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# Correlation between serotonin receptor 5HT3A and serotonin transporter expression in the gastric glands and symptoms of patients with functional dyspepsia

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**Background** : *Functional dyspepsia (FD) is one of the most common syndrome of gastrointestinal disorders. The sensation of pain or burning of the epigastrium including postprandial fullness and early satiation are the symptoms of FD, which were defined by ROME III criteria. Several factors have been suggested as the causes of FD including the functions of serotonin neurotransmission which has currently been featured in this study.*

**Objective** : *The purpose of this study was to determine the association between the expression of 5HT3A receptor and serotonin transporter on gastric gland cells in patients with FD.*

**Methods** : *Small pieces of the fundus, body and antrum of the stomach from twelve FD patients were collected by gastroscopy technique. The expression of 5HT3A receptor and serotonin transporter were analyzed by immuno-histochemistry technique.*

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**Results** : *The expressions of 5HT3A receptor on the parietal cells and chief cells in the antrum were significantly different in FD patients with different symptoms. Patients with 2 symptoms (gastric distension and stomachache) showed an increase of 5HT3A receptor expression. At the body region, serotonin transporters were significantly increased in enterochromaffin cells of FD patients with no stomachache when compared with FD patients with stomachache. Moreover, the expression of serotonin transporter on enterochromaffin cell in FD patients with distension was also found significantly higher than the FD patients with stomachache.*

**Conclusion** : *These results provided evidence to support that serotonin neurotransmission may play an important role in FD. The expression of 5HT3A receptor and serotonin transporter may be specifically related to specific symptoms in each patient which may be useful for developing individual treatment of FD in the future.*

**Keywords** : *Functional dyspepsia, ROME III, serotonin receptor, serotonin transporter, immunohistochemistry.*

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**กาญมณี แก้วกลิ่น, วิโรจน์ ชนม์สูงเนิน, สุทิสา ถาน้อย, เสมอ ถาน้อย. การศึกษาความสัมพันธ์ระหว่างการแสดงออกของตัวรับซีโรโทนิน 5HT3A และตัวขนส่งซีโรโทนินในต่อมของกระเพาะอาหารและอาการแสดงของผู้ป่วยโรคท้องอืดโดยไม่ทราบสาเหตุ. จุฬาลงกรณ์เวชสาร 2561 พ.ค. – มิ.ย.; 62(3): 499 – 510**

**เหตุผลของการทำวิจัย** : *โรคท้องอืดโดยไม่ทราบสาเหตุจัดเป็นโรคที่พบได้บ่อยที่สุดในกลุ่มของโรคที่พบความผิดปกติของระบบทางเดินอาหาร ซึ่งอาการปวดแสบร้อนบริเวณลิ้นปี่ รวมทั้งอาการจุกแน่นท้องภายหลังรับประทานอาหารและอาการอิ่มเร็วจัดเป็นอาการที่พบได้ในผู้ป่วยโรคนี้ โดยใช้เกณฑ์การวินิจฉัยโรคของระบบทางเดินอาหาร ROME III ในการจำแนกอาการของผู้ป่วย มีหลายปัจจัยที่เป็นสาเหตุของโรคนี้ รวมทั้งบทบาทของซีโรโทนินซึ่งถูกให้ความสำคัญในการศึกษานี้*

**วัตถุประสงค์** : *การศึกษานี้ต้องการศึกษาหาความสัมพันธ์ระหว่างการแสดงออกของตัวรับซีโรโทนิน และตัวขนส่งซีโรโทนินในต่อมของกระเพาะอาหารในผู้ป่วยโรคท้องอืดโดยไม่ทราบสาเหตุ*

**วิธีการทำวิจัย** : *ผู้ป่วยจำนวน 12 ราย จะได้รับการส่องกล้องทางเดินอาหาร และตัดชิ้นเนื้อจากกระเพาะอาหารบริเวณ fundus, body และ antrum จากนั้นนำมาศึกษาการแสดงออกของตัวรับซีโรโทนิน และตัวขนส่งซีโรโทนินด้วยวิธีอิมมูโนฮิสโตเคมี*

**ผลการศึกษา** : *การศึกษานี้พบการแสดงออกของจำนวนตัวรับซีโรโทนินที่ parietal cells และ chief cells บริเวณ antrum โดยพบความแตกต่างอย่างมีนัยสำคัญทางสถิติในระหว่างกลุ่มผู้ป่วยที่มีอาการแสดงที่ต่างกัน โดยกลุ่มของผู้ป่วยที่มีอาการแสดง 2 อาการ ทั้งอาการอืดแน่นท้อง และอาการปวดท้องพบการแสดงออกที่มากของตัวรับซีโรโทนิน บริเวณ body พบการแสดงออกของตัวขนส่งซีโรโทนินมากอย่างมีนัยสำคัญทางสถิติบน enterochromaffin cells ของกลุ่มผู้ป่วยที่ไม่พบอาการปวดท้องเปรียบเทียบกับกลุ่มผู้ป่วยที่พบอาการปวดท้อง นอกจากนี้ยังพบการแสดงออกของตัวขนส่งซีโรโทนินบน enterochromaffin cell ของผู้ป่วยในกลุ่มที่มีอาการอืดแน่นท้องซึ่งพบมากอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มของผู้ป่วยที่มีอาการปวดท้องอีกด้วย*

**สรุป** : *ผลจากการศึกษานี้แสดงให้เห็นว่าซีโรโทนินอาจมีบทบาทสำคัญในโรคท้องอืด โดยไม่ทราบสาเหตุ การพบการแสดงออกของตัวรับซีโรโทนินและตัวขนส่งซีโรโทนินบนต่อมของกระเพาะอาหารของผู้ป่วยสัมพันธ์กับอาการที่เฉพาะในผู้ป่วยแต่ละราย ซึ่งอาจเป็นประโยชน์ในการพัฒนาการรักษาผู้ป่วยได้ต่อไปในอนาคต*

**คำสำคัญ** : *โรคท้องอืดโดยไม่ทราบสาเหตุ, เกณฑ์การวินิจฉัยโรคของระบบทางเดินอาหาร (ROME III), ตัวรับซีโรโทนิน, ตัวขนส่งซีโรโทนิน, อิมมูโนฮิสโตเคมี.*

Functional dyspepsia (FD) is the one of most common syndrome of gastrointestinal disorders commonly found worldwide. FD symptoms were defined by ROME III criteria which include one of these symptoms, i.e., bothersome postprandial fullness, early satiation, epigastric pain and epigastric burning. FD patients show no pathology by gastroscopy diagnosis. [1, 2] It is therefore difficult for the treatment of FD due to limitation of treatment and there are no specific drugs for the direct treatment. As a result, FD is still a problem that affect the quality of life of the patients. The reported incidence of FD in the Western is 25% and 10 - 25% in the US, in 2008. [3] In addition, in Asian population in 2011 the incidence of FD was about 8 - 23% including Thailand. [4, 5] Currently, antidepressants are used in the treatment of FD for reducing the severity of symptoms in FD patients. [6] Also, selective serotonin reuptake inhibitors (SSRIs) as antidepressant drug is one of pharmacological treatment that has been used for FD. [7, 8] However, a good response to the treatment and its side effects are different between each individual. [9 -11] The role of serotonin that functions in FD symptoms is still inadequately known. While, the mechanism of SSRIs has been known to relate with serotonin neurotransmitter which is an important neurotransmitter in the brain. Moreover, serotonin has a relationship with brain-gut axis that also affects the gastrointestinal tract. [12]

Serotonin or 5-Hydroxytriptamine (5HT) is found in the gastrointestinal (GI) tract that has a major role in GI. [13] About 95% of 5HT in the body are produced by enterochromaffin cells and secreted into the GI lumen and blood vessels. [14, 15]

5-Hydroxytryptamine 3A (5HT3A) receptor is one of serotonin receptor subtypes, which is found in the central and peripheral nervous systems. Moreover, it has been suggested that the function of 5HT3A receptor involves in the process associated with gut motility. [16] In addition, serotonin transporter (SERT) is also one of the serotoninergic system which related to 5HT recycling. [17] There have been many reports about the pharmacological properties of serotonin neurotransmission associated with gut motility, peristalsis and pain from the GI tract. [18, 19] It is, therefore, hypothesized that the expression of 5HT3A receptors and SERT in stomach may be related to FD symptoms.

Therefore, the purpose of this study was to determine the association between the expression of 5HT3A receptor and serotonin transporter on gastric gland cells in patients with FD.

## Materials and Methods

### *Patient Selection*

Twelve patients with FD symptoms according to Rome III criteria for diagnosis of FD, their ages ranged between 26 to 66 years old were recruited in the study. The protocols of this research have been approved by Human Ethics Committee, Naresuan University, Thailand, at the certificate of analysis no. 113/2015 before the start of this study. All patients have signed the informed consent forms before recruitments. All patients underwent left gastroscopy after fasting by medical specialists at the Fort Somdet Phranaresuan Maharat Hospital, Phitsanulok province, Thailand.

### *Study design*

Small pieces of gastric biopsie from 3 areas

namely, the fundus, body and antrum were collected and fixed in 2.5% glutaraldehyde. The biopsies obtained during endoscopy were processed, embedded in paraffin and sectioned. Then, expression of 5HT3A receptor and SERT were studied by immunohistochemistry in through detection of the antigen-antibody binding reaction. The expressions of 5HT3A receptor were detected using rabbit polyclonal antibody to 5HT3A receptor (diluted at 1:50; ab13897, Abcam) for 3 hours and the biotinylated universal antibody; horse anti-mouse/rabbit IgG (diluted at 1:100; BA-1400, Vector Ltd), as secondary antibody for 2 hours in this protocol. Rabbit anti-serotonin transporter (SERT) polyclonal antibody (diluted at 1:50; ab174770, Abcam) was identified the expression of SERT on gastric gland cells for 2 hours and overnight incubation at 4 $^{o}$C. Biotinylated goat anti-rabbit IgG (diluted at 1:200; BA-1000, Vector Ltd), secondary antibody was used for 2 hours in SERT protocol. Then, all sections were incubated for enhancing signal with avidin-biotinylated horseradish peroxidase complex; ABC kit (PK-4000, Vector Ltd) for 60 minutes. Finally, chromogen 3,3'-Diaminobenzidine (DAB) (SK-4100, Vector Ltd) was used for specific protein visualizing for 10 minutes. Immunopositive cells in the gastric glands were captured under light microscope (Olympus CX31) and identified the type of cells for qualitative study.

### *Statistical Analysis*

SPSS 17.0 statistical analysis software was used for statistical analysis. For normally distributed comparison between 2 groups and the comparison among 3 groups were done using *t*-test and ANOVA, respectively; $P < 0.05$ was considered statistical significance.

## Results

Characteristics of FD patients are shown in Table 1. The results demonstrated that the expressions

**Table 1.** Characteristics of FD patients recruited for this study.

| Characteristics of FD patients | |
|---|---|
| Total number of FD Patients | 12 |
| Male | 1 |
| Female | 11 |
| Age range (yr) | 26 - 66 |
| Number patients classified by symptom types | |
| Group 1 | |
| Distension | 9 |
| Non-distension | 3 |
| Group 2 | |
| Stomachache | 8 |
| Non-stomachache | 4 |
| Patients grouped by number of symptoms | |
| 1 symptom | 4 |
| 2 symptoms | 8 |

of immunopositive cells, 5HT3A receptor and SERT were found in gastric gland cells in all regions of stomach of twelve FD patients. Qualitatively, the expressions of the 5HT3A receptor were detected on the parietal cells and chief cells (Figure1). The percentages of the number of the 5HT3A receptors that expressed on the parietal cells in the antrum region were significantly higher in FD patients with distension when compared with to those no distension ($P$ = 0.004) (Figure 2). Also, the percentages of the number of the expressions of the 5HT3A receptors on chief cells in the antrum region were significantly higher in FD with 2 symptoms (gastric distension and stomachache) when compared with those with 1 symptom ($P$ = 0.047) (Figure 3). Qualitatively, the expressions of the SERT were detected in the enterochromaffin cells (Figure 4). In the body region, the percentages of the number of SERT that expressed on enterochromaffin cells were significantly increased in FD patients with no stomachache when compared with those with stomachache ($P$ = 0.044) (Figure 5). Moreover, the expression of SERT on enterochromaffin cells in FD patients with distension was also found significantly higher than those with stomachache ($P$ = 0.036) (Figure 6) in the body region.

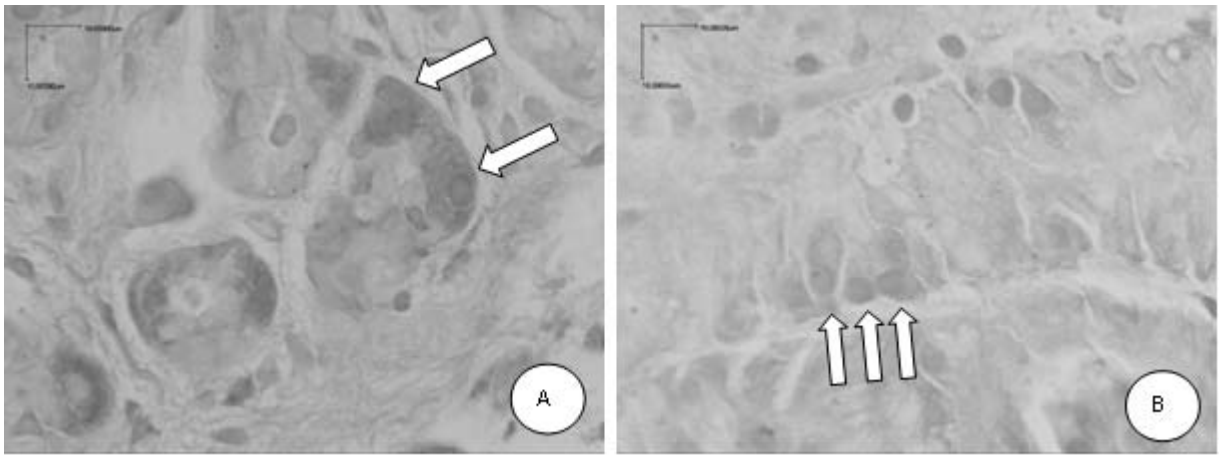


**Figure 1.** 5HT3A receptor expressions on gastric gland cells.
A; Parietal cell (arrow) B; Chief cell (arrow)

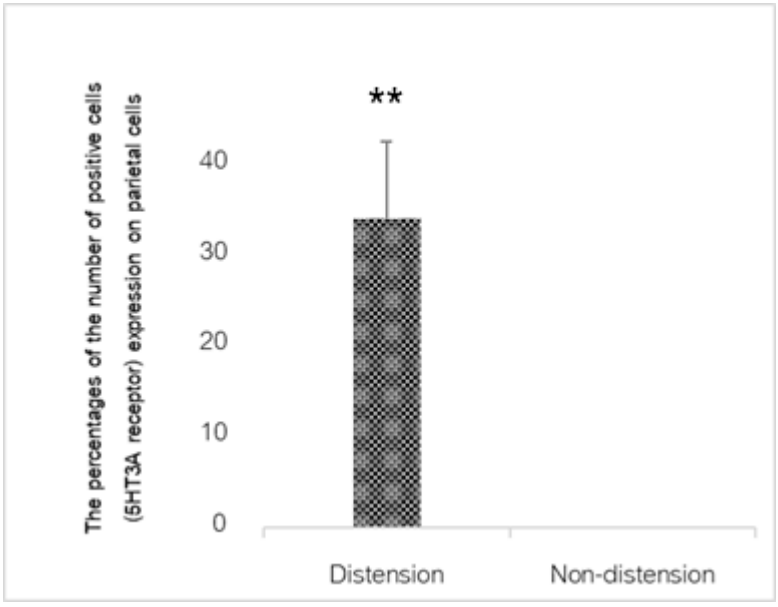


**Figure 2.** The percentages of the number of positive cell (5HT3A receptor) expression on parietal cells in antrum of FD patients with distension when compared with FD patients with no distension. Data are presented as mean ± SEM. ** Significantly different at $P < 0.01$ from independent *t*-test.

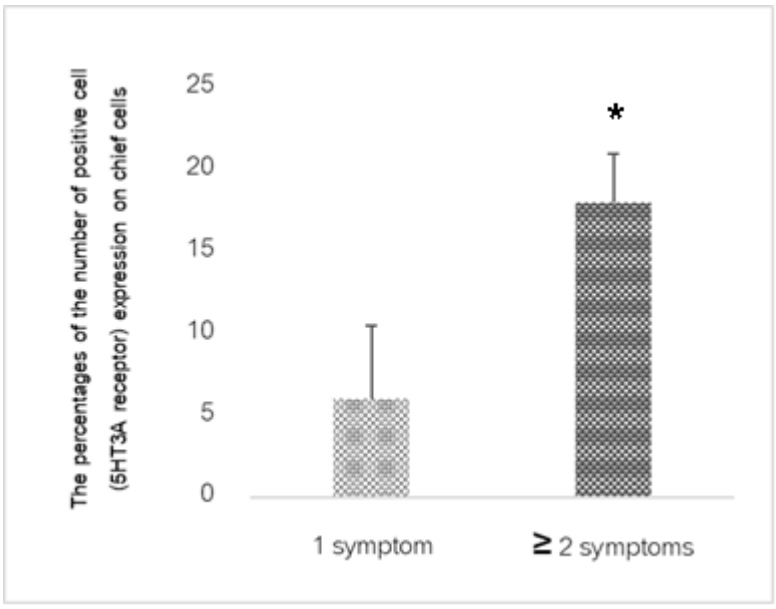


**Figure 3.** The percentages of the number of positive cell (5HT3A receptor) expression on chief cells in antrum of FD patients with 1 symptom when compared with FD patients with 2 symptoms. Data are presented as mean ± SEM. * Significant difference at $P < 0.05$ from independent *t*-test.

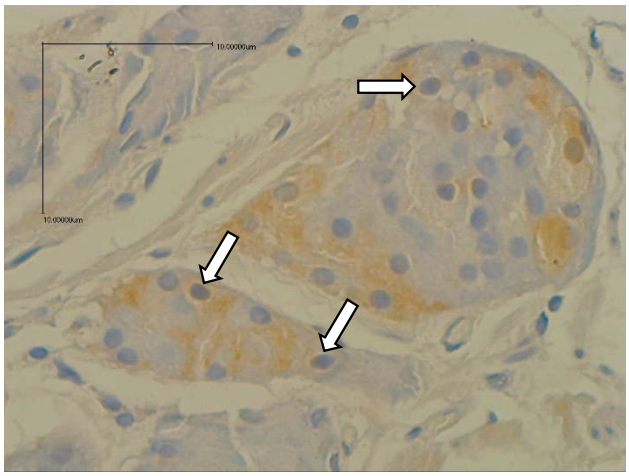

**Figure 4.** SERT expression on gastric gland cells.
Enterochromaffin cell (arrow)

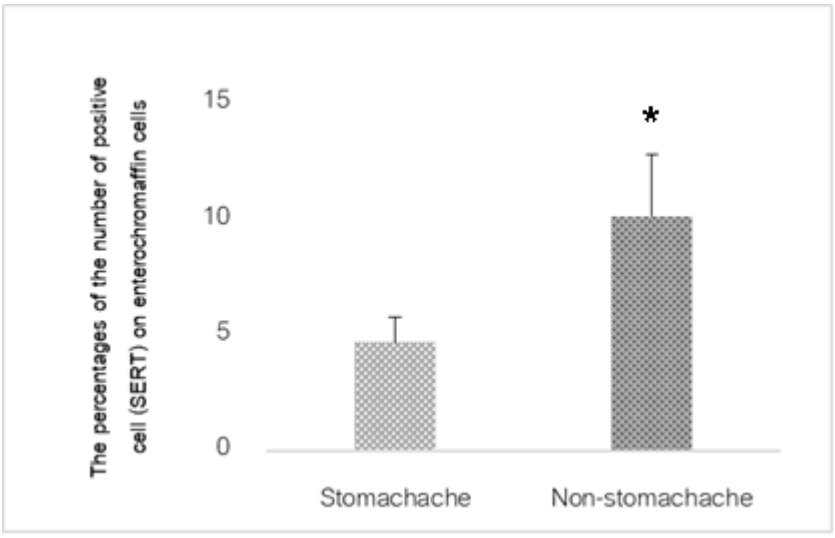


**Figure 5.** The percentages of the number of positive cell (SERT) expression on enterochromaffin cells in body of FD patients with stomachache when compared with FD patients with no stomachache. Data are presented as mean ± SEM. * Significant difference at $P < 0.05$ from independent *t*-test.

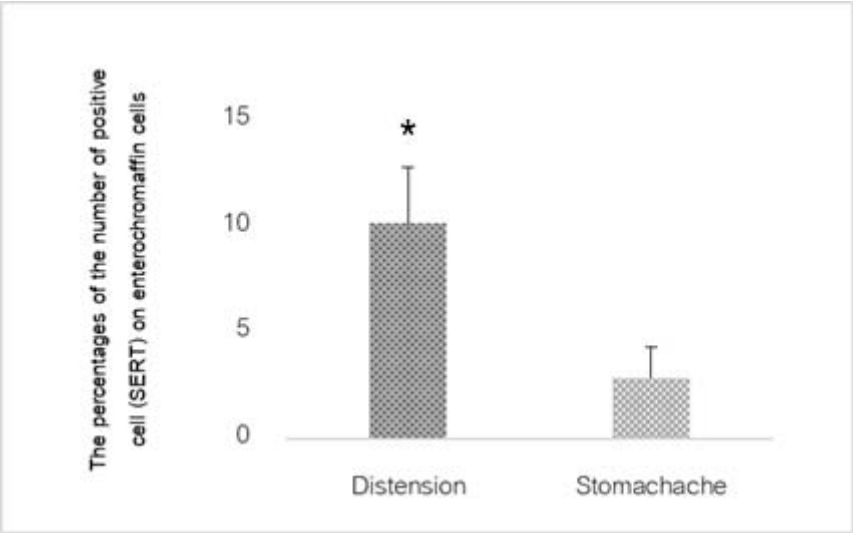


**Figure 6.** The percentages of the number of positive cell (SERT) expression on enterochromaffin cells in body of FD patients with distension when compared with FD patients with stomachache. Data are presented as mean ± SEM. * Significant difference at $P < 0.05$ from independent *t*-test.

## Discussion

This study has initially revealed the expression of 5HT3A receptors and SERT in gastric glands of the patients with FD. At the antrum region, FD patients with distension showed the expression of 5HT3A receptors on parietal cells. Moreover, 5HT3A receptors were also expressed on chief cells of FD patients with 2 symptoms. Commonly, parietal and chief cells are important cells in the gastric glands of which their functions involve the production and secretion of hydrochloric acid (HCl) and pepsinogen into the gastric lumen, respectively.[20] Therefore, the expressions of 5HT3A receptor on parietal and chief cells may involve increase serotonin to bind the receptor on those cells read to abnormalities of HCl and pepsinogen secretion which may relate to FD symptoms such as epigastric pain and burning sensation. Abnormal hypersecretion of HCl and gastric acid were reported to be associated with gastro-esophageal reflux disease.[21, 22] Similarly, distension or flatulence leads to increased abdominal pressure which may also cause acid reflux.[23] Moreover, our results showed the number of FD symptoms are correlated with the expressions of 5HT3A receptor in gastric gland cells as these receptors are abundantly appeared in patients with more than one symptom. At the body region, the expressions of SERT were found on enterochromaffin cells in FD patients with no stomachache and patients with distension. Enterochromaffin cells is an important cell in the GI tract; their functions involve in the production and secretion of 5HT to GI lumen.[24] Therefore, SERT expressions on enterochromaffin cells may be related to 5HT recycling. Especially, the expressions of SERT on enterochromaffin cells in FD patients with no stomachache could reflect an increase in the retention of 5HT in the body of stomach in order to increase the amount of 5HT in the GI tract and consequently reduced the pain symptom of FD patients.[25] Similarly, the higher expressions of SERT on enterochromaffin cells

in FD patients with distension when compared to the FD patients with stomachache may also reflect its effect on pain relief in these patients consistent with pain response as mentioned above.

**Conclusion**

In conclusion, the results support that serotonin neurotransmission may play an important role in FD. The expressions of 5HT3A receptor and serotonin transporter (SERT) seem to be specifically related to specific symptoms occurred in each patient. The expressions of 5HT3A receptor and SERT in each gastric cell type; they, may be useful for developing individual treatment of FD patient in the future.

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