Research Article
The Construction and Analysis of a ceRNA Network Related to Salt-Sensitivity Hypertensives

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Background. Salt-sensitivity hypertensives (SSH) are an independent risk factor for cardiovascular disease. However, the mechanism of SSH is not clear. This study is aimed at constructing a competing endogenous RNA (ceRNA) network related to SSH.

1. Background
Hypertension, one of the most common diseases in humans, is one of the most well-known major risk factors for cardiovascular disease (CVD) and stroke. Accumulating study confirmed a strong relationship between sodium intake and blood pressure. Studies have confirmed that reducing the intake of salt in the diet can effectively reduce blood pressure. However, individuals have different responses to dietary salt. Some people will significantly increase their blood pressure after increasing their dietary salt intake, whereas another part of the population does not show significant changes in blood pressure. This phenomenon is called salt sensitivity [1]. Salt sensitivity is related to multiple factors, including the physiological environment, genetics, and demographic factors. Common demographic factors usually include sex, race, and age. However, the mechanism of salt-sensitivity hypertensives (SSH) is not clear. The mechanism of SSH has become the focus of many researchers.

SSH can be defined as elevated blood pressure caused by relatively high salt intake [2]. The mechanism behind the rise in blood pressure caused by increased salt intake is complex. Current studies have found that it is related to an increase in blood volume caused by osmotic pressure, impaired endothelial function, and an imbalance in the regulation of nitric oxide and endothelin [3]. Furthermore,
abnormal activation of the renin-angiotensin-aldosterone system, enhanced sympathetic nervous system, insulin resistance, and renal mechanism are also contribute [4]. Genetics, nutritional, and environmental factors are also involved in the development of salt-sensitive hypertension [5]. However, the BP of salt sensitivity differs among individuals. The blood pressure of salt-sensitive people can be divided into SSH and salt-sensitive normotension (SSN). Although both are sensitive to salt, their blood pressure is different. Its internal mechanism has yet to be studied while few people pay attention to their differences.

Herein, we collect hypertension expression data for these three RNAs from the GEO database and construct a competing endogenous RNA (ceRNA) network related to SSH based on the base of DEGs (differentially expressed genes) and DELs (differentially expressed IncRNAs). This is the first study to investigate the ceRNA network related to SSH. After the analysis of DEGs and DELs, we found that some RNAs were tightly related to SSH. The DEGs and pathways predicted in our study may reveal the potential molecular mechanism of SSH. The workflow of this study is shown in Figure 1.

2. Methods

2.1. Data Collection and Processing. First, the GSE135111 microarray data, including 5 SSH blood samples and 5 SSN blood samples, was downloaded from the GEO database [2]. The microarray data were normalized by R software such as probe identification transformation and log2 transformation. Finally, the normalization data were used for further analysis.

2.2. Difference Analysis. In this study, the Limma package [6] was used to identify DEGs (log2 FC ≥ 0.5 and P value < 0.05), DELs (log2 FC ≥ 1 and P value < 0.05), and DECs (log2 FC ≥ 1 and P value < 0.05). Then, DEGs and DELs were used in the next analysis.

2.3. GO and KEGG Pathway Enrichment Analyses of DEGs. Gene ontology (GO) is widely used to annotate genes, gene products, and sequences. KEGG is a comprehensive database for the biological interpretation of genome sequences and other high-throughput data. To represent the characteristics of DEGs, the enrichment analysis of the GO and KEGG pathways of DEGs was performed using a clusterProfiler package with the following criterion: P value < 0.05.

2.4. Construction of the PPI Network and Screening of Hub Genes. To select hub genes related to SSH herein, the DEGs were mapped into PPIs (protein-protein interaction) by the STRING database, and a combined score of >0.4 was set as a threshold value. In addition, nodes with higher degrees of interaction from the PPI network were considered as hub nodes. As everyone knows, cytoHubba [7] is a tool for screening hub genes in the Cytoscape software [8]. Therefore, in this study, the hub gene modules (top 50 genes) were screened out by all 12 methods in Cytoscape software. Only the remaining overlapping genes in all 12 methods were selected as hub genes related to SSH.

Figure 1: Workflow of this study. Notes: workflow of the study.
Figure 2: Continued.
Group
- Down-regulated
- Not-significant
- Up-regulated

**Figure 2**: Continued.
Figure 2: Difference analysis of mRNAs and lncRNAs. Notes: (a) expression of mRNAs between two sets of samples (SSH vs. SSN). Blue represents the downregulated genes and red represents the upregulated genes, and the names of the 10 genes with the lowest P value have been indicated. (b) Expression of lncRNAs between two sets of samples (SSH vs. SSN). Blue represents the downregulated lncRNAs and red represents the upregulated lncRNAs, and the names of the 10 lncRNAs with the lowest P value have been indicated. (c) Hierarchical clustering heat map of DEGs (top 57), DELs (65), and DECs (4). Firebrick indicates that the relative expression of mRNAs/lncRNAs/circRNAs were upregulated, navy indicates that the relative expression of mRNAs/lncRNAs/circRNAs were downregulated.
2.5. Construction of lncRNA-miRNA-mRNA Pairs. As we know, lncRNA-miRNA pairs and miRNA-mRNA pairs can form lncRNA-miRNA-mRNA pairs. miRNA can bind to targeted mRNA to promote the degradation of mRNA, while lncRNA can bind to targeted miRNA to inhibit the degradation of mRNA. Herein, we used ggalluvial R package [9] to construct lncRNA-miRNA-mRNA pairs through miRcode (version 11; http://www.mircode.org/mircode/), miRDB (version 7.0; http://mirdb.org/), miRTarBase (http://mirtarbase.mbc.nctu.edu.tw/index.html), and TargetScan (version 7.2; http://targetscan.org/vert_72/) on the base of DEGs and DELs. miRcode provides “whole transcriptome” human microRNA target predictions based on the comprehensive GENCODE gene annotation, including > 10,000 long noncoding RNA genes. Coding genes are also covered, including atypical regions such as 5’UTRs and CDS. miRDB is an online database for miRNA target prediction and functional annotations. All miRDB targets were predicted by a bioinformatics tool, MirTarget, which was developed by analyzing thousands of miRNA-target interactions from high-throughput sequencing experiments. miRTarBase is a database of experimentally validated microRNA targets. TargetScan predicts biological targets of miRNAs by searching for the presence of conserved 8mer, 7mer, and 6mer sites that match the seed region of each miRNA. Firstly, we predicted the lncRNA-miRNA pairs through the miRcode database on the base of DELs. The target genes for these miRNA signatures were then obtained using the miRDB, miRTarBase, and TargetScan databases. Genes present in all three databases were regarded as the target genes for these miRNAs. Comparing predicted target genes with DEGs, only the remaining overlapping genes and their interaction pairs were used for constructing the lncRNA-miRNA-mRNA pairs.

2.6. Construction of the ceRNA Network. In this study, the overlapping genes of hub genes and lncRNA-miRNA-mRNA pairs were used as potential key genes related to SSH. In addition, we construct a ceRNA network on hub genes and lncRNA-miRNA-mRNA pairs.

3. Results

3.1. Identification of DEGs and DELs. After preprocessing and data integration, 163 DEGs (86 upregulated and 77 downregulated), 65 DELs (34 upregulated and 31 downregulated), and 4 DECs (1 upregulated and 3 downregulated) were screened (Figures 2(a) and 2(b)). Herein, the heat map of the lncRNAs, circRNAs, and mRNAs showed that the SSH clustered separately from the paired SSN (Figure 2(c)).

3.2. Enrichment Analysis of DEGs. As shown in Table 1 and Figure 3(a). GO and KEGG pathway enrichment analyses of DEGs were mainly enriched in metabolism (e.g., insulin secretion and cellular response to glucagon stimulus and peptidyl-tyrosine dephosphorylation) and plasma membrane signaling (e.g., cell adhesion and chemical synaptic transmission and integral component of membrane).

3.3. PPI Network Construction and Screening of Hub Genes. In this study, these DEGs demonstrated significant interactions. A total of 59 nodes of the 163 DEGs were mapped in the PPI network (Figure 3(b)). Four genes (7 ≥Degree ≥4), including ADCY2, TAS2R38, TNFSF11, and ADCY6, were located in the center of the PPI network. Additionally, 31 hub genes were selected, including EGLN3, TNFSF11, and DPPA4, and were screened (Table 2 and Figure 4(a)). Furthermore, the top 10 hub genes (radiality method) are shown in Figure 4(b).

| Category | Term | Count/10 | -log10 (P value) |
|----------|------|----------|----------------|
| BP       | Cell adhesion | 0.9      | 1.613392753    |
|          | Chemical synaptic transmission | 0.6      | 1.434237533    |
|          | Cellular response to glucagon stimulus | 0.3      | 1.428932795    |
|          | Peptidyl-tyrosine dephosphorylation | 0.4      | 1.39799905     |
|          | Adenylate cyclase-inhibiting G-protein coupled receptor signaling pathway | 0.3      | 1.30207393     |
| BP       | Plasma membrane | 4.4      | 1.791142931    |
|          | Intrinsic component of plasma membrane | 0.3      | 1.711353914    |
|          | Collagen trimer | 0.4      | 1.469118021    |
|          | Integral component of membrane | 5.1      | 1.467740938    |
|          | Transport vesicle membrane | 0.3      | 1.463466518    |
|          | Integral component of plasma membrane | 1.8      | 1.359780188    |
| CC       | Transmembrane receptor protein tyrosine phosphatase activity | 0.3      | 2.104180723    |
|          | Protein tyrosine phosphatase activity | 0.4      | 1.343534176    |
| MF       | Glutamatergic synapse | 0.5      | 1.867759405    |
|          | Insulin secretion | 0.4      | 1.500852037    |
|          | Tight junction | 0.4      | 1.475102357    |
### Table 1: GO and KEGG Pathway Enrichment Analyses of DEGs

| GO Term                                      | P-Value | Count |
|----------------------------------------------|---------|-------|
| Adenylate cyclase                            |         |       |
| Inhibiting G-protein coupled receptor signaling pathway |         |       |
| Protein tyrosine phosphatase activity        |         |       |
| Peptidyl-tyrosine diphosphorylation          |         |       |
| Cellular response to glucagon stimulus       |         |       |
| Chemical synaptic transmission               |         |       |
| Transport vesicle membrane                   |         |       |
| Integral component of membrane               |         |       |
| Intrinsic component of plasma membrane       |         |       |
| Collagen trimer                              |         |       |
| Tight junction                               |         |       |
| Insulin secretion                            |         |       |
| Cell adhesion                                |         |       |
| Integral component of plasma membrane        |         |       |
| Plasma membrane                              |         |       |
| Glutamatergic synapse                        |         |       |
| Transmembrane receptor protein tyrosine phosphatase activity | | |

### Figure 3: Enrichment analysis of DEGs and PPI network construction

Notes: (a) GO and KEGG pathway enrichment analyses of DEGs. (b) PPI network. Red represents upregulated DEGs. Navy blue represents downregulated DEGs, and yellow represents other genes associated with DEGs.
Table 2: The first 50 hub genes provided by MCC method.

| MCC       | Betweenness | BottleNeck | Closeness | ClusteringCoefficient | Degree | DMNC   | Eccentricity | EPC | MNC | Radiality | Stress |
|-----------|-------------|------------|-----------|-----------------------|--------|--------|--------------|-----|-----|-----------|--------|
| ADCY6     | PSMC4       | TNFSF11    | ADCY6     | HTR1F                 | ADCY6  | HTR1F  | GCG          | ADCY6| ADCY6| TNFSF11   | PSMC4  |
| ADCY2     | TNFSF11     | PSMC4      | ADCY2     | NTPCR                 | ADCY2  | TAS2R38| TNFSF11      | ADCY2| ADCY2| PSMC4     | TNFSF11|
| TAS2R38   | CSF2        | ADCY2      | PSMC4      | ADCY2                 | ADCY2  | ADCY2  | ADCY2        | ADCY2| ADCY2| PSMC4     | TNFSF11|
| HTR1F     | GTSE1       | GRIN2A     | ADCY2     | TASSR38               | TNFSF11| ADCY2  | DPPA4        | HTR1F| HTR1F| TAS2R38   | GTSE1  |
| TNFSF11   | ADCY6       | KCNT1      | CSF2      | GCG                   | KCNT1  | GCG    | CSF2         | GCG  | GCG  | EGLN3     | GTSE1  |
| KCNT1     | OBPP2       | PTTRB      | GTSE1      | ADCY6                 | OBPP2  | NTPCR  | EGLN3        | NTPCR| NTPCR| OTSTAMP   | GCG    |
| OBPP2     | EGLN3       | CSF2       | TASSR38    | REP15                 | CSF2   | REP15  | GTSE1        | TNFSF11| REP15| SPIB      | OBPP2  |
| CSF2      | GCG         | ADCY6      | GCG        | CLDN24                | GCG    | CLDN24| OTSTAMP      | PSMC4| CLDN24| OBPP2     | NTSR1  |
| GCG       | NTSR1       | GCG        | OBPP2      | CLDN22                | HTR1F  | NTPCR  | GYY1         | CYY1 | GCG  | EGLN3     | NTSR1  |
| PTTRB     | C7orf72     | GTSE1      | EGLN3      | NTPA                  | NTPA   | NTPA   | NTPA         | NTPA | NTPA| NTPA      | NTPA   |
| PSMC4     | KIF14       | DPPA4      | HTR1F      | TNN13                 | TNN13  | TNN13  | TNN13        | TNN13| TNN13| GASL23    | TASSR38|
| GTSE1     | KCNT1       | OBPP2      | NTPCR      | ETV5                  | ETV5   | NTSR1  | GYY1         | CYY1 | GCG  | EGLN3     | GTSE1  |
| DPPA4     | TASSR38     | NTSR1      | KIF14      | FUT1                  | DPPA4  | FUT1   | FUT1         | ADCY6| KIF14| FUT1      | DPPA4  |
| FUT1      | PLCX2D      | EGLN3      | OCSTAMP    | RASIP1                | FUT1   | RASIP1| RASIP1       | C4BA | RASIP1| ADCY2     | PLCX2D |
| GRIN2A    | ADCY2       | FUT1       | SPIB       | GRIN2A                | CYY1   | GYY1  | CYY1         | CYY1 | GCG  | KIF14     | CYY1   |
| C7orf72   | GRIN2A      | C7orf72    | NTSR1      | SLC16A4               | C7orf72| C7orf72| C7orf72      | SLC16A4| C7orf72| FCR1      | GRIN2A |
| NTSR1     | PTTRB       | SPRY1      | C7orf72    | ZNRF3                 | ZNRF3  | ZNRF3  | ZNRF3        | ZNRF3| ZNF577| PTPRR     | PTPRR  |
| SPRY1     | FUT1        | CMTM6      | PTTRP      | PTTRP                 | PTTRP  | PTTRP  | PTTRP        | PTTRP| PTTRP| PTPRR     | PTPRR  |
| CMTM6     | DAO         | TASSR38    | AKAP5      | GRIN2A                | GRIN2A | GRIN2A| GRIN2A       | GRIN2A| HTR1F| DAO       | CMTM6  |
| DAO       | CMTM6       | KIF14      | SDC2       | FAMIL124A             | FAMIL124A| FAMIL124A| FAMIL124A    | FAMIL124A| ARHGAP44| SPRY1     | ARHGAP44|
| KIF14     | DPPA4       | PLCX2D     | EGLN3      | FASL23                | FASL23 | FASL23| FASL23       | FASL23| FASL23| PLCX2D    | TNN13  |
| PLCX2D    | REP15       | REP15      | TCL1A      | C7orf72               | C7orf72| C7orf72| C7orf72      | C7orf72| C7orf72| FCR1      | TCL1A  |
| NTPCR     | CLDN24      | ZNF577     | OBPP2      | NTPR                 | NTPR   | NTPR   | NTPR         | NTPR | NTPR| NTPR      | NTPR   |
| REP15     | CLDN22      | CLDN22     | C4BA       | FCR1                  | FCR1   | FCR1   | FCR1         | FCR1 | FCR1| FCR1      | FCR1   |
| NTPCR     | CLDN24      | NPPA       | NPPA       | NPPA                  | NPPA   | NPPA   | NPPA         | NPPA | NPPA| NPPA      | NPPA   |
| NPPA      | CLDN22      | CLDN22     | C4BA       | FCR1                  | FCR1   | FCR1   | FCR1         | FCR1 | FCR1| FCR1      | FCR1   |
| NPPA      | NPPA        | NPPA       | NPPA       | NPPA                  | NPPA   | NPPA   | NPPA         | NPPA | NPPA| NPPA      | NPPA   |
| NPPA      | NPPA        | NPPA       | NPPA       | NPPA                  | NPPA   | NPPA   | NPPA         | NPPA | NPPA| NPPA      | NPPA   |
| NPPA      | ETV5        | ETV5       | C7orf72    | TNN13                 | TNN13  | TNN13  | TNN13        | TNN13| TNN13| GASL23    | C7orf72|
| TNN13     | RASIP1      | RASIP1     | PTTRB      | C7orf72               | C7orf72| C7orf72| C7orf72      | C7orf72| C7orf72| ARHGAP44  | C7orf72|
| ET5V      | CYRY1       | CYRY1      | GRIN2A     | ARHGAP44              | ETV5   | ARHGAP44| ARHGAP44     | ARHGAP44| ARHGAP44| GRIN2A    | CYRY1  |
| RASIP1    | SLC16A4     | SLC16A4    | FUT1       | KCNMB3                | RASIP1 | KCNMB3| CLDN24      | ZNF577| KCNMB3| FUT1      | SLC16A4|
| CYRY1     | ZNRF3       | ZNRF3      | FAMIL124A  | C4BA                  | CYRY1  | C4BA   | C4BA         | C4BA | C4BA| C4BA      | C4BA   |
| SLC16A4   | PTTRK       | PTTRK      | DAO        | APOC1                 | SLC16A4| APOC1  | APOC1        | APOC1| APOC1| APOC1     | APOC1  |
| ZNRF3     | FAMIL124A   | FAMIL124A  | SPRY1      | SLC17A4               | SLC17A4| SLC17A4| SLC17A4      | APOC1| APOC1| APOC1     | FAMIL124A|
| PTTRK     | GTSF1       | GTSF1      | CMTM6      | ZNF577                | PTTRK  | ZNF577| CYRY1        | SIGLEC14| ZNF577| PTTRK     | GTSF1  |
| MCC      | Betweenness | BtLeck | Closeness | ClusteringCoefficient | Degree | DMNC | EcCentricity | EPC | MNC | Radiality | Stress |
|----------|-------------|--------|-----------|-----------------------|--------|------|--------------|-----|-----|-----------|--------|
| FAM124A  | TCL1A       | TCL1A  | DPPA4     | SDC2                  | SDC2   | SLC16A14 | KCNMB3       | SDC2 | HOGA1 | TCL1A     |        |
| GTSF1    | FCRL1       | FCRL1  | KCNMB3    | CSF2                  | CSF2   | ZNRF3 | FAM124A      | CSF2 | SPRY1 | FCRL1     |        |
| TCL1A    | PTPRR       | PTPRR  | SIGLEC14  | AKAP5                 | TCL1A  | AKAP5 | PTPRK        | CDC175 AKAP5 | CMTM6 | PTPRR     |        |
| FCRL1    | GAS2L3      | GAS2L3 | RASIP1    | NTSR1                 | FCR1   | NTSR1 | FAM124A      | SDC2 | NTSR1 | SIGLEC14 | GAS2L3 |
| PTPRR    | CCDC175     | CCDC175| HOGA1     | SPRY1                 | PTPRR  | GTSF1 | ET5          | SPRY1 | REP15 | CCDC175   |        |
| GAS2L3   | ARHGAP44    | ARHGAP44| REP15     | PTPR5                 | GAS2L3 | PTPR5 | TLC1A        | REP15 | PTPR5 | ETV5      | ARHGAP44|
| CCDC175  | KCNMB3      | KCNMB3 | ETV5      | SIGLEC14             | CCDC175 | SIGLEC14 | C4BPA | DPPA4 | KCNMB3    |        |
| ARHGAP44 | C4BPA       | C4BPA  | GTSF1     | CMTM6                | ARHGAP44 | CMTM6 | APOC1        | DNMT3B | CMTM6 | GTSF1     | C4BPA  |
| KCNMB3   | APOC1       | APOC1  | TCL1A     | TNFSF11              | KCNMB3  | TNFSF11 | SLC17A4      | TCL1A | TNFSF11 | TCL1A     | APOC1  |
| C4BPA    | SLC17A4     | SLC17A4| DNMT3B    | PSMC4                | C4BPA  | PSMC4 | SPRY1        | HOGA1 | PSMC4 | DNMT3B    | SLC17A4|
| APOC1    | ZNF577      | ZNF577 | CLDN24    | EGLN3                | APOC1  | EGLN3 | SIGLEC14     | AQP6 | EGLN3 | CLDN24    | ZNF577 |
| SLC17A4  | SDC2        | SDC2   | CLDN22    | GTSE1                | SLC17A4 | GTSE1 | CMTM6        | PVRL3 | GTSE1 | CLDN22    | SDC2   |
| ZNF577   | AKAP5       | AKAP5  | NPPA      | HOAG1                | ZNF577 | HOAG1 | RPL22L1      | ZNRF3 | HOAG1 | NPPA      | AKAP5  |
| SDC2     | HTR1F       | HTR1F  | TNN13     | DAO                   | SDC2   | DAO   | MRPL15       | PTPRK | DAO   | TNN13     | HTR1F  |
| AKAP5    | SIGLEC14    | SIGLEC14| CYR1      | RPL22L1              | AKAP5  | RPL22L1 | DNMT3B       | CLDN24 | RPL22L1 | CYR1      | SIGLEC14|
Figure 4: Continued.
3.4. Construction of lncRNA-miRNA-mRNA Pairs. Herein, lncRNA-miRNA-mRNA pairs based on DEGs and DELs, including 2 mRNAs (EGLN3 and TNFRSF21), 2 miRNAs (hsa-miR-17-5p and hsa-miR-20b-5p), and 1 lncRNA (C1orf143) was constructed. As shown in Figure 5(a).

3.5. Construction of the ceRNA Network. In this study, an overlapping gene (EGLN3) between hub genes and lncRNA-miRNA-mRNA pairs was screened out and was identified as a potential key gene related to SSH (Figure 5(b)). Then, based on the potential key genes, a ceRNA network was successfully constructed, including 1 lncRNA (C1orf143), 2 miRNAs (hsa-miR-17-5p and hsa-miR-20b-5p), and 1 mRNA (EGLN3).

4. Discussion

It is generally known that salt sensitivity of blood pressure (SSBP) is an independent risk factor for cardiovascular disease. Although they are both sensitive to salt, their blood pressure is different. The blood pressure of salt-sensitive people can be divided into SSH and salt-sensitive normotension (SSN) [10]. As for SSH, salt sensitivity is related to multiple factors, including physiological environment, genetics, and demographic factors. Common demographic factors generally include gender, race, and age [11]. However, the pathogenic mechanisms of SSBP are still uncertain. Therefore, the mechanism of salt-sensitivity hypertensives (SSH) has become the focus of many researchers.

Nowadays, it has become a popular method for evaluating DEGs through gene expression analysis to explore the causes of diseases [12]. These different genome-wide expression profiling techniques make them more valuable on account of complementary results. In this study, we obtained the analysis of the expression of mRNAs and lncRNAs of whole blood samples from patients with SSH and SSN with active disease and explored potential RNAs related to SSH by the bioinformatic analysis. Then, we selected 163 DEGs and 65 DELs as our subsequent research object. Furthermore, an enrichment analysis of the GO, KEGG pathway, and the construction of DEG PPI networks were performed, and we found that these 163 genes may participate in the process of SSH through metabolism (e.g., insulin
Figure 5: Continued.
To change the phosphorylation state of channel proteins and enzymes can act on channel protein synthesis and transport. Some protein contains multiple tyrosine residues [17]. Studies have not yet been reported in the results of our study such as tyrosine dephosphorylation. Tyrosine dephosphorylation remains to be verified by experiments.

In plasma membrane signaling, at present, many types of research are thorough. Such as G-protein coupled receptor signaling pathway and insulin secretion [18]. Note that we constructed an SSH-related ceRNA network, including 1 mRNA (EGLN3), 2 miRNAs (hsa-miR-17-5p and hsa-miR-20b-5p), and 1 lncRNA (C1orf143). Among the ceRNA regulatory network, 1 lncRNA (C1orf143) is associated with two miRNAs (hsa-miR-17-5p and hsa-miR-20b-5p), and we proposed that possible competition for C1orf143 binding to hsa-miR-17-5p and hsa-miR-20b-5p influences the downstream regulation of EGLN3. However, this requires experimental verification.

EGLN3 (also named Proly-hydroxylase 3, PHD3, HPH1, and SM-20) belongs to the EGLN family of prolyl hydroxylases and can catalyze hydroxylation. Studies have shown that EGLN3 is involved in the metabolism and angiogenesis of oxygen from tumor cells, which in turn affects tumor cell proliferation [19]. However, there is no report about EGLN3 participating in SSH. It has been reported that EGLN3 is regulated by miRNA to play a role in different biological processes. However, so far, there has been no research on the EGLN3 ceRNA network in SSH. What is more, lncRNAs play a significant role in the development of the disease according to many studies. In our study, we found that C1orf143 could lncRNAs play important biological roles by regulating gene expression (a ceRNA network) in SSH. It is worth noting that there is no research report on C1orf143. This is a new gene. Our research results indicate that C1orf143 may play an important role in SSH. This gives us great hints. However, whether PTP participates in SSH through the dephosphorylation of K⁺ channel protein remains to be verified by experiments.

In metabolism, such as previous studies have demonstrated that single nucleotide polymorphisms (SNPs) of the sodium-bicarbonate cotransporter gene (SLC4A5) are associated with hypertension [13]. Furusho et al. found that mutations of with-no-lysine kinase 1 (WNKI) could lead to abnormally increased salt reabsorption and salt-sensitive hypertension [14]. Based on the epidemiological baseline survey, fasting blood glucose was found to be an independent and dose-dependent related factor of blood pressure salt sensitivity [15]. At the same time, it is well known that the ion channel is closely related to SSH. It has been found that there are many pathways mediated phosphorylation of ion channels in the body, including PKA-mediated phosphorylation, PKC-mediated phosphorylation, PI-3-K/PKB and PI-3-K/SGK3 pathway phosphorylation, MAPKs pathway-mediated phosphorylation, and Src channel regulation. However, there are still some signaling pathways that have not yet been reported in the results of our study such as tyrosine dephosphorylation. Tyrosine dephosphorylation is regulated by protein tyrosine phosphatase (PTP). Studies have found that the PTP gene mainly expresses 112 PTP in the human genome. It is not only an indispensable regulator in cell signal transduction but also a key signal molecule. It plays a key role in the regulation of different physiological events and is related to many diseases such as metabolism, cardiovascular diseases, cancer, and autoimmune diseases [15].

The composition of its catalytic sites determines the sensitivity of dephosphorylation [16]. The same PTP can also be divided into two categories: the receptor tyrosine phosphatase and the nonreceptor tyrosine phosphatase. It is worth noting that the K⁺ channel is closely related to SSH. At the same time, previous articles have reported that K⁺ channels contain multiple tyrosine residues [17]. Some protein enzymes can act on channel protein synthesis and transport to change the phosphorylation state of channel proteins and regulate channel functions. Meanwhile, our results suggest that PTP may be involved in the occurrence of SSH. This gives us great hints. However, whether PTP participates in SSH through the dephosphorylation of K⁺ channel protein remains to be verified by experiments.

On the whole, based on comprehensive bioinformatics analysis of multiple cohort datasets of SSH and SSN patients, 163 DEGs were identified. The enrichment analysis of DEGs involving related molecules or pathways may deepen our understanding of SSH. Additionally, the SSH-related ceRNA

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**Figure 5: ceRNA network construction.**

Notes: (a) Sankey diagram for the lncRNA-miRNA-mRNA pairs. Each rectangle represents a mRNA, miRNA, or IncRNA, and the connection degree of each gene is visualized based on the size of the rectangle. (b) Venn diagram of the hub gens and the IncRNA-miRNA-mRNA pairs. The overlapping genes in both represent the potential key genes related to SSH. (c) A ceRNA network. Red represents IncRNAs. Navy blue represents miRNAs. Violet represents mRNAs.
network, including 1 mRNA (EGLN3), 2 miRNAs (hsa-miR-17-5p and hsa-miR-20b-5p), and 1 lncRNA (C1orf143) was successfully constructed. However, some limitations still existed in this research. The main method of our research was bioinformatics technology, a useful tool to understand interactions, pathways, and networks.

In conclusion, these findings could enrich DEG expression profile between SSH and SSN and provide novel information on the occurrence of SSH. Other scientific researchers are expected to verify this at the genetic level.

Data Availability
The datasets used during the current study are available from the corresponding authors upon reasonable request (GSE135111).

Consent
There is no conflict of interest that exists in the submission of this manuscript, and the manuscript is approved by all authors for publication.

Conflicts of Interest
The authors have declared that no competing interests exist.

Authors’ Contributions
XJL performed a comparative analysis using bioinformatics tools. HLY and Yan Li participated in the data analysis and discussion. XJL and HH interpreted the results and wrote the manuscript. QLZ and Yang Liu organized and supervised the project. All authors read and approved the final manuscript.

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