary microalbumin (MA), urinary α 1-microglobulin, urinary β 2-microglobulin, urinary immunoglobulin (IGU), and urinary N-Acetyl- β -D-glucosaminidase enzyme using the nephelometry method and an analyzer (DCA Vantage analyzer, Siemens). Urinary protein Cr ratio (MA/CR) was calculated by dividing MA by CR. eGFR was obtained with simplified MDRD Chinese formula [12]: $\text{eGFR}(\text{mL}/[\text{min} \cdot 1.73 \text{ m}^2]) = 186 \times (\text{scr}[\mu\text{mol/L}] \times 0.011312) - 1.154 \times \text{age} - 0.179$ ($\times 0.762$ if female) ($\times 1.233$ if Chinese).

QAH-GF-1 Protein Array Operation

We numbered the total 329 enrolled people and selected 36 subjects randomly from them, with 15 in the SS group, 15 in the NSS group, and 6 in the normal group. Then, we obtained serum samples of the 36 subjects and had them tested with QAH-GF-1 protein array. The protein array applies matrix antibody array technology and can determine 40 growth factors at the same time with corresponding 40 growth factor antibodies riveted in a 1-cm² chip.

KEGG Enrichment Analysis

We imported the growth factor proteins detected by protein array to KEGG database and obtained biological information pathways. Through the enrichment process, possible pathways on which growth factors were related to the pathogenesis of SS hypertension were shown prominently.

Table 2. 24-h ambulatory blood pressure-related indicators (median $\pm$ IQ range)

	SS group (131)	NSS group (148)
24hASBP, mm Hg	135 $\pm$ 34 ^a	131 $\pm$ 32 ^b
DASBP, mm Hg	137 $\pm$ 35 ^a	133 $\pm$ 34 ^b
NASBP, mm Hg	127 $\pm$ 29 ^a	118 $\pm$ 28 ^b
24hADBP, mm Hg	82 $\pm$ 22 ^a	72 $\pm$ 15 ^b
DADBP, mm Hg	83 $\pm$ 24 ^a	74 $\pm$ 15 ^b
NADBP, mm Hg	76 $\pm$ 19 ^a	65 $\pm$ 14 ^b
24hAPP, mm Hg	100 $\pm$ 25 ^a	92 $\pm$ 18 ^b
DAPP, mm Hg	102 $\pm$ 26 ^a	94 $\pm$ 17 ^b
NAPP, mm Hg	92 $\pm$ 22 ^a	82 $\pm$ 16 ^b
24hSBPV	13 $\pm$ 6 ^a	10 $\pm$ 5 ^b
DSBPV	11 $\pm$ 6 ^a	9 $\pm$ 6 ^b
NSBPV	15 $\pm$ 7 ^a	12 $\pm$ 6 ^b
24hDBPV	14 $\pm$ 7 ^a	10 $\pm$ 4 ^b
DDBPV	13 $\pm$ 7 ^a	9 $\pm$ 4 ^b
NDBPV	17 $\pm$ 7 ^a	13 $\pm$ 4 ^b
24SBPL (%)	44 $\pm$ 38 ^a	31 $\pm$ 28 ^b
DSBPL (%)	41 $\pm$ 39 ^a	28 $\pm$ 28 ^b
NSBPL (%)	46 $\pm$ 36 ^a	33 $\pm$ 30 ^b
24DBPL (%)	46 $\pm$ 30 ^a	31 $\pm$ 24 ^b
DDBPL (%)	45 $\pm$ 30 ^a	30 $\pm$ 24 ^b
NDBPL (%)	49 $\pm$ 29 ^a	35 $\pm$ 25 ^b
NSBPRR (%)	13 $\pm$ 11	13 $\pm$ 8
NDBPRR (%)	10 $\pm$ 10	11 $\pm$ 8

When Adj. *p* is smaller than 0.05, the difference is significant. 24hASBP: Adj. *p* is 0.018; DASBP: Adj. *p* is 0.013; NASBP: Adj. *p* is 0.024; 24hADBP: Adj. *p* is 0.000; DADBP: Adj. *p* is 0.000; NADBP: Adj. *p* is 0.000; 24hAPP: Adj. *p* is 0.000; DAPP: Adj. *p* is 0.000; NAPP: Adj. *p* is 0.000; 24hSBPV: Adj. *p* is 0.000; DSBPV: Adj. *p* is 0.000; NSBPV: Adj. *p* is 0.000; 24hDBPV: Adj. *p* is 0.000; DDBPV: Adj. *p* is 0.000; NDBPV: Adj. *p* is 0.000; 24SBPL: Adj. *p* is 0.006; DSBPL: Adj. *p* is 0.006; NSBPL: Adj. *p* is 0.007; 24DBPL: Adj. *p* is 0.004; DDBPL: Adj. *p* is 0.004; NDBPL: Adj. *p* is 0.003; NSBPRR: Adj. *p* is 0.272; NDBPRR: Adj. *p* is 0.660. 24hASBP, 24-h average systolic pressure; DASBP, daytime systolic blood pressure; NASBP, night average systolic blood pressure; 24hADBP, 24-h amplitude of diastolic blood pressure; DADBP, daytime average diastolic blood pressure; NADBP, night average diastolic blood pressure; 24hAPP, 24-h average pulse pressure; DAPP, daytime average pulse pressure; NAPP, night average pulse pressure; 24HSPV, 24-h systolic pressure variability; DSBPV, daytime systolic blood pressure variability; NSBPV, night systolic blood pressure variability; 24HDBPV, 24-h diastolic blood pressure variability; DDBPV, daytime diastolic blood pressure variability; NDBPV, night diastolic blood pressure variability; 24SBPL, 24-h systolic pressure load; DSBPL, daytime systolic pressure load; NSBPL, night systolic blood pressure load; 24DBPL, 24-h diastolic blood pressure load; DDBPL, daytime diastolic blood pressure load; NDBPL, night diastolic blood pressure load; NSBPRR, night systolic blood pressure reduction rate; NDBPRR, night diastolic blood pressure reduction rate; Adj. *p*, adjusted *p*; SS, salt-sensitive; NSS, nonsalt-sensitive. ^a Adj. *p* < 0.05 compared with the NSS group. ^b Adj. *p* < 0.05 compared with the SS group.

Fig. 1. 24-h ambulatory blood pressure-related indicators in the SS group and NSS group. All data are expressed as median. *The significant differences are evidenced by an asterisk. 24hASBP, 24-h average systolic pressure; DASBP, daytime systolic blood pressure; NASBP, night average systolic blood pressure; 24hADBP, 24-h amplitude of diastolic blood pressure; DADBP, daytime average diastolic blood pressure; NADBP, night average diastolic blood pressure; 24hAPP, 24-h average pulse pressure; DAPP, daytime average pulse pressure; NAPP, night average pulse pressure; 24HSBPV, 24-h systolic pressure variability; DSBPV, daytime systolic blood pressure variability; NSBPV, night systolic blood pres-

sure variability; 24HDBPV, 24-h diastolic blood pressure variability; DDBPV, daytime diastolic blood pressure variability; NDBPV, night diastolic blood pressure variability; 24SBPL, 24-h systolic pressure load; DSBPL, daytime systolic pressure load; NSBPL, night systolic blood pressure load; 24DBPL, 24-h diastolic blood pressure load; DDBPL, daytime diastolic blood pressure load; NDBPL, night diastolic blood pressure load; NSBPRR, night systolic blood pressure reduction rate; NDBPRR, night diastolic blood pressure reduction rate; SS, salt-sensitive; NSS, nonsalt-sensitive.

Statistical Analysis

Statistical analysis was performed with the SPSS software, version 13.0 (SPSS, Chicago, IL). With all measurement data tested with the Kolmogorov-Smirnov test (K-S test), we found that not all of them conformed to normal distribution. Hereby, we adopted the Kruskal-Wallis *H* test which belongs to nonparametric tests to analyze all data. Measurement data were expressed as median $\pm$ interquartile range in the following tables, and the difference was statistically significant when adjusted *p* (Adj. *p*) ≤ 0.05 . All data were rounded to the nearest integer.

Results

Patient Characteristics

Clinical characteristics of the total 329 participants by group are shown in Table 1. The medians and interquartile range of age, course of disease, BMI, abdominal perimeter, and neck circumference are included. There was no significant difference in gender and age between groups. The SS group presented significantly longer course of disease than the NSS group. The values of BMI and abdominal perimeter of the SS group were signifi-

Table 3. eGFR and urinary microprotein levels (median $\pm$ IQ range)

	SS group (n = 131)	NSS group (n = 148)	Normal group (n = 50)
MA, mg/L	69 $\pm$ 43 ^{a,b}	39 $\pm$ 24 ^{b,c}	19 $\pm$ 18 ^{a,c}
α 1-M, mg/L	8 $\pm$ 10 ^{a,b}	3 $\pm$ 7 ^{b,c}	1 $\pm$ 2 ^{a,c}
MA/CR	298 $\pm$ 159 ^{a,b}	115.4 $\pm$ 103 ^{b,c}	26 $\pm$ 17 ^{a,c}
NAG, mg/L	9 $\pm$ 6	8 $\pm$ 4	8 $\pm$ 3
IGU, mg/dL	4 $\pm$ 2 ^{a,b}	2 $\pm$ 1 ^c	1 $\pm$ 1 ^c
eGFR, mL/min	59 $\pm$ 14 ^{a,b}	70 $\pm$ 23 ^c	83 $\pm$ 35 ^c

When Adj. *p* is smaller than 0.05, the difference is significant. MA: Adj. *p* between SS and NSS groups is 0.001, Adj. *p* between SS and normal groups is 0.008, Adj. *p* between NSS and normal groups is 0.034; α 1-M: Adj. *p* between SS and NSS groups is 0.031, Adj. *p* between SS and normal groups is 0.004, Adj. *p* between NSS and normal groups is 0.011; MA/CR: Adj. *p* between SS and NSS groups is 0.000, Adj. *p* between SS and normal groups is 0.012, Adj. *p* between NSS and normal groups is 0.024; IGU: Adj. *p* between SS and NSS groups is 0.001, Adj. *p* between SS and Normal groups is 0.004; eGFR: Adj. *p* between SS and NSS groups is 0.012, Adj. *p* between SS and normal groups is 0.000. MA, urinary microalbumin; α 1-M, urinary α 1-microglobulin; MA/CR, urinary protein Cr ratio; NAG, N-Acetyl- β -D-glucosaminidase; IGU, urinary immunoglobulin; eGFR, estimated glomerular filtration rate; Adj *p*, adjusted *p*; SS, salt-sensitive, NSS, nonsalt-sensitive. ^a Adj. *p* < 0.05 compared with the NSS group. ^b Adj. *p* < 0.05 compared with the normal group. ^c Adj. *p* < 0.05 compared with the SS group.

cantly higher than those of the normal group, and the value of neck circumference of SS and NSS groups were both significantly higher than those of the normal group.

24-h Ambulatory Blood Pressure

The results of 24-h ambulatory blood pressure are shown in Table 2, Figure 1. Except night systolic blood pressure reduction rate and night diastolic blood pressure reduction rate, the SS group showed significantly higher values than the NSS group including 24-h average systolic pressure, daytime systolic blood pressure, night average systolic blood pressure, 24-h amplitude of diastolic blood pressure, daytime average diastolic blood pressure, night average diastolic blood pressure, 24-h average pulse pressure, daytime average pulse pressure, night average pulse pressure, 24-h systolic pressure variability, daytime systolic blood pressure variability, night systolic blood pressure variability, 24-h diastolic blood pressure variability, DDBPV, night diastolic blood pressure variability, 24-h systolic pressure load, daytime systolic pressure load, night systolic blood pressure load, 24-h diastolic

blood pressure load, daytime diastolic blood pressure load, and night diastolic blood pressure load.

Renal Function and Urinary Microprotein

Results of eGFR and urinary microproteins are shown in Table 3. eGFR of the SS group was significantly lower than that of NSS and normal groups, and there was no difference between the NSS group and normal group. The values of urinary MA, ALM, and MA/CR of the SS group were significantly higher than those of NSS and normal groups, and the values of the normal group were the lowest. In addition, patients in the SS group had significantly more urinary IGU than the other 2 groups. When it came to urinary N-Acetyl- β -D-glucosaminidase enzyme, there was no difference between groups.

Growth Factors with Protein Array

The results of growth factors with QAH-GF-1 protein array are shown in Table 4. The SS group presented significantly higher AR, IGFBP1, GH, NT4, VEGFR2, VEGFR3, and VEGF-D than both NSS and normal groups and significantly higher BMP5 than the NSS group. The values of EG-VEGF, HGF, IGFBP2, IGFBP6, MCSFR, NT-4, PDGF-AA, SCF, and SCFR of the SS group were prominently elevated than the normal group.

KEGG Analysis of Differential Proteins between 3 Groups

The differential proteins between the 3 groups were respectively enriched with KEGG database, and the results are shown in Figure 2.

Discussion

Our study showed that most statistics of 24-h ambulatory blood pressure in the SS group were significantly higher than those in the NSS group. Especially when it was night, the SS group presented much worse blood pressure, which indicated that SS hypertensive patients had clear autonomic nervous regulation disorder, and suffered higher night blood pressure due to decreasing night sympathetic tension and increasing vagal tension. Meanwhile, renal injury of the SS group was more serious than that of the NSS group, which was distinctly elucidated by the lower eGFR and higher MA, α 1-M, MA/CR, and IGU in the SS group. We intended to discover the underlying pathogenesis from growth factors and pathways, so we applied protein array and found out the significantly elevated growth factors in the SS group were

Table 4. Growth factor analysis with protein array (median $\pm$ IQ range)

	SS group (n = 15)	NSS group (n = 15)	Normal group (n = 6)
AR	472 $\pm$ 777 ^{a,b}	273 $\pm$ 341 ^c	83 $\pm$ 96 ^c
BDNF	414 $\pm$ 507	311 $\pm$ 145	457 $\pm$ 312
bFGF	82 $\pm$ 44	54 $\pm$ 30	66 $\pm$ 63
BMP-4	31 $\pm$ 44	17 $\pm$ 33	2 $\pm$ 20
BMP-5	69,828 $\pm$ 49,014 ^a	37,480 $\pm$ 20,812 ^c	52,472 $\pm$ 100,792
BMP-7	19,972 $\pm$ 15,577	17,998 $\pm$ 9,059	23,412 $\pm$ 18,190
b-NGF	11 $\pm$ 11	11 $\pm$ 10	9 $\pm$ 9
EGF	132 $\pm$ 107	109 $\pm$ 60	187 $\pm$ 94
EGFR	6,012 $\pm$ 2,531	5,244 $\pm$ 1,499	4,745 $\pm$ 891
EG-VEGF	77 $\pm$ 56 ^b	75 $\pm$ 38	30 $\pm$ 51 ^c
FGF-4	1,780 $\pm$ 1,711	1,232 $\pm$ 1,445	1,028 $\pm$ 2,540
FGF-7	81 $\pm$ 113	60 $\pm$ 72	30 $\pm$ 146
GDF-15	1,099 $\pm$ 795	1,039 $\pm$ 745	935 $\pm$ 654
GDNF	35 $\pm$ 30	21 $\pm$ 22	12 $\pm$ 13
GH	1,441 $\pm$ 1,862 ^{a,b}	418 $\pm$ 416 ^c	45 $\pm$ 85 ^c
HB-EGF	12 $\pm$ 12	6 $\pm$ 7	5 $\pm$ 9
HGF	336 $\pm$ 201 ^b	260 $\pm$ 131	159 $\pm$ 127 ^c
IGFBP-1	10,382 $\pm$ 8,506 ^{a,b}	5,738 $\pm$ 5,695 ^c	8,017 $\pm$ 3,214 ^c
IGFBP-2	10,114 $\pm$ 2,461 ^b	9,506 $\pm$ 2,604	7,720 $\pm$ 1,449 ^c
IGFBP-3	86,335 $\pm$ 33,473	83,949 $\pm$ 30,463	82,884 $\pm$ 24,127
IGFBP-4	16,451 $\pm$ 11,646	6,691 $\pm$ 18,781	3,375 $\pm$ 10,500
IGFBP-6	98,199 $\pm$ 25,677 ^b	92,162 $\pm$ 31,293 ^b	73,062 $\pm$ 15,938 ^{a,c}
IGF-1	83 $\pm$ 517	0 $\pm$ 360	0 $\pm$ 20
Insulin	4,301 $\pm$ 2,612	4,902 $\pm$ 2,149	5,480 $\pm$ 2,456
MCSFR	20,810 $\pm$ 8,486 ^b	20,360 $\pm$ 5,201	15,536 $\pm$ 5,730 ^c
NGFR	1,179 $\pm$ 735	597 $\pm$ 513	905 $\pm$ 1,755
NT-3	292 $\pm$ 141	157 $\pm$ 184	187 $\pm$ 275
NT-4	365 $\pm$ 332 ^{a,b}	219 $\pm$ 168 ^c	117 $\pm$ 169 ^c
OPG	98 $\pm$ 69	115 $\pm$ 117	72 $\pm$ 163
PDGF-AA	586 $\pm$ 191 ^b	453 $\pm$ 342	303 $\pm$ 163 ^c
PIGF	107 $\pm$ 81	54 $\pm$ 44	55 $\pm$ 57
SCF	143 $\pm$ 123 ^b	71 $\pm$ 185	49 $\pm$ 28 ^c
SCFR	8,240 $\pm$ 2,461 ^b	6,534 $\pm$ 3,038	5,198 $\pm$ 3,502 ^c
TGF α	60 $\pm$ 101	30 $\pm$ 134	14 $\pm$ 41
TGF β 1	29,248 $\pm$ 20,757	30,036 $\pm$ 13,516	33,413 $\pm$ 26,358
TGF β 3	21 $\pm$ 69	12 $\pm$ 31	14 $\pm$ 97
VEGF	45 $\pm$ 22	25 $\pm$ 38	30 $\pm$ 20
VEGFR2	1,921 $\pm$ 1,014 ^{a,b}	1,209 $\pm$ 636 ^c	838 $\pm$ 598 ^c
VEGFR3	762 $\pm$ 462 ^{a,b}	244 $\pm$ 256 ^c	175 $\pm$ 70 ^c
VEGF-D	229 $\pm$ 175 ^{a,b}	110 $\pm$ 84 ^c	72 $\pm$ 29 ^c

When Adj. *p* is smaller than 0.05, the difference is significant. AR: Adj. *p* between SS and NSS groups is 0.038, Adj. *p* between SS and normal groups is 0.001; BMP5: Adj. *p* between SS and NSS groups is 0.034; EG-VEGF: Adj. *p* between SS and normal groups is 0.028; GH: Adj. *p* between SS and NSS groups is 0.000; HGF: Adj. *p* between SS and normal groups is 0.012; IGFBP-1: Adj. *p* between SS and NSS groups is 0.004, Adj. *p* between SS and NSS groups is 0.000; IGFBP2: Adj. *p* between SS and normal groups is 0.031; IGFBP6: Adj. *p* between SS and normal groups is 0.036, Adj. *p* between NSS and normal groups is 0.035; MCSFR: Adj. *p* between SS and normal groups is 0.02; NT4: Adj. *p* between SS and NSS groups is 0.011, Adj. *p* between SS and normal groups is 0.004; PDGF-AA: Adj. *p* between SS and normal groups is 0.008; SCF: Adj. *p* between SS and normal group is 0.024; SCFR: Adj. *p* between SS and normal group is 0.034; VEGFR2: Adj. *p* between SS and NSS groups is 0.028, Adj. *p* between SS and normal groups is 0.000; VEGFR3: Adj. *p* between SS and NSS groups is 0.005, Adj. *p* between SS and normal groups is 0.003; VEGF-D: Adj. *p* between SS and NSS groups is 0.005, Adj. *p* between SS and normal groups is 0.000. Adj. *p*, adjusted *p*; SS, salt-sensitive; NSS, nonsalt-sensitive. ^a Adj. *p* < 0.05 compared with the NSS group. ^b Adj. *p* < 0.05 compared with the normal group. ^c Adj. *p* < 0.05 compared with the SS group.

AR, BMP-5, EG-VEGF, GH, HGF, IGFBP-2, IGFBP-3, IGFBP-6, MCSFR, NT-4, PDGF-AA, SCF, SCFR, VEGFR2, VEGFR3, and VEGF-D, compared to both other 2 groups and one of them. With all differential proteins imported in KEGG database, PI3K-AKT (ID: hsa04151) may account for the specific manifestation of the SS group.

As for growth factors, IGFBP-3 and IGFBP-6 of the SS group were significantly higher than those of NSS and

normal groups. IGFBP-3 is one of the 6 binding proteins in the insulin-like growth factor (IGF) signaling system and is the main carrier protein for IGF in blood. IGFBP-3 have high affinity with IGF-1 and about 70–90% IGF-1 binds with IGFBP-3 to form stable complexes as reserves. Only 1% IGF-1 binds to IGF-1R and activates insulin receptor substrate and downstream PI3K-AKT pathway, which can regulate cell growth and apoptosis [13]. The higher IGFBP-3 in the SS group implies that patients with SS hypertension reserve more IGF-1, which implicates the number of IGF-1 links with IGF-1R also increases. It can be inferred that patients in the SS group secreted more IGF-1 than patients in the NSS group which resulted in more activated PI3K-AKT signaling pathway and more serious renal damage as a result.

Compared to patients in the NSS group, patients in the SS group also presented higher VEGFR2, VEGFR3, and VEGF-D, which can partially account for their relatively higher blood pressure. Previous studies have shown that hyperosmosis of local tissues in SS patients can induce the invasion and activation of monocytes/macrophages which can increase the expression of tonicity-responsive enhancer binding protein and secretion of vascular endothelial growth factor (VEGF), leading to the growth of lymph vessels. The dilated lymphatic system drains excess sodium and water back into circulation and raises blood pressure [14]. In order to remove excess water and sodium from the body, the kidney will take on heavier work, which further aggravates the damage and forms a vicious circle. Basing on the opinion, higher VEGF-related growth factors in the SS group may explain their higher blood pressure and worse renal function.

In addition, recent studies have demonstrated that SS hypertension and related renal damage are closely related to immune mechanisms. In terms of innate immunity, low inflammatory status in the body has been proved to stimulate immune regulation, leading to elevated blood pressure and related kidney damage, in which macrophages resident in the dermis can regulate salt sensitivity by affecting the storage of nonpermeable sodium in the skin [15]. Hypertonic state can also drive macrophages from anti-inflammatory phenotype to pro-inflammatory phenotype and then infiltrate the kidney in large quantities, release inflammatory factors, and cause kidney damage [16]. In cellular immunity, activated T lymphocytes have been illuminated to raise blood pressure by inducing oxidative stress injury and renal reabsorption of sodium. On the other hand, the lack of T cells in animals has been observed to reduce oxidative stress and RAS activation, causing lower blood pressure [17]. In our study, higher

Fig. 2. KEGG analysis of differential proteins between the SS group and normal group (**a**); KEGG analysis of differential proteins between the SS hypertension group and NSS group (**b**); KEGG analysis of differential proteins between the NSS group and normal group (**c**). KEGG, Kyoto Encyclopedia of Genes and Genomes, SS, salt-sensitive, NSS, nonsalt-sensitive.

MCSFR in SS group compared to normal group indicates that high blood pressure and kidney damage in SS patients may be associated with the activation of the immune system. But there was no significant difference in

MCSFR between the SS and NSS group, indicating that the higher blood pressure and more serious renal damage in the SS group than those in the NSS group could not be explained by the enhanced MCSFR alone. Independent of

the immune mechanism, pertinent studies have found that increased renal circulation pressure can also lead immune cells to infiltrate the kidney. This can induce cytokines and consequent renal damage, water and sodium retention, increased vascular pressure, and further exacerbation of hypertension. But its mechanism which may be linked to tissue damage and direct stimulation of salt still remains unclear [18].

Our study also showed that AR in the SS group appeared significantly higher than in the other 2 groups and HGF higher than the normal group. Both factors can regulate cell proliferation and have been confirmed to be positively correlated with tumor formation and development. The mechanism may be related to promoting tumor invasion and metastasis and inducing angiogenesis and lymphangiogenesis [19, 20]. Higher AR and HGF in SS patients could be a reason for heavier renal injury, but it still acquires further study to demonstrate.

As for the activated PI3K-AKT pathway in the SS group, not only it has to do with the higher GH but also with the raised IGF-1. Thirone [21] found that GH deficient nephrotic rats suffered milder renal impairment than GH sufficient rats due to low activity of PI3K-AKT pathway, indicating that high expression of GH aggravated renal damage through PI3K-AKT pathway. Similarly, he found over-expressed IGF-1 binding proteins in rats could cause glomerular hypertrophy and glomerulosclerosis by increasing GH level and activating PI3K-AKT signaling pathway. In our study, patients in the SS group showed both elevated GH and IGF-1, which could be a solid confirmation to their worse renal damage.

However, there remains certain indeterminate points in the study. As presented in Table 1, the SS group showed significantly longer duration of hypertension than the NSS group. This could be the result of salt sensitivity leading to an earlier onset of hypertension and thus a longer duration. But, as the course of hypertension provided by each patient cannot be exact due to their potential low awareness rate at the onset of the disease, sodium sensitivity could also be a consequence of renal damage caused by the long duration of arterial hypertension. Plus, recruited patients did not test their salt sensitivity and renal function at the very beginning of hypertension. Therefore, we could not ensure the sequence of renal damage and elevated blood pressure in the SS group. There have existed 3 main possibilities of relationships between renal damage and blood pressure. First, some hidden mechanisms may lead to renal damage and salt sensitivity simultaneously. With the aggravation of renal injury and salt sensitivity, blood pressure begins to rise. Second, a patient

unconsciously finds his/her blood pressure rises. Meanwhile, kidney damage and salt sensitivity are also discovered. Third, clinical blood pressure rises, while renal damage and salt sensitivity are not prominent at first. With the enhancement of blood pressure and the passage of time, renal damage and typical salt sensitivity gradually appear. Therefore, elevated blood pressure and renal damage could bolster each other and form a vicious cycle in a long term. In reality, SS test is seldom applied to hypertensive patients, let alone common people who may take a potential risk of being hypertensive. If the SS test could be contained in a regular health examination, we doctors will have a greater chance to discover further correlations among renal damage, salt sensitivity, and blood pressure. At that time, doctors can guide people even including those who are temporarily under normal blood pressure to limit their salt intake if they are confirmed to be SS.

Conclusion

Patients with SS hypertension tend to suffer higher blood pressure and more serious renal damage. This may be closely correlated with more activated PI3K-AKT signaling pathway, which can be induced by enhanced IGFBP-3, IGF-1, and GH. Plus, higher growth factors in the SS group including EG-VEGF, VEGFR2, VEGFR3, and VEGF-D can account for the increased blood pressure due to the dilated lymphatic system, which drains excess sodium and water back into circulation. The SS group also presented higher AR and HGF, which may worsen renal function by regulating cell proliferation and tumor formation. Besides, higher MCSFR in both SS and NSS groups indicates that high blood pressure and severe kidney damage may be associated with the activation of the immune system. However, due to the potential low awareness rate of hypertension at the very beginning, we cannot ensure the exact occurrence order of blood pressure, renal damage, and salt sensitivity in each patient. Therefore, further studies which can track data about renal functions, blood pressure, and salt sensitivity from the onset of hypertension are needed.

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Statement of Ethics

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Subjects have given their written informed consent. The study protocol was approved by Ethics Committee of Guang'anmen Hospital, Chinese Academy of traditional Chinese Medicine, Beijing, China (2018-039-SQ).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Yuguang Chu contributed to the trial design, subjects recruitment, and manuscript refinement; Yan Zhou contributed to data collection and analysis and manuscript drafting and refining; Shihua Lu contributed to data analysis and manuscript refinement; Feng Lu contributed to manuscript refinement; and Yuanhui Hu contributed to supervision and offering guides during the whole process.

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