Anxiety-induced overactive bladder: The role of oxidative stress and NF-κB signaling pathway with Hsp90 as a potential biomarker

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Abstract. Overactive bladder (OAB) is a common condition that affects lower urinary tract symptoms and markedly affects the physical and mental health of individuals. While the cause of OAB is unclear, some studies suggest a possible link to psychological factors, particularly anxiety. Despite this, research on the connection between anxiety and OAB is limited. The present study aimed to explore anxiety-induced OAB by analyzing clinical data and identifying key genes and pathways *in vivo*, ultimately providing new insights for diagnosing and treating OAB. Clinical data were analyzed to explore the relationship between anxiety and OAB. A chronic restraint stress model was used to induce anxiety, with histological scoring and cystometry assessing bladder function. Bladder transcriptomics identified key genes and pathways in OAB development. Differences in oxidative stress and NF-κB pathway activity were validated using immunohistochemistry, enzyme-linked immunosorbent assay and quantitative PCR. Clinical data showed a positive link between overactive bladder symptom scores and general anxiety disorder scale-7, with higher urination urgency scores in OAB patients with anxiety. Analysis confirmed anxiety as an independent risk factor for OAB. *In vivo* experiments showed that anxiety induced OAB-like symptoms in mice through oxidative stress and NF-κB pathway activation, with RNA sequencing revealing key hub genes included heat shock protein 90 (*Hsp*90) aa1, *Hsp*90ab1 and *Hsp*90b1. The present study demonstrated that anxiety may precipitate the onset of OAB by activating oxidative stress and the NF-κB signaling pathway. Hsp90 may serve as a potential biomarker for diagnosing anxiety-induced OAB. Retrospectively registered on 1 April 2025, The present study received the identifier ChiCTR2500100548 from the Chinese Clinical Trial Registry.

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Introduction

Overactive bladder (OAB) is a common, benign urinary disorder. It causes lower urinary tract symptoms (LUTS). It is characterized by urinary urgency, often accompanied by increased frequency and nocturia, with or without urinary incontinence, excluding urinary tract infections and other pathological changes (1-3). The primary distinction between the diagnosis of OAB and other lower urinary tract disorders causing LUTS is the presence of urinary urgency. In the Asia-Pacific region, the overall prevalence of OAB is as high as 20.8% and prevalence rises with age (4). Among Chinese women aged 31-40, the rate already exceeds 20% (5). OAB, often referred to as 'social cancer', markedly affects the physical and mental well-being of affected individuals.

The etiology of OAB is incompletely understood, with neurogenic, myogenic, epitheliogenic and urogenic hypotheses proposed. Established risk factors include advanced age, elevated BMI, pelvic surgery, vaginal delivery, diabetes and chronic constipation (6,7). Psychological factors such as depression and anxiety also play a role (8,9). Research on the OAB-anxiety link is scarce and inconclusive. The underlying mechanisms of OAB remain inadequately understood, leading to limited efficacy of various treatment modalities, including behavioral therapy, pharmacotherapy and sacral neuromodulation. Consequently, investigating the mechanisms underlying OAB is of substantial clinical importance for the prevention and treatment of this condition.

Anxiety is one of the commonest mental disorders. It can both trigger and worsen OAB, and severe OAB can intensify anxiety (10-12). An *in vivo* study conducted by Tanyeri *et al* (13) demonstrated that administration of mirabegron, a first-line treatment for OAB, to anxious

mice resulted in a significant reduction in anxiety levels. This finding suggested the potential relationship between anxiety and OAB. Anxiety also provoked oxidative stress. It activated the hypothalamic pituitary adrenal axis, raising glucocorticoids and damaging mitochondria, leading to malondialdehyde (MDA) rise, while superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) fell (14,15). Furthermore, oxidative stress can induce neuroinflammation and the release of pro-inflammatory cytokines, including IL-6, IL-1β and TNF-α, which influence neuronal excitability and synaptic transmission, thereby exacerbating anxiety (16,17). In colitis, multiple sclerosis and Alzheimer models, oxidative stress turns on the NF-κB pathway, amplifying inflammation and tissue injury (18-20). Although research suggests a correlation between anxiety, oxidative stress and NF-κB activation, the relationship between these factors and OAB remains unclear.

At present, no clinical research has investigated the causal relationship between anxiety and OAB, nor the potential mechanisms involved in anxiety-induced OAB *in vivo*. The mechanism of anxiety-induced OAB is categorized under the aforementioned neurogenic hypothesis. The present study aimed to explore the possibility of anxiety-induced OAB and identify key genes *in vivo*, with the objective of offering new perspectives and foundational research for the diagnosis of this condition.

Materials and methods

Research design and clinical data collection. The present study was conducted at Fujian Provincial Hospital and received approval from the Ethics Committee (approval no. K2024-10-019). Informed written consent was obtained from all participants. Inclusion criteria: All patients who meet the diagnostic criteria for OAB and healthy volunteers. Patients with any one of the following conditions were ineligible: urinary tract infection, various cystitis, urinary urogenital cancer, urinary tract stones, neurogenic bladder, pelvic organ prolapse, pregnancy and reluctance to participate in the present study. Between July 2023 and July 2024, a total of 83 patients presenting with LUTS and 40 healthy volunteers serving as controls were recruited from Fujian Provincial Hospital. Of the 83 patients, 43 were excluded due to LUTS resulting from other causes, leaving 40 patients who were diagnosed with OAB and included in the disease cohort. Comprehensive clinical data were successfully collected for 80 participants, encompassing the overactive bladder symptom scores (OABSS; Table SI) and the generalized anxiety disorder scale-7 (GAD-7; Table SII). Based on the OABSS scale, a diagnosis of OAB is made when urinary urgency has a score of ≥2 and the overall OABSS score is ≥3. When OABSS with a total score range of 3-5, mild OAB; 6-11, moderate OAB; ≥12, severe OAB. When GAD-7 with a total score range of 0-4, normal; 5-9, mild anxiety; 10-13, moderate anxiety; 14-18, moderately severe anxiety; 19-21 points, severe anxiety. These assessment tools are primarily utilized for the screening and evaluation of OAB severity and anxiety levels, respectively (21). Based on the OABSS and GAD-7 results, participants were categorized into four groups: OAB with anxiety (n=33), OAB without anxiety (n=7), control with anxiety (n=5), and control without anxiety (n=35). The recruitment process for the study participants is illustrated in Fig. 1.

Animal anxiety model. Animal experiments were conducted at the Nanfang Hospital's Animal Research Center, approved by its Institutional Animal Ethical Care Committee (approval no. NFYY-2021-0572) and followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals (22). A total of 60 female C57BL/6 mice (5-8 weeks old, 16-19 g) were obtained from SPF Biotechnology Co., Ltd. Mice were maintained under specific pathogen-free conditions in individually ventilated cages with 0.22-μm filter tops at 21-23˚C, 40-55% humidity, under a 12-h light/dark cycle, with autoclaved food and water available *ad libitum*. All handling was performed under HEPA-filtered air conditions to maintain barrier integrity. Before the establishment of the model, all the animals were first marked on their ears for identification and then randomly assigned to groups. For three weeks, mice underwent chronic restraint stress (CRS) by being immobilized in a transparent cylinder for six hours daily, from 09:00 to 15:00, without access to food or water (n=6). The control group could move, eat, and drink freely (n=6) (23). Prior to the tests, the groups were randomly shuffled and unrelated staff were invited to perform the operations. All behavioral tests were repeated three times per mouse. Humane endpoints were predetermined and approved by the Institutional Animal Care and Use Committee, including >20% weight loss, severe dehydration, persistent hunched posture, inability to ambulate, or any signs of restraint-induced injury. Animals were monitored twice daily; no animals reached these endpoints. Mice were deeply anesthetized with pentobarbital (60-80 mg/kg, intraperitoneal) and subsequently euthanized by cervical dislocation.

Elevated plus maze (EPM) test. The EPM test consists of two open and two closed arms, each measuring 50x10 cm, elevated 50 cm above the ground. The closed arms have 30 cm high opaque walls, and the intersection of the arms forms a 10x10 cm center area. Mice are placed in the center facing an open arm, and their movements are recorded for 5 min using a video system (Shanghai Jiliang Software Science & Technology Co., Ltd.). Anxiety-like behavior is indicated by increased entries and prolonged time spent in the closed arms.

Open field (OF) test. The OF test involves a 60x60x40 cm open box with a video system (Shanghai Jiliang Software Science & Technology Co., Ltd.) to track movement. The box is divided into 25 squares, with 9 central and 16 peripheral squares. During the 5-min test, the total distance traveled, distance in the central area, and time spent in the central area are recorded. A significant reduction in these measures indicates anxiety in mice.

Cystometry. Mice were anesthetized with isoflurane (3.0-4.0% for induction, 1.0-1.5% for maintenance) and placed in a supine position. A midline incision exposed the lower abdomen, and a 0.6x15 mm needle was inserted into the bladder to remove residual urine. Bladder pressure was measured using the BL-420N BioSignal Acquisition System (Chengdu Techman

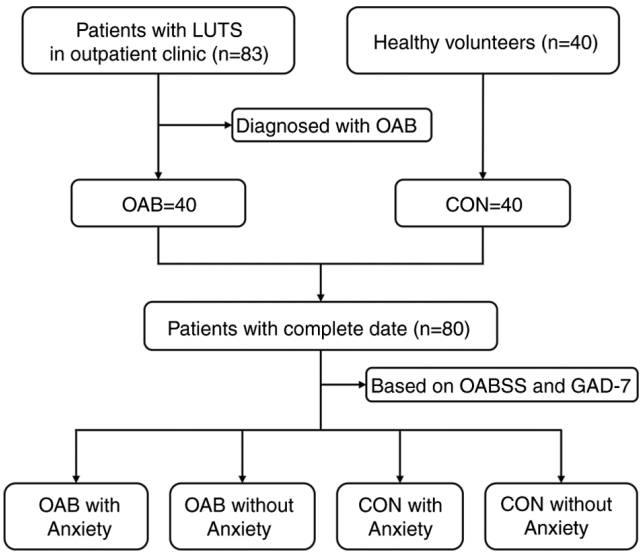

Figure 1. Flowchart of the present study. LUTS, lower urinary tract symptoms; OAB, overactive bladder; CON, control; OABSS, overactive bladder symptom scores; GAD-7, generalized anxiety disorder scale-7.

Software Co., Ltd.), with sterile saline infused at 1 ml/h. Once a stable micturition waveform was achieved, intravesical pressure data were recorded for 30 min or at least five voiding cycles. Urine outflow was monitored, and bladder contractions were identified by increased intravesical pressure, indicating micturition. Bladder contractions without urination were labeled as non-micturition contractions, it reflects the involuntary contraction of the bladder and is one of the core indicators for diagnosing and describing OAB (24). The peak intravesical pressure during urination was measured and maximum bladder capacity was determined by multiplying infusion time by infusion rate.

RNA-seq analysis. Bladder tissues were stored at -80˚C. Total RNA was extracted with the Eastep Super Kit (cat. no. LS1040; Promega Corporation). Poly-A mRNA was selected on oligo(dT) beads and converted to cDNA with the NEBNext Ultra kit (cat. no. 7530; New England Biolabs, Inc.). The cDNA was amplified and sequenced on the Illumina Novaseq6000 instrument (Illumina, Inc.), which was owned by Gene Denovo Biotechnology Co. The specific parameters can be found in Appendix S1.

To identify differential gene expression, a comparative analysis was performed using the DESeq2 software package v 1.48.1 (https://bioconductor.org/packages/release/bioc/html/DESeq2.html). Genes with FDR ≤0.05 and |fold change|≥1.5 were called significant (25).

Analysis of gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG). This part of the analysis was conducted using clusterProfiler software v 3.81 (26).GO enrichment analysis connects differentially expressed genes (DEGs) to significant biological functions by identifying enriched GO terms compared with the genome background (27).

After Bonferroni correction, P-values were adjusted using FDR correction, with a threshold of FDR ≤0.05. KEGG aids in understanding gene functions through pathway-based analysis. After using the same multiple testing corrections, the Q-value, which is the P-value after FDR correction, defined as the markedly enriched pathways.

Generation of protein-protein interaction (PPI) and discovery of key genes. The PPI network was built with STRING v10 (http://string-db.org), with genes as nodes and interactions as edges (28). Cytoscape v3.7.1 visualized the network, highlighting core and hub genes (www.cytoscape.org) (29). Bioinformatics analyses were conducted using Omicsmart, an online platform for data analysis (http://www.omicsmart.com). The details of analysis parameters are listed in Appendix S2.

Reverse transcription-quantitative (RT-q) PCR. The primers used in the present study are listed in Table SIII. cDNA synthesis and qPCR were performed according to the manufacturer's protocols. RNA was extracted from the bladder using the Animal Total RNA Isolation Kit (Foregene Co., Ltd.) following the manufacturer's instructions. cDNA was synthesized from total RNA with HiScript III RT SuperMix for qPCR (Vazyme Biotech Co., Ltd.) at 50˚C for 15 min and 85˚C for 5 sec. RT-qPCR was conducted on a LightCycler480 system (Roche Diagnostics, Ltd.) using ChamQ SYBR qPCR Master Mix (Vazyme Biotech Co., Ltd.) at 95˚C for 30 sec; followed by 40 cycles at 95˚C for 10 sec and 60˚C for 30 sec; and a final melt curve analysis at 60-95˚C. *GAPDH* was the internal reference, and gene expression was measured using the $2^{-\Delta\Delta Cq}$ method (30).

Determining the levels of oxidative stress and pro-inflammatory cytokines. Each mouse had a blood sample of ~1 ml collected by cardiac puncture. Serum and bladder levels of MDA (cat. no. S0131S), total superoxide dismutase (T-SOD; cat. no. S0101S) and GSH (cat. no. S0052) were measured using specific assay kits from Beyotime Biotechnology. ELISA kits from R&D Systems, Inc. were used to assess IL-6 (cat. no. M6000B), IL-1β (cat. no. MLB00C) and TNF-α (cat. no. MTA00B) levels. All procedures followed the provided instructions.

Immunohistochemistry. Bladder tissues were immersed in 4% paraformaldehyde for fixation at 4˚C for 8 h, and sequentially underwent dehydration with an ethanol gradient (25˚C, 5 h), permeabilization with 0.3% Triton X (25˚C, 20 min), paraffin dipping (58˚C, 1.5 h) and embedding (58˚C to 4˚C, 30 min); after which, the tissues were cut into 4-μm sections. The sections were cleaned and sealed with drops of goat serum (cat. no. BL210A; Biosharp Life Sciences) for 30 min at room temperature. Subsequently, the blocking solution was discarded and diluted rabbit anti-Hsp90aa1 (1:200; cat. no. BF0084-50; Affinity Biosciences), anti-Hsp90ab1 (1:200; cat. no. BF0215; Affinity Biosciences), anti-Hsp90b1 (1:200; cat. no. AF5287; Affinity Biosciences) and anti-NF-κB (1:200; cat. no. YM8209; ImmunoWay Biotechnology Company) antibodies were added dropwise to the sections and incubated overnight at 4˚C. The next day, goat anti-rabbit secondary antibody (1:200; cat. no. LF102; Epizyme; Ipsen Pharma) was dropped into the sections and incubated at 37˚C for 30 min. DAB chromogen concentrate was mixed with diluent to prepare a working solution according to the instructions of

the DAB kit (cat. no. BL732A; Biosharp Life Sciences) and applied to the tissue sections, which were washed, re-stained with hematoxylin at room temperature for 3 min and washed again. Finally, the sections were dehydrated, permeabilized with xylene and sealed. Scanning and viewing of the images was performed using NanoZoomer Digital slide scanner (Hamamatsu Photonics K.K.) and NDP View2 Plus Image viewing software (version U12388-01; Hamamatsu Photonics K.K.). The average optical density was quantified using Image-Pro Plus software (version 6.0; Media Cybernetics, Inc.), with measurements taken from three randomly selected fields of view at x10 magnification for every sample.

Hematoxylin and eosin staining. Paraffin-embedded sections (4 μm) were dewaxed in xylene (3x5 min) and rehydrated through a descending ethanol series (100, 95 and 70%, 2 min each). Nuclei were stained with Mayer's hematoxylin (cat. no. MHS16-100ML; MilliporeSigma) for 90 sec at room temperature, differentiated in 1% acid alcohol (1 sec), blued in 0.2% ammonia water (30 sec) and counterstained with 1% eosin Y (cat. no. 318906-25G; MilliporeSigma) for 45 sec. After dehydration in ethanol (95 and 100%, 2 min each) and permeabilization in xylene (2x3 min), slides were mounted with neutral balsam (cat. no. 10050041; Sinopharm Chemical Reagent Co., Ltd.). Digital images were acquired at x20 magnification using a NanoZoomer S60 scanner (Hamamatsu Photonics K.K.).

Statistical analysis. Continuous variables were summarized as mean ± standard deviation or median (Q1-Q3) for normal and non-normal distributions, respectively, while categorical variables were shown as frequency and percentage (GraphPad Prism software version 10; Dotmatics). When comparing the difference in means between two samples, the unpaired Student's t-test was used when data had a normal distribution, whereas the Mann-Whitney U test was employed when data had a non-normal distribution. χ^2 or Fisher's exact tests compared categorical variables. Correlations were analyzed using Pearson's or Spearman's coefficients. Stepwise multivariate logistic regression identified key predictors, reporting odds ratios (OR) with 95% confidence intervals (CI). Model fit was checked with the Hosmer-Lemeshow test, and predictive ability was evaluated using the ROC curve. All candidate variables with P<0.10 in univariate analysis were entered into the initial model. Multicollinearity was assessed by variance inflation factor (VIF); all retained variables showed VIF <5. The final model exhibited good calibration (Hosmer-Lemeshow test P=0.616). All the data underwent normality tests before being subjected to statistical analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

Demographic characteristics of the participants. Demographic characteristics of participants were assessed in a study involving 40 patients with OAB and 40 healthy controls (CON). As shown in Table I, there were no significant differences in age, BMI, sex, history of pelvic surgery, diabetes, or hypertension between the groups. OABSS were markedly higher in the OAB group; intriguingly, GAD-7 was notably elevated at 7.18±3.56 compared with 2.00 (1.00, 3.00) in the CON group, indicating a potential link between anxiety and OAB.

The relationship between anxiety and OAB. To elucidate the relationship between anxiety and OAB, and to determine whether anxiety can induce OAB, the present study conducted correlation and regression analyses. The findings indicated a positive correlation between OABSS and the GAD-7 (Fig. 2A). Additionally, urinary urgency, a key symptom of OAB, was positively correlated with GAD-7 (Fig. 2B). Subsequently, all OAB patients were categorized into two groups based on the presence or absence of anxiety and compared their OABSS and urinary urgency scores. The results demonstrated that OAB patients with anxiety exhibited markedly higher OABSS and urinary urgency levels compared with those without anxiety (Fig. 2C and D). To further explore the causal relationship between anxiety and OAB, the present study initially performed a univariate logistic regression analysis on GAD-7, which yielded an odds ratio (OR) of 1.81 (95% CI: 1.43-2.44), with a crude P-value of <0.001. This was followed by a multivariate logistic regression analysis, incorporating all baseline variables, which resulted in an adjusted OR of 1.91 (95% CI: 1.48-2.68), with an adjusted P-value of <0.001 (Fig. 2E). In conclusion, receiver operating characteristic (ROC) curves were used to evaluate the potential of anxiety as an independent risk factor for predicting the occurrence of OAB. The GAD-7 demonstrated a notably high predictive accuracy, with an area under the curve (AUC) of 0.87 (95% CI: 0.79-0.95) (Fig. 2F), the specificity was 0.875, the sensitivity was 0.825 and the Youden's index was 1.7. This aspect of the present study effectively demonstrated, at a clinical level, that anxiety can be employed as an independent risk factor for forecasting the onset of OAB.

Anxiety can induce OAB-like symptoms in vivo. In the present study, an anxiety model was developed using 21 days of CRS (Fig. 3A).

In the open-field (OF) test (Fig. 3B and C), the time spent (11.98±1.41 sec) by the CRS mice in the central area, the distance traveled (983±179 mm) during activity, and the total distance traveled (7,803±727.10 mm) were all markedly lower than those of the control group (24.41±2.63 sec), (2,209±106.80 mm), (1,7926±722.10 mm), (Fig. 3D-F). In the elevated-plus maze (EPM), The number of times the CRS mice entered the open arm (1.83±0.54)and the duration they stayed (11.20±2.97 sec) there were markedly lower than those of the control group (5.50±0.56), (24.35±2.00 sec) while the number of times they entered the closed arm (12.17±0.60) and the duration they stayed (257.10± 5.83 sec) there were markedly higher than those of the control group (6.16±1.01), (190.90±10.60 sec), (Fig. 3G-J). These changes confirmed that CRS induced anxiety-like behavior, as the mice exhibited behaviors resembling anxiety, such as reduced exploration desire and avoidance of conflicts.

Furthermore, cystometry was conducted to assess bladder function (Fig. 4A) and it was and found that CRS mice had more non-voiding (4.16±0.47 vs. 1.33±0.21), voiding contractions (3.67±0.95 vs. 1.83±0.31) and lower peak pressure (28.33±1.26 vs. 32.67±1.02 cm H_2O), whereas bladder

Table I. Comparisons of characteristics between OAB group and CON group.

Characteristic	OAB (n=40)	CON (n=40)	P-value
Age	35.90±7.65	37.45±12.70	0.51
BMI	21.74±2.43	22.65±2.72	0.12
Sex			0.82
Male	21 (53)	20 (50)	
Female	19 (47)	20 (50)	
History of pelvic surgery	8 (20)	6 (15)	0.56
Diabetes	7 (17.5)	8 (20)	0.76
Hypertension	9 (22.5)	8 (20)	0.79
GAD-7	7.18±3.56	2.00 (1.00, 3.00)	<0.001
OABSS	7.00 (4.25, 8.00)	1.00 (0.00, 2.00)	<0.001

Data are showed as mean (SD) or median (interquartile ranges) for continuous variables or n (%) for counts. OAB, overactive bladder; CON, control; BMI, body mass index; OABSS, overactive bladder symptom scale; GAD-7, generalized anxiety disorder scale-7.

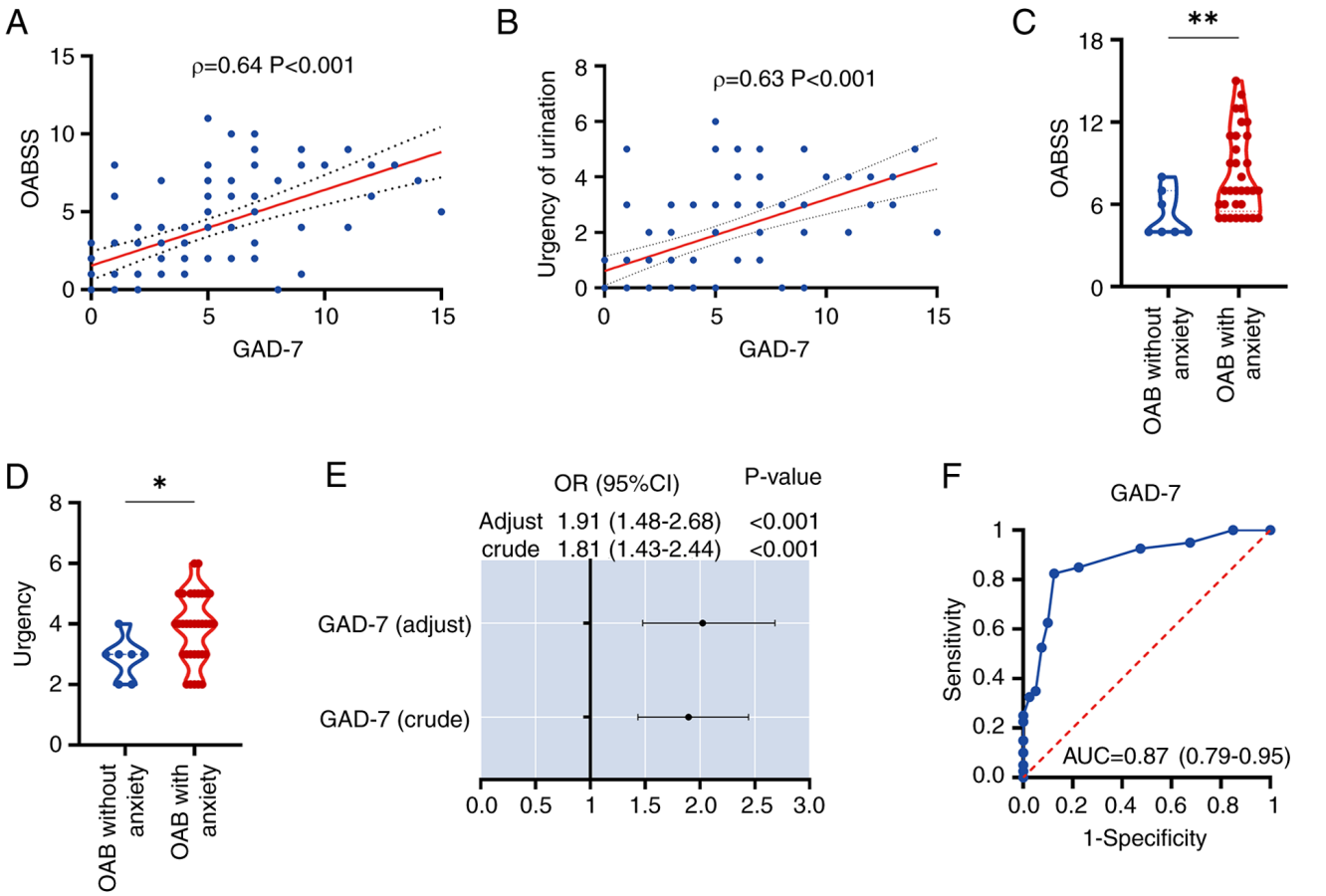

Figure 2. Anxiety is an independent risk factor for predicting OAB. (A) Correlation analysis between OABSS and GAD-7. (B) Correlation analysis between urgency of urination and GAD-7. (C) OABSS between OAB patients with and without anxiety. (D) Urgency of urination between OAB patients with and without anxiety. (E) Logistic regression model for predicting OAB based on anxiety. (F) ROC curve analysis for the diagnostic performance of anxiety in identifying OAB. Data were presented as Mean ± SD or median. T-test or Mann-Whitney *U*-test was used to compare continuous variables between two groups, Correlation analysis was performed using Spearman's rank correlation coefficient, a stepwise regression approach was applied to identify the most significant predictors. The model's goodness-of-fit was assessed using the Hosmer-Lemeshow test (*P<0.05, **P<0.01, ns, no statistical difference). OAB, overactive bladder; OABSS, overactive bladder symptom scores; GAD-7, generalized anxiety disorder scale-7; ROC, receiver operating characteristic.

capacity was unchanged (0.38±0.02 vs. 0.39±0.03 ml), (Fig. 4B-E). These results indicate that CRS-induced anxiety may cause OAB-like symptoms in mice through involuntary contractions of the detrusor muscle. Subsequently, the present study conducted hematoxylin and eosin staining on bladders and calculated the bladder-to-body weight ratio, finding no

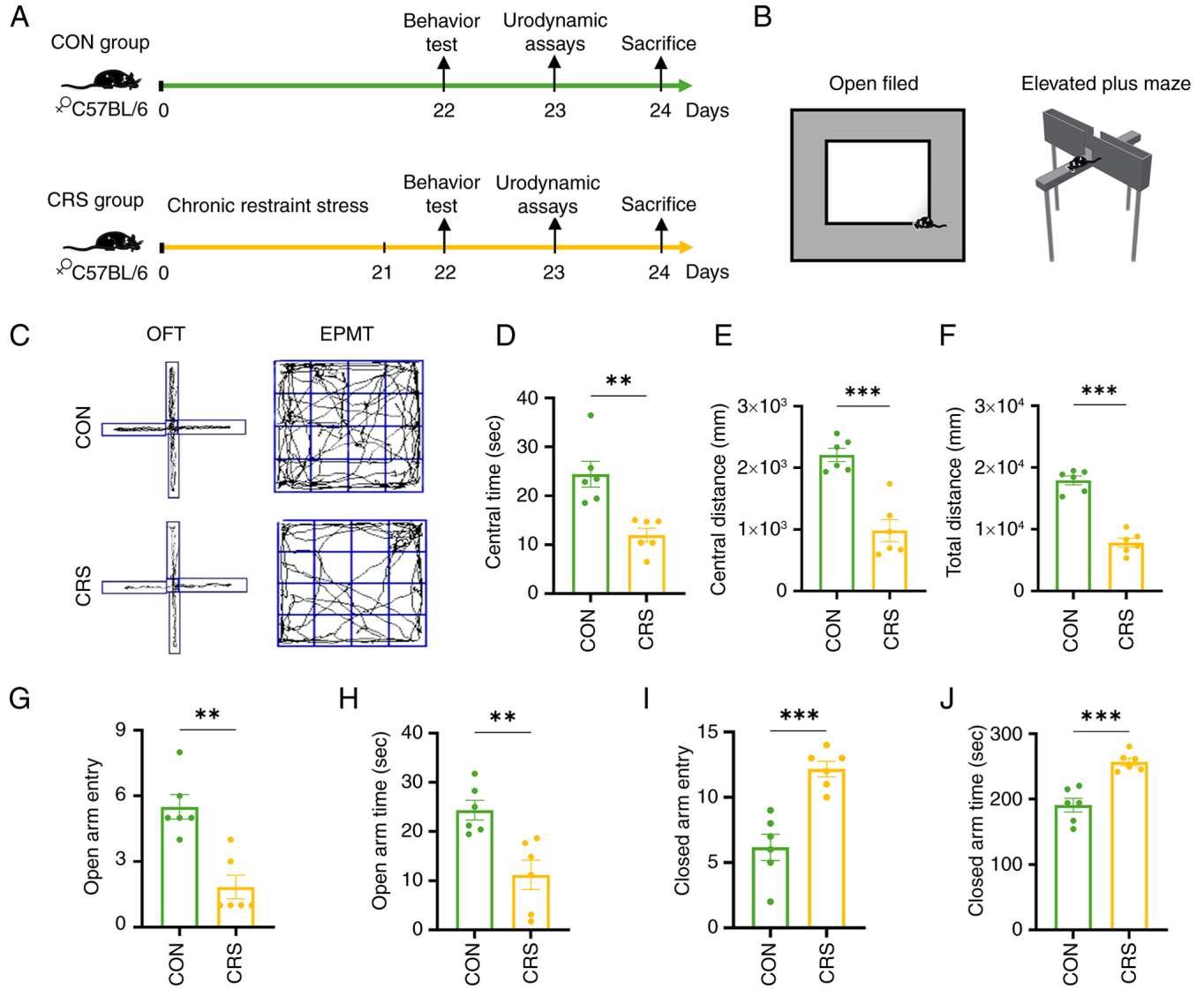

Figure 3. CRS induced anxiety-like behavior in mice. (A) Flow chart of *in vivo* procedures. (B) Schematic diagram of the behavioral experiment. (C) Display of OFT and EPMT in CRS group and CON group mice. (D) The time spent in the central area in the OFT. (E) The distance traveled in the central area in the OFT. (F) The total distance traveled in the OFT. (G) Times into the open arm in the EMPT. (H) Time spent in the open arm in the EMPT. (I) Times into the closed arm in the EMPT. (J) Time spent in the closed arm in the EMPT. Data were presented as mean ± SD (n=6). P-values were calculated using a two-tailed unpaired Student's t-test (**P<0.01, ***P<0.001, ns, no statistical difference). CRS, chronic restraint stress; OFT, open field test; EPMT, elevated plus maze test; CON, control.

difference in bladder-to-body weight ratio or lamina propria thickness between groups, mirroring human OAB (31) (Fig. 4F-H). The bladder mucosal layer was thinner in CRS mice compared with controls (Fig. 4I), suggesting that mucosal layer changes might contribute to OAB symptoms.

Exploration of differentially expressed genes (DEGs). The present study conducted RNA-seq analysis to identify DEGs between CRS mice and control mice. After processing and normalizing the raw data, 551 DEGs were found, with 58 upregulated and 493 downregulated (Fig. S1). This meant that certain pathways are activated, while numerous others are suppressed.

Enrichment analysis of function and pathways in DEGs. GO analysis identified the most enriched categories among downregulated DEGs as 'positive regulation of biological process', 'Binding', and 'Cellular anatomical entity' (Fig. 5A-C). For upregulated DEGs, the top categories were 'Regulation of reactive oxygen species metabolic process', 'lipid transporter activity', and 'P granule' (Fig. 5D-F). KEGG pathway analysis revealed 'Antigen processing and presentation' as the most markedly associated with downregulated DEGs (Fig. 6A), while 'Base excision repair' was most significant for upregulated DEGs (Fig. 6B).

Construction of a PPI network and identification of key hub genes. The PPI network comprised 50 nodes and 533 edges (Fig. 7). The top three hub genes, identified by combined score, were *Hsp90aa1*, *Hsp90ab1*, and *Hsp90b1*, all from upregulated DEGs.

Anxiety-induced OAB activated oxidative stress and NF-κB pathways in vivo. Based on GO analysis and previous studies,

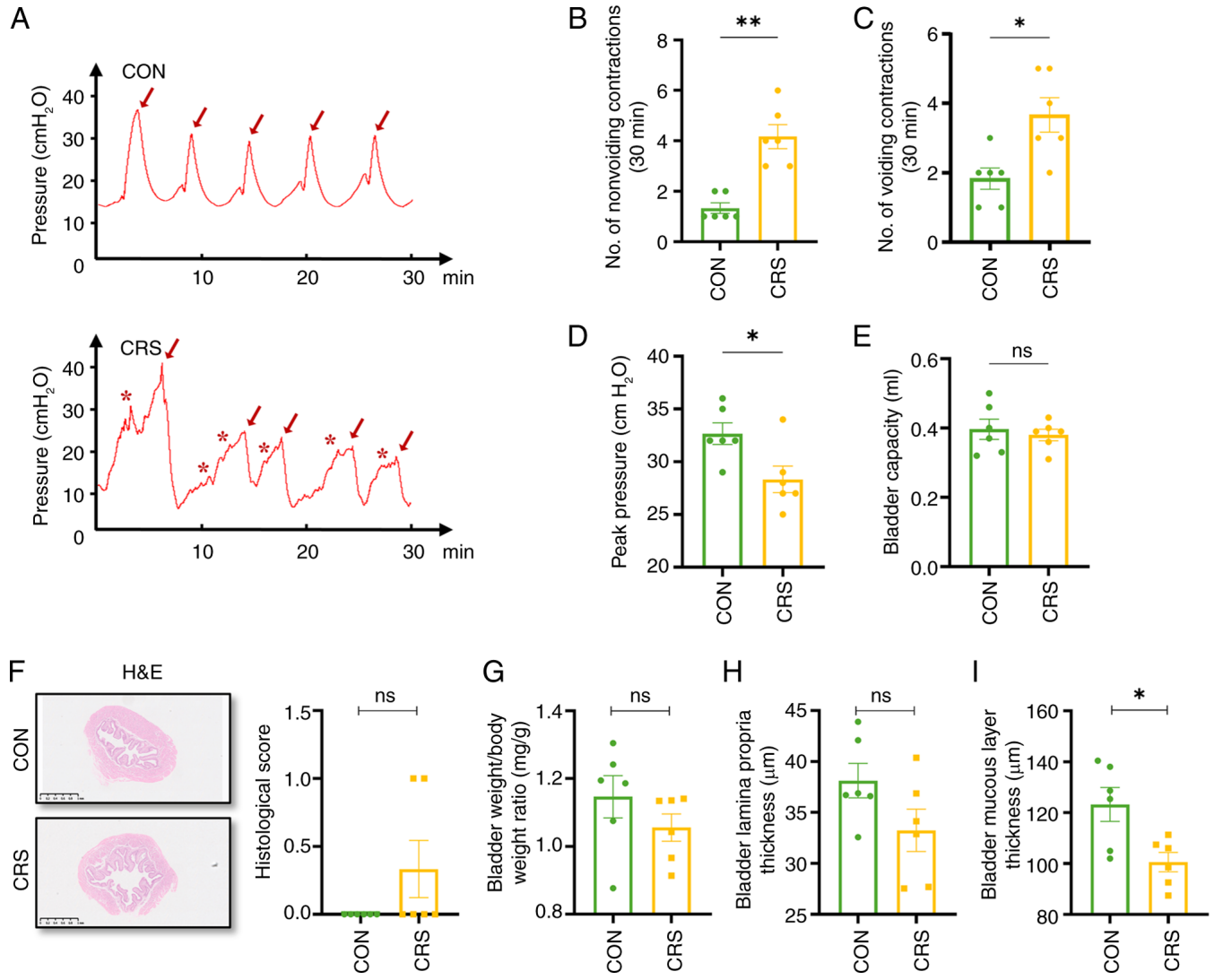

Figure 4. Anxiety caused OAB-like symptoms in mice. (A) Representative trace of cystometry urodynamic assay during a 30 min continuous monitoring performed in the CON and CRS group. Arrows indicate voiding contractions of the bladder, and asterisks represent non-voiding contractions of the bladder. (B) The number of bladder non-voiding contractions. (C) The number of bladder voiding contractions. (D) Bladder peak pressure. (E) Maximum bladder capacity. (F) H&E staining and histological score of bladder tissues (scale bar, 1 mm). Data were presented as the median with interquartile range. (G) Bladder weight/body weight ratio. (H) Thickness of lamina propria of the bladder. (I) Thickness of mucosal layer of the bladder. Data were presented as mean ± SD. (n=6) P-values were calculated using a two-tailed unpaired Student's t-test (*P<0.05, **P<0.01, ns, no statistical difference). CRS, chronic restraint stress; CON, control.

it was hypothesized that the disease mechanism might involve oxidative stress, which could activate the NF-κB pathway (32,33). To investigate, the present study measured serum levels of MDA, GSH, and T-SOD in mice, as these indicate oxidative stress activation. Serum MDA was higher in CRS mice, whereas GSH and T-SOD were lower (Fig. 8A-C). The same pattern was seen in bladder tissue (Fig. 8G-I). Next, we examined downstream inflammation. Serum IL-6, IL-1β and TNF-α were all elevated (Fig. 8D-F), and identical increases were found in the bladder (Fig. 8J-L).

To further verify the status of NF-κB in the bladder, the gene and protein expression of NF-κB in the bladder of each group were examined and the results showed that the gene expression of *Nf-κb1* and the protein expression levels of NF-κB as well as IL-6, IL-1β and TNF-α were markedly increased in the CRS group compared with the control group (Fig. 8M-O). These findings might indicate that anxiety could trigger the NF-κB pathway through oxidative stress and pro-inflammatory mediators, causing bladder overactivity.

*Identification of key genes and proteins in bladder. Hsp*90 has been linked to NF-κB in various disease models (34,35). In the present study, *Hsp90* genes were identified during PPI network construction. The present study used qPCR and IHC on mouse bladder tissue to confirm Hsp90 mRNA and protein levels. Results (Fig. 8P-T) showed significant upregulation of Hsp90aa1, Hsp90ab1, and Hsp90b1 in the OAB group compared with controls, with protein expression mainly in the mucosal layer.

Discussion

OAB is a common cause of LUTS. It markedly affects patients' quality of life, leading to mobility issues, urinary tract

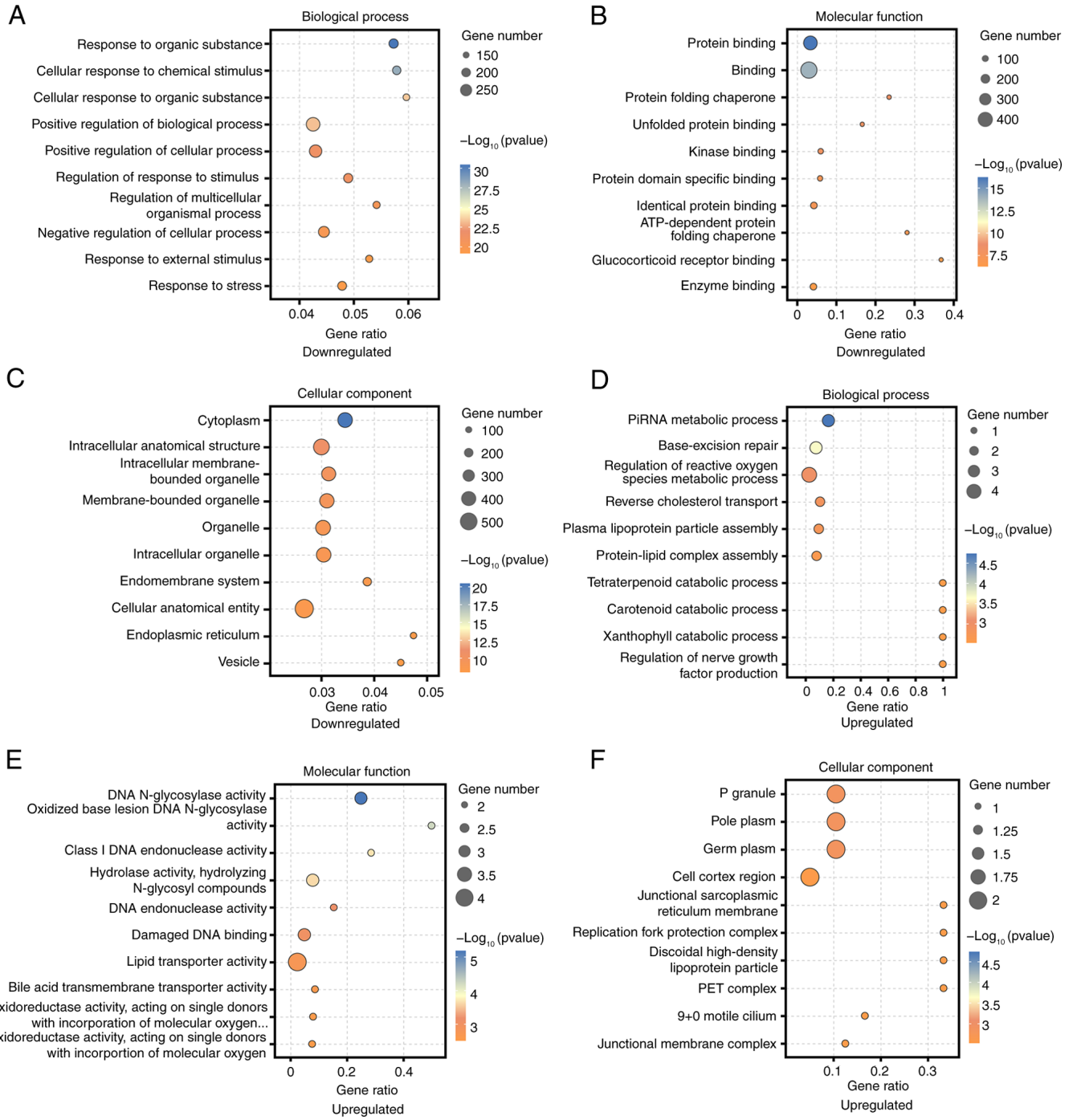

Figure 5. GO analysis for DEGs. (A) Biological process for downregulated genes. (B) Molecular function for downregulated genes. (C) Cellular component for downregulated genes. (D) Biological process for upregulated genes. (E) Molecular function for upregulated genes. (F) Cellular component for upregulated genes. (n=3). The differential gene filtration threshold was FDR <0.05, |log2FC|≥1.5. GO, Gene Ontology; DEGs, differentially expressed genes.

infections, anxiety, depression and loss of self-confidence (36). The unclear pathogenesis of OAB results in a lack of targeted treatments. Thus, understanding OAB's underlying mechanisms is crucial for effective prevention and treatment.

Studies have established a positive correlation between OAB and factors such as age and BMI (37-39). Additionally, conditions such as diabetes mellitus, hypertension, history of pelvic surgery and reproductive history can lead to the onset or progression of OAB (40,41). Regarding psychological factors, limited research suggests a correlation between the severity of OAB and anxiety (42-44). Nonetheless, there is insufficient evidence to conclude whether anxiety independently induces OAB or serves as a standalone risk factor for its development. The current study identified a positive correlation between the level of anxiety and the severity of OAB and urinary urgency, corroborating previous findings. Furthermore, through multivariate logistic regression analysis and the construction of ROC curves, it established that anxiety serves as an independent risk factor for the development of OAB.

To explore anxiety-induced OAB mechanisms, the present study conducted *in vivo* experiments after analyzing clinical data. OAB modeling methods include drug injection and

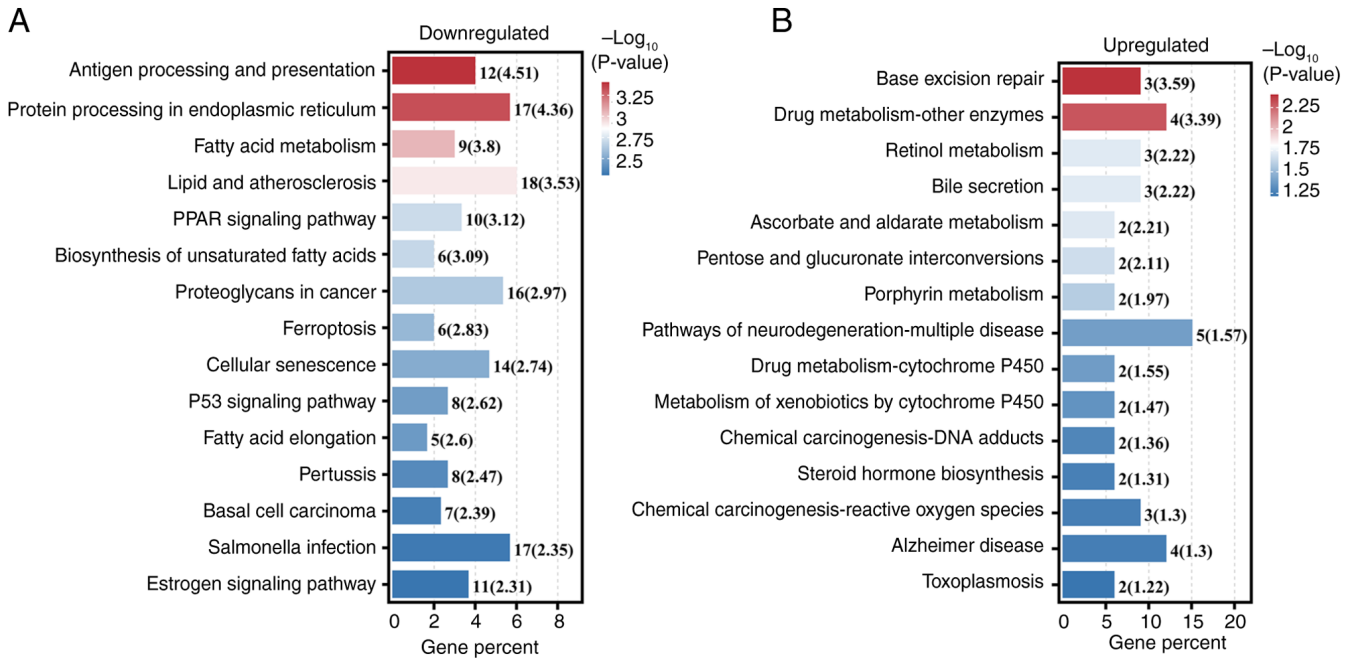

Figure 6. KEGG pathway analysis for DEGs. (A) KEGG pathway analysis for downregulated genes. (B) KEGG pathway analysis for upregulated genes. (n=3). The differential gene filtration threshold was FDR <0.05, |log2FC|≥1.5. KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, Differentially expressed genes.

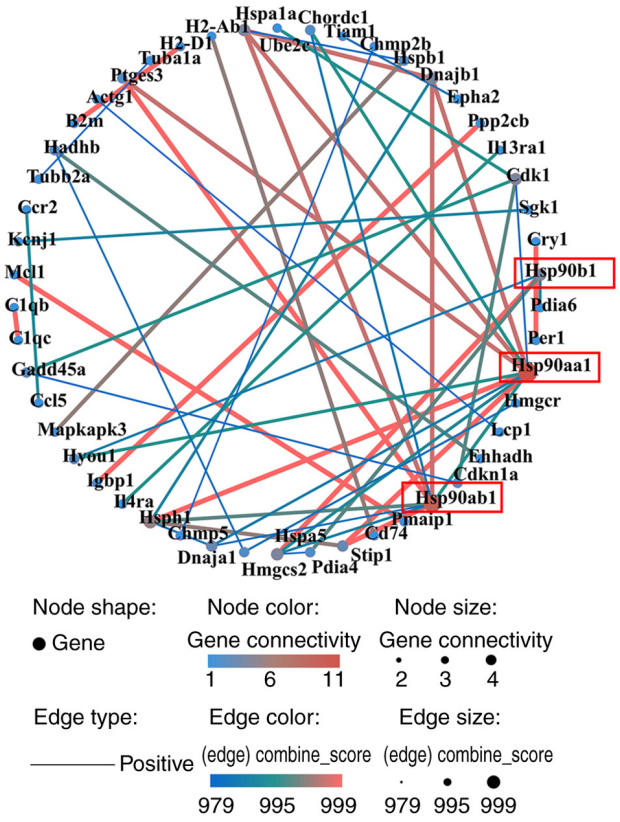

Figure 7. PPI network based on DEGs. The selected hub genes were marked in red. (n=3). The differential gene filtration threshold was FDR <0.05, |log2FC|≥1.5. PPI, protein-protein interaction; DEGs, differentially expressed genes.

bladder outlet obstruction (BOO), with BOO being the most common (45-47). BOO can lead to issues such as collagen redistribution, muscle hypertrophy and changes in bladder capacity, potentially skewing OAB mechanism studies (48-50). The present study uniquely applied CRS, typically used for anxiety models, to induce OAB in mice (51). This method minimized surgical impact on bladder physiology, reducing confounding factors and realistically replicated anxiety-induced OAB showed in clinical settings.

Having confirmed anxiety as an independent OAB risk factor and generated a murine anxiety-OAB model, the present study performed bladder transcriptomics to dissect mechanisms, biomarkers and targets. RNA-seq revealed 551 DEGs, with 58 upregulated and 493 downregulated. It was hypothesized that this transcriptional imbalance represents a cellular 'functional trade-off', where the extensive downregulation of homeostatic pathways serves to conserve energy under chronic anxiety stress. Concurrently, the selective upregulation of genes related to ROS metabolism and base excision repair indicated a prioritization of essential survival mechanisms over routine physiological functions, such as antigen presentation. GO analysis highlighted enriched terms such as 'regulation of reactive oxygen species metabolic process', 'lipid transporter activity', and 'P granule' among the upregulated genes. Reactive oxygen species (ROS) are highly reactive compounds involved in various biological processes (52). The excessive accumulation of ROS induces oxidative stress within cells, resulting in damage to DNA, proteins and lipids. This damage is implicated in the pathogenesis of various diseases, including cancer, cardiovascular diseases and neurodegenerative disorders. Furthermore, ROS accumulation has been associated with NF-κB signaling and ferroptosis, as documented in several studies (53-55). These findings establish a molecular framework for mechanistic inquiry. Notably, KEGG pathway analysis revealed a significant enrichment of upregulated genes in the 'Base Excision Repair' pathway. Base excision repair is a crucial mechanism by which cells address

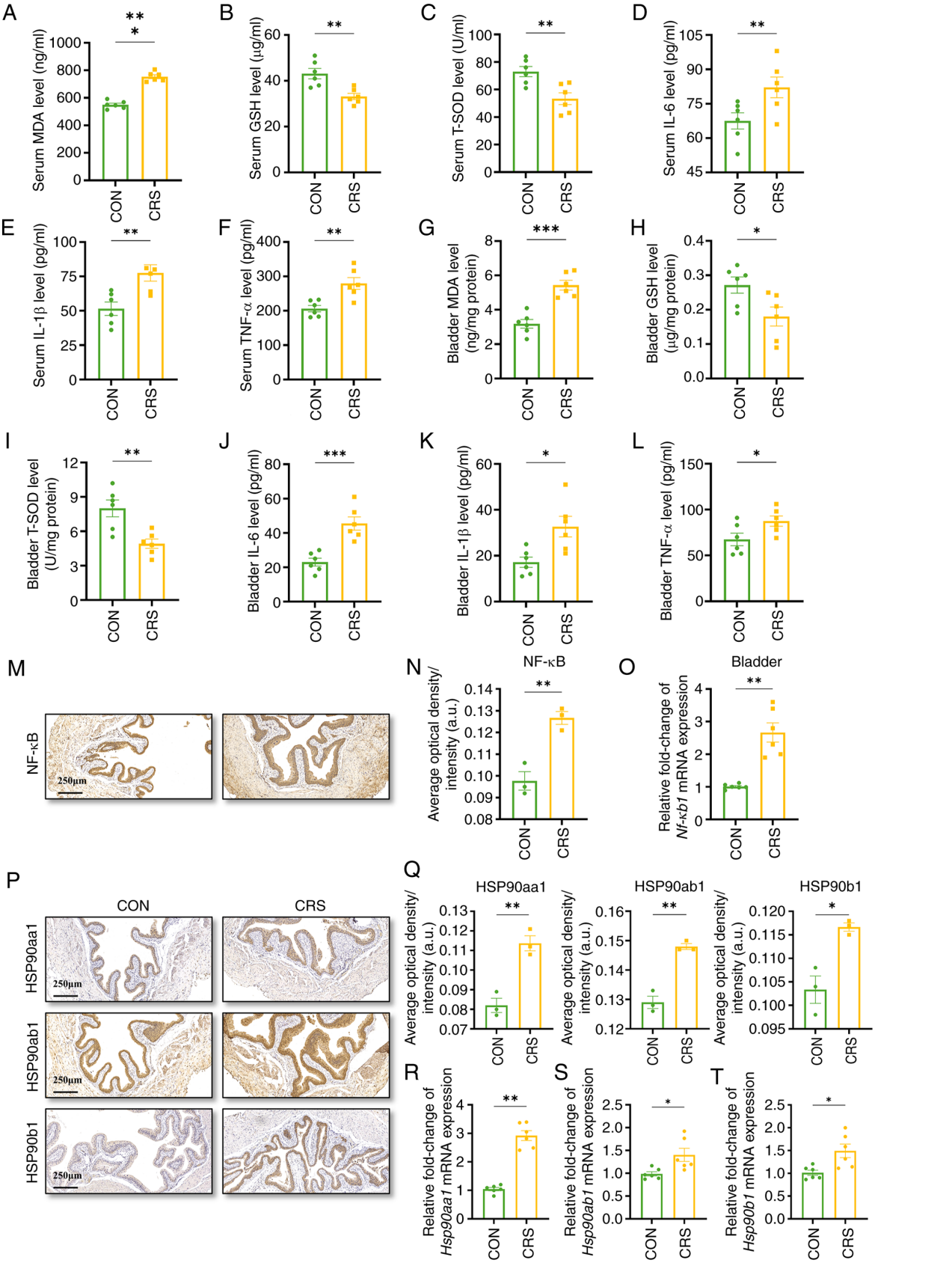

Figure 8. Anxiety active oxidative stress and NF-κB signaling. The concentration of (A) MDA, (B) GSH, (C) T-SOD, (D) IL-6, (E) IL-1β and (F) TNF-α in the serum. The concentration of (G) MDA, (H) GSH, (I) T-SOD, (J) IL-6, (K) IL-1β and (L) TNF-α in the bladder. (M) Representative images of NF-κB in the bladder, scale bar, 250 μm. (N) Average optical density of NF-κB in the bladder. (O) Relative mRNA level of *Nf-κb1* in the bladder. (P) Representative immunohistochemistry of Hsp90aa1, Hsp90ab1 and Hsp90b1 in the bladder, scale bar, 250 μm. (Q) The average optical density analysis results of Hsp90aa1, Hsp90ab1 and Hsp90b1 in the bladder. (R) Relative mRNA levels of *Hsp90aa1* in bladder tissues. (S) Relative mRNA levels of *Hsp90ab1* in bladder tissues. (T) Relative mRNA levels of *Hsp90b1* in bladder tissues. Data were presented as the mean ± SD. n=3 in M, N, P and Q, n=6 in A-L, O and R-T for each group. P-values were calculated using a two-tailed unpaired Student's t-test (*P<0.05, **P<0.01, ***P<0.001). MDA, malondialdehyde; GSH, glutathione; T-SOD, total superoxide dismutase.

DNA damage and preserve genomic stability, thereby playing an essential role in maintaining cellular health and preventing disease progression (56).

Through GO analysis and literature review, the present study concentrated on oxidative stress and the NF-κB signaling pathway. Anxiety boosts ROS by stimulating the neuroendocrine, autonomic and inflammatory systems (57). Excess ROS damages DNA, proteins and mitochondria, depletes SOD/GSH and raises MDA, IL-6, IL-1β and TNF-α (58-60). Oxidative stress switches on NF-κB, releasing inflammatory mediators and neurotransmitters that amplify anxiety. In bladder ischemia-reperfusion models, the same ROS burst sensitives bladder nerves and triggered overactivity (61). Our previous study found that both oxidative stress and NF-κB are involved in OAB models induced by a high-salt diet (62). The present study confirmed oxidative stress and NF-κB pathway activation in an anxiety-induced OAB model, aligning with previous findings.

To identify DEGs, a PPI network was constructed through data mining and integration. The present study found that the upregulated DEGs *Hsp*90aa1, *Hsp*90ab1, and *Hsp*90b1 were markedly elevated in the bladder tissues of the CRS group, confirmed by q-PCR, with increased protein levels.

Heat shock protein 90 (Hsp90) is a key molecular chaperone involved in protein folding, stability, and signal transduction. The main homologs in the Hsp90 family are Hsp90aa1, Hsp90ab1, and Hsp90b1 (63). Hsp90aa1, found on chromosome 14, encodes a chaperone that forms a homodimer to assist in protein folding using ATPase activity (64,65). Hsp90ab1 stabilizes various proteins in the cytoplasm, maintaining cell homeostasis under stress (66). Hsp90b1, located in the endoplasmic reticulum (ER), aids in protein folding and secretion, crucial during ER stress for maintaining protein balance (67). In cancers such as osteosarcoma and breast cancer, NF-κB, JAK/STAT and autophagy pathways often upregulate Hsp90 and fuel tumor growth (68-72). However, few studies connect Hsp90 to urologic diseases such as bladder cancer and acute kidney injury, with unclear mechanisms (73,74). While HSP90 is important in various diseases, its connection to OAB has not been reported until now. The present study is the first, to the best of the authors' knowledge, to show increased Hsp90 expression in an anxiety-induced OAB model, potentially linked to oxidative stress and the NF-κB signaling pathway. Perhaps in the future, we may be able to predict the occurrence and severity of OAB by detecting the content of HSP90 in the patient's bladder tissue or develop drugs that inhibit the expression of HSP90 to treat OAB.

The present study successfully identified anxiety as an independent risk factor for the onset of OAB for the first time to the best of the authors' knowledge, developed an *in vivo* model of anxiety-induced OAB, and made preliminary discoveries regarding the underlying mechanisms. However, several limitations must be acknowledged. First, the sample sizes collected from clinical settings were relatively small, limiting the generalizability of the findings. Although the overall sample (n=80) provided 88% power to detect a medium-to-large main effect of anxiety on OAB (partial $\rho^2 \geq 0.20$, $\alpha=0.05$), post-hoc analysis revealed that pairwise comparisons within the smallest subgroups (OAB without anxiety, n=7; controls with anxiety, n=5) remain under-powered (<50%). Consequently, negative findings in these strata should be interpreted cautiously and future prospective studies with pre-specified balanced allocation are warranted. Second, the conclusions derived from the animal model may not accurately represent the human condition, and it is recommended that tissue samples be obtained via cystoscopic biopsy to further validate these findings. Additionally, the results of the present study cannot conclusively prove the causality between anxiety and OAB. Further research using pharmacologic inhibition or genetic knockdown are needed to confirm causal relationship.

The present study was the first, to the best of the authors' knowledge, to identify anxiety as an independent risk factor for OAB, using *in vivo* experiments to create an anxiety-induced OAB model. Bioinformatic analyses, qPCR and IHC confirmed increased Hsp90 expression in the bladder. The study also suggested that anxiety-induced OAB activates oxidative stress and the NF-κB pathway. *Hsp90* could become a potential biomarker and target for diagnosing and treating anxiety-induced OAB, pending clinical trial confirmation.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author. The raw sequence data generated in the present study may be found in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2025), National Genomics Data Center (Nucleic Acids Res 2025), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences under accession number GSA: CRA034777 or at the following URL: https://ngdc.cncb.ac.cn/gsa/browse/CRA034777.

Authors' contributions

HH conceived and designed the study. ZZ and LX conducted experiments and prepared samples, with ZZ and HW handling RNA-seq analysis. ZZ and HW confirmed the authenticity of all the raw data. HW verified the data. ZZ and LX wrote the paper, while HH and HW offered critical advice and revised it extensively. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study received ethical clearance from the Institutional Review Board of Fujian Provincial Hospital, with the protocol number K2024-10-019. Informed consent was obtained in writing from all participants. Animal experiments

were performed at the Animal Research Center of the Nanfang hospital, following approval from the Institutional Animal Care and Use Committee (approval no. NFYY-2021-0572).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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