

**mRNA Expression of aquaporin1,7,8 in colonic mucosa of rat models with slow transit constipation**Li Zhen<sup>1</sup>, Zhang Shuijun<sup>2</sup>, LI Guobin<sup>1</sup>, ZHI Hui<sup>1</sup>, Wang Guixian<sup>1</sup>, Song Junmin<sup>1</sup>, Yuan Weitang<sup>1\*</sup>.<sup>1</sup>Department of Colorectal and Anal Surgery, Surgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China<sup>2</sup> Department of Hepatobiliary Surgery, Surgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China\*Corresponding author. E-mail: [dryuanweitang@126.com](mailto:dryuanweitang@126.com).

**Abstract: BACKGROUND:** The relationship between melanosis coli (MC) and aquaporin (AQP) has not yet been elucidated. The aim of this research was to investigate the relationship between the expression of aquaporin 1,7,8 and the pathological mechanism of MC. **METHODS:** A rat model of slow transit constipation was designed, and the correlated changes of aquaporin protein 1, 7 and 8 at the mRNA level were examined. The rat model of slow transit constipation was produced with diphenoxylate administration. The mRNA expression of AQP1 and AQP 7 from STC rats and control rats were examined with reverse transcription polymerase chain reaction (RT-PCR). AQP1 was expressed in ascending and descending colon of both control and STC rats. **RESULTS:** For the ascending colon, the AQP1 grayscale ratio in control and STC rats were  $0.546 \pm 0.064$  and  $0.279 \pm 0.074$  ( $P < 0.05$ ), respectively; these were  $0.574 \pm 0.075$  and  $0.571 \pm 0.078$  ( $P > 0.05$ ), respectively in descending colon. AQP7 was also expressed in ascending and descending colon of both control and STC rats, with  $0.495 \pm 0.053$  and  $0.503 \pm 0.060$  in ascending colon ( $P > 0.05$ ), and  $0.521 \pm 0.082$  and  $0.522 \pm 0.062$  in descending colon ( $P > 0.05$ ), respectively; but AQP8 had little expression in both proximal and distal colon of trial and control groups. **Conclusions:** Expression of AQP1 in proximal colon of rats with STC was down-regulated, and it may play a regulation role in water absorption; the expressions of AQP7 and AQP8 had a little alteration in proximal and distal colon of both groups.

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**Introduction**

Constipation is a common clinical symptom, which presents defecation reduces, difficult defecation, dry feces with little defecation, which influences the quality of life severely. As the aging population coming, constipation is attracting more attention as tumor, but it is still at the exploratory stage when dealing with constipation. Constipation is induced by many factors, including gastrointestinal disease, and other system disease in relation to gastrointestinal disease; many kinds of drug also induce constipation. Functional constipation (FC) is in the majority in constipation. For the diagnosis of functional disease, firstly organic disease and drug-induced disease must be excluded. Detailed clinical history, physical examination, and the examination of laboratory, iconography can provide important evidence for the diagnosis of constipation. After the organic disease is excluded, functional constipation can be divided into Slow transit constipation (STC), Outlet obstructive constipation (OOC) and MIX according to the examination of intestinal motility and anorectal function. STC is a type of obstipation what feature are weaken of the gastrointestinal power and the lengthen of the time of slow transit of the content in

gastrointestinal tract. Some researches on pathogenesis about STC reveal that STC is induced by many factors including the degeneration or reduction of enteric nerve, the degeneration of smooth muscle, the exception of gastrointestinal hormone, unreasonable diet, the degeneration of cajal pacemaking cell and the factors of mental and hereditary et al. AQPs is a kind of major intrinsic protein [1]; More and more researches on many systems found that the pathophysiology process of many diseases had related to AQPs, including cataract, cerebral edema, nephrogenic diabetes insipidus (NDI), Sjogren syndrome, pneumoedema, hepatocirrhosis et al. So it provided new target spot for treatment of some diseases. Reported in the literature, AQP1,3,4,7, 8 are existing in the colon and has deep relationship with the water absorption and secretion in colon [2-5] Slow transit constipation (STC) is characterized by slow transiting of gastrointestinal contents. In the process of formation, there is a change in water absorption and secretion in the colon [6-7]. It is unclear the change of expression of AQP in STC colon. The role of AQP in the process of formation of constipation is unclear too. This study established STC rat model. RT-PCR was used to measure the expression of AQP mRNA in the STC rat model. According to this, we investigated the

relationship between AQP and STC.

## Materials and Methods

### Animals

Clean grade rats (32: male or female, weight 150 g) from Zhengzhou University Experimental Animal Research Center were randomly assigned into STC group and control group with 16 rats in each. After 1 week of adaptation to the environment, the animals were subjected to intragastric administration of distilled water at 0.32 ml/kg for 5 days for adaptation to the experimental manipulations to the gastrointestinal system. The experiments have been approved by animal research committee in Zhengzhou University. During the experiment, the STC group was fed with rat chow containing diphenoxylate (Tianlei, He Nan) and the control group was fed with normal chow. The content of diphenoxylate was pre-adjusted according to the daily food consumption to have 8 mg/kg body weight (BW). For the monitoring of the successful model (every 5 days), the animals were fasted from food, but not water for 12 h, then allowed free food intake for 30 min before the intragastric administration of ink. The successful modeling was determined by significant differences ( $P < 0.01$ ) of time length it took to find the first black particle from excretion between control and STC groups. The diphenoxylate was removed from the food chow and the difference could last for another week ( $P < 0.01$ ).

### Reverse transcription polymerase chain reaction (RT-PCR)

After the successful establishment of the model, the rats were fasted from food, but not water for another 12 h. Then the animals were sacrificed and the ascending as well as descending colon were obtained, cleaned with saline and frozen in liquid nitrogen. The total RNA was extracted with TRIZOL kit (Shenggong, Shanghai), and then transcribed into cDNA with reverse transcription kit (Shenggong, Shanghai) following the quality examination (Shenggong, Shanghai). The RNA purity was determined by

absorbance measurement A260/A280, with the value of 1.97. The primers for AQP1 and AQP7 was designed and synthesized by Shenggong Bio Co (Shanghai). The primers for AQP1: upstream: 5'-AAAG TGGCAAGGAAGG GACA-3' and 5'- GCTG TG GATGTTGGGAA AGAG-3' (705 kp). The primers for AQP7: upstream: 5'-GTCA TGGCGAAGAGACA CAGC-3'; downstream: 5'-GTGA TGGCGAAGAGAC AGAGC -3' (537 bp). AQP8 upstream: 5'-ATGCTCTTGGTATTG GCTGTGTG-3'; downstream: 5'-CCTGTCTCCTTTTCTA TGTGG T-3'; (556bp); The internal control was beta-actin.  $\beta$ -actin upstream: 5'-AGAG GGAAATCGTGCGTGAC-3'; downstream: 5'-GGACTCATCGT AC TC CTG CTTG-3'; (482bp). The reaction conditions: 94°C for 3 min; 94°C for 30 s, Tm (AQP1 58°C, AQP7 56°C, AQP8 60°C and beta-actin 61°C) for 30s, 72°C for 1 min for 35 cycles; extension at 72°C for 5 min. The PCR products were collected and stored in the fridge.

PCR products of 5  $\mu$ l with 1  $\mu$ l 5X loading buffer were put on 2% agarose gel electrophoresis at 120 V for 30 min before ethidium bromide staining and imaging with the gel imaging system (BioXtal, US).

### Statistics

The data were represented as mean  $\pm$  standard deviation (SD) and then was analyzed by Statistical Package for Social Sciences (SPSS) 13.0 software (Chicago, US). T test was used to examine the differences between two groups and  $P < 0.05$  was determined as statistically significant. AQP8 in the STC group and the control group had a small amount of expression or no expression.

### RESULTS

The RT-PCR results of AQP1 expression in ascending and descending colon of both groups are as shown in Table 1 and Figure 1. In ascending colon of STC group animals, the AQP1 expression was lower than control group ( $P < 0.05$ ).

**Table 1.** The AQP1 gray scale ratio in STC and control groups ( $x \pm s$ ).

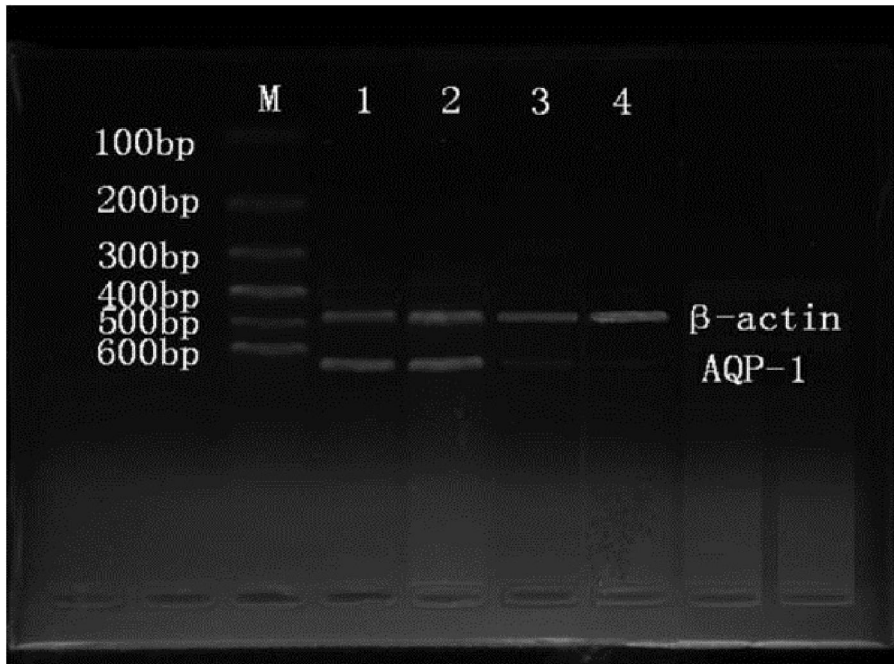
Group	n	Ascending colon	Descending colon	t	P
STC	16	0.279 $\pm$ 0.074	0.571 $\pm$ 0.078	-10.861	<0.05
Control	16	0.546 $\pm$ 0.064	0.574 $\pm$ 0.075	1.144	>0.05
t		-10.793	0.127		
P		<0.05	>0.05		

The RT-PCR results of AQP7 expression in ascending and descending colon of both groups are as shown in Table 2 and Figure 2. There were no significant differences between two groups, both ascending and descending colons ( $P > 0.05$ ).

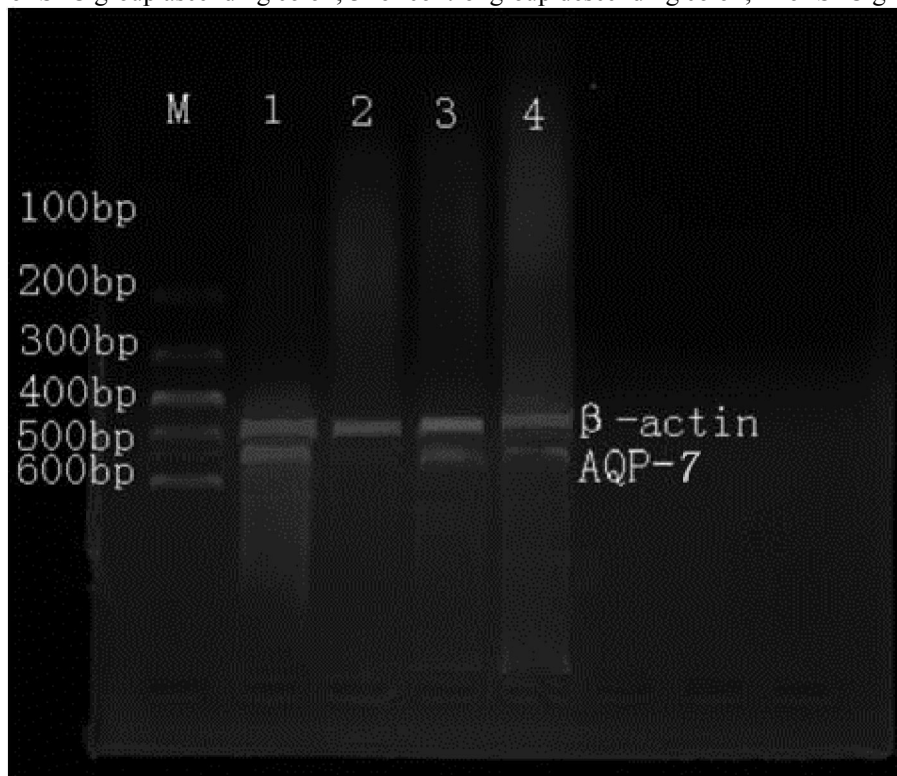
**Table 2.** The AQP7 gray scale ratio in STC and control groups ( $x \pm s$ ).

Group	n	Ascending colon	Descending colon	t	P
STC	16	0.503 $\pm$ 0.060	0.522 $\pm$ 0.062	-0.897	>0.05
Control	16	0.495 $\pm$ 0.053	0.521 $\pm$ 0.082	0.049	>0.05
t		0.366	-1.041		
P		>0.05	>0.05		

附图



**Figure 1.** The RT-PCR results of AQP1 expression. M represents the marker; 1 for control group ascending colon; 2 for STC group ascending colon; 3 for control group descending colon; 4 for STC group descending colon



**Figure 2.** The RT-PCR results of AQP7 expression. M represents the marker; 1 for control group ascending colon; 2 for STC group ascending colon; 3 for control group descending colon; 4 for STC group descending colon

## DISCUSSION

Constipation results from the increasing stress in

current society and could contribute to the development of some severe cardiovascular disease disorders. The

understanding in the pathological mechanisms of constipation is therefore important to improve the life quality of these patients. This study employed the rat model of STC induced by diphenoxylate administration, in order to observe the potential changes in water channel protein expressions of the colon. Diphenoxylate is composed of hydrochloride diphenoxylate and atropine sulfate, which could act on intestinal opioid receptors to inhibit the movement of gastrointestinal smooth muscle, and finally lead to the delayed emptying. The current raSTC model shows neither changes in physiological functions, nor the food consumption process, with high clinical relevance. Moreover, it has been found that the changes in opioid peptide activity were involved in the pathogenesis of STC [8-9].

The expression changes of AQPs can be regulated upon the slowdown of the movement of gastrointestinal smooth muscle, and has been at least partially attributed to the dysfunction of ascending and descending colons. This could either be directly resulted from the pharmacological administration of diphenoxylate compound, or indirectly modulated by the movement of the smooth muscle as a compensatory mechanism. We believe that it was the latter case as there were differences between ascending and descending colon at the same time. After the slowdown of the transportation, the expression of AQP1 was downregulated in the ascending colon to reduce the water re-absorption, which facilitates the intestinal transportation. This is consistent with results obtained in a previous study showing that with 80% removal of the small intestine, the AQP1 expression in the remaining small intestine and colon increased to enhance the water reabsorption [10]. The expression of AQP7 was not changed in this study, and its role in constipation is yet to be investigated in further studies. The current research literature on colon AQP8 is still rare. Yang [11] who took the advantage of AQP8 knockout rats found that the intestinal metabolism of the liquid did not change significantly. This study didn't observe significant expression of AQP8 in the rat colon in the two groups. That may need further study.

One limiting factor in this study is that only the mRNAs of the AQPs were examined, leaving the protein level changes of these proteins uninvestigated. However the changes at transcriptional level could also directly reflect the cellular responses to environmental factors, suggesting for the future changes at the protein

level. This can be clarified through western blotting of the AQP proteins in future studies.

Conclusively, this study provides some evidences that AQP1 is involved in the pathogenesis of STC. This could be a potential drug target in future experimental studies.

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