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Differentially expressed genes of esophageal tissue in male acute and chronic sleep deprivation mice

Jing Li[1,2] | Yifan Lu[3] | Dingding Yang[3] | Mudan Ren[1,2] | Yan Yin[1,2] | Yan Zhao[1,2] | Shuixiang He[1,2]

[1]Department of Gastroenterology, First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China

[2]Shannxi Clinical Research Center of Digestive Disease (Cancer Devision), Xi'an, China

[3]Department of Neurobiology, School of Basic Medicine, Fourth Military Medical University, Xi'an, China

Correspondence
Yan Zhao and Shuixiang He, Department of Gastroenterology, The First Affiliated Hospital of Xi'an Jiaotong University, 277 West Yanta Road, Xi'an 710061, China.
Email: yanzhao211@163.com; dyyyjxk@mail.xjtu.edu.cn

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Abstract

Gastroesophageal reflux disease (GERD) is associated with sleep disturbances. However, mechanisms underlying these interactions remain unclear. Male acute and chronic sleep deprivation (SD) mice were used for this study. Mice in the chronic SD group exhibited anxiety- and depression-like behaviors. We further performed high-throughput genome sequencing and bioinformatics analysis to screen for featured differentially expressed genes (DEGs) in the esophageal tissue. The acute SD group, comprised 25 DEGs including 14 downregulated and 11 upregulated genes. Compared with the acute SD group, more DEGs were present in the chronic SD group, with a total of 169 DEGs, including 88 downregulated and 81 upregulated genes. Some DEGs that were closely related to GERD and associated esophageal diseases were significantly different in the chronic SD group. Quantitative real-time polymerase chain reaction verified the downregulation of Krt4, Krt13, Krt15 and Calml3 and upregulation of Baxl1 and Per3. Notably, these DEGs are involved in biological processes, which might be the pathways of the neuroregulatory mechanisms of DEGs expression.

KEYWORDS
differentially expressed genes, gastroesophageal reflux disease, network analysis, neuroregulatory, sleep deprivation

1 | INTRODUCTION

Gastroesophageal reflux disease (GERD) is a chronic disease characterized by typical symptoms, including recurrent and troublesome heartburn and acid regurgitation.[1] In addition, some atypical symptoms seriously plague patients, including difficulty swallowing, throat discomfort, chest pain, dyspepsia, postprandial fullness, early satiation and epigastric pain.[2] Gastroesophageal reflux is strongly associated with the development of multiple esophageal diseases, including Barrett's esophagus (BE) and esophageal carcinoma (ESCA). Recently, the

Abbreviations: ASD, acute sleep deprivation; BE, Barrett's esophagus; BP, biological processes; CC, cell component; CSD, chronic sleep deprivation; DEGs, differentially expressed genes; EAC, esophageal adenocarcinoma; EPM, elevated plus maze experiment; ESCA, esophageal carcinoma; FDR, false discovery rate; GEO, gene expression omnibus; GEPIA, gene expression profiling interactive analysis; GERD, gastroesophageal reflux disease; GO, gene ontology; HE, hematoxylin–eosin; KEGG, Kyoto encyclopedia of genes and genomes; logFC, $\log_2$-fold change; MF, molecular function; OFT, open field test; PD, Parkinson disease; PPI, proton pump inhibitor; qRT-PCR, quantitative real-time polymerase chain reaction; SD, sleep deprivation; SPT, sucrose preference test; TST, tail suspension test.

Jing Li and Yifan Lu contributed equally to this study.

population-based prevalence of GERD has increased in Asian countries (15%) and China (17.8%).[3,4] Although proton pump inhibitor (PPI) therapy based on acid suppression is recognized as the first-line therapy for GERD, its efficacy rate is still less than 50%.[5] Drug tolerance and an increased susceptibility to enteric infections are the major limitations of PPI therapy.[6,7]

Circadian rhythms affect the basic functions of various organ systems in the human body and overall quality of life. Sleep disturbances are commonly reported in patients presenting to gastroenterology clinic.[8] Sleep disturbances are prevalent in patients with gastrointestinal disorders including GERD, irritable bowel syndrome, and inflammatory bowel disease.[9] Notably, majority of patients (75%) with GERD have reported that nighttime heartburn affects sleep quality.[10] Moreover, some patients experience atypical symptoms such as dry cough, choking and chest pain, which also affect sleep quality of patients with GERD.[11] Resolving sleep disturbances and circadian misalignment can help relieve gastrointestinal symptoms and improve well-being.[12,13] However, sleep disturbances and circadian misalignments are frequently overlooked during visits and treatments.

GERD adversely affects sleep quality through nocturnal heartburn in patients awakening from sleep.[14] Alternatively, gastroesophageal reflux events may cause short, amnestic arousal, leading to sleep fragmentation and deprivation.[15] In turn, sleep disturbances aggravate heartburn and acid regurgitation at night by enhancing the perception of intra-esophageal stimuli (esophageal hypersensitivity) and increasing the risk of acid exposure.[16,17] The bidirectional vicious cycle between GERD and sleep disturbances worsens quality of life, suggesting that, further research may develop novel strategies for GERD treatment to break this cycle.

Currently, few studies have investigated the host genetic factors involved in the development of GERD and its associated esophageal diseases and sleep disturbances. In this study, we established a connection between sleep disturbances and mental disorders verified by behavioral tests using the male sleep deprivation (SD) mouse model, and performed histological analysis. We then performed high-throughput genome sequencing and analyzed the differentially expressed genes (DEGs) through bioinformatics analysis to screen the featured genes that play a key role in the association between GERD and sleep disturbances, further providing novel targets for developing effective therapies.

2 | MATERIALS AND METHODS

2.1 | Animals

Male C57BL/6 mice (8–10 weeks old) of different litters obtained from the Fourth Military Medical University were used in all experiments. All experimental animals were maintained at a constant temperature of 22–26°C under a 12 h light–dark cycle fed with free standard food and water. All experimental procedures abided by the ARRIVE guidelines and the National Research Council's Guide for the Care and Use of Laboratory Animals with the approval by the Institutional Animal Care and Use Committee at the Fourth Military Medical University.

Mice ($n = 6$ per group) were randomly divided into an acute sleep deprivation (ASD) group, a chronic sleep deprivation (CSD) group and the corresponding control group. Experimental mice were moved to a separate water-filled holding area (23 ± 2°C) up to 1.5 cm high to induce SD. The water tank had nine narrow platforms on which the mice were placed. Mice could not sleep or maintain a resting position to avoid falling into water. As previous studies, mice were kept awake continuously for a one-time 6 h in the ASD model, whereas repeated SD period (6 h/day for 14 consecutive days) in the CSD model.[18] After the 6 h SD period, the mice were returned to the holding facility. During the SD period, control mice were left untreated. Once the SD intervention was terminated in each group, behavioral tests were immediately performed on the mice and their corresponding controls.

2.2 | Behavioral tests

Male mice were acclimated by placing them into the experimental cage for 30 min before the start of the experiment. All the behavioral tests were carried out by a same researcher who was blinded to the groups to avoid expectation bias.

2.2.1 | Open field test (OFT)

The specific steps were mainly referring to the previous studies.[18,19] The mouse was placed in an enclosed white plastic box with a length, width and height of 40 cm × 40 cm × 40 cm under the full-light of 1000 lx. The mouse was placed in the center of the plastic box while being filmed and recorded lasted for 5 min. Later, clean the inner wall and bottom of the box, replace the animal and repeat the test. The time spent in the center area was analyzed by the automated analysis system (SMART 3.0, Panlab S.L.U.), evaluating the level of anxiety.

2.2.2 | Elevated plus maze experiment (EPM)

The specific steps were mainly referring to the previous studies.[18,19] The experimental system consisted of four arms with the length and width of 50 cm and 5 cm, of which the two closed arms had 15-cm-high walls whereas the two open arms without walls. The maze was placed on a platform 1 m from the ground. The mouse was placed in the central area of the maze facing one of the open arms while being tracked and recorded lasted for 5 min. The time spent in the two open arms was analyzed by the automated analysis system (SMART 3.0, Panlab S.L.U.), reflecting the level of anxiety.

2.2.3 | Tail suspension test (TST)

The experimental procedures were as described in the previous studies.[18,20] The mouse taped at one-third of the way from the tip of the tail was suspended from a platform 50 cm above the ground lasted for 6 min.

The camera background contrasted with the color of mouse fur. The stationary time during the last 4 min reflected the level of depression.

2.2.4 | Sucrose preference test (SPT)

The experimental procedures were as described in the previous studies.[18,21] All mice were undergoing the adaptation period of sugar water and the deprivation period, respectively. After deprivation of food and water lasted for 15 h, the mouse was randomly transferred into chambers of the apparatus with a cup of sucrose water and a cup of water lasted for 10 h. The electronic SPT device and the software MDA were applied to complete the test. The sucrose preference (the ratio of sucrose water intake to the sum of sucrose water and water intake) was used to evaluate the level of depression.

2.3 | Hematoxylin–eosin (HE) staining

Mice were anesthetized with 1.5% isoflurane in oxygen (1 L/min), and esophageal tissues were separated. Histological changes of the esophagus were identified in HE staining. Esophageal tissues were fixed in 10% neutral buffered formalin. Then, tissues were decalcified in 8% formic acid and paraffin embedding. Staining 4 mm sections were produced through hematoxylin and eosin.

2.4 | Analysis of DEGs profile in esophagus of SD model

High-throughput genome sequencing of the esophagus was performed after SD, and bioinformatics analysis was subsequently used to analyze the obtained gene data to extract information relevant to involved pathways based on Gene Expression Omnibus (GEO) datasets. R software was used to identify and analyze DEGs. To account for the false-positive results, the false discovery rate (FDR) and adjusted *P* values using RMA method were examined. A $\log_2$-fold change (logFC) value higher than 1.5 or lower than −1.5 was respectively considered as significantly upregulation or downregulation. Volcanic plot was used to show the differences in gene expression. Gene ontology (GO) analysis is a popular technique for analyzing the function of genes, mainly including biological processes (BP), cell component (CC), and molecular function (MF) categories. Metascape was used to perform further enrichment analysis of system-level datasets.[22] Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis was applied to analyze functional information functions of DEGs. Gene expression profiling interactive analysis (GEPIA) was used to analyze the expression of DEGs in human.[23]

2.5 | Analysis of mRNA expression by quantitative real-time polymerase chain reaction (qRT-PCR)

RNA from the esophagus was extracted by the phenol/chloroform extraction method. Reversed transcription to cDNA was performed using the High-Capacity cDNA Reverse Transcription Kit. qRT-PCR (Bio-Rad, Hercules, CA, USA) was performed based on the following 25 mL reaction mixtures: 1 μL of PCR forward primer and 1 μL of PCR reverse primer (Sangon, Shanghai, China), 2 μL of template DNA, 12.5 μL of SYBR Premix Ex Taq II (Tli RNaseH Plus) (2×) and 8.5 μL of sterilized water. The PCR profile was 30 s at 95°C, 40 cycles of 5 s at 95°C, and 30 s at 60°C. The forward and reverse PCR primers were as Table S1.

2.6 | Statistical analysis

Quantitative data were presented as means and standard deviation. The unpaired *t*-test and two-sided *t*-test were used to evaluate the statistical significance. Statistical significance was set to the final 2-sided $p < 0.05$. The statistical data were analyzed using the SPSS 26 software (IBM, Armonk, NY, USA) and GraphPad Prism 8 (San Diego, CA, USA).

3 | RESULTS

3.1 | SD model induces anxiety- and depression-like behaviors

In the ASD model, no significant difference was observed in the time remaining immobile in the TST (Figure S1).

In the CSD group, anxiety- and depression-like behaviors were observed. Anxiety-like behavior was evaluated by the OFT and EPM. The time spent in the center area of the plastic box in the OFT (Figure 1A) and in the two open arms in the EPM (Figure 1B) in CSD mice was significantly less than that in the corresponding control group (both $p < 0.01$), indicating that CSD mice exhibited anxiety-like behaviors.

Depression-like behavior was evaluated using TST and SPT. More time was spent immobile in the TST (Figure 1C) and sucrose was consumed less in the SPT (Figure 1D) in CSD mice than that in the corresponding control group (both $p < 0.01$), indicating that CSD mice exhibited distinct depression-like behavior.

3.2 | Pathology of esophagus

The microscopic histologic performance of the esophageal mucosa stained with HE and imaged at 200× magnification in the ASD and CSD groups and the corresponding control groups is shown in Figure S2. No significant pathological changes were observed in the esophageal mucosa between the SD and control groups, including the ASD and CSD groups.

3.3 | Analysis of DEGs profile in esophageal tissue of SD model

A total of 25 genes, including 14 downregulated and 11 upregulated genes, were present in the ASD group (all $p < 0.05$). However, a total

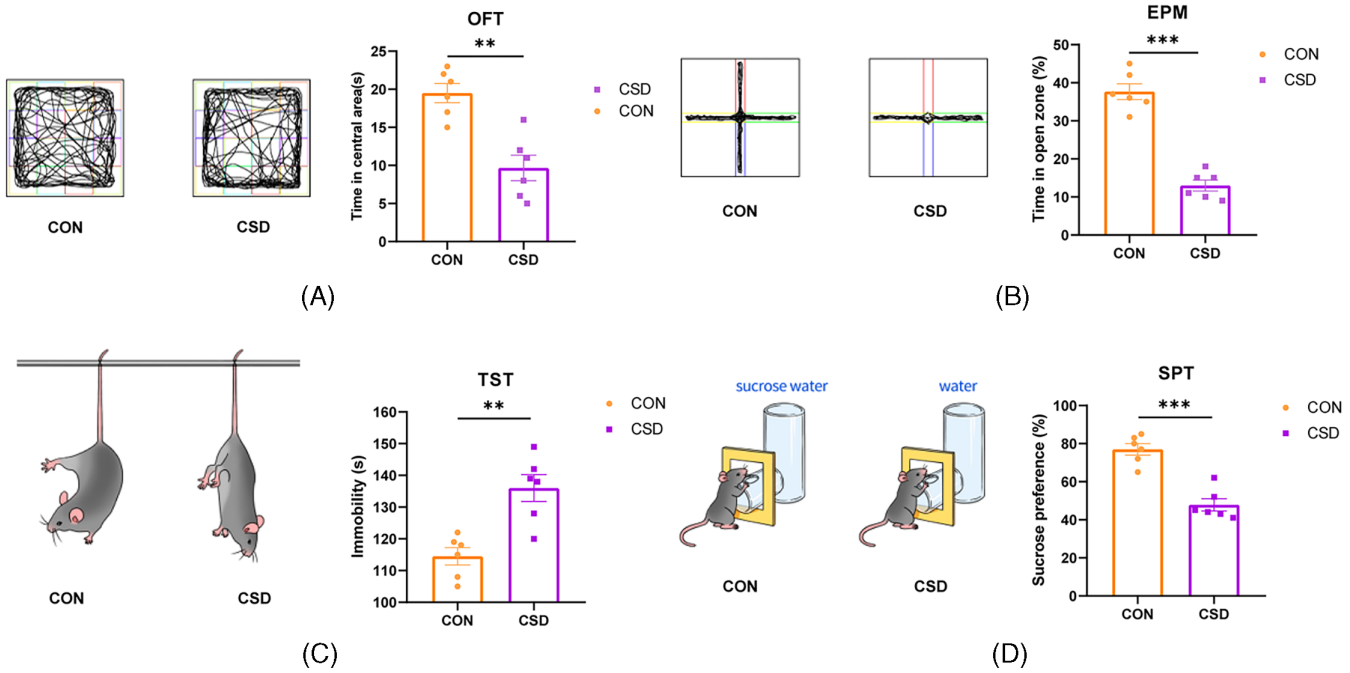

FIGURE 1 Behavioral tests in CSD mice. (A) Compared with the corresponding control group, the time spent in the center area of the plastic box in the OFT was significantly less. (B) Compared with the corresponding control group, the time spent in the two open arms in the EPM was significantly shortened. (C) Compared with the corresponding control group, the time remaining immobile in TST was significantly increased. (D) Compared with the corresponding control group, the consumption of sucrose in SPT was significantly decreased (mean ± SEM for $n = 6$ mice per group, Student's *t*-test. $^{**}p < 0.01$, $^{***}p < 0.001$). ASD, acute sleep deprivation; CSD, chronic sleep deprivation; EPM, elevated plus maze experiment; OFT, open field test; SPT, sucrose preference test; TST, tail suspension test.

TABLE 1 The top 20 upregulated and downregulated differentially expressed genes in male acute sleep deprivation mice.

Gene symbol	Gene location	Fold change	Log_2Fold change	*p* value	Adjusted *p* value	Regulation
Krt17	chr11:100256217–100261029:–	0.214510865	−2.220877375	6.08E-08	0.000184657	Down
Krt6a	chr15:101689932–101694307:–	0.220990877	−2.177941282	9.18E-06	0.007340985	Down
Krt16	chr11:100246091–100248902:–	0.266767848	−1.906343295	4.21E-06	0.005285023	Down
Cnfn	chr7:25367620–25369724:–	0.280767544	−1.832551922	1.17E-07	0.000254913	Down
Sprr3	chr3:92456502–92458720:–	0.323627892	−1.627592141	8.01E-06	0.006762361	Down
Krt6b	chr15:101676034–101680289:–	0.33260508	−1.588117892	5.95E-06	0.005826871	Down
Tgm1	chr14:55700009–55713492:–	0.346096555	−1.530753513	7.85E-05	0.0458797	Down
Chgb	chr2:132781278–132,795,079:+	0.371369254	−1.429073718	5.13E-07	0.000975228	Down
Fosl1	chr19:5447703–5455945:+	0.433918083	−1.204505386	4.26E-06	0.005285023	Down
Hdc	chr2:126593667–126619299:–	0.469521551	−1.090736717	1.09E-07	0.000254913	Down
Noxa1	chr2:25085667–25095149:–	2.783614778	1.476959572	1.77E-06	0.002991561	Up
Soat2	chr15:102150526–102163474:+	2.924784431	1.548330296	2.38E-05	0.017236824	Up
Sprr2a3	chr3:92285417–92291405:+	3.151640211	1.656102847	1.83E-08	9.27E-05	Up
Entpd8	chr2:25080304–25085716:+	3.690917313	1.883979417	1.66E-09	1.26E-05	Up
Apol7a	chr15:77388221–77399110:–	3.70065167	1.887779346	7.38E-05	0.04581013	Up
Slc9a3	chr13:74121457–74169442:+	3.80800692	1.9290361	3.42E-06	0.005191017	Up
Pla2g4c	chr7:13324655–13360672:+	4.624368866	2.20925648	7.73E-06	0.006762361	Up
Chil4	chr3:106201490–106219507:–	4.741652173	2.245389837	6.70E-05	0.044245768	Up
Clca1	chr3:145003817–145032776:–	5.040979412	2.333704062	1.31E-09	1.26E-05	Up
Atp6v0d2	chr4:19876841–19922605:–	5.908504625	2.562793047	3.31E-08	0.000125692	Up

TABLE 2 The top 20 upregulated and downregulated differentially expressed genes in male chronic sleep deprivation mice.

Gene symbol	Gene location	Fold change	Log_2Fold change	*p* value	Adjusted *p* value	Regulation
Chil4	chr3:106201490–106219507:−	0.058528843	−4.094708424	0.001665554	0.049249318	Down
Lor	chr3:92080271–92083142:−	0.078839598	−3.664935764	0.000131275	0.011064058	Down
Krt4	chr15:101918536–101924735:−	0.08504661	−3.555602454	0.000101347	0.009703799	Down
Igha	chr12:113254830–113260236:−	0.121456688	−3.041486158	3.79E-19	5.33E-15	Down
Mcpt1	chr14:56017964–56020391:+	0.153285091	−2.705710712	0.00023519	0.01638761	Down
Jchain	chr5:88519809–88527891:−	0.165027719	−2.599219729	2.57E-09	3.29E-06	Down
Krt13	chr11:100117327–100,121,566:−	0.189570671	−2.399192318	0.001165234	0.040915256	Down
Mcpt2	chr14:56042123–56044634:+	0.205489248	−2.282865186	0.001537876	0.047341599	Down
Krt78	chr15:101946004–101954287:−	0.250741757	−1.99572582	4.49E-09	5.27E-06	Down
Thbs4	chr13:92751590–92794818:−	0.286688607	−1.802443523	1.70E-07	9.55E-05	Down
Tnnt2	chr1:135836354–135852260:+	2.223041957	1.152535177	3.86E-07	0.000175355	Up
Nrbp2	chr15:76085595–76090013:−	2.259322178	1.175890013	1.48E-10	2.97E-07	Up
mt-Co3	chrMT:8607–9390:+	2.292498496	1.196920787	5.86E-05	0.007191412	Up
Meg3	chr12:109541001–109571726:+	2.296080364	1.199173138	1.19E-08	1.28E-05	Up
Rgs11	chr17:26202951–26211324:+	2.435800277	1.284395844	1.63E-09	2.33E-06	Up
mt-Nd3	chrMT:9459–9806:+	2.458409579	1.297725293	3.67E-06	0.001014015	Up
Ttc6	chr12:57564113–57737928:+	2.700414545	1.433180894	2.72E-08	2.25E-05	Up
Ifi27l2a	chr12:103442167–103443680:−	2.92412239	1.548003697	1.69E-07	9.55E-05	Up
Fes	chr7:80377756–80387946:−	3.753266616	1.908146777	1.25E-11	3.51E-08	Up
Ghrl	chr6:113716119–113719880:−	3.791754258	1.922865467	1.66E-09	2.33E-06	Up

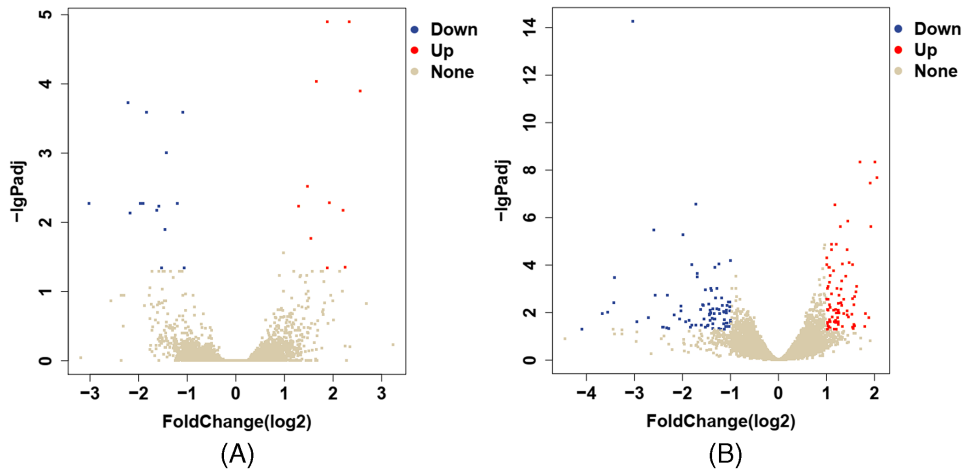

FIGURE 2 A volcano plot of the whole DEGs in the ASD group (A) and CSD group (B) ($n = 3$ mice per group). ASD, acute sleep deprivation; CSD, chronic sleep deprivation; DEGs, differentially expressed genes.

of 169 genes including 88 downregulated and 81 upregulated genes had difference in the CSD group (all $p < 0.05$), of which, 36 genes were significantly downregulated (logFC < −1.5) and 19 genes, significantly upregulated (logFC > 1.5) (Table S2). The top 20 upregulated and downregulated DEGs in the ASD and CSD groups are shown in Tables 1 and 2, respectively. A volcano plot shows the whole DEGs in the ASD and CSD groups in Figure 2.

The obtained DEGs and protein data were analyzed to extract information relevant to the involved pathways. For ASD mice, 21 GO terms were enriched, including 15 terms of BP, five terms of CC, and one term of MF (Figure 3A,B and Table S3). For CSD mice, an overview of the GO enrichment analysis and a network of enriched terms are shown in Figure 3C,D. In total, 103 GO terms were significantly enriched in Table S4. A total of 88 GO terms were mainly related to BP, such as intermediate filament cytoskeleton organization, positive regulation of protein secretion, and glucose homeostasis; nine GO terms were related to CC, such as intermediate filament, cornified envelope and perikaryon; and six GO terms were connected with MF, such as structural constituent of skin epidermis, immunoglobulin receptor binding, and structural molecule activity. Majority of significantly enriched terms were classified as BP in both ASD and CSD mice.

Considering that the SD mice had various DEGs, KEGG pathway analysis of the ASD and CSD groups was performed (Table S5). No

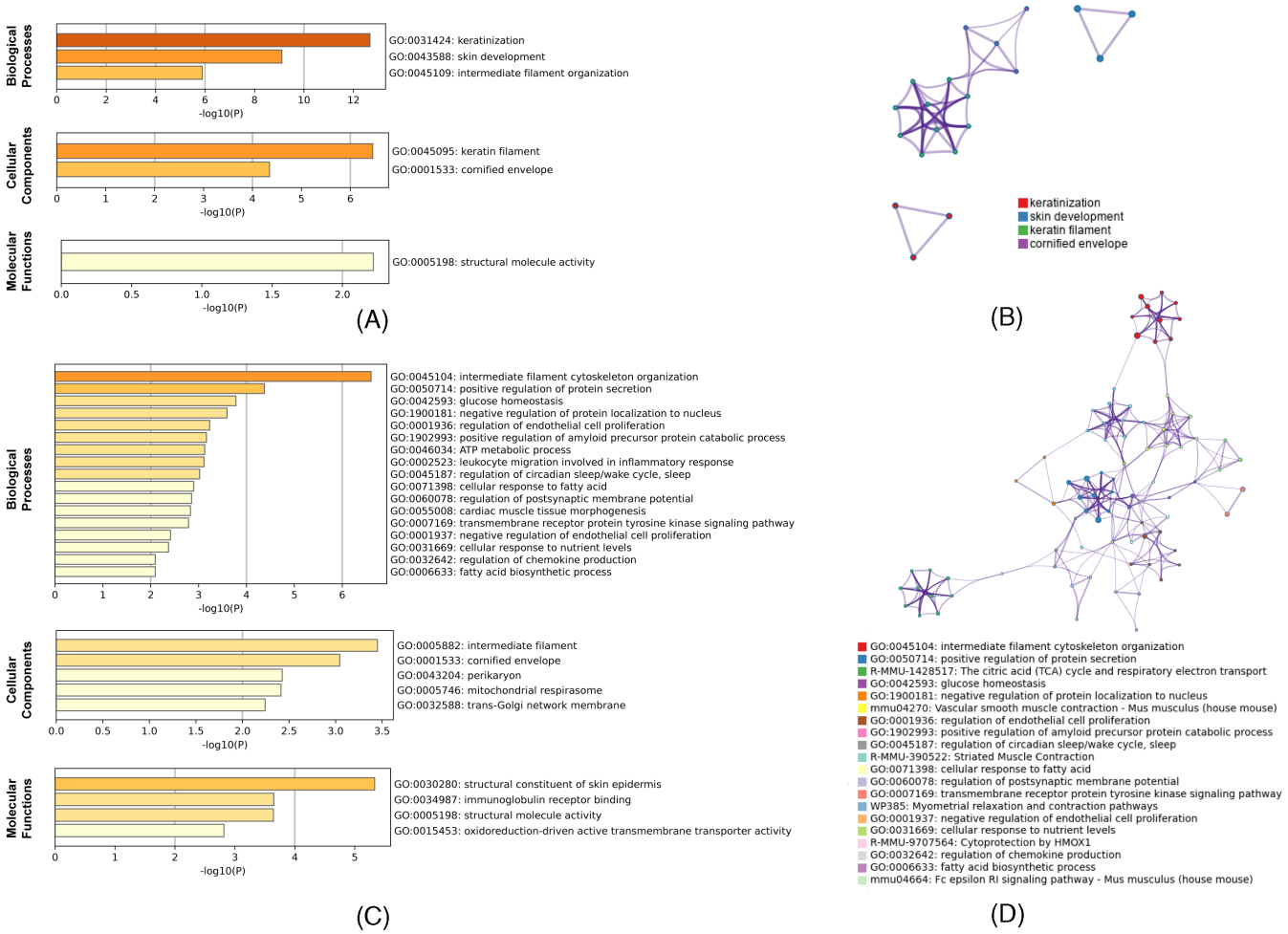

FIGURE 3 GO enrichment analysis of identified DEGs in three categories including biological processes, cellular component and molecular function, and the network of enriched terms in the ASD group (A, B) and CSD group (C, D) ($n = 3$ mice per group). ASD, acute sleep deprivation; CSD, chronic sleep deprivation; DEGs, differentially expressed genes; GO, gene ontology.

significant pathways were identified in the ASD group. For the CSD group with long-term SD cycles, five significant pathways are shown in Figure 4 and Table 3, including parkinson disease (PD), cardiac muscle contraction, oxidative phosphorylation, estrogen signaling pathway, and thermogenesis. Notably, most of these genes were related to PD.

3.4 | qRT-PCR of mRNA expression

Among the DEGs, we found that Krt4, Krt13, Krt15, Calml3, Baxl1 and Per3 were associated with esophageal disorders in previous studies; therefore, we performed qRT-PCR validation for these DEGs. Among them, Krt4, Krt13, Krt15 and Calml3 showed lower expression in 182 patients with ESCA than that in 286 normal from GEPIA in Figure 5A–D (all $p < 0.05$). The mRNA expression levels of some DEGs, including Krt4, Krt13, Krt15, Calml3, Baxl1 and Per3, are shown in Figure 5E–J. Squamous epithelium-specific genes including Krt4, Krt13 and Krt15, and Calml3 as a specific marker of normal epithelial development, were significantly downregulated in CSD mice compared with control mice (all $p < 0.05$), whereas Baxl1 and Per3 were significantly upregulated in CSD mice (both $p < 0.01$).

4 | DISCUSSION

Increasing evidence has demonstrated a close relationship between sleep disturbances and GERD.[24] The vicious cycle between the typical symptoms of GERD and SD seriously affects the life quality of patients. In addition, prevalent mental disorders, including anxiety and depression, are associated with an increased risk of GERD and esophageal hyperalgesia.[25] While improving sleep, the esophageal mucosa can be protected and the frequency of sleep-related reflux events can be decreased.[26–28] However, few studies have investigated the underlying mechanisms of the interaction between SD and esophageal diseases. In the current study, we established the SD model with anxiety- and depression-like behaviors in the CSD group. We first performed high-throughput genome sequencing and bioinformatics

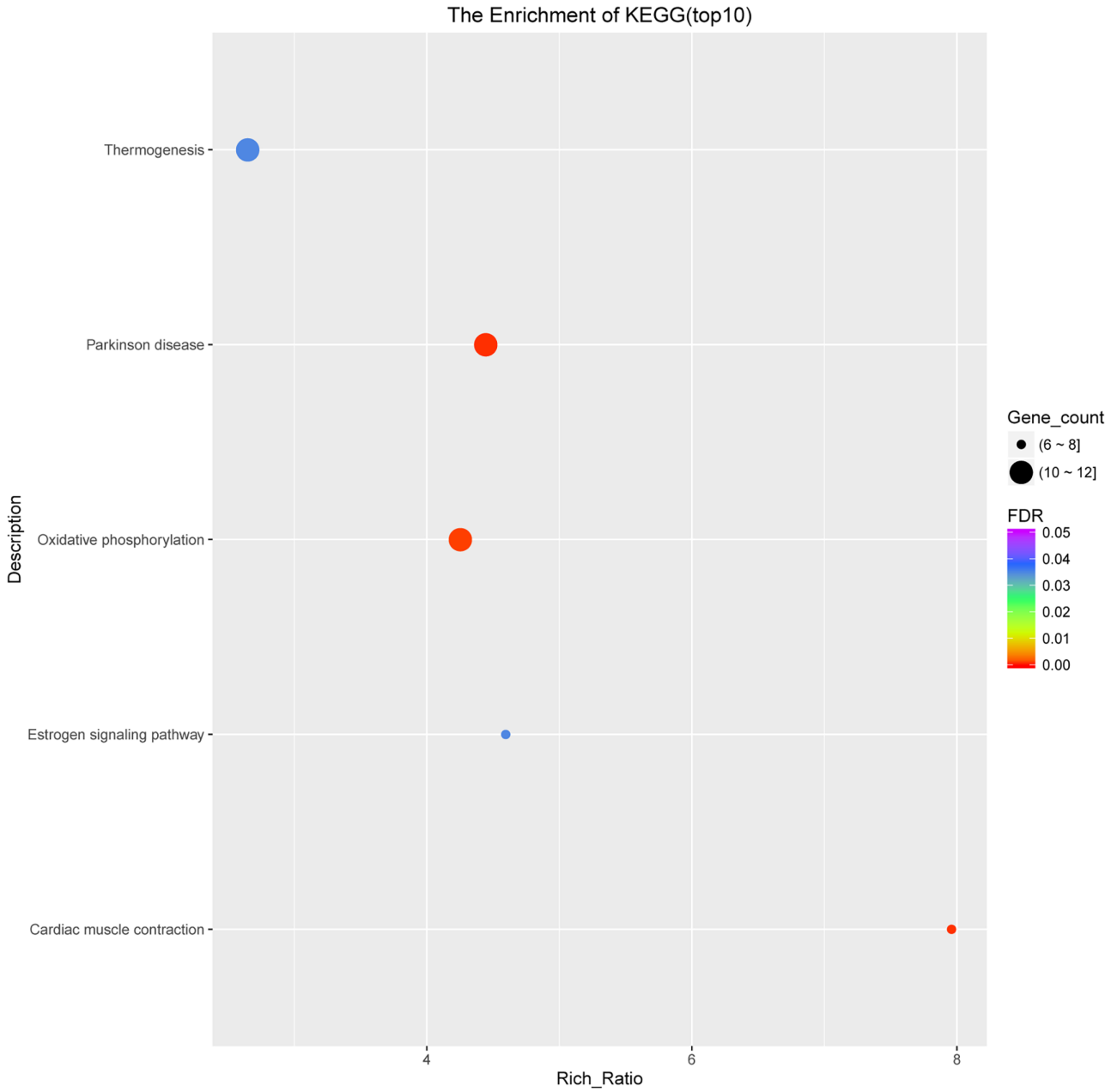

FIGURE 4 Distribution of KEGG pathway analysis in the CSD group ($n = 3$ mice per group). CSD, chronic sleep deprivation; KEGG, Kyoto encyclopedia of genes and genomes.

analysis of esophageal tissues after SD to screen featured DEGs verified by qRT-PCR, providing novel insights into the mechanisms of the interaction between sleep disorders and esophageal diseases.

Sleep plays a crucial role in maintaining neuronal circuits and signaling, and SD has a negative impact on the brain and behavioral functions, resulting in various neurological disorders.[29] The short-term SD model could not reflect the cumulative effect of the activation of brain regions, and long-term SD was closer to the real-life situation in humans.[18,30] In the current study, we failed to observe significant pathological changes in the esophageal epithelium between the SD and control groups, which might have been due to the short duration of the SD intervention. Mice in the CSD group exhibited obvious anxiety and depression. A greater number of DEGs were identified in the CSD group than in the ASD group. A total of 169 genes, including 88 downregulated and 81 upregulated genes, were differentially expressed in the CSD mice. To some extent, changes in these genes reflect a possible mechanism of the link between SD and GERD.

Our study demonstrated an evident increase in the expression of clock genes Per3 and Barx1 in CSD mice. These clock genes are closely associated with esophageal diseases. Hashimoto et al. observed changes in clock gene Per3 expression in the esophagus of rats with reflux esophagitis, and the mRNA expression level of Per3 was upregulated on day 21 in the reflux esophagitis model group compared with that in the control group, which was similar to the

TABLE 3 KEGG pathway analysis of differentially expressed genes in male chronic sleep deprivation mice.

Pathway	*p* value	FDR	Gene name	Up/down count	Gene count
Parkinson disease	1.85019E-06	0.000640167	Uchl1|K05611; mt-Atp6|K02126; mt-Co3|K02262; mt-Nd3|K03880; mt-Nd6|K03884; Gm13340|K02256; Gm10925|K02126; Gm28437|K02262; Gm29216|K02256; Gm28438|K03880; Gm28661|K02261	11/0	11
Cardiac muscle contraction	4.0987E-06	0.000709075	Tnnt2|K12045; mt-Co3|K02262; Gm13340|K02256; Gm28437|K02262; Gm29216|K02256; Gm28661|K02261; Actc1|K12314	6/1	7
Oxidative phosphorylation	9.39547E-06	0.001083611	mt-Atp6|K02126; mt-Co3|K02262; mt-Nd3|K03880; mt-Nd6|K03884; Gm13340|K02256; Gm10925|K02126; Gm28437|K02262; Gm29216|K02256; Gm28438|K03880; Gm28661|K02261	10/0	10
Estrogen signaling pathway	0.000531785	0.034807921	Raf1|K04366; Calm4|K02183; Krt13|K07604; Krt15|K07604; Calml3|K02183; Gm12346|K04079	0/6	6
Thermogenesis	0.000603606	0.034807921	mt-Atp6|K02126; mt-Co3|K02262; mt-Nd3|K03880; mt-Nd6|K03884; Gm13340|K02256; Gm10925|K02126; Gm28437|K02262; Gm29216|K02256; Gm28438|K03880; Gm28661|K02261	10/0	10

Abbreviations: FDR, false discovery rate; KEGG, Kyoto encyclopedia of genes and genomes.

results of our study.[31] In addition, Barx1 allows esophageal epithelial differentiation and controls tracheoesophageal septation by restricting Wnt signaling in accurate esophageal development, and dysregulation of genes involved in esophageal development increases susceptibility to BE and esophageal adenocarcinoma (EAC), probably by reducing anatomical antireflux mechanisms.[32,33] A study by Argyrou et al. that included 160 patients with GERD found that Barx1 was a genetic risk locus for the development of GERD and its complications.[34] Therefore, the role of these two clock genes in the interaction between sleep and GERD should be further investigated in future studies.

GERD is one of the most important etiological components of BE, which is a premalignant condition of the distal esophagus. GERD promotes the differentiation of squamous esophageal cells into the columnar epithelium, contributing to the precancerous lesions of BE. GERD has a shared genetic effect on BE and EAC based on a significant overlap of 77% between GERD and BE and 88% between GERD and EAC.[35] Notably, nocturnal gastroesophageal reflux is associated with an increased risk of erosive esophagitis, BE, and EAC.[36] Keratin, the major constituent of the esophageal epithelium, changes generally during the development of esophageal diseases.[37] In our study, we found that the expression levels of Krt4, Krt13, and Krt15 consistently decreased in the esophageal tissues of CSD mice. This change is consistent with a previous finding that keratin genes, including Krt4, Krt13 and Krt15, were significantly downregulated in rat and human BE, whereas columnar and intestinal epithelium-specific genes were significantly upregulated.[38] These DEGs indicate a possible link between SD and esophageal diseases. Besides, as a specific marker of normal epithelial development, Calml3 with a calmodulin-like function is downregulated in malignant tumor cells, which is a tumor suppressor of gastric cancer, tongue, and esophageal squamous cell carcinoma.[39] In our study, Calml3 was also downregulated in CSD mice, although its function in GERD remains unclear.

Several notable gene expression changes were observed in the ASD group. Sprr3 coding the cornified-envelope structural precursor, Tgm1 as a miRNA-mediated hub gene owing to its high degree in the protein–protein interaction network, Chgb as one of the prognosis-related hub genes, and Fosl1 as a MAP Kinase target related to CTHRC1-mediated regulation of proliferation and motility, were all reported to be downregulated in esophageal squamous cell carcinoma.[40–43] In the present study, we observed that these DEGs were downregulated in the ASD mice. These similar changes after ASD in esophageal tissues deserve further study to explore their regulatory mechanisms and downstream effects.

The results of the GO analysis indicated that the proteins encoded by the DEGs were involved in a wide variety of BP, such as intermediate filament cytoskeleton organization, positive regulation of protein secretion, glucose homeostasis, and keratinization, suggesting the possible involvement of these proteins. KEGG analysis revealed that the most active pathway was cardiac muscle contraction, the complex process initiated by the electrical excitation of cardiac myocytes based on Ca^{2+} binding to troponin C. Lower esophageal sphincter relaxation is the major mechanism of GERD, and the esophageal and lower esophageal sphincter circular muscles utilize different Ca^{2+} sources, phospholipid pools, and signal transduction pathways to contract in response to acetylcholine.[44]

Notably, the pathway with the most related genes was PD, which is a progressive neurodegenerative movement disorder characterized by the loss of dopaminergic neurons in the pars compacta of the substantia nigra.[45] Gastrointestinal disorders are the most commonly observed non-motor symptoms of PD, and their incidence increases over time, reaching 65% at 4 years after PD diagnosis.[46] The

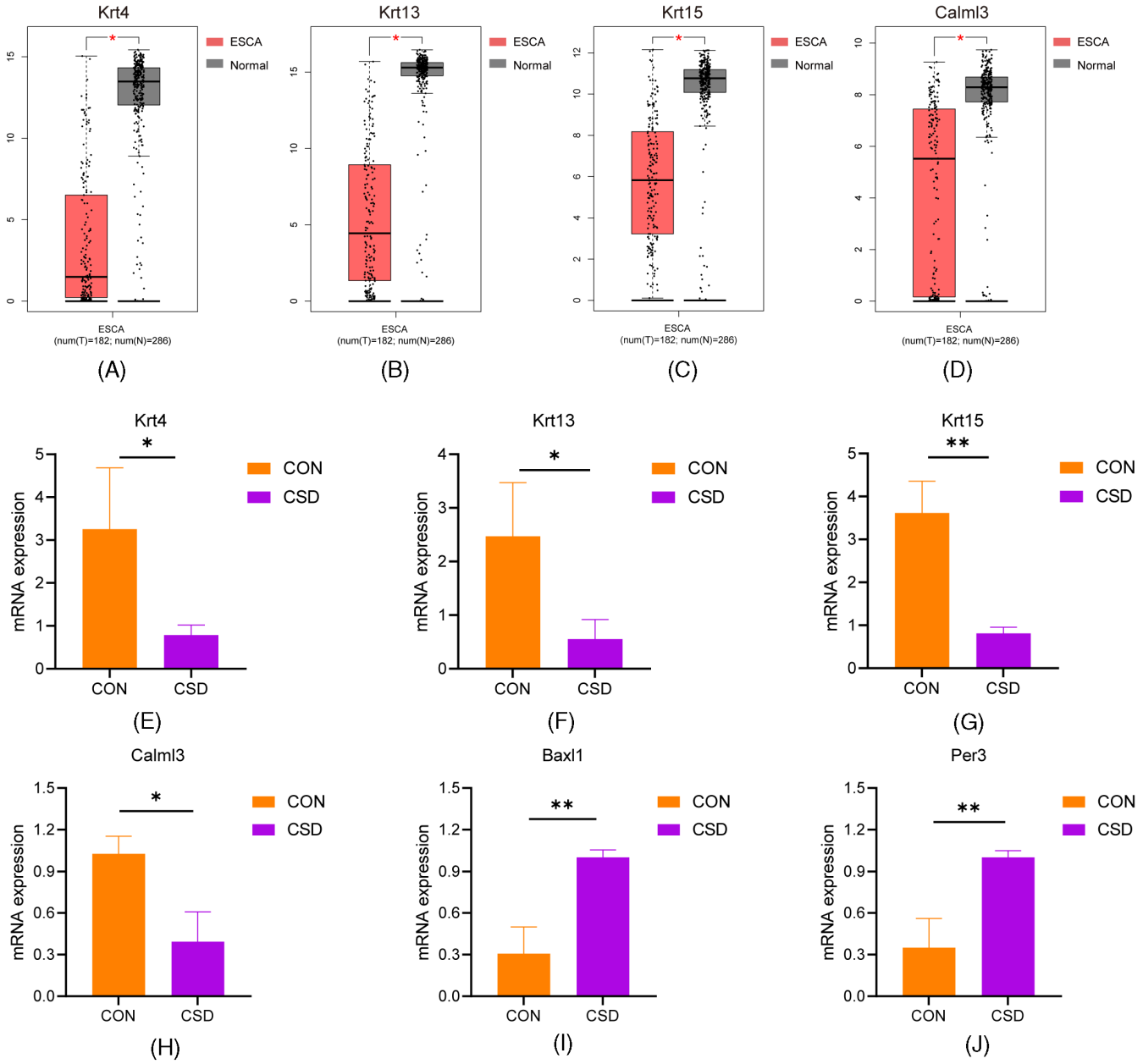

FIGURE 5 The results of analysis from GEPIA revealed that lower expression of Krt4 (A), Krt13 (B), Krt15 (C) and Calml3 (D) in patients with ESCA than that in normal. Quantitative real-time polymerase chain reaction verified the downregulation of Krt4, Krt13, Krt15 and Calml3 (E–G) and upregulation of Baxl1 and Per3 (H–J) in the CSD group ($n = 3$ mice per group, Student's t-test. $^{*}p < 0.05$, $^{**}p < 0.01$). CSD, chronic sleep deprivation; DEGs, differentially expressed genes; ESCA, esophageal carcinoma; GEPIA, gene expression profiling interactive analysis.

prevalence of GERD was 26.5% in patients with PD and heartburn was a prevalent symptom.[47] In addition, some patients with PD frequently experience psychiatric symptoms including depression, anxiety, apathy, and sleep disturbances.[45] In our study, behavioral tests showed that CSD induced mental disorders, including depression and anxiety. Patients with GERD also experienced these symptoms. These overlapping symptoms and pathways suggest an intrinsic link between GERD and PD, which implies a possible neuroregulatory mechanism of the brain-gut.

5 | CONCLUSION

In conclusion, our findings are the first to provide data on the transcriptome of the esophageal tissue after SD. We found that genes closely related to the development of GERD were significantly altered in the CSD group, and these DEGs were also involved in BP. These results promote further understanding of the neuroregulatory mechanisms of genes involved in the interaction between SD and the development of GERD and its related esophageal diseases, as well as

provide insights into potential targets for the treatment of GERD in the future.

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CONFLICT OF INTEREST STATEMENT

All authors have no financial or personal conflict of interest to declare. All authors approved the final version of the article, including the authorship list.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

ETHICS STATEMENT

All experimental procedures abided by the ARRIVE guidelines and the National Research Council's Guide for the Care and Use of Laboratory Animals with the approval by the Institutional Animal Care and Use Committee at the Fourth Military Medical University.

ORCID

Shuixiang He https://orcid.org/0000-0002-4832-1896

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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