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Novel insights into cytochrome P450 enzyme and solute carrier families in cadmium-induced liver injury of pigs

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ABSTRACT

Cadmium (Cd) is a typical pollutant and carcinogen in environment. Exposure assessment of contaminants is an important component of occupational and environmental epidemiological studies. Early studies of Cd have focused on aquatic animals, chickens and rats. However, toxicological evaluation of Cd in pigs has not been reported. Therefore, twelve pigs were randomly divided into two groups (n = 6): the control group and the Cd group (Cd content: 15 ± 0.242 mg/kg feed) in this study, the experimental period was 30 d, and the toxic effects of Cd on the liver of weanling piglets were examined by antioxidant function, liver function, Cd content, histological examination and transcriptomics. The results showed that the changes of antioxidant function, liver function and Cd content were significant in the liver. Transcriptional profiling results showed that 399 differentially expressed genes (DEGs) were significantly up-regulated while 369 DEGs were remarkably down-regulated in Cd group, and which were concentrated in three ontologies: molecular function, cellular component and biological processes. Interestingly, significant changes in some genes of the cytochrome P450 enzyme (CYP450) and solute carrier (SLC) families have been observed and were consistent with qRT-PCR results. In conclusion, Cd could cause liver injury in weanling piglets and change the transcriptomic characteristics of liver. CYP450 and SLC families play an indispensable role in Cd-mediated hepatotoxicity. Importantly, changes in mRNA levels of CYP2B22, CYP7A1, CYP8B1, SLC26A8, SLC11A1, SLC27A2 and SLC22A7 induced by Cd have been reported for the first time. Our findings will provide a new insight for better assessing the mechanism of Cd toxicity to the liver.

1. Introduction

Cadmium (Cd) is a typical environmental pollutant and has been listed as a human carcinogen by the International Agency for Research on Cancer (Suzuki et al., 2017). Cd in the environment mainly comes from smelting, mining, fertilizer use, coal combustion and battery manufacturing (Ju et al., 2020). Cd levels in the environment vary widely because Cd released into the environment is usually transported continuously between the three main environmental units, air, water and soil. When the environment is polluted by Cd, Cd can be enriched in organisms and enter the human body through the food chain, causing chronic poisoning. The Cd has a half-life of about 20 years in living organisms, it cannot be degraded and is not readily excreted from the body, thus causing great harm to animal and human health (Strungaru et al., 2016; Abu-El-Zahab et al., 2019; Gustin et al., 2020). The Americas and Asia are main producers of feed and feed ingredients, where average soil Cd concentrations range from 0.1 to 2.6 mg/kg, with maximum concentrations exceeding 30 mg/kg (Ni et al., 2020). The "Itai-itai disease" in Toyama, Japan, and the "Cd Rice incident" in Hunan, China, were caused by Cd contamination in the environment (Baba et al., 2013; Hu et al., 2016). Cd has toxic effects on a variety of animals, such as fish (Dave and Kwong, 2020), chickens (Tang et al., 2019), mice (Nan et al., 2019; Adil et al., 2020), toads (Wu et al., 2017), turtles (Huo et al., 2020), snails (Dallinger et al., 2020) and quails (Suljevic et al., 2019). These data showed that liver and kidney were the target organs of Cd poisoning. In addition, Cd accumulates in other organs such as spleen, pancreas, thyroid gland and hair to a certain extent. Therefore, studies on the toxic effects and mechanisms of Cd on organisms at the top of the food chain and model animals have been widely concerned by researchers in the field of environmental toxicology in recent years (Qu et al., 2019; Sánchez et al., 2020).

The liver is an important detoxification organ for environmental

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pollutants, and one of the most important target organs for Cd toxicity (Mitkovska et al., 2020). The exposure routes of Cd include inhalation, intake of food and drinking water, and skin absorption (Rahman et al., 2017; Tucovic et al., 2018). When Cd enters the body, it can be transported to the liver through the blood and combined with cysteine-rich cytoplasmic proteins to form metallothionein, which can play its toxic role to the liver (Smalinskiene et al., 2009). Cd can induce the changes of liver structure, liver function, metallothionein level, detoxifying enzyme activity, DNA damage and some factors in mRNA, microRNA and protein levels (Shi et al., 2019; Zoidis et al., 2019; Mitkovska et al., 2020). However, studies on the toxic effect of cadmium on pig liver and its mechanism have not been reported.

Recently, pig as a conventional animal model has been widely used in biomedical, pharmaceutical and toxicological fields, because the cardiovascular system, digestive system, skin, nutritional needs, bone development, and mineral metabolism are all similar to humans, and the pig's size and domestication allow repeated sampling (Fu et al., 2020). However, due to the cost of pig as experimental animal is more expensive than chicken, fish and mouse, only a few literatures have reported the toxic effect of Cd on pig liver. Transcriptome technology can be used to study the function and structure of genes and reveal the molecular mechanism of specific biological processes and diseases. It is widely used because of its high efficiency and easy operation (Chen et al., 2019; Wang et al., 2019; Jing et al., 2020). In order to further understand the toxicological effects of Cd on liver, it is necessary to evaluate the toxic effects of Cd on liver of weaned piglets by transcriptomics.

2. Materials and methods

2.1. Experimental model

All procedures used in our experiment were approved by the Animal Care and Use Committee of Northeast Agricultural University. Weaned piglets aged 30 d were housed in the animal house at the Experimental Animal Center in Northeast Agricultural University. Twelve 30-days-old three-way cross (Large White × Duroc × Min Pig) male piglets with a similar weight (8.96 ± 0.42 kg) from the third born sow were randomly divided into two groups (n = 6): the control group and the Cd group. The piglets in the control group were feed basal diets (Table 1a). The piglets in the Cd group were feed basal diets with $CdCl_2$ (Cd: 15 ± 0.242 mg/kg) (Ni et al., 2020). Cd content in the feed was measured by Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (iCAP Q, Thermo, USA). In the experimental environment, the pre-feeding was 10 d, and the formal experiment was 30 d, during which the piglets were allowed to feed and drink freely. For a pig, a total of 7 kg of feed is required during 40–50 days of age, 10 kg during 50–60 days of age, and 15 kg during 60–70 days of age. At the end of the experiment, blood was taken from the anterior vena cava after 24 h on an empty stomach, the animals were euthanized, and liver tissues were quickly collected. The liver was rinsed with normal saline and then dried with filter paper. The collected tissues were rapidly divided into five parts under ice conditions: the first part was homogenized for antioxidant assay at low temperature, the second part was fixed in 10% formalin solution for histological examination, the third part was sub-packed into RNA-free cryopreservation tubes and EP tubes and placed in liquid nitrogen for 1 h, then stored at −80 °C for transcriptome. The fourth part and fifth part were used for determination of quantitative real-time PCR (qRT-PCR) and Cd content in tissues.

Table 1a
Composition of conservation piglet feed.

Composition	%	Composition	%
Corn	61	Oil	1
Peel bean meal	5	Stone powder	1.15
Puffed soybean	7.5	Calcium bicarbonate	0.5
Fish mean	1.5	Trace elements	0.2
Fermented soybean meal	10	L-lysate (98.5%)	0.3
Rice bran	3.70	Methionine (99%)	0.1
NaC1	0.2	Threonine (98.5%)	0.2
White sugar	2	Choline (50%)	0.15
Whey powder	2	Other	3.50

2.2. Measurement of antioxidant indicators

The collected liver samples were homogenized in a ratio of 1:9 to cold physiological saline. After centrifugation at 3500 rpm for 10 min at 4 ℃, the liver supernatant was collected for the determination of the activities of glutathione peroxidase (GSH-P_X), catalase (CAT) and superoxide dismutase (SOD) and the contents of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) according to the detection kits provided by Nanjing Jiancheng Bioengineering Institute. Then, the absorbance (OD) values were measured by enzyme-labeled instrument at 412, 240, 450, 532 and 415 nm, respectively. The coefficient of variation (CV) of oxidative stress was calculated and its maximum CV was 6.3%. The results showed good repeatability and robustness.

2.3. Measurement of liver function

Serum isolated from anterior vena cava blood was used to determine alanine transaminase (ALT) and aspartate transaminase (AST) activities by Microplate assay kits (Shanghai Hengyuan Bioengineering Co., Ltd., China) according to manufacturer's protocol. Serum ALT and AST levels were determined by double antibody sandwich method. First, the purified ALT or AST antibody of pigs was coated with the microporous plate, and then serum was added to the micropore to form the antibody-antigen-elisa antibody complex. After thorough washing, substrate TMB was added for color development. The OD values were measured by an enzyme-labeled instrument at the wavelength of 450 nm. The concentration of ALT or AST in the sample was calculated by the standard curve.

2.4. Determination of Cd content in the liver

The Cd content in the liver was measured according to "National Food Safety Standard for the determination of multiple elements in food" in China (GB 5009.268-2016). The 5 g tissue samples were put into a microwave digestion tank and 5 mL of concentrated nitric acid (65%, Millipore, Germany) was added. The tissue sample with concentrated nitric acid was put into a microwave digestion device (MARS6, CEM, USA) for digestion according to certain digestion procedures, and the air was removed after cooling. It was then placed on a temperature-controlled electric heating plate and heated at 100 °C for 30 min. After cooling, the liquid was transferred to a 50 mL volumetric flask and diluted to scale with pure water. The Cd content were determined by ICP-MS (iCAP Q, Thermo, USA). The standard solutions for Cd were 0, 1.00, 5.00, 10.0, 30.0, and 50.0 ng/mL. Each standard solution was measured three times. The CV (Y = 9290.344X-12.547, R2 = 0.9999) was plotted on the basis that the measured value (the average of the three readings) was in ordinate and the concentration was in abscissa. There was a good linear relationship between the intensity response of Cd and its concentration. The detection limit of Cd was 0.0061 μg/kg. Under the same conditions, 6 parallel samples of the same batch were tested, and the test results showed good repeatability.

2.5. Histological examination

Histological examination was performed according to traditional methods. Concisely, the tissues fixed in 10% formalin solution were dehydrated in a series of ethanol steps, washed with xylene, and embedded in paraffin. The 5–6 mm thick sections were stained with haematoxylin–eosin slices and examined under a Leica DME 100 light microscope (Fu et al., 2020).

2.6. RNA-Seq

The RNA in tissue samples was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The total RNA with qualified quality (RIN ≥ 7, 28S/18S ≥ 1.5, 1.9 < $OD_{260/280}$ < 2.1) was selected for library construction. mRNA was purified by the interaction of the poly (A) tails and magnetic oligo (dT) beads. After the synthesis of first strand cDNA and second strand cDNA, the purification and clonal amplification of library fragments was performed, then the Agilent Bioanalyzer 2200 system (Agilent Technologies) was used to assess purified libraries. Unqualified reads were removed by the cutadapt quality control tool. The clean reads were used for downstream analysis. DESeq software and Q-value were used to evaluate differentially expressed genes (DEGs). Here, only DEGs with an absolute value of log2 ratio ≥2 and FDR significance score <0.05 were used for subsequent analysis. Subsequently, all DEGs were subjected to Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis and Gene Ontology (GO) enrichment analysis to evaluate the effects of Cd on biological systems and functional classes at the molecular level.

2.7. Validation of the qRT-PCR

Total RNA from each sample was extracted using TRIzol reagent. The primers of detected mRNA were shown Table 1b and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). β-actin was used as a reference gene of mRNA. The reverse transcription of mRNA was performed using a reverse transcription reaction system (60 μL) as directed by the manufacturer. qRT-PCR was performed on Light Cycler®480 System (Roche, Basel, Switzerland). The transcript level was analyzed by the $2^{-\triangle\triangle Ct}$ method.

2.8. Statistical analysis

The statistical analysis of antioxidant indicators, Cd content and liver function was performed using SPSS for Windows (version 23 SPSS Inc., Chicago, IL). All values were normally distributed and expressed as means ± standard deviation. An unpaired *t*-test was used to elucidate if there were significant differences between the control groups and the Cd group. A $P < 0.05$ was considered significant.

3. Results

3.1. Effects of Cd on antioxidant indicators, Cd content, liver function and histological structure

To assess Cd-induced liver damage, the antioxidant markers, Cd content in tissues, liver function and histological structure were measured. The results were shown in Fig. 1. Compared with the control group, the activities of GSH-Px, SOD and CAT in the Cd group were significantly decreased by 29.11%, 27.69% and 36.78% ($P < 0.05$), respectively; and the contents of H_2O_2, MDA and Cd and the activities of AST and ALT were significantly increased 45.67%, 34.78%, 29.85%, and 39.97% ($P < 0.05$), respectively. For histological structure, the hepatocytes had normal structure, clear morphology, no degeneration and necrosis, and the chorda hepatis is radially arranged with the central vein as the center in the control group; degeneration and swelling of hepatocytes, loose and light staining of cytoplasm, infiltration of lymphocytes around portal area and local bile duct hyperplasia were observed in the Cd group.

Table 1b
Gene-specific primers for qRT-PCR.

Gene	Primer sequence (5′-3′)	Size of product (bp)
CYP1A1	5-GGACAAGAGGATGGACGAGAATGC-3 5-GGAGATGGCGGTTGTGACTGTG-3	112
CYP2B22	5-GGAGGAGCGGATTCAGGAGGAG-3 5-ATGGAGCAGATGATGTTGGCAGTG-3	120
CYP7A1	5-AAGTCGCTCCTCGCTGTCCTC-3 5-GCTTCAGGGCTCCTAATCGTTTGG-3	92
CYP8B1	5-GCAGGCAAGAAGATCCACCACTAC-3 5-TGACCATGAGCAGCACAAAGAGC-3	112
SLC22A7	5-GACACCAAGGCTCCGACACATC-3 5-ACCCGACACATCCAGGCTCAG-3	94
SLC26A8	5-TTGCATCTCTGGTAAGCGCA-3 5-AGGTAGGGTAGGACGTTGCT-3	121
SLC27A2	5-AGGACGAGACGCTCACCTATGC-3 5-CCAGCCAGAGCCACACATAAGC-3	144
SLC11A1	5-ACAGCAGCCTCCACGACTACG-3 5-CAGAAGACCCACCGCCCAAATG-3	137
β-actin	5-CTCCATCATGAAGTGCGACGT-3 5-GTGATCTCCTTCTGCATCCTGTC-3	114

3.2. Quality control results of RNA-Seq

Transcriptome sequencing results of two groups were showed in Table 2. A total of 50,743,324 (C1), 44,839,756 (C2) and 51,128,470 (C3) raw reads were obtained from control group, respectively. In the Cd group, 38,804,034 (Cd1), 40,892,854 (Cd2), 39,397,358 (Cd3) raw reads were obtained, respectively. After removing reads containing adapter, undetermined base, and low-quality from raw reads, valid data reads from control group (46,812,190 (C1), 42,595,720 (C2) and 47,993,444 (C3)) and Cd groups (37,488,904 (Cd1), 38,707,374 (Cd2) and 37,097,512 (Cd3)) were obtained, respectively. The ratios of Q20 and Q30 were greater than 99.97% and 98.25%, respectively. GC content was between 50% and 51%.

3.3. Differential expression analysis of RNA-Seq

The effect of Cd on the molecular level of liver toxicity in weanling piglets was studied by RNA-Seq. The DEGs between control and treatment groups were identified and were showed in Fig. 2. We obtained 768 DEGs in the liver according to the absolute values of fold change >2 and $P < 0.05$. Among them, the significantly up-regulated and down-regulated DEGs were 399 and 369, respectively.

3.4. Orientation of DEGs in metabolic pathways

The results of the obtained DEGs after enrichment analysis of the KEGG signal pathway were shown in Fig. 3. The signal pathway listed here were the top 20 signal paths with the most significant differences.

3.5. Orientation of DEGs in functional profiles

All DEGs between Cd group and control group were annotated with the GO analysis to study the transcriptome composition (Fig. 4). Significant GO-terms enriched by DEGs include three ontologies that describe the molecular function, its cellular component, and related biological processes of the gene (Fig. 4A). The major annotation categories in biological, molecular, and cellular processes were oxidation-reduction process, extracellular space, and protein binding, respectively. Fig. 4B showed the top 20 GO-terms with the most significant differences.

3.6. Effects of Cd on the mRNA expression of CYP and SLC families

The results of RNA-Seq showed that many genes of CYP and SLC families had been changed significantly (Fig. 5). It was preliminarily confirmed that these two families were involved in Cd-induced hepatotoxicity. In order to further reveal the roles of these two families in cadmium-induced hepatotoxicity, some genes of CYP and SLC families were verified by qRT-PCR (Fig. 5). CYP1A1, CYP2B22, CYP7A1, SLC26A8 and SLC22A7 were significantly increased ($P < 0.05$) at mRNA level, while CYP8B1, SLC11A1 and SLC27A2 were significantly decreased ($P < 0.05$) at mRNA level compared with the control group.

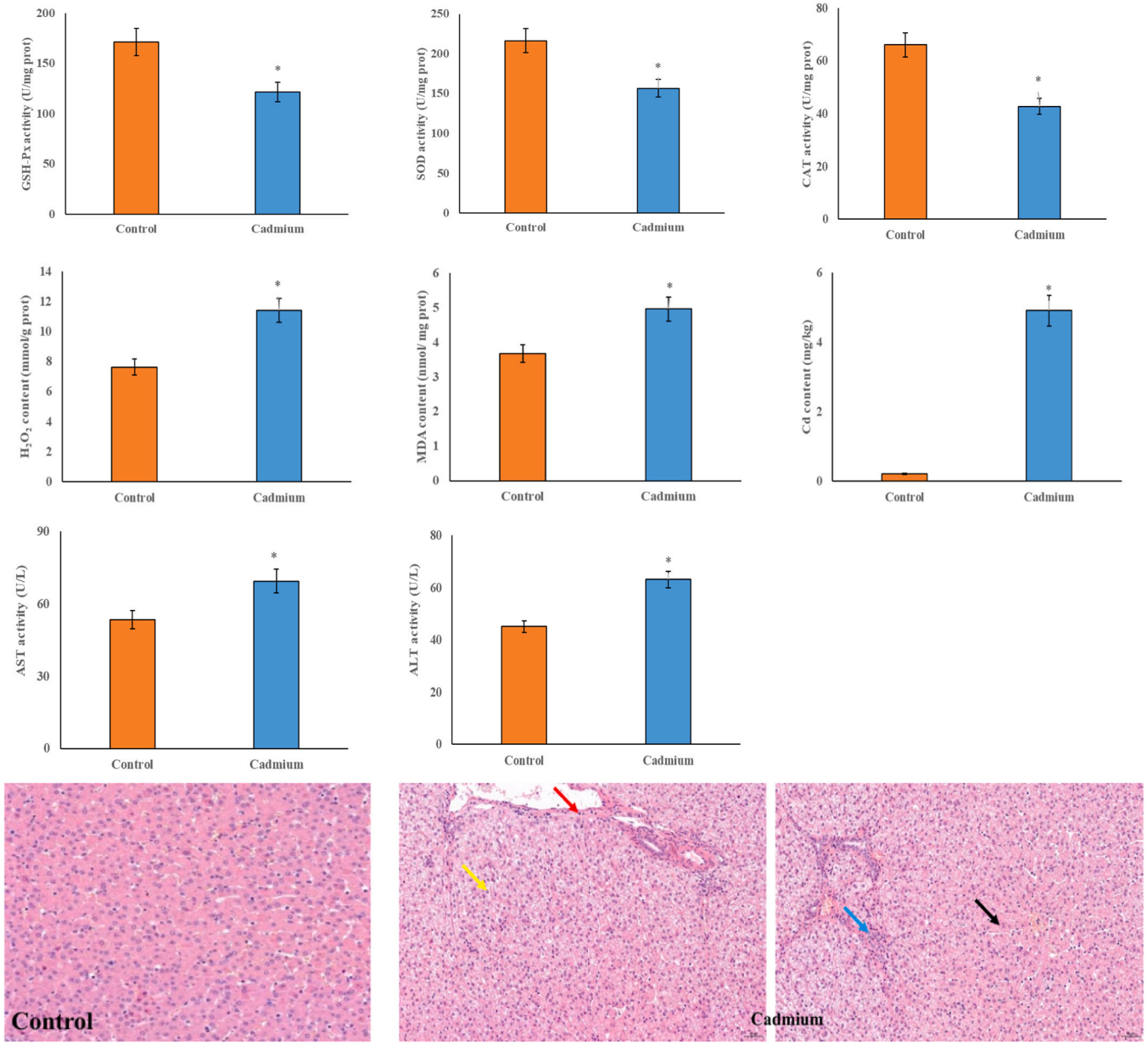

Fig. 1. Effects of Cd on antioxidant indicators, Cd content, liver function and histological structure. Antioxidant indicators include GSH-Px, SOD, CAT, MDA and H_2O_2. Indicators of liver function include ALT and AST. Each value in the histogram represents the mean ± SD of 6 individuals. *Indicated that the difference between the Cd group and the control group was significant ($P < 0.05$). An unpaired t-test was used to elucidate if there were significant differences between the control group and the Cd group. Histological structure: control group (×400); Cd group (×200), yellow arrow: hepatocytes loose; black arrow: ballooning degeneration; red arrows: lymphocyte infiltration; blue arrow: bile duct hyperplasia (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Table 2
Summary statistics of the transcriptome sequencing for pigs from control group (C) and Cd group.

Sample	Raw data reads	Base	Valid data reads	Base	Valid ratio (%)	Q20 (%)	Q (30%)	GC content (%)
C1	50,743,324	7.61G	46,812,190	7.02G	92.25	99.98	98.44	50
C2	44,839,756	6.73G	42,595,720	6.39G	95.00	99.98	98.33	50
C3	51,128,470	7.67G	47,993,444	7.20G	93.87	99.98	98.25	51
Cd1	38,804,034	5.82G	37,488,904	5.62G	96.61	99.98	98.45	51
Cd2	40,892,854	6.13G	38,707,374	5.81G	94.66	99.98	98.41	50
Cd3	39,397,358	5.91G	37,097,512	5.56G	94.16	99.98	98.35	51

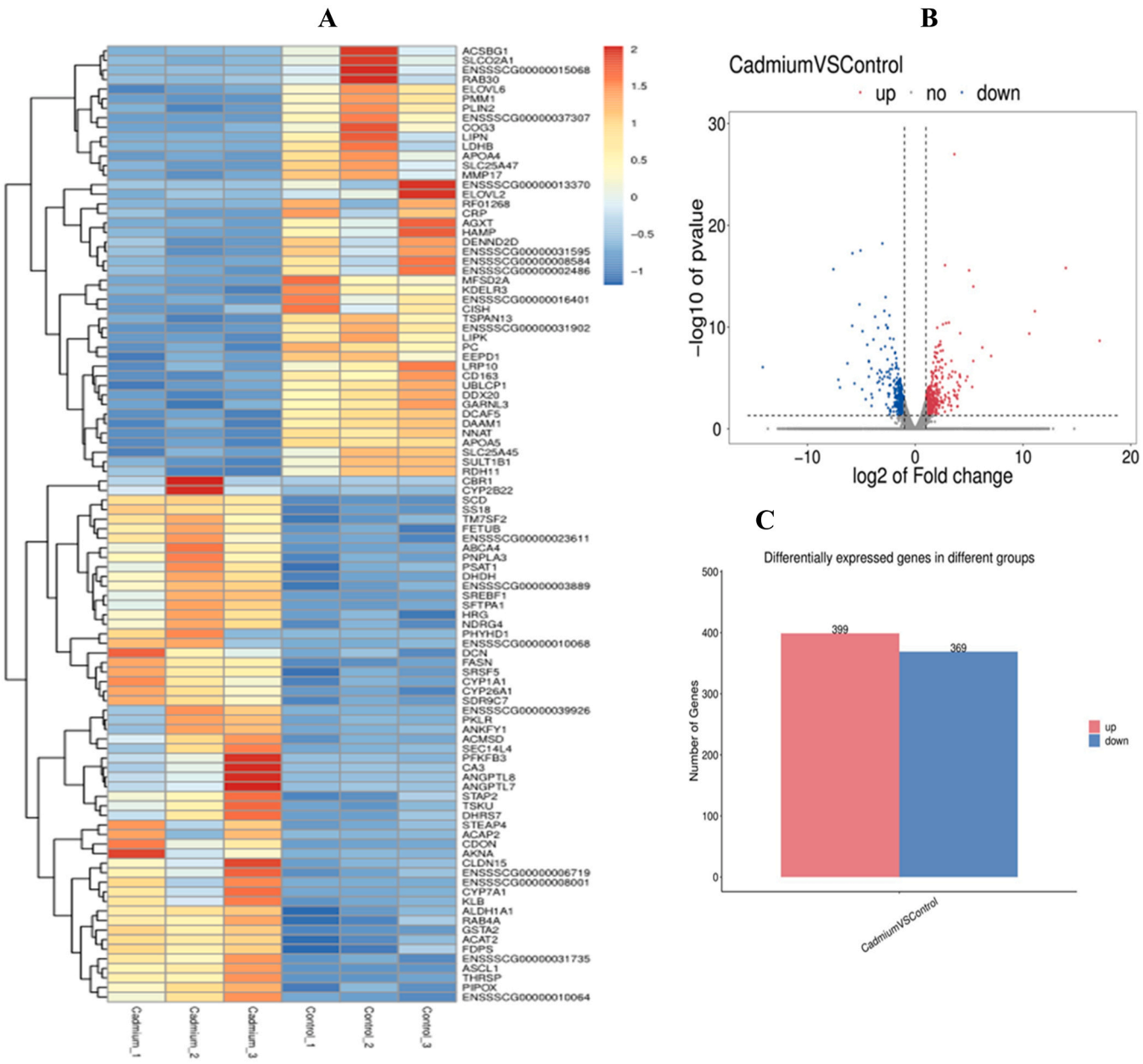

Fig. 2. The result of DEGs by RNA-Seq. (A) DEG heat map; (B) DEG volcano map; (C) DEG histogram.

The result of RNA-Seq was consistent with that of qRT-PCR.

4. Discussion

According to data from the World Health Organization, the toxic element Cd has been found in 10 major public health chemicals (WHO, 2019). It has been reported that Cd has been detected in many water, soil and air environments around the world (Gustin et al., 2020). After Cd enters the body, it has the toxic effect to many organs, especially the most serious damage to liver or kidney (Fowler, 2009). As the liver is an important detoxifying organ of the body and a target organ for the toxic effects of Cd on animals, it is of great significance to study the toxicological mechanism of cadmium on the liver. Oxidative stress is one of the toxic mechanisms of Cd (Tang et al., 2019). Cd accumulates in the organism and can indirectly produce free radicals, which combine with GSH and lead to lipid peroxidation (Shi et al., 2019). Scientific research showed that cancer, aging or other diseases are mostly associated with the production of excessive free radicals. Enzymatic and non-enzymatic antioxidant systems play a crucial role in protecting against oxidative damage caused by exogenous substances (Zhao et al., 2018; Wang et al., 2018). Apparently, the influence of Cd on antioxidant function in pig liver is worth understanding. In the present study, Cd markedly inhibited antioxidant enzyme activities (GSH-Px, SOD and CAT) and increase of lipid peroxidation levels (MDA) in pig liver. These results were consistent with other studies, confirming that Cd can cause cytotoxicity and tissue and organ damage by inducing oxidative stress (Zhang et al., 2018; Li et al., 2019). AST and ALT were the main markers for the diagnosis of hepatocyte damage (Goodarzi et al., 2020). When the activities of AST and ALT increased, liver injury was suggested. In our study, Cd content in the Cd group increased significantly, indicating that Cd in the liver has been enriched, and will have adverse effects on the liver. The increase of AST and ALT activities, and the change in histological structure further suggested that Cd caused liver damage and dysfunction in weaned piglets.

Since the physiological structure and function of pigs are highly similar to that of humans, it is of practical significance to select pigs as

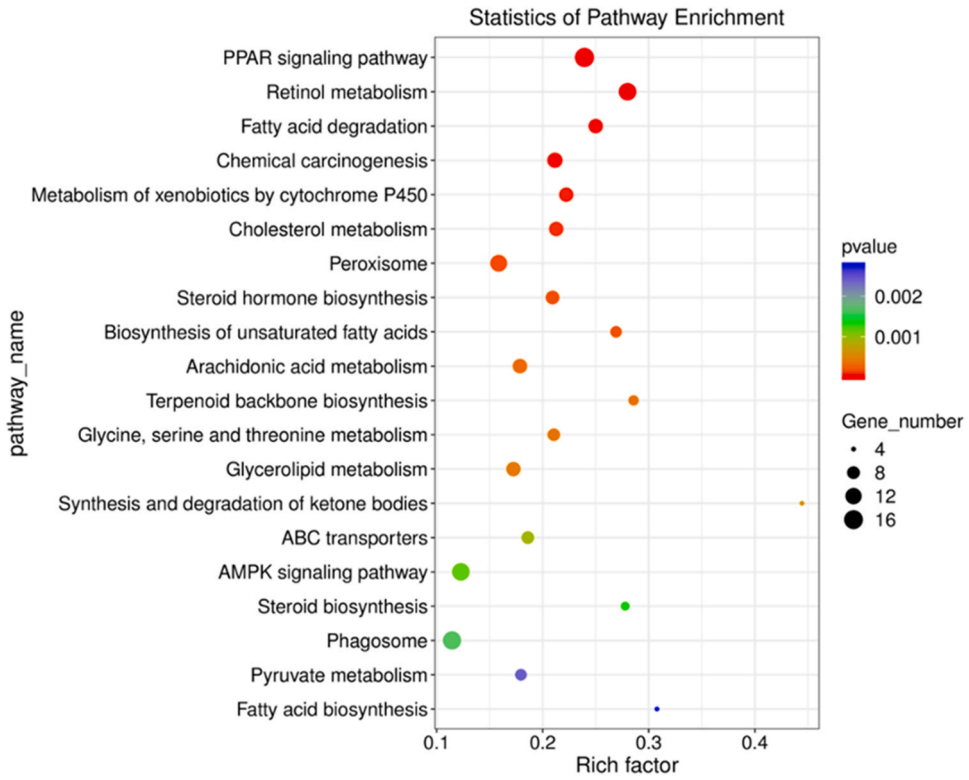

Fig. 3. KEGG pathway bubble diagram of DEG enrichment.

animal models to study the toxicity mechanism of Cd. Transcriptome is a high throughput sequencing technique developed in recent years, which plays an important role in the study of toxicity mechanism, transcriptome data analysis can provide important information and direction for the study of toxicity mechanism of environmental pollutants (Wang et al., 2019). In our study, 768 DEGs were found in the liver tissues of pigs in the Cd group, among which 369 DEGs were significantly down-regulated and 399 DEGs were significantly up-regulated. These DEGs were revealed by GO analysis. The major annotation categories in biological, molecular, and cellular processes were oxidation-reduction process, extracellular space, and protein binding, respectively.

CYP450 system are the most important toxicant metabolizing enzyme families (Baxter et al., 2015). They are mainly found in the liver and intestinal tract and catalyze the metabolism of various endogenous and exogenous substances (including poisons). CYP450 can pass the iron ion in heme in its structure, oxidize the foreign substance, enhance the water-solubility of the foreign substance, make them easier to expel from the body. Previous studies in other species have suggested that CYP450 may be involved in Cd metabolism in the body (Lewis et al., 2006; Nair et al., 2013). Recent studies in chicken and fish have shown that Cd can cause an increase in the CYP1A1 mRNA level (Guo et al., 2020; Jiaxin et al., 2020), which is consistent with our study. However, the increase of CYP2B22, CYP7A1 and CYP8B1 found in our study has not been reported. Our transcriptional data showed that DEGs contain the genes from the CYP450 family, which were enriched in the related GO entry. Subsequently, some genes of CYP450 family were identified by qRT-PCR, which indicated that CYP450 was involved in the Cd metabolism and its toxicity mechanism in the liver of weaned piglets.

The SLC family is mainly located in mammalian cell membrane and are responsible for the absorption and transport of several substances on the cell membrane, including amino acids, nucleotides, sugars, inorganic ions and drugs (Palmieri et al., 2020). Recent studies have found that SLC family play an important role in neurodegeneration, rheumatoid arthritis, bowel cancer, and drug metabolism, but the biological function of SLC family has not been widely understood, which has attracted the attention of researchers (Omura et al., 2020; Ayka and Şehirli, 2020; Zhang et al., 2020; Wang et al., 2020). Our transcriptome data suggested that the genes of the SLC family underwent significant changes during Cd-induced liver injury. To verify this result, we conducted a qRT-PCR test and found that the results of the selected DEGs (SLC26A8, SLC22A7, SLC27A2, and SLC11A1) were consistent with the results of the transcriptome data. It suggested that the SLC family were involved in Cd-induced liver injury. SLC26 isoforms are a conservative family of anion transporters, including SLC26A1, SLC26A2, SLC26A3, SLC26A4, SLC26A5, SLC26A6, SLC26A7, SLC26A8, SLC2 6A9, and SLC26A11, which constitute important regulators of pH and HCO concentration (Huang et al., 2019; Touré, 2019). The SLC25 family transports a variety of solutes such as ATP synthase reagents (ATP, ADP, and phosphates), tricarboxylic acid cycle intermediates, cofactors, amino acids, and carnitine esters of fatty acids. SLC25 genes are highly conserved and the introns are located at exactly superimposable positions in most SLC25 genes of zebrafish, chicken, mouse, and human (Panaro et al., 2020). Changes in SLC25 genes will affect the biochemical reactions and transport of organisms, leading to substrate accumulation, mitochondrial dysfunction and cell metabolism (Palmieri et al., 2020). There were few literatures about SLC11A family. Previous studies showed that SLC11A family were involved in immune response in vivo (Nairz et al., 2009; Cossu et al., 2013). It can be concluded that Cd exerts its toxic effect by altering the SLC family genes in the liver, which affects the material transport, biochemical reaction and immune reaction.

5. Conclusion

In conclusion, our results showed that Cd could cause liver injury in weanling pigs and change the transcriptomic characteristics of liver. Cd can cause changes in the CYP450 and SLC family gene networks in the liver of weanling pigs, indicating that CYP450 and SLC families play an indispensable role in Cd-mediated hepatotoxicity. Our study provides a new way to better reveal the mechanism of Cd toxicity.

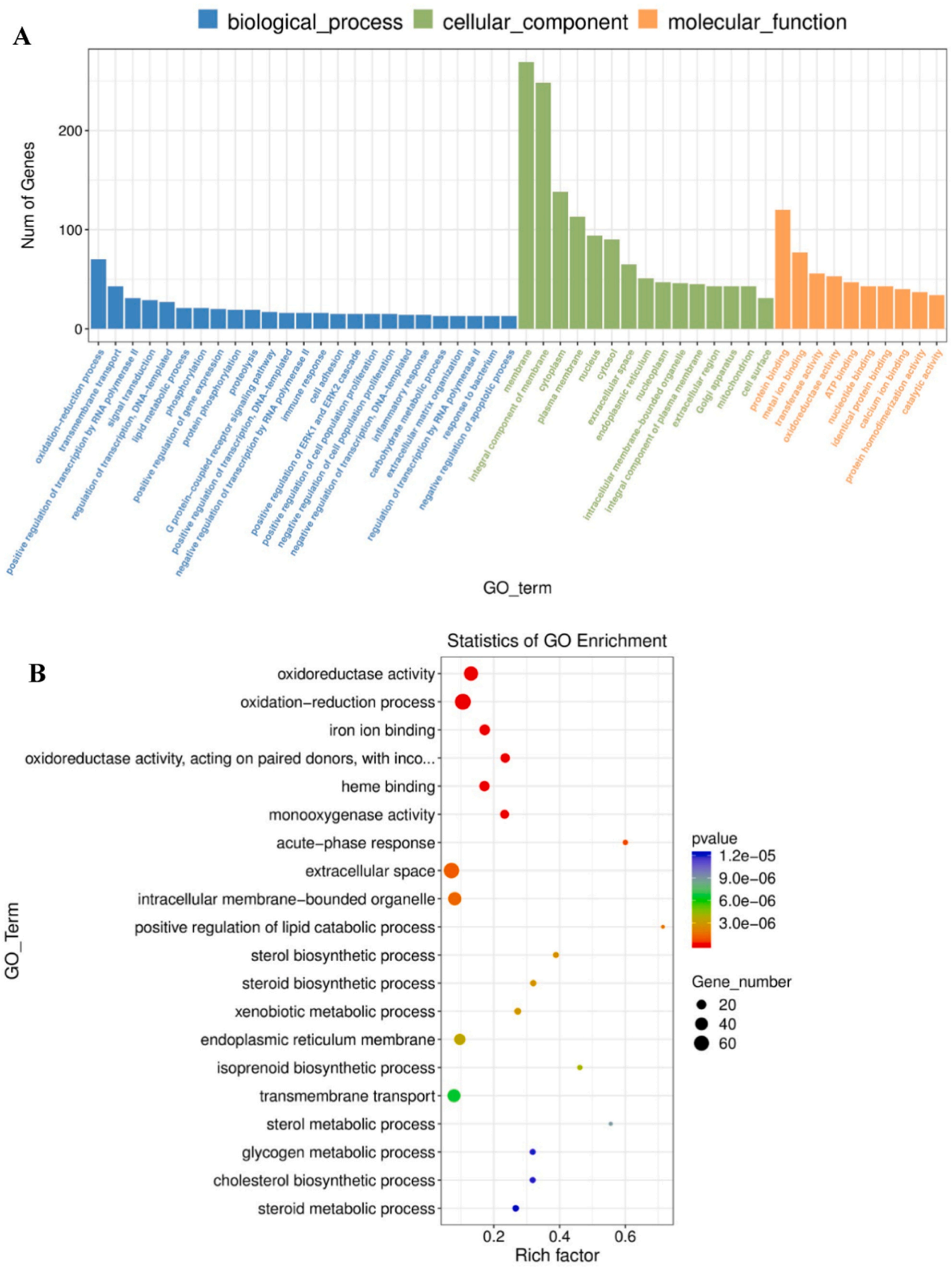

Fig. 4. Functional analysis of DEGs in GO. (A) Three ontologies of GO analysis (molecular function, cellular component and biological process); (B) Bubble maps of the first 20 significantly enriched GO terms.

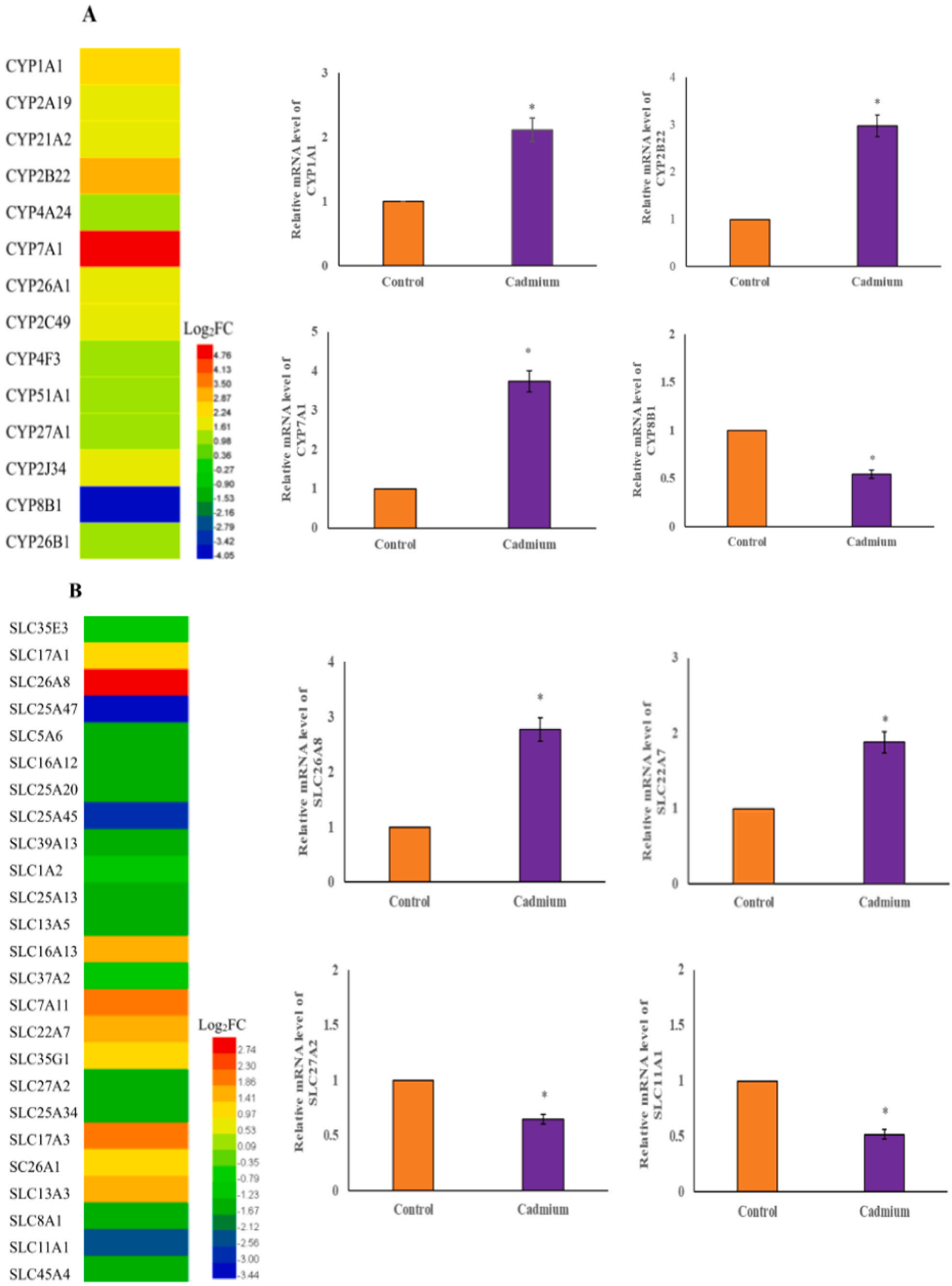

Fig. 5. Heat map of the selected DEGs and validation of qRT-PCR. (A) RNA-Seq results and qRT-PCR verification results of major CYP family genes; (B) RNA-Seq results and qRT-PCR verification results of major SLC family genes. Each value represents the mean ± SD of 6 individuals. An unpaired t-test was used to elucidate if there were significant differences between the control group and the Cd group. *Indicated that the difference between the Cd group and the control group was significant ($P < 0.05$).

CRediT authorship contribution Statement

Huan Wang: Data curation, Writing - original draft, Formal analysis. **Qi Han:** Investigation, Formal analysis, Writing - review & editing. **Yongjie Chen:** Visualization, Formal analysis, Writing - review & editing. **Guanghui Hu:** Writing - review & editing, Supervision, Validation, Project administration. **Houjuan Xing:** Writing - review & editing, Supervision, Validation, Project administration.

Declaration of Competing Interest

None of the authors have any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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