Research Article

Jiangzhi Ligan Decoction Inhibits GSDMD-Mediated Canonical/Noncanonical Pyroptosis Pathways and Alleviates High-Fat Diet-Induced Nonalcoholic Fatty Liver Disease

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 Increasing evidence suggests that gasdermin D (GSDMD) mediated pyroptosis signaling pathways play a vital role in the pathogenesis of nonalcoholic fatty liver disease (NAFLD). Jiangzhi Ligan Decoction (JZLGD) has been verified to prevent NAFLD, but its specific mechanism has not been determined. In this study, an NAFLD model was established in Sprague-Dawley rats by a high-fat diet (HFD). After 12 weeks, JZLGD was orally administered once a day for 6 additional weeks. We investigated the effects of JZLGD on NAFLD rats and determined the GSDMD pathway-associated proteins to explore whether such effects were associated with pyroptosis. Our data show that JZLGD significantly reduced the liver index; improved serum lipid levels, liver function parameters, and lipid droplet content; and relieved NAFLD. We further found that the serum levels of the proinflammatory factors interleukin-1β (IL-1β), IL-18, tumor necrosis factor-α (TNF-α), and IL-6 were obviously decreased in the JZLGD group. HFD rats treated with GSDMD exhibited NLRP3, caspase-1, lipopolysaccharide (LPS), and caspase-11 activation; however, these effects were blunted by JZLGD treatment. Taken together, JZLGD may exert hepatoprotective effects against NAFLD in a rat HFD model by regulating GSDMD-mediated canonical/noncanonical pyroptosis pathways.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a global health problem, and its incidence is rapidly increasing, especially in the United States, where its prevalence is 20–30% [1]. Its prevalence in Asia is 7–20% [2]. With the changes in people’s diet and lifestyle, the incidence of NAFLD has gradually increased among the younger population, causing a burden on public healthcare systems. In some patients, NAFLD will progress to hepatitis, liver fibrosis, and cirrhosis, increasing the risk of hepatocellular carcinoma. However, the pathological mechanism underlying NAFLD is complex, and there is no effective drug at present [3], so it is very important to identify therapeutic targets for NAFLD treatment.

The pathogenesis of NAFLD is related to lipid metabolism disorder, lipid accumulation, insulin resistance, and inflammation [4, 5]. In particular, hepatic inflammation is an essential pathophysiological process during NAFLD [6]. Additionally, a study showed that inflammatory activation results in hepatic insulin resistance and steatohepatitis [7]. The occurrence and development of inflammation will aggravate the deterioration of NAFLD and make NAFLD progress into cirrhosis. Pyroptosis is a form of proinflammatory cell death, and gasdermin D (GSDMD) is the executive molecule [8]. Cleavage of GSDMD by caspase-1 and caspase-4/5/11 leads to translocation of the N-terminal fragment to the plasma membrane, which then forms pores (10–15 nm in diameter) that facilitate pyroptotic cell death to release interleukin-1β (IL-1β), IL-18, tumor necrosis factor-α, and IL-6 were obviously decreased in the JZLGD group. HFD rats treated with GSDMD exhibited NLRP3, caspase-1, lipopolysaccharide (LPS), and caspase-11 activation; however, these effects were blunted by JZLGD treatment. Taken together, JZLGD may exert hepatoprotective effects against NAFLD in a rat HFD model by regulating GSDMD-mediated canonical/noncanonical pyroptosis pathways.
and bind lipopolysaccharide (LPS), in which case the com-

pound activates GSDMD to cause noncanonical pyroptosis

[13–15]. Recent studies have shown that pyroptosis mediates

the death of hepatocytes and aggravates the processes of inflam-
mation and fibrosis in the pathological development of NAFLD,

which has become an important potential target for NAFLD

treatment [16].

In traditional Chinese medicine (TCM) theory, the path-

ogenesis of NAFLD includes spleen vacuity, liver stagnation,

and phlegm-damp obstruction [17]. TCM is effective in

treating NAFLD, but there are few related studies. Jiangzhi

Ligan Decoction (JZLGD), a classical Chinese herbal for-

mula, is a widely used prescription in clinical practice. JZLGD

is composed of six kinds of Chinese medicines, including

Alisma orientale (Zexie), Salvia miltiorrhiza (Dan-

shen), Cassis Tora (Juemingzi), Curcumae Radix (Yujin),

Sargassum (Haizao), Lotus Leaves (Heye). Previous experi-

ments showed that JZLGD regulates lipid metabolism, exerts

hepatoprotective effects, and contributes to weight loss. It has

been demonstrated that JZLGD plays an important role in

NAFLD treatment, but the underlying mechanisms are not

clear. Pyroptosis is closely related to the inflammatory

response and plays an indispensable role in the develop-

ment of NAFLD. Inhibition of pyroptosis in NAFLD can protect

the liver from inflammation and delay the progression of liver
disease; hence, we hypothesized that the beneficial effects of JZLGD on the progression of NAFLD may be related to the canonical and noncanonical pyroptosis path-

ways. Therefore, an NAFLD rat model was established to

evaluate the effects of JZLGD on NAFLD and explore the

underlying molecular mechanisms.

2. Materials and Methods

2.1. Materials. Sodium pentobarbital (57-33-0) was pur-

chased from TCI Shanghai; lysis buffer for Western blot

and immunoprecipitation (IP) analysis (P0013) was pur-

chased from Beyotime Biotechnology; Protease inhibitor

cocktail (without EDTA, 100× stock solution in DMSO)

(B14002) and phosphatase inhibitor cocktail (100× stock

solution) (B15002) were purchased from Bimake. The rat

IL-1β ELISA Kit (GN-R30172), the rat IL-18 ELISA Kit

(GN-R30168), the rat TNF-α ELISA Kit (GN-R31092), and

and the rat IL-6 ELISA Kit (GN-R30201) were purchased from

Gaining Biological. The LPS detection kit (H178) was pur-

chased from NanJing JianCheng Bioengineering Institute.

2.3. JZLGD. JZLGD is composed of Alisma orientale (Zexie)

(10 g), Cassis Tora (Juemingzi) (30 g), Salvia miltiorrhiza

(Danshen) (10 g), Curcumae Radix (Yujin) (10 g), Sargassum

(Haizao) (30 g), and Lotus Leaves (Heye) (10 g). All compo-

nents were purchased from the First Hospital of Hunan Uni-

versity of Chinese Medicine, soaked in eight volumes of water

for 30 min, boiled for 1 h, and filtered. Six volumes of water

were added, and samples were boiled for 1 h and filtered. We

mixed the constituents and concentrated the mixture to

2 g/ml using a rotary evaporator at 65°C and stored it at

4°C. Before use, it was diluted to the required concentration

with normal saline.

2.4. Model Establishment. According to a previous study, the

rats received a high-fat diet (HFD) (40 kcal% fat, 20 kcal%
sucrose, and 2% cholesterol) for 12 weeks to develop NAFLD.

The HFD was provided by Research Diets, Inc. (batch num-

ber: D09100301).

2.5. Experimental Design. Sprague-Dawley rats were ran-

domly divided into five groups: the normal control group,

the NAFLD model group, and three JZLGD-treated NAFLD

groups (receiving 2.3, 4.6, and 9.2 g/kg of body weight,

respectively). Rats in the normal control group were fed a

normal diet (ND, 0.275 ppm cholesterol, 5.1% fat, 23.5% pro-

tein, and 50.3% carbohydrate), while the rats in the other
groups received an HFD during the experimentation period

(12 weeks + 6 weeks). After 12 weeks, rats in the JZLGD-
treated NAFLD groups were administered different doses of

JZLGD by oral gavage once daily for another period of 6

weeks; rats in the other two groups were given the same dos-

age of normal saline. Food intake was monitored daily, and

body weight was measured weekly. After the intervention,

the rats were euthanized by peritoneal injection of 1%
sodium pentobarbital (40 mg/kg body weight). The blood

and liver from each rat were rapidly removed for further

studies.

2.6. Liver Index. After 18 weeks, the body weight and liver

weight were measured, and the following formula was used
to determine the liver index: liver index = (liver weight/body

weight) × 100%.
Figure 1: Continued.
was drawn to calculate the concentration of LPS. The absorbance was measured at 450 nm. A standard curve

For histopathological analysis, we collected the same liver tissue segment from each rat. Tissue seg-

2.7. Histopathology. For histopathological analysis, we collected the same liver tissue segment from each rat. Tissue segments were washed with ice-cold saline once or twice and kept in 4% paraformaldehyde. Tissues were cut into slices with a thickness of 4 μm and stained with hematoxylin–eosin (H&E). Three practiced pathologists who were blinded to the study design performed all histopathological examinations. The ballooning degeneration score and the NAFLD activity score (NAS) were recorded. The scoring criteria were in accordance with the guidelines of the National Institutes of Health Clinical Research Network on nonalcoholic steatohepatitis.

2.8. Biochemical Analysis. The blood samples were centrifuged at 3000 rpm for 10 min to isolate the serum. We used a fall-automatic biochemical analyzer to evaluate the activity of liver enzymes in serum (alanine transaminase enzyme (ALT) and aspartate transaminase (AST)) and the serum levels of the triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), and free fatty acid (FFA).

2.9. Assessment of Serum IL-1β, IL-18, TNF-α, and IL-6 Levels. Blood was obtained from the abdominal aorta and centrifuged to obtain the serum. The serum levels of IL-1β, IL-18, tumor necrosis factor-α (TNF-α), and IL-6 were determined by ELISA according to the manufacturer’s instructions.

2.10. Serum LPS Levels. Blood was obtained from the abdominal vein, placed at room temperature for 30 min, and centrifuged to isolate the serum. Serum LPS levels were measured with a kit according to the manufacturer’s instructions. Firstly, samples were added to antibody-coated enzyme-labeled wells, and then, labeled antigens were added. The mixture was incubated at room temperature for 1 h. Afterwards, the plate was washed with PBST three times, avidin-HRP was added, and plates were incubated at 37°C for 1 h. The absorbance was measured at 450 nm. A standard curve was drawn to calculate the concentration of LPS.

2.11. Western Blot Analysis. We randomly selected five mice from each group, and 100 mg of the same segment of liver tissue was collected. Then, 1 ml RIPA buffer containing protease and phosphatase inhibitor cocktail was added. After homogenization on ice with a glass homogenizer for 30 min, the homogenate was centrifuged to obtain the hepatic tissue proteins. The protein concentration was determined by BCA assay. For each group, 40 μg of protein was taken for the following experiments. Protein samples were separated by SDS-PAGE and transferred to PVDF membranes. After blocking with 5% nonfat milk in TBST for 1 h, membranes were incubated at 4°C overnight with the following primary antibodies: anti-NLRP3 (1 : 1000), anti-apoptosis-associated speck-like protein containing a CARD (ASC) (1 : 1500), anti-caspase-1 (1 : 500), anti-caspase-11 (1 : 500), anti-GSDMD (1 : 1000), anti-GSDMD N-terminus (GSDMD-N) (1 : 1000), anti-IL-1β (1 : 1000), anti-IL-18 (1 : 1000), and anti-β-actin (1 : 5000). After washing the membranes with TBST, the membranes were incubated at room temperature with secondary antibodies for 1 h. The protein bands were visualized with Western Bright ECL. Finally, the Quantity One software was used for the quantitative analysis of the protein bands.

2.12. Statistical Analysis. The grouped data were analyzed with SPSS 22.0. Comparison of two groups was performed by two-tailed Student’s t-test. Comparisons among three or more groups were performed with one-way ANOVA. Results are expressed as the mean ± standard deviation (SD). The GraphPad Prism 6.0 software was used for statistical evaluations. P < 0.05 was considered to indicate a statistically significant difference.

3. Results

3.1. JZLGD Reduced Liver Index and Serum Lipid Levels. To evaluate the therapeutic effects of JZLGD on NAFLD in HFD-fed rats, we measured the body weight, liver weight, liver index, serum lipid levels, and liver function parameters. The body weight, liver weight, and liver index were
Figure 2: Effects of JZLGD on liver histology and hepatic inflammation-related parameters in HFD-fed NAFLD rats. (a) H&E staining (200x magnification) showed that inflammatory cell infiltration, balloon-like degeneration, and liver steatosis were obvious in the HFD group and inhibited in the JZLGD-treated NAFLD groups. Immediately afterwards, the NAFLD activity score (b) and (c) ballooning pathology score were recorded to evaluate the lesions better. (d–g) IL-1β, IL-18, TNF-α, and IL-6 serum levels were analyzed by ELISA. Data are expressed as the mean ± SD (n = 10); **P < 0.01 vs. control; *P < 0.05, **P < 0.01 vs. HFD group.
significantly higher in the NAFLD group than in the control group. After 6 weeks of JZLGD intervention (Figure 1), the body weight, liver weight, and liver index had improved significantly in the JZLGD-treated NAFLD groups, which proves that JZLGD blocks NAFLD development. Serum analysis results were in agreement with these observations. Serum TG, TC, FFA, and LDL levels were significantly higher in HFD-fed rats than in control rats. Compared to the HFD group, serum TG, TC, FFA, and LDL levels were greatly ameliorated in the JZLGD-treated NAFLD groups. High ALT and AST serum levels are associated with NAFLD progression; indeed, ALT and AST levels were higher in HFD rats than in control rats. Treatment with JZLGD reduced serum ALT and AST levels, indicating that JZLGD can protect rats from HFD-induced liver injury.

3.2. JZLGD Alleviated Pathological Morphology of Liver Tissue and Inflammation. To further confirm the effects of JZLGD on the liver histopathology of NAFLD rats, we performed H&E staining, and the NAS and ballooning score were recorded. As shown in Figure 2, in the normal group, the hepatic lobules were intact, and the hepatocyte cords were arranged in an orderly fashion, with the central vein as the center in a radial arrangement. Hepatocytes were polygonal in shape, with large and round nuclei located centrally in the hepatocytes. A few lymphocytic infiltrates and no fibroproliferation were observed in the hepatic sinusoids and in the pooled areas. In the HFD group, the liver cells were disorderly arranged and accompanied by ballooning degeneration, infiltration of inflammatory cells, and marked hepatic steatosis, and hepatocytes were swollen and round, with large lipid droplets. Various degrees of steatosis were observed in the JZLGD-treated NAFLD groups, but the ballooning degeneration was reduced compared with that in the HFD group. These distinguishing features implied that HFD feeding can induce NAFLD and hepatic steatosis. Upon JZLGD treatment, hepatic steatosis and lipid accumulation gradually decreased. JZLGD treatment also effectively reduced the ballooning score and the NAS in NAFLD rats, in a concentration-dependent manner. This indicates that JZLGD has a positive protective effect on the liver.

We also detected the related inflammatory factors to assess the degree of hepatic inflammation in rats fed with HFD. Higher IL-1β, IL-18, TNF-α, and IL-6 levels were observed in the serum of HFD rats relative to the normal group. In JZLGD-treated NAFLD rats, IL-1β, IL-18, TNF-α, and IL-6 levels were significantly decreased, illustrating that JZLGD inhibits hepatocyte inflammation and prevents the progression of NAFLD.

3.3. JZLGD Inhibited the Expression of GSDMD and GSDMD-N. The levels of the key protein GSDMD, which is the ultimate executor and the critical factor in pyroptosis, were measured by Western blot analysis. Moreover, the levels

Figure 3: Effects of JZLGD on the levels of the pyroptosis executive protein GSDMD and GSDMD-N. (a) Western blot results. (b, c) Densitometric analysis of the relative band intensities of GSDMD and GSDMD-N. β-Actin was used as the internal control. Data are expressed as the mean ± SD (n = 10); **P < 0.01 vs. control; #P < 0.05, ##P < 0.01 vs. HFD group.
of its active form, GSDMD-N, in liver tissue were also evaluated. As shown in Figure 3, GSDMD and GSDMD-N levels were significantly higher in livers in the NAFLD group compared with the control group, confirming our conjecture that the occurrence of NAFLD is closely related to pyroptosis. Moreover, upon JZLGD treatment, the levels of GSDMD and GSDMD-N in the liver were significantly lower in the JZLGD-treated NAFLD groups. This effect was concentration-dependent. Therefore, JZLGD can effectively prevent the process of pyroptosis and protect hepatocytes.

3.4. JZLGD Suppressed the Levels of NLRP3, ASC, Caspase-1, IL-1β, and IL-18.

To further explore the effects of JZLGD on the GSDMD-mediated canonical pyroptosis pathway, we measured the levels of NLRP3, caspase-1, and their adaptor protein ASC. As shown in Figure 4, the protein expression levels of NLRP3, caspase-1, and ASC in liver tissue were significantly higher in the HFD group compared with the normal group. Medium or high concentrations of JZLGD significantly decreased the NLRP3, caspase-1, and ASC protein levels. We also examined the levels of the

![Figure 4: JZLGD reduced the expression levels of inflammasome-related proteins and proinflammatory factors involved in canonical pyroptosis signaling in HFD rats. (a) Representative blot and (b–f) quantitative analyses of (b) NLRP3, (c) ASC, (d) caspase-1, (e) IL-1β, and (f) IL-18 protein levels. Data are expressed as the mean ± SD (n = 10); **P < 0.01 vs. control; #P < 0.05, ##P < 0.01 vs. HFD group.](image-url)
proinflammatory factors IL-1β and IL-18, which act downstream of the pyroptosis pathway. The IL-1β and IL-18 protein levels of HFD rats were higher than those of normal rats, and JZLGD successfully suppressed the expression of IL-1β and IL-18. These results are consistent with our previous study.

3.5. JZLGD Suppressed the Expression of Caspase-11 and LPS. We also measured the levels of caspase-11 and LPS, which are related to the GSDMD-mediated nonclassical NAFLD pathway. As shown in Figure 5, the protein expression of caspase-11 in the liver tissue and the serum LPS levels were both significantly higher in the HFD group compared to the normal group. This suggests that the GSDMD-mediated nonclassical pathway also plays an important role in the development of NAFLD. Upon JZLGD treatment, hepatic caspase-11 levels and serum LPS levels were significantly reduced.

4. Discussion

NAFLD is rapidly developing into the main cause of liver cancer and liver cirrhosis in the world, yet there is no approved treatment drug [18]. Contrarily, JZLGD is a multi-ingredient pharmacon that affects multiple pathways, and its therapeutic effects for NAFLD treatment have been well documented [19, 20]. In the present study, the mechanisms underlying the effects of JZLGD on NAFLD were explored using an HFD-induced NAFLD rat model. HFD rats exhibited increased body weight, liver weight, and liver index; upon JZLGD treatment, these indicators were effectively improved. Besides, JZLGD regulated blood lipid levels. ALT and AST activities also tended to be lower in JZLGD-treated rats, which suggested that JZLGD contributed to the prevention and alleviation of NAFLD in HFD-fed rats. Subsequently, histological examinations showed that JZLGD markedly mitigated liver lipid droplets. Furthermore, the NAS and the ballooning score were decreased in JZLGD-treated HFD rats, suggesting that JZLGD can slow down lipid accumulation. All these findings illustrate that JZLGD indeed exerts beneficial therapeutic effects on NAFLD.

HFD markedly increased the expression of hepatic inflammatory factors such as IL-1β, IL-18, TNF-α, and IL-6. Treatment with JZLGD obviously decreased these levels compared to the NAFLD model group, which suggests JZLGD attenuates hepatocellular inflammation. As the core of the pathogenesis of NAFLD, the inflammatory response plays an important role in the occurrence and development of NAFLD [21, 22]. Therefore, we speculate that the protective effect of JZLGD against NAFLD was probably mediated by the anti-inflammatory response. Recent studies have shown that pyroptosis can be regarded as a potential therapeutic target, as an inflammation-related and controllable cell death method [23] accelerates the process of hepatic
inflammation and fibrosis [24, 25]. Notably, pyroptosis is a key pathogenetic factor in the development of NAFLD [23, 26], and suppression of inflammasome-dependent GSDMD-mediated cell pyroptosis could attenuate hepatic injury in liver diseases [27]. Therefore, we focused on the influence of JZLGD on pyroptosis in this study. We first detected the major executor protein GSDMD, which is a generic substrate of inflammatory caspases. Its cleaved form GSDMD-N acts as a pyroptosis executor, which triggers pyroptosis and aggravates the release of proinflammatory factors like IL-1β and IL-18 through the GSDMD pores [24]. Furthermore, inhibition of the activation of GSDMD blocks the progression of NAFLD. Hence, GSDMD is considered as a potential target for the treatment of NAFLD [9, 28]. Here, our experimental data show that the protein expression of GSDMD and its activated form GSDMD-N were significantly increased in HFD-induced NAFLD rats, which was consistent with previous studies [9] and further verified that GSDMD plays an important role in the occurrence and development of NAFLD. However, the fact that JZLGD treatment reversed the HFD-induced increase in the GSDMD and GSDMD-N expression indicated that JZLGD could regulate the activation of GSDMD, so as to achieve the purpose of treating NAFLD.

As shown in Figure 6, GSDMD protein was cleaved to generate GSDMD-N, depending on the proinflammatory caspase-1 and caspase-4/5/11 via the canonical and noncanonical inflammasome signaling pathways [29]. Caspase-1 is the initiating factor of the canonical pyroptosis pathway, and its activation requires NLRP3 [30]. Furthermore, caspase-1 directly cleaves pro-IL-1β and pro-IL-18 to produce mature cytokines and controls their secretion to induce pyroptosis [31]. The expression levels of NLRP3, caspase-1, IL-1β, and IL-18 are key indicators for evaluating the activation of the NLRP3 inflammasome [32, 33]. Our results show that NLRP3, ASC, caspase-1, LPS, caspase-11, GSDMD, GSDMD-N, IL-1β, and IL-18 levels were elevated in HFD-induced NAFLD rats, which suggests that the NLRP3 inflammasome is also activated in NAFLD. JZLGD treatment decreased NLRP3, ASC, caspase-1, LPS, caspase-11, GSDMD, GSDMD-N, IL-1β, and IL-18.

Besides, in the noncanonical pyroptosis signaling pathway, GSDMD is cleaved by caspase-11, and caspase-11 is directly activated by LPS [34, 35]. Our results show that the levels of cleaved caspase-11 in liver tissue of HFD-induced NAFLD rats were significantly increased, while in the JZLGD
treatment groups, we observed a significance decrease in caspase-11 levels, implying that JZLGD may regulate the activation of GSDMD through inhibiting the expression of caspase-11. It has previously been shown that disorder of the intestinal flora and damage of the intestinal mucosa can lead to the dissolution of Gram-negative bacilli in the intestinal tract, resulting in the release of LPS into the portal vein, which aggravates liver inflammation and accelerates NAFLD progression [36, 37]. Nicole et al. showed that HFD may induce changes or even disorders in the structure of intestinal flora, and Moreira et al.’s research showed that HFD can cause an imbalance in intestinal flora and lead to an increase in serum LPS levels [38, 39]. Moreover, some studies have revealed that JZLGD can regulate the intestinal flora and intervene with the progression of NAFLD [40]. Here, our data show that the LPS content in the hepatic portal vein of NAFLD rats was significantly increased, and JZLGD treatment could significantly reduce it. Based on the above results, we speculate that JZLGD may regulate serum LPS levels in NAFLD rats to prevent the activation of caspase-11 and GSDMD, thereby reducing the release of proinflammatory factors and attenuating the process of NAFLD. These findings illustrate that JZLGD treatment prevented steatosis, inflammation, and pyroptosis in HFD rats, which may be related to the LPS/caspase-11/GSDMD-mediated pyroptosis pathway.

5. Conclusions
In conclusion, our findings demonstrate that the canonical and noncanonical pyroptosis pathways are activated in HFD-induced NAFLD rats. JZLGD inhibited the NLRP3/caspase-11/GSDMD-mediated canonical pyroptosis pathway and the LPS/caspase-11/GSDMD-mediated noncanonical pyroptosis pathway, exerting beneficial effects in HFD-fed NAFLD rats. In future studies, the specific mechanism by which JZLGD regulates pyroptosis needs to be elucidated. For example, does JZLGD reduce serum LPS levels by changing intestinal permeability? Further investigations of the specific mechanisms of action of GSDMD might lead to the development of effective drugs to treat NAFLD.

Data Availability
The data generated during this study are included in this article and results as shown in figures.

Disclosure
This manuscript was previously published online as a preprint at Research Square (doi: 10.21203/rs.3.rs-250732/v1).

Conflicts of Interest
The authors declare that there are no conflicts of interest.

Authors’ Contributions
Biao Tang conceived the study and designed the experiments. Kangkang Yin, Xiao Zhou, Wei Jiang, and Ziwei Dai performed the experiments. Kangkang Yin, Xiao Zhou, Linlin Wang, and Wei Jiang analyzed the data and drafted the manuscript. Biao Tang, Linlin Wang, and Xiao Zhou revised and revised the manuscript. All authors contributed to the article and approved the submitted version. Kangkang Yin, Xiao Zhou, Wei Jiang, and Linlin Wang contributed equally to this paper.

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