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# Revealing the contribution of Cytochrome P450 to salt-sensitive hypertension using DNA microarray

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**Abstract. – BACKGROUND: Salt sensitivity is an important cause of hypertension which is a major public health problem. This study aimed to investigate the contribution of Cytochrome P450 (CYP) to salt-sensitive hypertension with microarray data and bioinformatics analysis.**

**METHODS: Gene expression data set GSE4800 was downloaded from Gene Expression Omnibus database, including 6 gene chips from 3 Dahl salt sensitive (DS) rat samples and 3 Lewis (LEW) rat samples. Raw data were preprocessed and normalized, and then differentially expressed genes (DEGs) were identified with Limma package. Interaction network was constructed by employing STRING (Search Tool for the Retrieval of Interacting Genes) tool. GO (Gene Ontology) enrichment analysis was performed using FuncAssociate tool and pathway analysis was carried out by EASE (Expressing Analysis Systematic Explorer). BLAST (Basic Local Alignment Search Tool) was applied to explore the sequence homology among CYP3A genes in rat and human based on multiple alignments.**

**RESULTS: A total of 1264 DEGs, including 1082 up-regulated genes and 182 down-regulated genes were identified between DS and LEW samples. CYP3A2 and CYP3A9 were selected to construct the protein interaction network, which comprised 1653 pairs of interaction relationship among 100 genes. Functional analysis showed that CYP3A2 and CYP3A9 were most significantly related to oxidation reduction and metabolism of xenobiotics by cytochrome P450. Furthermore, the CYP3A2 and CYP3A9 genes in rats had high homology with genes CYP3A4and CYP3A5 in human beings.**

**CONCLUSIONS: CYP3A4 and CYP3A5 may contribute to salt-sensitive hypertension in human which may act as biomarkers for this disease.**

*Key Words:*
Salt-sensitive hypertension, Differentially expressed genes, Cytochrome P450, Interaction network, Functional analysis.

## Introduction

Hypertension is a major public health problem and contributes to deaths from stroke, myocardial infarction and kidney failure[1]. Salt sensitivity is the causative agent for an important subgroup of humans with essential hypertension[2]. Salt sensitive hypertension refers to increases in blood pressure as a response to eating increased amounts of sodium. In salt sensitive individuals, fluctuations in blood pressure in response to increased or decreased sodium are greater than normal fluctuations. Clinical studies show that the cardiovascular and renal morbidity and mortality induced by hypertension are markedly reduced through timely diagnosis and early clinical intervention[3]. However, since the molecular mechanism of the disease remains uncertain, its early diagnosis and treatment are largely symptomatic. Identification of novel pathways/genes related to salt sensitive hypertension may improve the diagnosis and therapy.

The role of cytochrome P450 (CYP) enzyme superfamily in the pathogenesis of salt-sensitive hypertension is a research hotspot. CYP-epoxygenase is highly expressed in the kidney and its metabolism of arachidonic acid plays important roles in regulating renal Na transport and in modulating vasoactivity in the kidney[4]. Early study has been revealed that renal CYP ω-hydroxylase- and epoxygenase activity are differentially modified by sodium chloride[5]. The expression of CYP members, CYP4A subfamily, CYP2C11 and CYP2C23, can be altered by high dietary salt that CYP4A proteins are down-regulated while CYP2C11 and CYP2C23 are up-regulated in the kidney[2,5(Stec, 1996 #17, 6]. Dysfunctional CYP4A10 gene causes a type of hypertension that is dietary


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salt sensitive and associated with alterations in the activity of the renal epithelial sodium channel (ENC) [7]. There are many other CYP isoforms in addition to above; however, little is known about whether they are involved in Salt sensitive hypertension or not. DNA microarray is a powerful technology that provides the expression profile of thousands of genes[8]. In addition to the many molecular biological and genomic research uses, DNA microarray covers applications of pharmacogenomics research and drug discovery, infectious and genetic disease and cancer diagnostics [9]. In this study, the microarray data of Dahl salt sensitive (DS) rats and Lewis (LEW) rats were obtained, and the differentially expressed genes (DEGs) between DS rats and LEW rats were identified. The differentially expressed CYP family members were selected for further analysis by bioinformatics methods. Our findings may further clarify the molecular mechanisms of human salt-sensitive hypertension and provide new potential biomarkers for this disease.

## Methods

All animal studies have been approved by China Ethics Committee and performed in accordance with the ethical standards.

### Affymetrix Microarray Data

The gene expression profile GSE4800 was obtained from a public functional genomics data repository Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/), which was based on the GPL1355 [Rat230_2] platform data (Affymetrix Rat Genome 230 2.0 Array). This database was deposited by Yasui et al[10]. Male Dahl salt-sensitive rats and Lewis (LEW) rats (n=3 each) were fed an 8% NaCl diet starting at 5 weeks of age for 8 weeks. Total RNA was isolated with TRIzol reagent from DS kidneys and LEW kidneys and was subjected to microarray analysis. Total VI chips were available for further analysis, including 3 chips of DS samples and 3 LEW samples.

### Data Preprocessing and DEGs Analysis

The original data in CEL files were converted into expression form measures firstly and then the missing data were imputed[11]. Finally, normalization was performed for these data[12]. The differentially expressed genes (DEGs) between DS and LEW samples were identified using Limma package[13] in R based on the normalized data. The $p$ value < 0.05 and |logFC| > 1 were selected as the cut-off criterion. And then, the CYP family genes from the DEGs were collected to further analysis.

### Construction of Interaction Network

The STRING (Search Tool for the Retrieval of Interacting Genes) [14] is an online database that provides uniquely comprehensive coverage and ease of access to both experimental as well as predicted protein interaction information. Interactions in STRING are provided with a confidence score which represents a rough estimate of the probability of a given association between two proteins. The CYP family genes we selected were mapped into the STRING database to construct the protein-protein interaction network which was visualized by Cytoscape software[15]. The interactions with confidence scores higher than 0.8 were selected for further analysis.

### Gene Ontology (GO) Enrichment Analysis

FuncAssociate[16] is a web application designed to facilitate the task of characterizing large collections of genes or proteins. The genes in the interaction network were inputted into the FuncAssociate to identify overrepresented GO categories. After Benjamini Hochberg (BH)[17] correction for multiple testing, the false discovery rate (FDR) less than 0.05 and gene count number larger than 10 were set as cut-off criterion.

### Pathway Enrichment Analysis of Network

EASE (Expressing Analysis Systematic Explorer)[18] is an online analysis tool developed for rapid biological interpretation of gene lists derived from the analysis of microarray, proteomics and other high-throughput genomic data. The EASE was applied to perform pathway enrichment analysis for the genes in the network based on the Fisher's test. The FDR less than 0.05 was chosen as the threshold.

### Homologous Alignment of CYP3A Between Rat and Human

Generally, molecules with high sequence homology share the similar biological function[19]. The BLAST (Basic Local Alignment Search Tool) is used to find homologous region among sequences, including nucleotide sequences and amino acid sequences[20]. The amino acid sequences of CYP3A family from rat and human were inputted into the BLAST for homology analysis.

## Results

### Differential Gene Expression Analysis

We obtained publicly available microarray dataset GSE 4800 from GEO database. After the preprocessing, those data with high degree of standardization (Figure 1A) were subjected to differential expression analysis. The individual with *p*-value less than 0.05 and |logFC| larger than 1 was chosen as the DEG between DS and LEW, and volcano plot of the results was shown in Figure 1B. Finally, we got 1264 DEGs, including 1082 up-regulated genes and 182 down-regulated genes. Interestingly, the two genes CYP3A2 and CYP3A9, both of which belong to the CYP3A gene family, showed opposite changes of expression that the CYP3A2 was down-regulated and the CYP3A9 was up-regulated in DS rats (Figure 1 C). Meanwhile, CYP3A2 and CYP3A9 presented significantly differential expression ($p = 0.039754$ and $p = 0.009562$, respectively). Therefore, they were selected for our further investigation.

### Construction of Network

Products of CYP3A2 and CYP3A9 were used to predict the proteins which may interact with

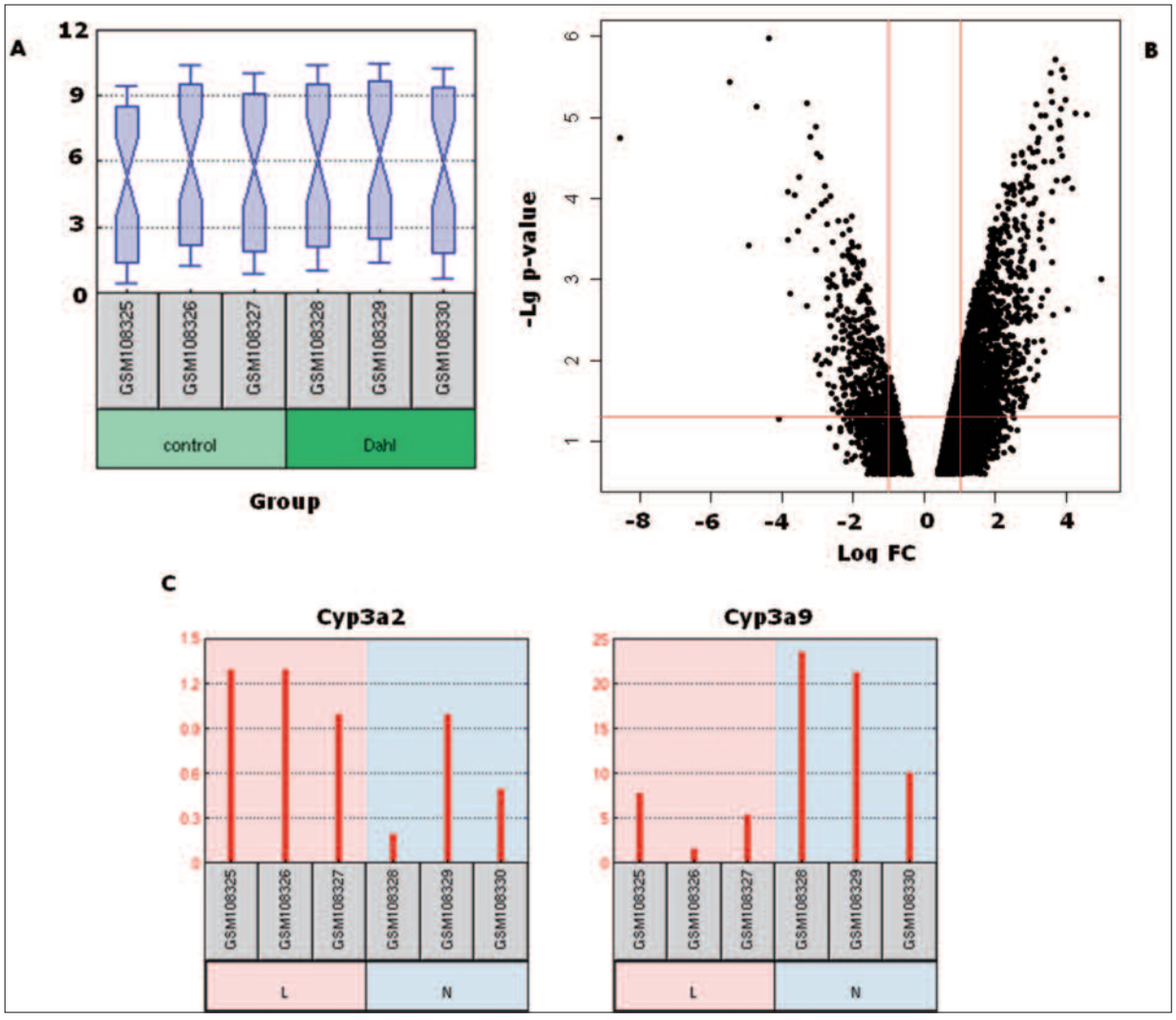


**Figure 1.** Data preprocessing and gene expression value analysis. ***A,*** Box plots displaying the data normalized result. Control and Dahl represent LEW rat and DS rat samples, respectively. ***B,*** Volcano plot showing the correlation between the *p* value and logFC. The horizontal axis is the fold change of gene expression between the two groups (on a log scale), and the vertical axis represents the *p*-value for a *t*-test of gene expression differences between samples. The red horizontal line represents the *p*-value at 0.05, and the red vertical lines represents the logFC at 1 or -1. ***C,*** Histogram displaying the expression value of CYP3A2 and CYP3A9, two DEGs identified in our study, in 6 rat samples. Red (L) and blue (R) boxes represent LEW rats and DS rats, respectively.

them by the STRING software. A total of 1653 pairs of interaction relationship among 100 genes were obtained and these interactions were visualized via Cytoscape software (Figure 2).

### GO Enrichment Analysis

GO functional annotation for the genes in the interaction network was performed using FuncAssociate (FDR less than 0.05 and gene count number larger than 10). As shown in Table I, these genes were significantly related to 6 GO categories. Among them the most significant GO term was oxidation reduction with a FDR of 1.23 E-29.

### Pathway Enrichment Analysis of Network

To further investigate the function of genes in the interaction network, EASE tool was used to carry out pathway enrichment analysis and finally 5 pathways with FDR < 0.05 were obtained (Table II). The most significant enrichment pathway was metabolism of xenobiotics by cy-

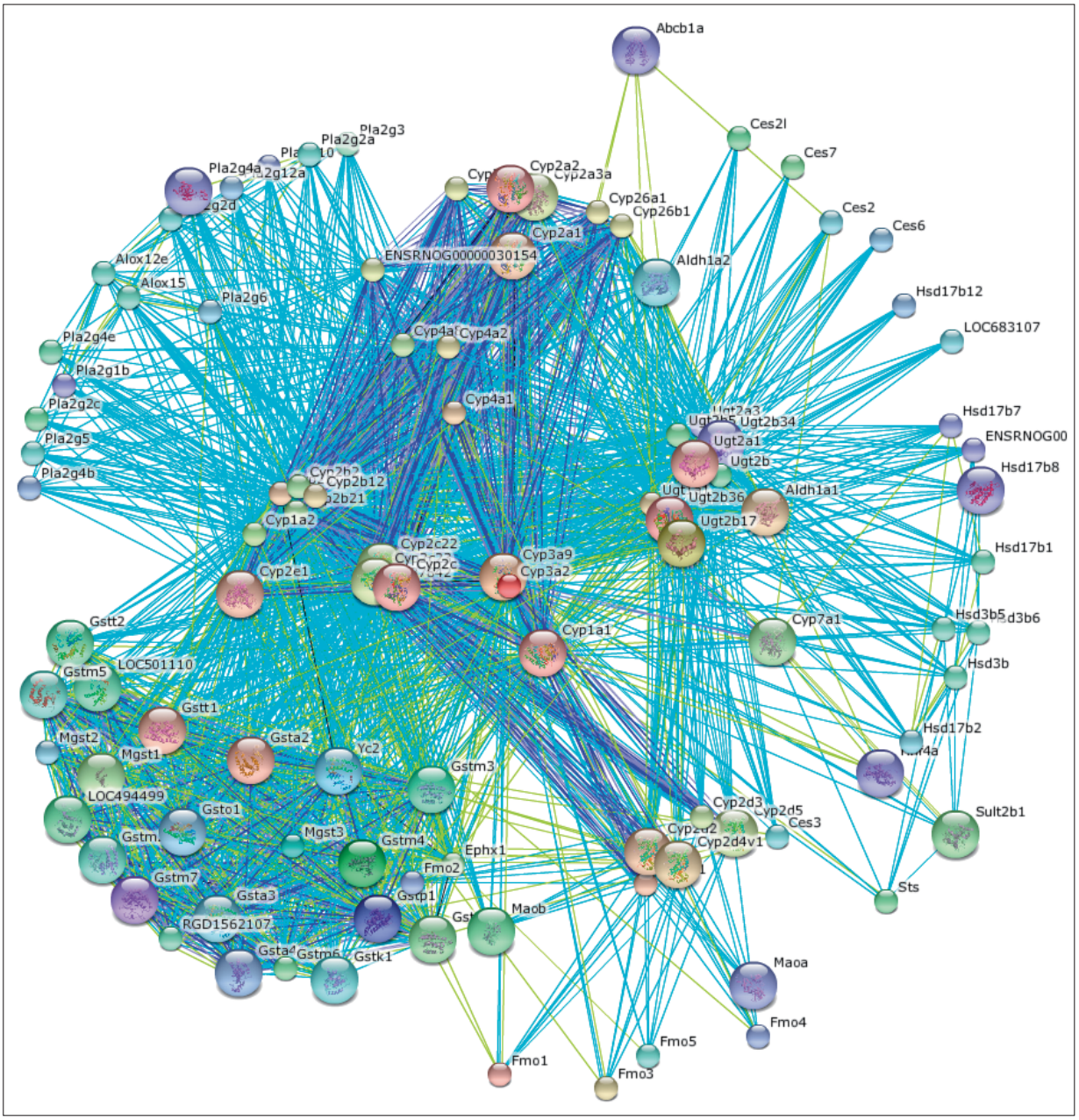


**Figure 2.** Interaction networks formed by the genes CYP3A2, CYP3A9 and their respective interacting partners.

**Table I.** GO enrichment analysis of genes in the interaction network.

| GO ID | Description | Count | FDR |
|---|---|---|---|
| GO:0055114 | Oxidation reduction | 42 | 1.23E-29 |
| GO:0020037 | Heme binding | 26 | 1.28E-24 |
| GO:0046906 | Tetrapyrrole binding | 26 | 3.65E-24 |
| GO:0009055 | Electron carrier activity | 28 | 7.39E-23 |
| GO:0005506 | Iron ion binding | 27 | 1.80E-18 |
| GO:0008395 | Steroid hydroxylase activity | 10 | 6.68E-11 |

tochrome P450 (FDR = 7.04 E-53) and the genes CYP3A2and CYP3A9 were involved in this pathway (Figure 3).

### *Homologous Alignment of CYP3A Between Rat and Human*

The sequence similarity of CYP3A between rat and human was shown in Figure 4. From the result of multiple alignments, we found that the amino acid sequences of CYP3A2/CYP3A9 from rat share high homology with CYP3A4/CYP3A5 from human.

## Discussion

To investigate the contribution of CYP to salt-sensitive hypertension, DEGs between male DS rats and LEW rats were identified and then the differentially expressed CYP members were used to construct interaction network. Further, GO enrichment and pathway enrichment analyses were performed to functionally characterize the interaction network. The results showed that the selected CYP3A2 and CYP3A9 were markedly related to the oxidation reduction and metabolism of xenobiotics by cytochrome P450. Furthermore, multiple alignment analysis indicated the high sequence homology shared by CYP3A2/CYP3A9 of rats and CYP3A4/CYP3A5 of human which suggested that they may have similar biological functions.

Enzymes produced from the CYP genes play roles in the formation[21] and breakdown[22] of various molecules and chemicals within cells. The biological behavior of CYP involved in biotransformation, drug metabolism and biological detoxification has been widely researched[23-25]. Results in this study showed the differential expression of CYP3A2 and CYP3A9 between DS samples and LEW samples which suggested that they may be related to salt-sensitive hypertension. This result agrees with previous studies which demonstrated the association of CYP proteins with blood pressure[26,27]. Pathway enrichment analysis uncovered the close relationship between the genes CYP3A2 and CYP3A9 and metabolism of xenobiotics by cytochrome P450. Evidence showed that the metabolism regulated by CYP-epoxygenase plays important roles in regulating renal Na transport and in modulating vasoactivity in the kidney[4]. CYP3A2 and CYP3A9 may contribute to salt-sensitive hypertension via participating in the metabolism pathway of xenobiotics.GO enrichment analysis indicated that the CYP3A2 and CYP3A9 were related to oxidation reduction, which provided base data for the research about the contribution of reactive oxygen species (ROS)[21] (termed oxidative stress) to hypertension. Furthermore, many factors have been demonstrated to implicate in the pathophysiology of hypertension such as perturbed G protein-coupled receptor signaling, altered T-cell function, up regulation of the renin-angiotensin-aldosterone (RAA) system, inflammation and activation of the sympathetic nervous system[22,28,29]. Common to these processes is in-

**Table II.** The significant pathway enrichment analysis of genes in the interaction network..

| Path ID | Description | FDR |
|---|---|---|
| rno00980 | Metabolism of xenobiotics by cytochrome P450 | 7.04E-53 |
| rno00830 | Retinol metabolism | 8.71E-33 |
| rno00591 | Linoleic acid metabolism | 1.39E-27 |
| rno00140 | Steroid hormone biosynthesis | 2.44E-22 |
| rno00983 | Drug metabolism | 6.43E-17 |

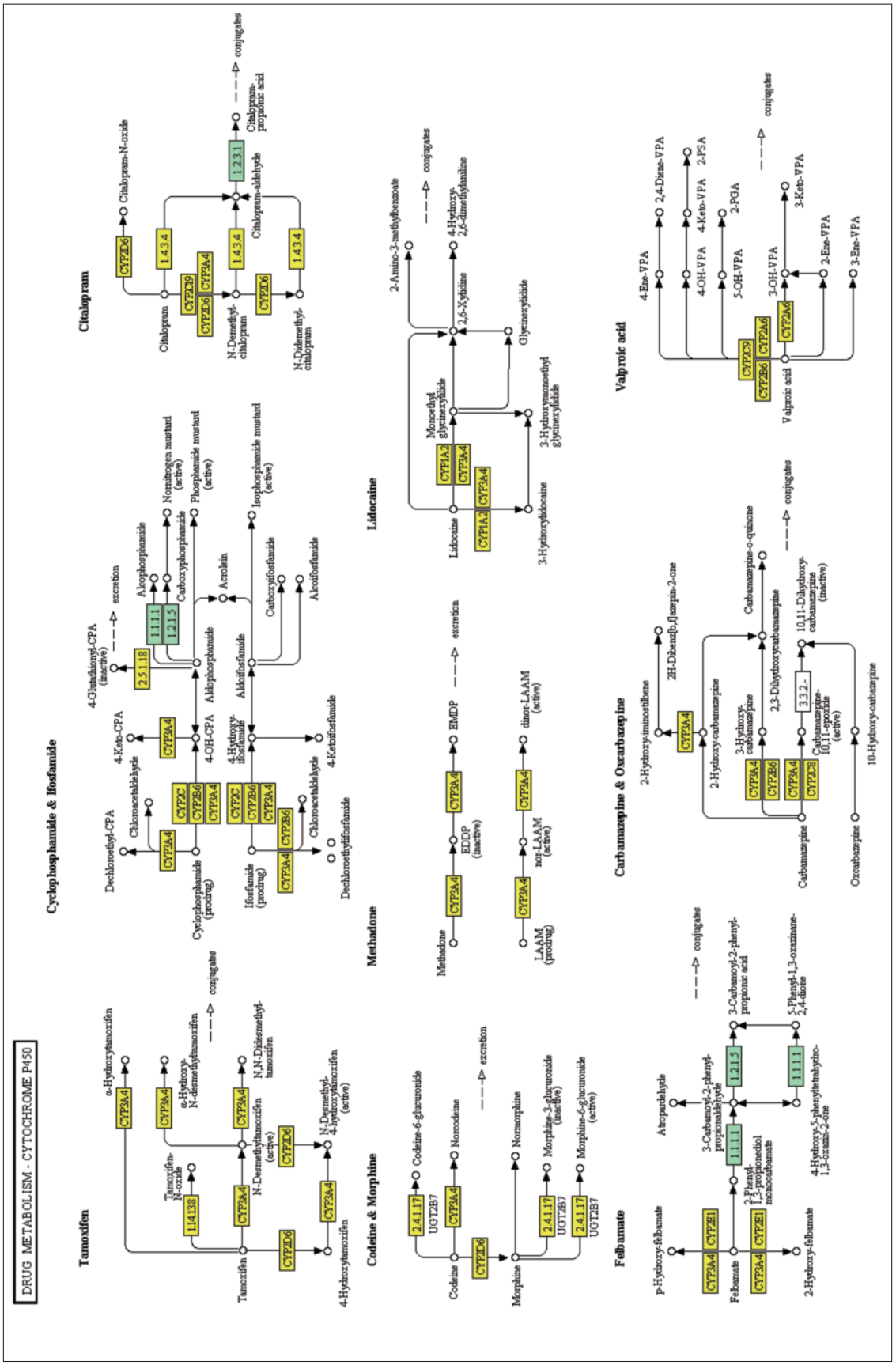


**Figure 3.** Pathway enrichment analysis for the interaction network. The yellow box stands for genes in interaction network.


**Figure 4.** Alignment analysis of genes CYP3A2 and CYP3A9 from rats and genes CYP3A4 and CYP3A5 from human. Identical amino acids among all sequences are indicated by"*", where as those with high or low similarity are indicated by ":" and "." respectively.

creased bioavailability of ROS[21] (termed oxidative stress), which leads to cardiovascular and renal damage. ROS (reactive oxygen species) also promote activation of the sympathetic nervous system associated with increase of blood pressure[30,31]. Although convincing data from experimental and animal studies support a causative role of ROS in the pathogenesis of hypertension, there is still no solid evidence that oxidative stress causes hypertension in humans[32]. However, many classical antihypertensive drugs have antioxidant capacity[33]. Thus, CYP3A2 and CYP3A9, the differentially expression genes in the disease samples may be involved in salt-sensitive hypertension through regulation of oxidation reduction.

Results showed that the amino acid sequences of CYP3A2/CYP3A9 from rats shared high homology with that of CYP3A4/CYP3A5 from human beings. CYP3A homologs are variably expressed in rat and human kidney, liver, anterior pituitary gland and adrenal gland[34-39]. Although the physiological consequences of CYP3A enzyme activity have not been defined, some observations support a role in blood pressure control. The CYP3A not only participate in the metabolism of bile acids and steroids (such as testosterone, aldosterone and estrogens), but also in the biotransformation of xenobiotics such as immunosuppressive drugs[40]. CYP3A4 is expressed at high levels in adult liver and small intestine, and CYP3A5 is also expressed in kidney[37]. Interestingly, it has been reported that CYP3A4/CYP3A5 activity could affect the risk of developing hypertension in pregnancy and have relations with the mortality in pregnant women and fetal death[21, 40-42]. So we deduced that CYP3A4/CYP3A5 may play important role in the pathogenesis of hypertension.

## Conclusions

We analyzed the differentially expressed genes between DS rats and LEW rats induced hypertension by high salt diet. The CYP3A2 and CYP3A9 were identified significant ones, which were related to the oxidation reduction. In addition, the amino acid sequences of CYP3A2/CYP3A9 shared high homology with that of CYP3A4/CYP3A5 from human beings. So we infer that the CYP3A4 and CYP3A5 may contribute to salt-sensitive hypertension through the process of the oxidation reduction and metabolism pathway of xenobiotics in human which may act as biomarkers for this disease. However, further experimental researches are needed to verify our results.

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### Conflict of Interest

The Authors declare that there are no conflicts of interest.

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