Article

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Effects of *PAR2* Gene Knockout on Visceral Sensitivity, Stress Behaviors, and Colonic Electrical Activities in Irritable Bowel Syndrome

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Background: Irritable bowel syndrome (IBS) could seriously affect the patient's health quality by its recurrence. There is a great medical and social need to explore the mechanisms and new treatment strategies of IBS.
Objective: To explore the influence of protease-activated receptor 2 (*PAR2*) gene knockout on IBS visceral sensitivity, stress behaviors, and colonic electrical activities.
Methods: The *PAR2* gene knockout and IBS model rats were generated and divided into $PAR2^{+/+}$ wild control (WC) group, $PAR2^{+/+}$ wild IBS model (WM) group, $PAR2^{-/-}$ knockout control (KC) group and $PAR2^{-/-}$ knockout IBS model (KM) group. The stress behaviors scores, minimum rectal fluid injection capacity threshold value of abdominal withdrawal reflex (AWR) = 3, and the colonic electrical activities indexes were recorded and the experimental results were analyzed statistically.
Results: (1) *PAR2* gene deletion and IBS models were successfully generated. (2) The scores of aggressive and exploratory behaviors in WM and KM groups were higher than WC and KC groups ($p < 0.05$). The grooming behavior scores in WC and KC groups were higher than the WM and KM groups ($p < 0.05$). WM group had the highest aggressive and exploratory behavior scores; WC group had the highest grooming behavior scores. (3) The minimum rectal fluid injection capacity threshold value of AWR = 3 in WM and KM groups were lower than WC and KC groups ($p < 0.05$); WM group had the lowest value. (4) The maximum amplitude and wave frequency of colonic fast and slow waves in WM and WC groups were greater than in the KM and KC groups, respectively ($p < 0.05$). The amplitude index and number of colonic contraction waves in WM and KM groups were greater than the WC and KC groups ($p < 0.05$). Colonic electrical activity indexes were highest in the WM group.
Conclusions: The *PAR2* gene deletion could have a beneficial effect on visceral sensitivity, stress behaviors and colonic electrical activities in rats with IBS.

Keywords: *PAR2*; IBS; visceral hypersensitivity; stress behavior; colonic electrical activity

Introduction

Irritable bowel syndrome (IBS) has many distinct biological features, such as abnormal visceral sensitivity, abnormal colonic electrical activities, and psychological abnormalities. One of the most important features is visceral hypersensitivity; its occurrence and developmental mechanisms are complex, involving a variety of altered biomolecules [1–7]. Protease-activated receptor 2 (*PAR2*) belongs to the G protein-coupled receptor family; it participates in many human pathophysiological processes, such as inflammation and pain through various signaling pathways. A number of human diseases are related to the imbalance of the *PAR2*-related signals, such as tumors, and skin, cardiovascular, and digestive diseases, making *PAR2* a hot spot of basic and clinical research in the medical field [8–11]. Research in IBS has shown that *PAR2* is related to an increase in IBS visceral sensitivity [12–14]. We found that the colon-positive expression of *PAR2* in IBS was greater than the control group after Nesfatin-1 administration in our previous research of IBS and visceral hypersensitivity in our preliminary experiment. Therefore, to clarify the relationship between *PAR2* and IBS and find out effective treatments to regulate the IBS symptoms, we studied the changes of visceral sensitivity, stress behaviors, and colonic electrical activities in IBS models with *PAR2* gene deletion. Using behavioral and electrophysiological methods, we probed into the influence of *PAR2* on visceral sensitivity, stress behaviors, and colonic electrical activities in IBS.

Animals, Materials and Methods

Animals and Materials

The electrophysiological detector was obtained from Taimeng (BL-420F, Chengdu, China). DNA Extraction kit (DP304) was purchased from Tiangen (Beijing, China).

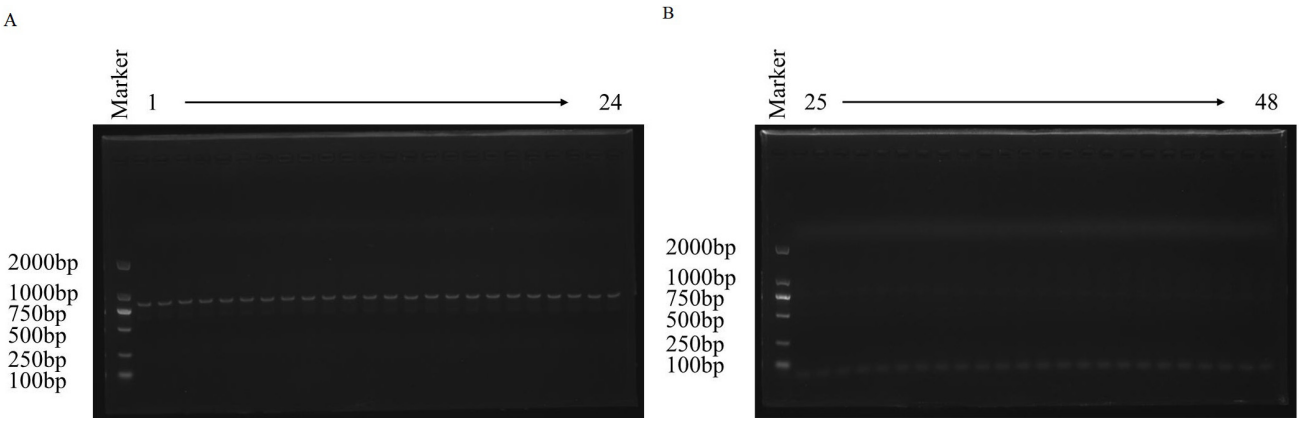

Fig. 1. Genotype identification. (A) Samples 1 to 24. (B) Samples 25 to 48. N = 48 per group.

Rats with global deletion of the *PAR2* gene ($PAR2^{-/-}$) and *PAR2* wild-type rats ($PAR2^{+/+}$) were purchased from Sai ye Model Biological Research Center Co., Ltd. (SCXK(SU) 2018-0003, Suzhou, China). The offspring rats were obtained by the breeding of male and female rats with *PAR2* gene deletion heterozygous ($PAR2^{+/-}$). The rats were genetically identified as $PAR2^{+/+}$, $PAR2^{-/+}$ and $PAR2^{-/-}$ rats; the $PAR2^{-/-}$ were selected for mating and generation, then the immature and successful model rats were obtained for the experiment. A total of 7 rats were not studied because of modeling failure or death.

Experiment Methods

Identification of the *PAR2* Genotype

The $PAR2^{-/-}$ and $PAR2^{+/+}$ rats were used for mating and reproduction. The required number of immature rats was obtained for the experiments. 0.3~0.4 cm of the tail was cut from the 3-week-old rats. Their DNA was extracted by alkaline lysis method; 1.5% agar gel electrophoresis was performed after PCR amplification. The genotype was determined according to the presence or absence of the expected bands.

Animal Groups and Establishment of IBS Models

(1) Control groups

The control groups were divided into $PAR2^{+/+}$ wild control group (WC group, n = 8) and $PAR2^{-/-}$ knockout control group (KC group, n = 8). The mice received 0.3 mL of 0.9% NaCl enema at 2 PM every day between the 8th and the 20th postnatal days. The rats were kept in a standard environment with room temperature of 22 °C~25 °C, humidity of 50%~60%, free drinking water, a standard diet, and a 12 h day/night cycle.

(2) IBS Model groups

The IBS Model groups were divided into $PAR2^{+/+}$ wild IBS model group (WM group, n = 8), $PAR2^{-/-}$ knockout IBS model group (KM group, n = 8). The IBS models were generated by maternal separation with 0.3 mL of 0.5% acetic acid enema; the models were validated through abdominal withdrawal reflex (AWR). The rats were kept in a standard environment with a room temperature of 22 °C~25 °C, humidity of 50%–60%, free drinking water, a standard diet, and a 12 h day/night cycle.

(3) Establishment of the IBS models

The IBS models were established by maternal separation combined with acetic acid enema, and the maternal separation method was used to introduce psychological stress and acetic acid enema to simulate local body stimulation in the pathogenesis of human IBS [15–18]. The specific methods were as follows. The offspring were separated from their mothers at 9:00~12:00 AM for 3 hours from the 2nd to the 21st postnatal days. Lactation was not allowed during the separation period. Acetic acid (0.5%, 0.3 mL) was given by enema once a day at 2 PM from the 8th to the 21st postnatal days. The dams were separated from the offspring on the 21st postnatal day. They were kept in a standard environment for 4 weeks.

Histological Identification

The experimental rats were euthanized after the experiments. A piece of colon was isolated and grossly observed. The tissues were fixed in 4% formaldehyde solution for 5 days, then trimmed, dehydrated, hyalinized, dipped in wax, embedded, sliced, baked, and stored in a 65 °C incubator for 12 hours. The tissues were then stored in boxes at room temperature and examined under a microscope.

Measurement of Emotional Stress Behaviors

The experimental rats were trained for 1 week, after 2 weeks of free feeding and adaptation. The rats were given water twice daily, from 9 AM to 9:20 AM and from 9 PM to 9:20 PM; water was withheld except these times. Then the rats received the emotional stress stimulation for 1 week. Emotional stress was induced by randomly giving empty bottles once daily for 10 minutes, instead of regular water to induce the rats' emotional stress response. A stress behavior was scored as 1 otherwise, 0 was recorded; the total score was recorded for each minute of emotional stress.

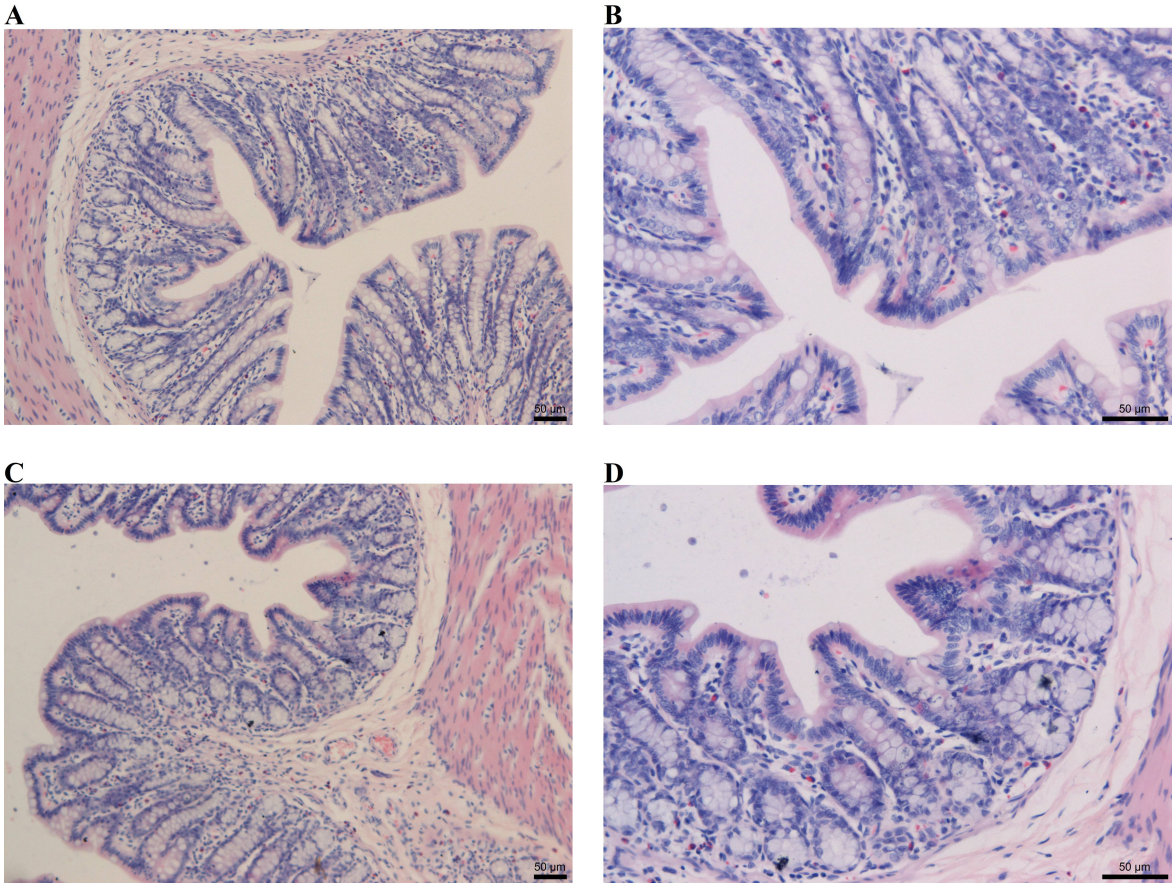

Fig. 2. HE staining of colonic tissue. (A) HE ×100 protease-activated receptor 2 ($PAR2$)$^{+/+}$ wild irritable bowel syndrome (IBS) model (WM) group. (B) HE ×200 (WM group). (C) HE ×100 $PAR2^{-/-}$ knockout IBS model (KM) group. (D) HE ×200 (KM group). N = 8 per group.

The total scores during the 1 week of emotional stress were recorded to quantify the emotional stress. The observed behaviors included aggressive behavior (biting or pushing against empty bottle and cage), exploratory behavior (moving from side to side and visiting the water bottle), and grooming behavior (grooming fur and washing face) [19].

Measurement of Visceral Sensitivity

The abdominal withdrawal reflex (AWR) score was used to assess visceral sensitivity [18]. The rats' rectums were dilated with a balloon by injecting 0.9% NaCl, the volume of the injected NaCl was from 0 to the volume of AWR = 3 gradually, then recorded the injection volume of AWR = 3; the balloon was kept for 10 s/per dilatation when AWR = 3. The rectum was dilated 3 times at 4 min intervals and collected the minimum rectal fluid injection capacity threshold value of AWR = 3. The calculated average value was the index for visceral sensitivity. The visceral sensitivity in the rats increased, which indicated that the IBS models were successfully established.

Measurement of Colonic Electrical Activities

The following indexes were measured and recorded by the electrophysiological function experiment system:

(1) The average maximum amplitude and frequency of fast wave: The 3 min curve with obvious waveform with regular waveform and maximum amplitude as one section in the colonic electrophysiological curve was taken. A total of 10 sections were collected; the average value of the maximum amplitude and wave frequency were taken as the average maximum amplitude and wave frequency of the colonic fast wave.

(2) The average maximum amplitude and wave frequency of slow wave: The 3 min curve had an obvious waveform, regular waveform, and minimum amplitude as one section in the colonic electrophysiological curve. A total of 10 sections were collected. The average value of maximum amplitude and wave frequency were taken as the average maximum amplitude and wave frequency of colonic slow wave.

(3) The average amplitude index and wave number of contraction wave: The 3 min curve with obvious waveform and regular waveform as one section in the colonic electrophysiological curve was taken. The total number of

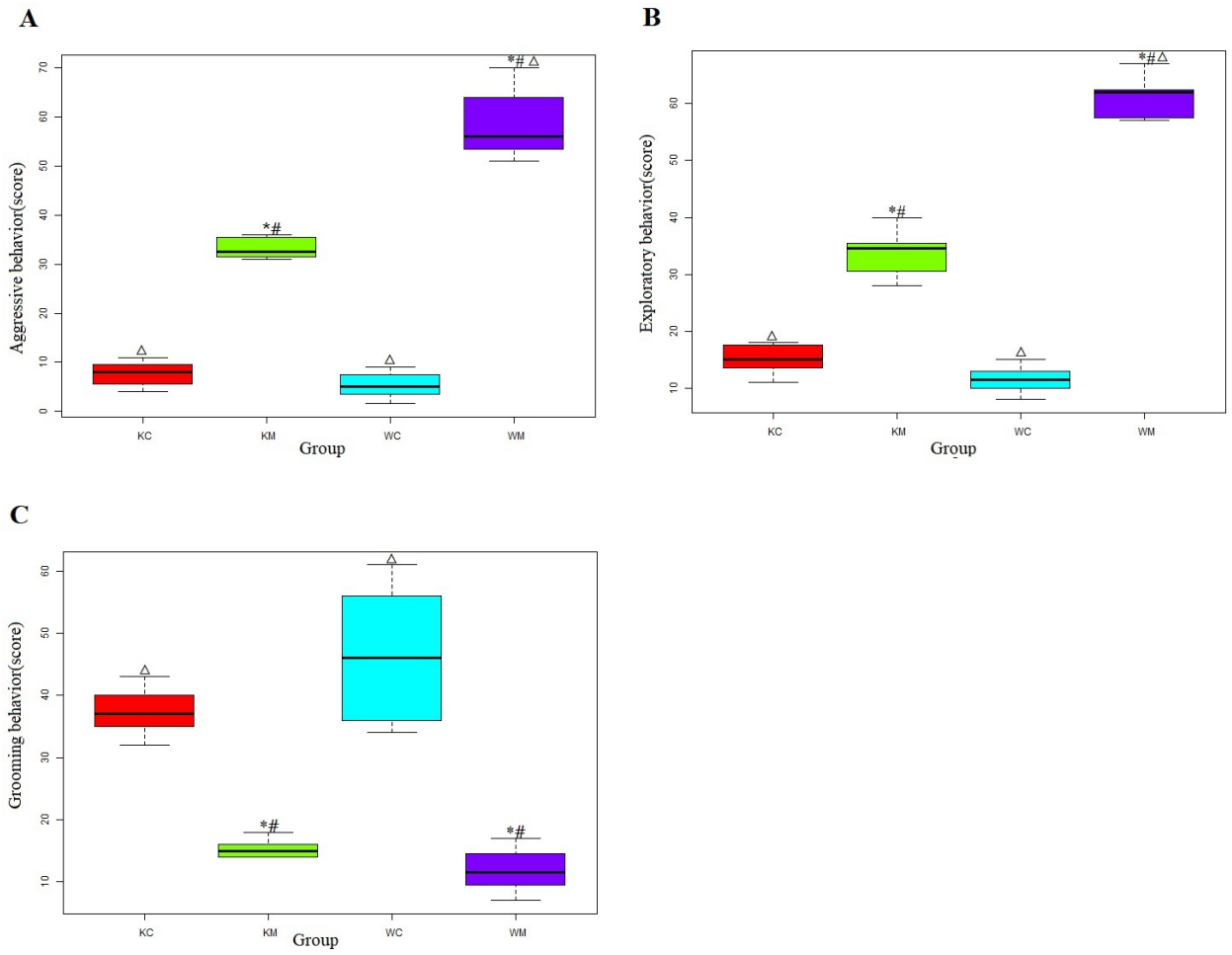

Fig. 3. Comparison of emotional stress behaviors. (A) Aggressive behavior. (B) Exploratory behavior. (C) Grooming behavior. N = 8 per group. $^{*}p < 0.05$, compared with the $PAR2^{-/-}$ knockout control (KC) group, $^{\#}p < 0.05$, compared with the $PAR2^{+/+}$ wild control (WC) group, $^{\triangle}p < 0.05$, compared with the KM group.

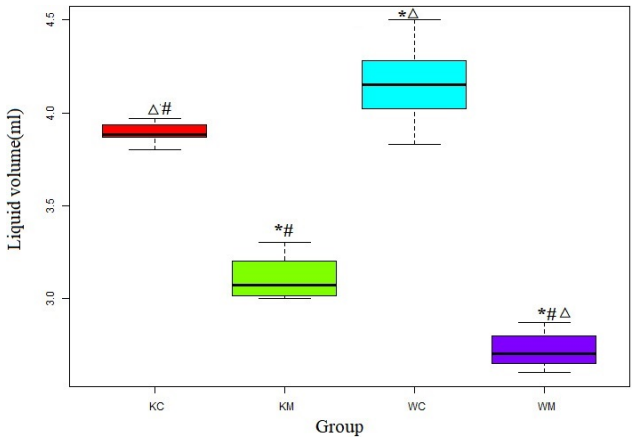

Fig. 4. Comparison of minimum rectal fluid volume threshold value to reach abdominal withdrawal reflex score = 3. N = 8 per group. $^{*}p < 0.05$, compared with the KC group. $^{\#}p < 0.05$, compared with the WC group. $^{\triangle}p < 0.05$, compared with the KM group.

contraction waves in this section was calculated; the amplitude index was the sum of all contraction wave amplitudes within 3 min. A total of 10 sections were collected, and the average value of contraction wave and amplitude index were taken as the number and amplitude index of the colonic contraction wave.

Anesthesia and Euthanasia Methods

For the experiments, anesthesia was induced by the intraperitoneal injection of 1% pentobarbital sodium (3 mg/100 g body weight). At the end of the experiment, the rats were euthanized by cervical dislocation.

Statistical Analysis

The data processing and analysis were performed by the statistical package EmpowerStats 5.0 (http://www.empowerstats.com/; X&Y Solutions, Inc., Boston, MA, USA).

The experimental data are presented as the mean ± standard deviation. The different groups were compared using one-way analysis of variance. $p < 0.05$ was considered statistically significant.

Results

Identification of PAR2 Gene Deletion Rats

The rat's genotype was identified (Fig. 1A,B), according to the agar gel electrophoresis; samples 1 to 24 are wild-type rats whereas samples 25 to 48 are homozygous rats.

Histological Identification

The WM and KM groups' colonic tissues were grossly normal; the tissues showed no obvious dilation, congestion, and other pathological changes. HE staining showed that the tissues had intact mucosa, submucosa, muscle, and serous layers, as well as the villus structure. They had no pathological changes, such as inflammation, erosion, ulcers, or abnormal cells (Fig. 2A–D).

Effects of PAR2 Gene Deletion on Non-Stress and Emotional Stress Behaviors in Rats

The observation of the general behaviors under the no-stress stimulation condition showed that the rats in WC and KC groups had normal daily performances, smooth hair, white color, lively, regular eating habits, and formed stool. The rats in WM and KM groups had normal daily activities, slightly coarse hair, white color, reduced food intake, and thin stool occasionally. The observation of the stress behaviors in all groups under the emotional stress stimulation condition showed that the scores of aggressive behaviors in WC, KC, WM, and KM groups were 5.32 $\pm$ 2.53, 7.63 $\pm$ 2.56, 58.5 $\pm$ 6.85, and 33.13 $\pm$ 5.08, respectively. The exploratory behavior scores of WC KC, WM, and KM groups were 11.50 $\pm$ 2.20, 15.13 $\pm$ 2.53, 61.00 $\pm$ 3.46 and 33.63 $\pm$ 4.03, respectively. The grooming behavior scores of WC, KC, WM, and KM groups were 46.38 $\pm$ 10.56, 38.13 $\pm$ 5.49, 11.88 $\pm$ 3.39, and 14.50 $\pm$ 2.93, respectively. Aggressive behavior was not significantly different between the WC and KC groups but there were significant differences among the other groups ($F = 233.10$, $p < 0.05$). Exploratory behavior comparison showed that the WC and KC groups were not significantly different, but there were significant differences in the WM and KM groups ($F = 414.75$, $p < 0.05$). Grooming behavior was similar between the WM and KM groups and between the KC and WC groups but less in the former than the latter groups ($F = 58.20$, $p < 0.05$) (Fig. 3A–C).

Effects of PAR2 Gene Deletion on Visceral Sensitivity in Rats

The minimum rectal fluid injection capacity of AWR = 3 threshold value was determined by the AWR scoring standard. The results showed that the minimum rectal fluid injection capacity threshold values of AWR = 3 in WC, KC, WM and KM groups were 4.15 $\pm$ 0.21 mL, 3.89 $\pm$ 0.06 mL, 2.72 $\pm$ 0.09 mL, and 3.12 $\pm$ 0.11 mL, respectively. There were significant differences among all groups, the minimum rectal fluid injection capacity of WC and KC were higher than WM and KM, respectively ($F = 203.94$, $p < 0.05$) (Fig. 4).

Effects of PAR2 Gene Deletion on Colonic Electrical Activities in Rats

Effects of *PAR2* Gene Deletion on Colonic Fast Wave in Rats

The fast wave maximum amplitude of the WC, KC, WM, and KM groups were 16.72 $\pm$ 0.71 µV, 12.42 $\pm$ 0.99 µV, 31.79 $\pm$ 1.91 µV, and 22.87 $\pm$ 2.20 µV, respectively. The fast wave frequency of WC, KC, WM, and KM groups were 5.30 $\pm$ 0.31 beats/min, 4.34 $\pm$ 0.40 beats/min, 7.75 $\pm$ 0.39 beats/min, and 5.55 $\pm$ 0.26 beats/min, respectively. The differences in fast wave maximum amplitude among all groups were significant ($F = 226.61$ $p < 0.05$). The fast wave frequency had no significant difference between the WC and KM groups, while the KC group had the lowest fast wave frequency and WM had the highest fast wave frequency ($F = 139.24$, $p < 0.05$) (Fig. 5A,B).

Effects of *PAR2* Gene Deletion on Colonic Slow Wave in Rats

We observed the slow wave electrical activities in all groups, the results showed that the slow wave maximum amplitude of WC, KC, WM, and KM groups were 158.11 $\pm$ 2.34 µV, 130.12 $\pm$ 1.72 µV, 179.42 $\pm$ 5.56 µV, and 152.42 $\pm$ 2.30 µV, respectively. The slow wave frequency of WC, KC, WM, and KM groups were 4.23 $\pm$ 0.22 beats/min, 3.05 $\pm$ 0.19 beats/min, 6.20 $\pm$ 0.26 beats/min and 4.77 $\pm$ 0.17 beats/min, respectively. The slow wave maximum amplitude was different among the groups ($F = 294.76$, $p < 0.05$). The slow wave frequency among all groups showed that WC and KM groups was not significantly different from each other, while the KC group had the least and the WM group had the most slow wave frequency ($F = 299.70$, $p < 0.05$) (Fig. 6A,B).

Effects of *PAR2* Gene Deletion on Colonic Contraction Wave in Rats

We observed the contraction wave electrical activities in all groups. The results showed that the contraction wave amplitude index of WC, KC, WM, and KM groups were 5.54 $\pm$ 0.36, 4.69 $\pm$ 0.20, 9.68 $\pm$ 0.43, and 6.01 $\pm$ 0.33, respectively. The contraction wave number of WC, KC, WM, and KM groups were 3.33 $\pm$ 0.35 beats/3 min, 2.97 $\pm$ 0.08 beats/3 min, 6.54 $\pm$ 0.41 beats/3 min, and 4.57 $\pm$ 0.35 beats/3 min, respectively. The differences in the contraction wave amplitude index among all groups were significant ($F = 358.35$, $p < 0.05$). The contraction wave number had no significant difference between the WC and KC groups, while the KC group had the least and the WM group had the most contraction wave number ($F = 199.56$, $p < 0.05$) (Fig. 7A,B).

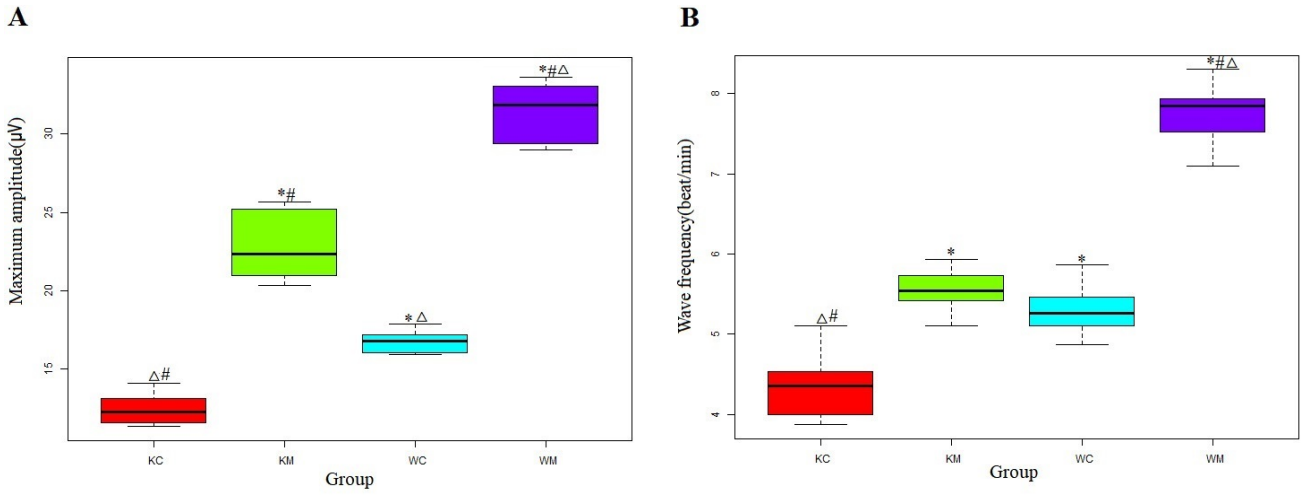

Fig. 5. Comparison of fast wave electrical activity. (A) The maximum amplitude of fast wave. (B) Frequency of fast wave. N = 8 per group. $^{*}p < 0.05$, compared with the KC group, $^{\#}p < 0.05$, compared with the WC group, $^{\triangle}p < 0.05$, compared with the KM group.

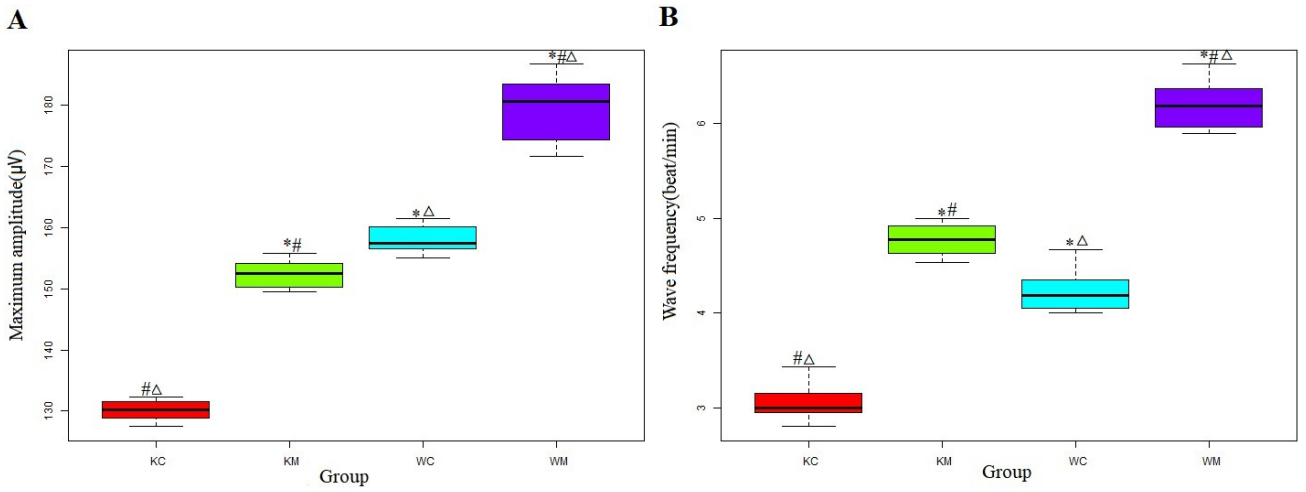

Fig. 6. Comparison of slow wave electrical activity. (A) The maximum amplitude of slow wave. (B) Frequency of slow wave. N = 8 per group. $^{*}p < 0.05$, compared with the KC group, $^{\#}p < 0.05$, compared with the WC group, $^{\triangle}p < 0.05$, compared with the KM group.

Discussion

The IBS morbidity has recently increased, and its repeated and prolonged course tormented the patients, even inducing the patients to become anxious and depressed, which seriously influenced the patients' health quality, daily work, and study; these also could lead to tremendous waste in health-care resources [20,21] There were many studies on the pathogenesis of IBS; some researchers have found that *PAR2* is strongly associated with IBS [22–33]. *PAR2* is widely expressed in epithelial, smooth muscle, and other cells. And *PAR2* can induce neurogenic pain through reception of the sensory neurons excitation. More and more research has focused on the relationship between *PAR2* and IBS [21–32]. We aimed to clarify the details of the relationship between *PAR2* and IBS and observe the role of *PAR2* gene on visceral sensitivity, stress behaviors, and colonic electrical activities in IBS. To this end, we generated rats with global knockout of *PAR2* gene by clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9) molecular biological technique. Then, we combined the mental stimulation method with physicochemical stimulation method to generate the $PAR2^{+/+}$ IBS and $PAR2^{-/-}$ IBS rat models. The *PAR2* gene of $PAR2^{-/-}$ rats has been identified. The colons of rats in the WM and KM groups were grossly normal in appearance with no obvious dilation, congestion, and other pathological changes. HE staining showed that the colon had intact mucosa, submucosa, muscle, and serosal layers and villus, with no inflammation, erosion, ulcers, and other pathological changes. The visceral sensitivity of the experimental rats was measured by AWR = 3 minimum rectal liquid injection capacity threshold value. The results showed that the threshold value was highest in the WC group and lowest in the WM group, and the threshold values were significantly different among WC, KC, WM, and KM groups.

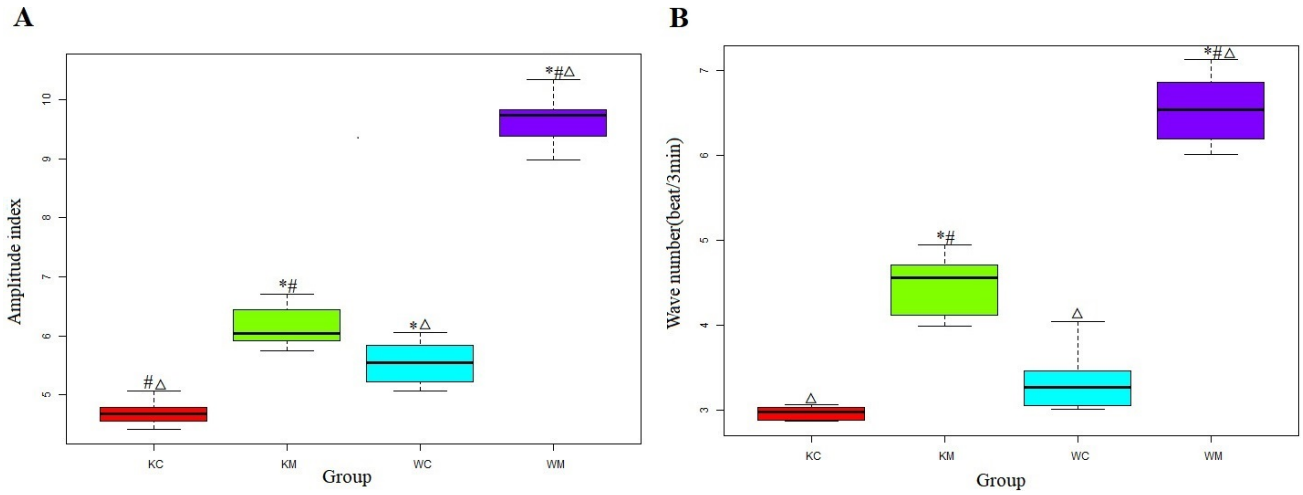

Fig. 7. Comparison of contraction wave electrical activity. (A) Amplitude index of contraction wave. (B) Wave number of contraction wave. N = 8 per group. $^{*}p < 0.05$, compared with the KC group, $^{\#}p < 0.05$, compared with the WC group, $^{\triangle}p < 0.05$, compared with the KM group.

The above experimental results showed that the IBS models were successfully established in the WM and KM groups, without visible pathological changes which could have led to an increase in visceral sensitivity. And the threshold values were significantly decreased in WM and KM groups, which proved that they had developed the visceral hypersensitivity phenomenon. Thus, our models developed gastrointestinal diseases, which had the typical characteristics, proving that our models were successfully generated.

In 1973, Ritchie proposed, for the first time, a hypothesis of visceral pain sensitivity [33]. Since then, visceral sensitivity has been a research hotspot in the IBS pathophysiological process. Some studies showed that *PAR2* activation can cause intestinal paresthesia. Based on this observation, we studied visceral sensitivity variation in the *PAR2* knockout IBS models; the studies in $PAR2^{+/+}$ rats showed that the minimum rectal fluid injection capacity threshold value of the WC group was higher than WM group, indicating that the rats in WM group had higher visceral sensitivity. KC group had a minimum rectal fluid injection capacity threshold value that was higher than the KM group, indicating that the KM group had higher visceral sensitivity. In the control group, the minimum rectal fluid injection capacity threshold value in the WC group was higher than KC group, indicating that KC group had higher visceral sensitivity than the WC group. In the model group, the minimum rectal fluid injection capacity threshold value in the KM was higher than that in WM group, indicating that the rats in WM group had higher visceral sensitivity than the KM group. Thus, we found that the visceral sensitivity of KC group with *PAR2* deletion increased significantly, and the visceral sensitivity of KM group with *PAR2* deletion reduced significantly. We, therefore, speculated that *PAR2* could play a key role in the development of visceral sensitivity formation, and positively regulate visceral sensitivity in IBS rats. Li *et al.* [21] found that metformin can down-regulate *PAR2* through blockade of mast cell activation and reduce IBS rat's visceral hypersensitivity. Du *et al.* [34] also found that *PAR2* can lead to immune activation and visceral hypersensitivity in mice with post-infectious IBS by increasing intestinal permeability. Xu *et al.* [35] also found that the down-regulation of tryptase (*TPSP*)/protease-activated receptor 2 (*PAR2*)/substance (*SP*)/calcitonin gene related peptide (*CGRP*) signals in post-infectious-IBS rats can alleviate visceral hypersensitivity symptoms. These studies have demonstrated that *PAR2* is closely related to the degree of visceral sensitivity in IBS in different experimental situations, which are consistent with our experimental results.

Our current study determined the effect of *PAR2* deletion on IBS behavior variation from a behavioral perspective. Under the no-stress stimulation condition, we observed that the rats in WC and KC groups had normal daily activities such as eating, drinking, and stool formation whereas the WM and KM groups rats had reduced food intake and had occasionally thin stools. It could be seen that the control group and the model group have basically normal daily behavior activities without stress stimulation. The variation comparison of the aggressive, exploratory, and grooming behaviors under the empty bottle-induced emotional stimulus stress can be summarized as follows: the $PAR2^{+/+}$ rats had aggressive and exploratory behaviors in the WC group that were lower than the WM group, and the scores of grooming behaviors in the WC group were higher than the WM group. In the $PAR2^{-/-}$ rats, the scores of aggressive and exploratory behaviors in the KC group were lower than that in the KM group, and the scores of grooming behaviors were higher in the KC group than that in the KM group. In the control rats, the scores of aggressive were similar but the exploratory behaviors were slightly lower in the

WC than in the KC group. The scores of grooming behavior were slightly higher in the WC than the KC group, but the difference was not significant. The scores of aggressive and exploratory behaviors were higher in the WM group than KM group. The scores of grooming behavior in the WM group were slightly lower than the KM group. Thus, it could be seen that the KC group with *PAR2* deletion has no obvious abnormalities in no-stress behavior, but with regards to the emotional stress behaviors, the aggressive and exploratory behaviors in the *PAR2* deletion group of KC and KM groups increased greatly, and the grooming behaviors decreased. We speculate that *PAR2* participates in the regulation of emotional stress behavior in IBS rats, which could increase the aggressive and exploratory behaviors, and reduce the grooming behavior. The empty bottle stimulation was based on the notion that giving an empty bottle to rats causes a chronic emotional response, and the rats' emotional response variation under stress condition through the aggressive, exploratory and grooming behaviors were observed. IBS patients often have anxiety, depression, and other psychological disorders. The anxiety and depression of IBS rats could be manifested as aggressive, exploratory, grooming, scratching, and other abnormal behaviors, which could reflect their abnormal psychology, indirectly [36–39]. Zhao *et al*. [39] found that the scratching behavior was relevant to the *PAR2*. Chao G *et al*. [37] found that *PAR2* could influence the IBS clinical symptoms and psychological manifestations by up-regulating *PAR2*. These publications are consistent with our findings. We speculate that *PAR2* may participate in the regulation of IBS stress behaviors, which is related to anxiety, depression, and other psychological disorders. However, the lack of quantitative scoring for the rats' general behaviors in our experimental design and effects of other stress stimuli were not observed. In view of these limitations, it is necessary to adjust the design and verify the results from other research perspectives.

Finally, our experiment studied the effect of *PAR2* deletion on IBS from the perspective of colon electrophysiology; the results of $PAR2^{+/+}$ IBS rat studies showed that the fast wave maximum amplitude, fast wave frequency, slow wave maximum amplitude, slow wave frequency, contraction wave amplitude index and contraction wave number were all lower in the WC group than WM group. The colonic electrical activity in WM group was obviously abnormal, the $PAR2^{-/-}$ IBS rat studies showed that the fast wave maximum amplitude, fast wave frequency, slow wave maximum amplitude, slow wave frequency, contraction wave amplitude index, and contraction wave number were all lower in the KC group than the KM group. However, the colonic electrical activity in KM group was obviously abnormal. The fast wave maximum amplitude, fast wave frequency, slow wave maximum amplitude, slow wave frequency, contraction wave amplitude index and contraction wave number in the WC group were higher than the KC group, but the contraction wave number difference was not significant between them. The model group results showed that the fast wave maximum amplitude, fast wave frequency, slow wave maximum amplitude, slow wave frequency, contraction wave amplitude index and contraction wave number were all higher in the WM group than the KM group, but the difference of fast wave maximum amplitude, fast wave frequency, slow wave maximum amplitude and slow wave frequency were not significantly different between WC and KM groups. Thus, it could be seen that the fast wave maximum amplitude, fast wave frequency, slow wave maximum amplitude, slow wave frequency, contraction wave amplitude index, and contraction wave number were lower in *PAR2* gene deletion groups than *PAR2* gene groups. The colonic electrical activity is an electrophysiological index which reflects the colonic physiological function, under physiological conditions; the rat's colonic motility is composed of I, II, III, and IV different phases of periodic electrical activity. The colonic electrical activity at different time phases is composed of fast wave, slow wave, and contraction wave with different characteristics, which in turn regulate the rhythm of the colon smooth muscle movement. The fast waves, slow waves, and contraction waves becoming disordered indicate the dysfunction of colonic motion. These could lead to the disharmony of intestinal contraction movement and cause weakness of contraction of the colon. The colonic peristalsis disorder and other manifestations eventually cause abdominal pain, abdominal discomfort, and other abdominal symptoms. Many studies have confirmed that the disorders of colonic motility and electrical activity are common in rats with visceral hypersensitivity models, but their regulatory mechanisms are not incompletely clear. Some studies have shown that *PAR2* activation depends on the sensory nerve pathways, NK1 and NK2 receptor pathways to participate in the movement of colon smooth muscle. In vitro studies have found that the *PAR2* activators could inhibit the rat colon circumferential muscle, contract the colon longitudinal muscle, or play a biphasic effect on initial excitation and later inhibition [40–42]. From the results of the above experiments, we could also speculate that *PAR2* gene participates in the regulation of colonic electrophysiological activities in rats with IBS, and it could negatively regulate IBS colonic fast and slow contractions. These studies have confirmed that *PAR2* is related to the colonic movement in IBS. The mechanism may be achieved by interfering with the colonic electrophysiological activity. However, the specific mechanism of *PAR2* gene involvement in the colon electrophysiology of IBS is still unclear. Therefore, it is necessary to perform further studies in the future.

Conclusions

In the view of visceral sensitivity, stress behavior, and colonic electrical activity perspectives, we found that in the IBS models in rats, the *PAR2* gene can negatively regu-

late visceral sensitivity and colon electrical activity; it could also increase the occurrence of aggressive and exploratory behaviors and decrease grooming behavior under conditions of stressful stimulation, at the same time. We speculated that *PAR2* gene plays a significant regulatory role in visceral sensitivity, stress behavior, and colonic electrical activity in an IBS rat model through its related signaling pathway. Because the pathogenesis of IBS involves the interaction of many complex factors, we still need to determine the specific mechanisms and signaling pathways of *PAR2* gene involvement in the regulation of visceral sensitivity, stress behavior, and colonic electrical activity in IBS. The abnormal changes of visceral sensitivity, stress behavior and colonic electrical activity in IBS mediated by *PAR2* need to be studied further, in order to elucidate the specific role and mechanisms of *PAR2* gene and its related molecules in the occurrence and progression of IBS. Meanwhile, these would provide valuable new ideas for finding the effective therapeutic targets for IBS.

Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author [Qiaoyan Gu], upon reasonable request.

Author Contributions

Conceptualization, QG; methodology, QG and HZ; software, validation, TH, YL and JL; formal analysis, QG and YL; investigation, QG and HZ; resources, JL and SS; data curation, TH, JL and SS; writing—original draft preparation, QG, HZ and TH; writing—review and editing, QG; visualization, QG and TH; supervision, QG and HZ; project administration, QG; funding acquisition, QG. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Our experiment was approved by the Animal Ethics Association of Yanan University Affiliated Hospital (Ethics No.2017-14).

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Conflict of Interest

The authors declare no conflict of interest.

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