

Original Article

Colonic mucosal gene expression in irritable bowel syndrome rats by the liquid chip technology

Guanqun Chao¹, Yingying Wang², Shuo Zhang²

¹Department of Family Medicine, Sir Run Run Shaw Hospital, Zhejiang University, Zhejiang, China; ²Department of Gastroenterology, The First Affiliated Hospital, Zhejiang Chinese Medical University, Zhejiang, China

Received July 18, 2016; Accepted August 27, 2016; Epub October 1, 2016; Published October 15, 2016

Abstract: Background: Irritable bowel syndrome (IBS) is one of the most frequent GI disorders. The etiology and pathogenesis underlying IBS are currently unclear. Gene influence a number of chronic disease processes and may also be involved in regulating disease activity in gastrointestinal disorders, but few studies of IBS have focused on gene expression. Objective: The objective of this study was to screen the differentially expressed colonic mucosal genes in IBS rats to build the expression profile of genes in the colon of IBS rats. Methods: Twenty SD rats were divided randomly into two groups: the rats of control group were normal rats; the rats of model group were induced by conditioned stimulus and unconditioned stimulus. The rats' visceral sensitivity was evaluated by abdominal withdrawal reaction. Then we screened differential expression of colonic mucosal gene by the liquid chip technology and verified by RT-PCR technology. Results: The IBS model was successfully established. Compared with control group, the dose of injection water was decreased in model group ($P < 0.01$). We screened *htr4*, *htr1a*, *2r13*, *nos1*, *Calca*, *npv*, *crhr2*, *il1b*, *p2rx3*, *nos2*, *tph1*, *crhr1*, *hmx1*, *trpv1*, *Vip*, *f2rl*, *tgfb1*, *htr3a*, *slc6a4*, *tff2*, *aqp8* from the colon, but we found that only the expression of *nos1*, *il1b*, *htr3a* in model group was up-regulated ($P < 0.05$). Conclusion: Colonic mucosal genes may be involved in the pathogenesis of IBS with the regulation in colon function.

Keywords: Irritable bowel syndrome, colonic mucosal, gene, regulate

Introduction

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disease without organic abnormalities characterized by recurrent abdominal pain or discomfort associated with abnormal bowel habits [1]. Evidence shows that the diagnosis of IBS can be confidently made for the patients fitting the symptoms-based criteria and having no concerning features for organic diseases, including symptom onset after age 50, severe or progressively worsening symptoms, unexplained weight loss, nocturnal diarrhea, GI bleeding, unexplained iron-deficiency anemia, and family history of colonic cancer, celiac disease, or inflammatory bowel disease [2]. Epidemiological surveys show that the prevalence of IBS in the western population is relatively high ranging from 10% to 18% [3]. The pathogenesis of IBS has not been completely understood until recently. Some patients with diarrhea-predominant IBS have increased intestinal permeability or low-grade inflammation [4, 5].

Some patients with IBS demonstrate enhanced responses to distension of the gut lumen, that is, visceral hypersensitivity accounting for symptoms of urgency, bloating and abdominal pain [6].

A heritable component of IBS has been demonstrated in twin and family studies, although heritability estimates have varied between 0 and 57% [7]. Genetic variations that alter gene expression cause phenotypic diversity and play an important role in disease susceptibility especially with regard to complex conditions [8]. One of the studies [9] found that 33% of IBS patients had a family member with IBS as compared to only 2% in the control group. In a recent large nationwide case cohort study from Sweden, a higher odds ratio of IBS was found in first, second and third degree relatives of IBS patients (OR for first degree relatives: 1.75-1.90, for second degree relatives: 1.10-1.78 and third degree relatives: 1.11) [10]. And there are also several candidate genes have been identified

Colonic mucosal gene expression in IBS rats

that are potentially linked to IBS [11]. Above all genes play an important role in IBS. Our research was to build IBS models, and to screen differential expression of colonic mucosal gene by the liquid chip technology so as to build colonic mucosal gene expression profile of IBS.

Materials and methods

Subjects

There were 20 adult female SD big rats, the weight of every rat was about 200 g. Feeding environment was provided by experimental animal center of Zhejiang Chinese Medical University. 20 SD rats were divided randomly into two groups. The control group was 10, model group was 10. They were put into the environment where the temperature was 22-24°C, the humidity <60%, the noise <50 db.

Experimental procedure and method

Control group: They were normal rats. The control group was injected by 1 ml saline for control. After 2 weeks of normal eating and drinking, we observed the condition of rats. Then the rats' visceral sensitivity was evaluated by abdominal withdraw reaction.

Model group: The conditioned stimulus was camphor ball special odor. The unconditioned stimulus was rectal distention pressure (>60 mmHg (1 mmHg=0.133 kPa)) combining with extremities constraint. Rats were put into the cage with camphor ball in it, we fixed the extremities and trunk of the rats for 45 min. At this time inserted catheter into rectum. The distance from air bladder distal end to anal was about 1 cm. The catheter was fixed at the root of the tail. The balloon volume was 1.6 ml (hydrostatic pressure in Bowman's space >60 mmHg) and lasted for 60 s, filled gas 10 times once. This was a stress process. There was one process in the first day, the same process was performed on the second day at the same time. The conditioned stimulus was done on the fourth day. The completed process was repeated once on the fifth day. The process same as the fourth day was performed on the sixth day. The conditioned stimulus was performed on the eighth day. Then the rats' visceral sensitivity was evaluated by abdominal withdraw reaction.

Model authentication: Visceral sensitivity was evaluated by abdominal withdraw reaction (AWR).

8 F urethral catheter which was lubricated by liquid paraffin was inserted per anum. The distance from air bladder distal end to anal was about 1 cm, and it was fixed at the root of the tail. The rats were put on the platform, after they accommodated the environment, we gradually affused water into sacculus, and recorded the water injection rate when the rats raised the abdomen and made the back like a bow. Rectal distention lasted for 30 s every time, and repeated 3 times. And then we took the mean number.

Experimental sample: After a laparotomy incision, a portion of the colon was removed and placed in an oxygenated Tyrode's solution. A segment of 2 cm length colon were mounted in a 10 ml organ bath containing Tyrode's solution that was bubbled with a 95% O₂ and 5% CO₂ mixture, and the temperature was held at 37°C.

Experimental procedure: After preparing total RNA, we did primer test and sample assay. Then we performed reverse transcription. After creating and setting up a plate document, we prepared the PCR reaction plate, after that we run the PCR reaction plate, then we analyzed the result. The main procedure was that: High-throughput sequencing was conducted on independent samples. Total RNA was extracted from duodenal tissues with TRIzol (Invitrogen, Gaithersburg, MD, USA) using the one-step method. After purification, RNA concentration was analyzed using Nanodrop (Nanodrop Technologies, Wilmington, DE) and quality testing was conducted using BioAnalyzer (Agilent Technologies, Palo Alto, CA). Small RNA was purified from total RNA to enrich molecules in the range of 16-30 nt, and then 3' and 5' linker sequences were attached, and SuperScript II reverse transcriptase was used to synthesize cDNA. PCR amplification was conducted.

Statistical analysis

The data was demonstrated as $X \pm s$. We used SPSS package as statistical method. Two samples' mean numbers were compared by T test. Significance level was $P < 0.05$.

Colonic mucosal gene expression in IBS rats

Table 1. Colonic mucosal gene expression in irritable bowel syndrome rats

Gene	Control group ($\bar{x} \pm s$)	Model group ($\bar{x} \pm s$)	P value
htr4	0.0683 ± 0.0287	0.0789 ± 0.410 [#]	0.52
htr1a	0.1723 ± 0.1334	0.2201 ± 0.1272 [#]	0.42
f2rl3	0.3443 ± 0.2234	0.5037 ± 0.3161 [#]	0.21
nos1	1.3214 ± 0.4338	1.7893 ± 0.5534 [*]	0.03
Calca	0.2707 ± 0.1685	0.3290 ± 0.1767 [#]	0.46
npy	0.2473 ± 0.0893	0.3244 ± 0.1556 [#]	0.20
crhr2	0.2302 ± 0.1433	0.3031 ± 0.1786 [#]	0.33
il1b	0.0053 ± 0.0019	0.0152 ± 0.0134 [*]	0.045
p2rx3	0.1578 ± 0.1041	0.2072 ± 0.1303 [#]	0.36
nos2	0.2510 ± 0.1640	0.3621 ± 0.2551 [#]	0.26
tph1	0.0377 ± 0.0241	0.0479 ± 0.0327 [#]	0.44
crhr1	0.0048 ± 0.0023	0.0045 ± 0.0022 [#]	0.81
hmox1	0.6324 ± 0.2561	0.7319 ± 0.5565 [#]	0.62
trpv1	0.0626 ± 0.0335	0.0755 ± 0.0346 [#]	0.41
Vip	0.9260 ± 0.6934	1.0079 ± 0.7608 [#]	0.80
f2rl1	0.3394 ± 0.1621	0.4400 ± 0.2819 [#]	0.34
tgfb1	0.2326 ± 0.0648	0.3192 ± 0.1847 [#]	0.19
htr3a	0.2121 ± 0.0607	0.2985 ± 0.1003 [*]	0.0345
slc6a4	0.2567 ± 0.1388	0.2897 ± 0.1786 [#]	0.65
tff2	0.0380 ± 0.0338	0.0563 ± 0.0356 [#]	0.25
aqp8	0.2465 ± 0.1542	0.2002 ± 0.1267 [#]	0.47

[#]Express P>0.05, ^{*}express P<0.05.

Results

The change of the model

After completing the models, we found that there was no change of their behavior. Rats of model group expressed excessive reaction when they were frightened or they were performed by intragastric administration. There was also no change of their stool.

Model authentication

The rectum effusion amount of the model rats (0.77 ± 0.08 ml) were lower than the control group (1.52 ± 0.09 ml), the difference had statistical significance (P<0.01), so the sensitivity of model rats was higher, it suggested that model building method was successful.

The screening of microRNAs in colon of IBS rats

The result of gene was listed in **Table 1**. We screened htr4, htr1a, 2rl3, nos1, Calca, npy,

crhr2, il1b, p2rx3, nos2, tph1, crhr1, hmox1, trpv1, Vip, f2rl, tgfb1, htr3a, slc6a4, tff2, aqp8 from the colon, but we found that only the expression of nos1, il1b, htr3a in model group was up-regulated (P<0.05).

Discussion

Irritable bowel syndrome (IBS) is the most common functional gastrointestinal disorder with a worldwide prevalence rates ranging between 7%-21% [12]. In the absence of reliable biomarkers or specific laboratory tests, consensus criteria towards a positive diagnosis have been developed: the symptom-based Rome III criteria [13]. Because of unclear etiology and pathogenesis and the lack of treatment in clinical, the symptoms of IBS patients are long-standing. IBS negatively affects quality of life, which reflects in increased work and school absenteeism, decreased work productivity, and higher utilization of the health care system, with considerable repercussions on health and socioeconomic systems [14]. There are a number of factors involved including the enteric nervous system, inflammatory processes, diet, and (recognized more recently) microbiota. Visceral hypersensitivity and altered pain perception also appear to be important features of IBS, as demonstrated in rectal sensitivity experiments [11].

It is traditionally believed that IBS is a result of multiple factors including hypersensitivity of the bowel, altered bowel motility, inflammation and stress [11]. However gene has been popular in researches of IBS. It was revealed that children of parents who had IBS made 20% more ambulatory care visits than the children of parents without IBS (P=0.0001) [15]. A research [16] found that a survey of people residing in Olmsted county of Minnesota with functional gastrointestinal disorders showed higher odds (OR=2.3; 95% CI: 1.3-3.9) of reporting a relative with gastrointestinal symptoms among these individuals. Another research [17] reported that genetic polymorphisms and haplotypes of CRHR2 are related to IBS. There is also a research reported that the single-nucleotide polymorphisms rs56109847 led to reduced microRNA binding and overexpression of the target gene in intestinal cells, thereby increasing IBS-D risk in the Chinese Han population [18]. Our

research was to build IBS models, and to screen differential expression of colonic mucosal gene by the liquid chip technology.

We found 21 genes in our research, those were: *htr4*, *htr1*, *a2rl3*, *nos1*, *Calca*, *npy*, *crhr2*, *il1b*, *p2rx3*, *nos2*, *tph1*, *crhr1*, *hmox1*, *trpv1*, *Vipf2rl*, *tgfb1*, *htr3a*, *slc6a4*, *tff2*, *aqp8*. However the expression of *nos1*, *il1b*, *htr3a* in model group was up-regulated ($P < 0.05$). HTR3A, one of the 5-HT₃ subunits (HTR3A, HTR3B, HTR3C, HTR3D and HTR-3E), plays a critical role in the formation of 5-HT₃ receptor function [19]. One research has been reported that HTR3E only expressed in the gastrointestinal tissues [20]. However, Serotonin (5-HT) is a brain-gut axis contact key neurotransmitter, and it plays an important role in the pathogenesis of IBS [21, 22]. As a result, HTR3A might be a special gene which plays a special role in IBS rats which expressed in the colonic tissue of IBS rats. Moreover, up to now we still did not find any research about *nos1* and *il1b* in IBS, so the further research is needed to detect the relationship. In conclusion, the present study indicated that genetic polymorphisms in *nos1*, *il1b* and *htr3a* were associated with IBS.

Conclusion

It is suspected in our study that different gene involved in the mechanism of IBS with the regulation in colon. The further researches will be done to detect the interacting and interadjusting mechanism of colonic mucosal in IBS patients, and we also want to build the expression profiling of colonic mucosal to be used for diagnosis and treatment of IBS.

Acknowledgements

This work was partially supported by the Fund of Traditional Chinese medicine science and technology plan projects of Zhejiang province (2016ZB071); and National Natural Science Foundation of China (81573760); and Traditional Chinese medicine science and technology plan projects of Zhejiang province (2015ZZ012); and medical health platform plan projects of Zhejiang province (2015-RCA020); and the national natural youth fund in China (81202828); Zhejiang Provincial Natural Science Foundation of China (LY16H03-0010).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Shuo Zhang, Department of Gastroenterology, The First Affiliated Hospital, Zhejiang Chinese Medical University, Zhejiang, China. E-mail: zhangshuo@medmail.com.cn

References

- [1] Xu XJ, Liu L, Yao SK. Nerve growth factor and diarrhea-predominant irritable bowel syndrome (IBS-D): a potential therapeutic target? *J Zhejiang Univ Sci B* 2016; 17: 1-9.
- [2] Chey WD, Kurlander J, Eswaran S. Irritable bowel syndrome: A clinical review. *JAMA* 2015; 313: 949-958.
- [3] Choung RK, Locke GR. Epidemiology of IBS. *Gastroenterol Clin N Am* 2011; 40: 1-10.
- [4] Zhou Q, Zhang B, Verne GN. Intestinal membrane permeability and hyper-sensitivity in the irritable bowel syndrome. *Pain* 2009; 146: 41-46.
- [5] Camilleri M, Nadeau A, Lamsam J, Nord SL, Ryks M, Burton D, Sweetser S, Zinsmeister AR, Singh R. Understanding measurements of intestinal permeability in healthy humans with urine lactulose and mannitol excretion. *Neurogastroenterol Motil* 2010; 22: e15-e26.
- [6] Zhou Q, Verne GN. New insights into visceral hypersensitivity-clinical implications in IBS. *Nat Rev Gastroenterol Hepatol* 2011; 8: 349-55.
- [7] Saito YA. The role of genetics in IBS. *Gastroenterol Clin North Am* 2011; 40: 45-67.
- [8] Montagnana M, Danese E, Lippi G. Genetic risk factors of atherothrombosis. *Pol Arch Med Wewn* 2014; 124: 474-82.
- [9] Whorwell PJ, McCallum M, Creed FH, Roberts CT. Non-colonic features of irritable bowel syndrome. *Gut* 1986; 27: 37-40.
- [10] Waehrens R, Ohlsson H, Sundquist J, Sundquist K, Zöller B. Risk of irritable bowel syndrome in first-degree, second-degree and third-degree relatives of affected individuals: a nationwide family study in Sweden. *Gut* 2015; 64: 215-21.
- [11] Makker J, Chilimuri S, Bella JN. Genetic epidemiology of irritable bowel syndrome. *World J Gastroenterol* 2015; 21: 11353-61.
- [12] Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. *Clin Gastroenterol Hepatol* 2012; 10: 712-721, e4.
- [13] Drossman DA. The functional gastrointestinal disorders and the Rome III process. *Gastroenterology* 2006; 130: 1377-90.

Colonic mucosal gene expression in IBS rats

- [14] Zucchelli M, Camilleri M, Andreasson AN, Bresso F, Dlugosz A, Halfvarson J, Törkvist L, Schmidt PT, Karling P, Ohlsson B, Duerr RH, Simren M, Lindberg G, Agreus L, Carlson P, Zinsmeister AR, D' Amato M. Association of TNFSF15 polymorphism with irritable bowel syndrome. *Gut* 2011; 60: 1671-7.
- [15] Levy RL, Whitehead WE, Von Korff MR, Feld AD. Intergenerational transmission of gastrointestinal illness behavior. *Am J Gastroenterol* 2000; 95: 451-6.
- [16] Locke GR 3rd, Zinsmeister AR, Talley NJ, Fett SL, Melton LJ 3rd. Familial association in adults with functional gastrointestinal disorders. *Mayo Clin Proc* 2000; 75: 907-12.
- [17] Komuro H, Sato N, Sasaki A, Suzuki N, Kano M, Tanaka Y, Yamaguchi-Kabata Y, Kanazawa M, Warita H, Aoki M, Fukudo S. Corticotropin-Releasing Hormone Receptor 2 Gene Variants in Irritable Bowel Syndrome. *PLoS One* 2016; 11: e0147817.
- [18] Zhang Y, Li Y, Hao Z, Li X, Bo P, Gong W. Association of the Serotonin Receptor 3E Gene as a Functional Variant in the MicroRNA-510 Target Site with Diarrhea Predominant Irritable Bowel Syndrome in Chinese Women. *J Neurogastroenterol Motil* 2016; 22: 272-81.
- [19] Kapeller J, Möller D, Lasitschka F, Autschbach F, Hovius R, Rappold G, Brüß M, Gershon MD, Niesler B. Serotonin receptor diversity in the human colon: Expression of serotonin type 3 receptor subunits 5-HT3C, 5-HT3D, and 5-HT3E. *J Comp Neurol* 2011; 519: 420-32.
- [20] Schuhmacher A, Mössner R, Quednow BB, Kühn KU, Wagner M, Cvetanovska G, Rujescu D, Zill P, Möller HJ, Rietschel M, Franke P, Wölwer W, Gaebel W, Maier W. Influence of 5-HT3 receptor subunit genes HTR3A, HTR3B, HTR3C, HTR3D and HTR3E on treatment response to antipsychotics in schizophrenia. *Pharmacogenet Genomics* 2009; 19: 843-51.
- [21] Zhan DW, Sun JH, Luo KT, Xu LZ, Zhou JL, Pei LX, Chen L, Wu XL, Zhang JW, Zhang W, Jiao DY, Zhu L. Effects and efficacy observation of acupuncture on serum 5-HT in patients with diarrhea-predominant irritable bowel syndrome. *Zhongguo Zhen Jiu* 2014; 34: 135-8.
- [22] Itagaki R, Koda K, Yamazaki M, Shuto K, Kosugi C, Hirano A, Arimitsu H, Shiragami R, Yoshimura Y, Suzuki M. Serotonin (5-HT3) receptor antagonists for the reduction of symptoms of low anterior resection syndrome. *Clin Exp Gastroenterol* 2014; 7: 47-52.