Chapter

Role of Gut Microbiota in Bile-Acid Metabolism

Yuji Naito, Tomohisa Takagi and Ryo Inoue

Abstract

The role of the gut microbiota in modifying the pathophysiology of various diseases, including neurodegenerative diseases, is increasingly becoming clear. Bile acids have been shown to be endogenous factors that affect gut microbiota, and bile-acid metabolites directly or indirectly affect host physiology and pathophysiology. The development of metagenomic analysis for gut microbiota and systematic bile-acid measurement using LC–MS/MS has triggered a breakthrough for research in this field. Clinically, an inhibitor of the ileal bile-acid transporter (Elobixibat) was used as a therapeutic agent for chronic constipation, which also paved the way for progress in bile-acid signal research. Additionally, this review emphasizes the importance of gut microbiota-bile acid-receptor signals when considering nutritional approaches to promote healthy longevity.

Keywords: *Akkermansia muciniphila*, bile acid, gut microbiota, ileal bile-acid transporter, TGR5

1. Introduction

Bile acids have been studied for more than 100 years, but recently, their interaction with intestinal flora has been drawing attention and is being increasingly clarified. Bile-acid research, which has been conducted mainly in the field of liver disease, has led to the development of ursodeoxycholic acid (UDCA), which is generally accepted to improve clinical and biochemical index values in patients with cholestatic liver disease. TGR5 (G protein-coupled bile-acid receptor 1, GPBAR-1), identified in 2002, is a G protein-coupled receptor with seven transmembrane domains and is widely distributed in various organs and tissues. TGR5 can be activated by primary and secondary bile acids, indicating the function of bile acids as signal transduction molecules and in regulating energy metabolism and glycolipid metabolism [1]. Bile acids undergo various metabolic processes such as deconjugation by intestinal bacteria; it has also been shown that the host response is regulated by the reaction between the metabolite and the receptor [2]. Under these circumstances, an inhibitor of the ileal bile-acid transporter (IBAT) localized at the terminal ileum has been shown to be effective in treating constipation [3, 4] and has become a topic of clinical research. This review focuses on the interaction between bile acids and gut microbiota.

2. Classification of bile acids and their metabolites

Bile acids are synthesized from cholesterol in the liver; in humans, cholic acid (CA) and chenodeoxycholic acid (CDCA) are typical primary bile acids [5]. In the rodent liver, chenodeoxycholic acid is further converted to muricolic acid (MCA). After biosynthesis, CA and CDCA undergo further glycine conjugation or taurine conjugation in the liver. Among human conjugated bile acids, taurine conjugates and glycine conjugates accumulate in the gallbladder at a concentration ratio of approximately 1:3 and are secreted from the bile duct into the duodenum in response to food intake. On secretion into the duodenum, taurine conjugates (tauro-CA, tauro-CDCA) and glycine conjugates (glyco-CA, glyco-CDCA) are deconjugated by the bile salt hydrolase (BSH) of the gut microbiota; these deconjugated bile acids are involved in the formation of micelles and the absorption of dietary fat (**Figure 1**).

Lactobacillus plantarum, *Lactobacillus johnsonii*, *Clostridium perfringens*, and *Bifidobacterium longum* have been reported to be specific bacteria carrying the BSH gene [6]. Because the BSH gene is expressed in many bacteria and the presence of BSH is favorable to the host, the presence of these bacteria can be interpreted to be a result of selection by the host. BSH has been suggested to play an important role in the colonization and survival of bacteria in the gut [7]. Jarocki et al. [8] first analyzed the occurrence of BSH in 14 strains belonging to the *Bifidobacterium* genus and purified and analyzed two BSHs from *B. pseudocatenulatum* and *B. longum* subsp. *suis* for their selected biochemical and molecular features. Deconjugation by BSH seems to be meaningful in human physiology and is involved in the lowering of cholesterol levels, maintenance of intestinal homeostasis, maintenance of the intestinal circadian rhythm, and supply of glycine and taurine to the surrounding bacteria [6]. In particular, free bile acids (CA, CDCA) have been reported to be directly involved in the expression of clock genes in intestinal epithelial cells that can control peripheral circadian rhythms in the intestinal tract and liver. Govindarajan et al. [9] demonstrated that unconjugated bile acids are potential chronobiological regulators of host circadian gene expression, especially in the intestine and liver. These data may indicate the potential role of microbiota-generated bile acids as chronological regulators of the peripheral circadian clock and

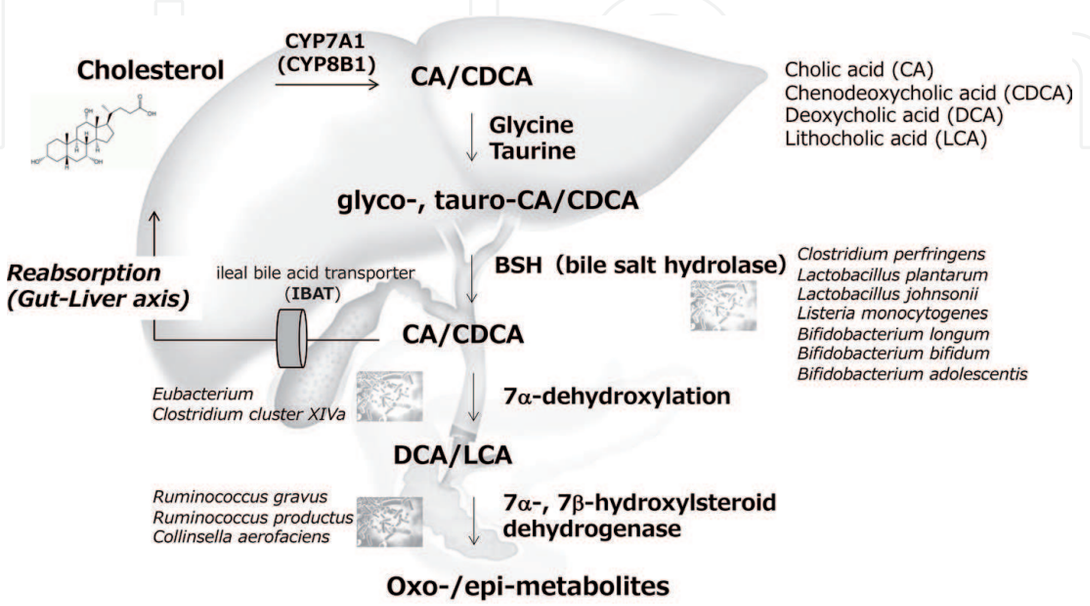

Figure 1.
Synthesis, conjugation, and metabolism of bile acids by gut microbiota.

suggest that intervention strategies that alter gut bile-acid profiles could influence the circadian clock. Joyce et al. [10] investigated the role bacterial BSH in the host physiology and have demonstrated that bacterial BSH activity significantly impacts the systemic metabolic processes and adiposity in the host and represents a key mechanistic target for the control of obesity and metabolic syndrome.

Primary bile acids are actively reabsorbed by the ileal bile-acid transporter (IBAT) present in the terminal ileum in addition to being passively absorbed. Consequently, bile acids secreted into the intestinal tract could return to the liver via the portal vein, 95% of which is reused. Bile acids are reported to be reused by enterohepatic circulation and circulated in the human body 4–12 times a day [11]. The details of the mechanism regulating IBAT expression have not yet been elucidated; however, IBAT expression appears to be affected by gut microbiota and is markedly enhanced in germ-free mice [12].

Bile acids that flow from the small intestine to the large intestine are further metabolized by abundant gut bacteria. First, multi-step reactions of specific bacteria result in the hydroxyl group at the C-7a position of the deconjugated bile acids (CA and CDCA) being dehydroxylated (7α-dehydroxylation) to form secondary bile acids, such as deoxycholic acid (DCA) and lithocholic acid (LCA). Specifically, CA is metabolized to deoxycholic acid (DCA), which in turn is metabolized to lithocholic acid (LCA). Specific *Eubacterium* and *Clostridium* cluster XIVa species, belonging to the Firmicutes phylum, are involved in this conversion in a complex manner, but the entire pathway has not yet been clarified. More than 90% of bile acids in feces are secondary bile acids, and in humans, DCA and LCA are the most abundant [13]. These bile acids affect host physiological function via the TGR5 receptor, which is a bile-acid receptor, and are involved in water secretion to the lumen of the large intestine and peristaltic movement of the tract. Therefore, secondary bile acids produced by the gut microbiota are essential for the physiological function of the host, and a decrease in their concentration could lead to a corresponding decrease in intestinal peristalsis. Thus, a decrease in bile-acid concentrations may not only induce constipation symptoms, but also adversely affect the gut-brain axis.

Furthermore, some amount of CDCA is further metabolized to UDCA by gut bacteria carrying the 7α- and 7β-hydroxylsteroid dehydrogenase (HSDH) genes. Bacteria such as *Ruminococcus gravus*, *Ruminococcus productus*, *Collinsella aerofaciens*, and *Clostridium absonum* have the HSDH gene but have not been studied in detail. Although the luminal concentrations of these bile-acid metabolites in the large intestine are low, recent reports have reported anti-inflammatory, anti-bacterial, and wound healing promoting effects of these metabolites [14]; thus, further studies are required.

As described above, primary bile acids secreted into the duodenum in a conjugated form are metabolized by various intestinal bacterial genes. This interaction is complex because bile acids also have a more direct effect on the survival of the gut microbiota. Islam et al. [15] have demonstrated that bile acid is a host factor that regulates the composition of the cecal microbiota in rats, and that CA feeding simplifies the composition of the microbiota, with outgrowth of several bacteria in the classes Clostridia and Erysipelotrichi. Furthermore, importantly, several bile-acid receptors have been discovered and each bile acid has differing binding ability to these receptors; these aspects should be considered to understand the bile acid-mediated host response. Metabolic disorders have an impact on longevity and the recent findings showed the relationship between bile acid metabolism and metabolic disorders [16–18]. Broeders et al. [16] have showed that CDCA promotes mitochondrial uncoupling via bile-acid receptor (TGR5) in human brown adipocytes and increases brown fat activity and energy expenditure in women.

Because it has been shown that TGR5 localizes in many cells and tissues, including enteroendocrine cells, neurons, macrophages, muscle and endothelial cells, the bile acid-mediated host response should be carefully analyzed.

3. Serum bile-acid profile as a biomarker

The profiles of various bile acids can be selectively measured using quantitative systematic liquid chromatography–tandem mass spectrometry (LC–MS/MS) for samples such as blood and stool. Furthermore, quantitative measurement using internal standard substances has made it possible to rapidly conduct research using a large number of clinical samples [19, 20]. Features of serum bile-acid profiles have been reported in patients with inflammatory bowel disease [19], colon cancer [21], irritable bowel syndrome [22], chronic constipation [23], liver diseases such as fatty liver and fatty hepatitis [24], and neurodegenerative diseases such as Alzheimer's disease and cognitive dysfunction [25, 26].

The relationship between colorectal cancer and the gut microbiota is a hot topic of research. *Fusobacterium nucreatum*, detected relatively specifically in colorectal cancer tissues, is a sulfate-reducing bacterium that can produce hydrogen sulfide, which is a gene mutagen. Taurine released from taurine-conjugated bile acids has been suggested to be a substrate for hydrogen sulfide production [27]; therefore, the carcinogenic-promoting effect of a high-fat diet may be partially explained by the increase in levels of taurine-conjugated bile acids caused by the diet. Bile-acid profile information for patients with colorectal cancer has also been reported. Uchiyama et al. [21] measured serum bile-acid profiles of healthy individuals, colorectal adenomatous polyps, and colorectal cancer at each clinical stage and analyzed the principal components of 30 types of bile acids. Free CA, 3epi-DCA, CDCA, 3-dehydro CA, glyco-CA, and tauro-CA were extracted as principal components (PC) 1 and free 3-dehydroDCA was extracted as PC 2 by canonical discriminant function coefficients. They concluded that the verification of discriminability using the cross-validation method revealed that the correct classification rate was 66.3% for the original data and 52.6% for the cross-validation data. Kuhn et al. [28] also demonstrated the association between serum concentrations of individual bile acids and colon cancer risk and was the first to show the importance of conjugated bile acids compared to that of unconjugated bile acids. They observed statistically significant positive associations between most conjugated primary bile acids (glyco-CA, tauro-CA, glyco-tauro-CDCA, tauro-CDCA, and glyco-hyocholic acid [GHCA]) and colon cancer risk.

The clinical usefulness of serum bile-acid profiles has also been analyzed in several diseases other than colorectal cancer. Recent reports have demonstrated that the concentration of conjugated secondary bile acids (glycol-DCA, glycol-LCA, tauro-DCA, and tauro-LCA), but not of the unconjugated forms, increases parallelly with the progression of the disease, especially in patients with Alzheimer's disease and dementia. Nho et al. [26] were the first to show that altered bile-acid profiles and increased ratios of glyco-DCA:CA, tauro-DCA:CA, and glyco-LCA:CDCA were significantly associated with structural and functional changes in the brain, as indicated by greater atrophy and reduced glucose metabolism. Higher levels of secondary conjugated bile acids (glyco-DCA, glycol-LCA, and tauro-LCA) were significantly associated with worse cognitive function in 1,464 subjects, including 370 cognitively normal older adults, 284 individuals with early mild cognitive impairment (MCI), 505 individuals with late MCI, and 305 patients with Alzheimer's disease [25]. The increase in the concentration of conjugated bile acids in the blood should be considered to be caused by the high-fat diet-stimulated

increase in the bile-acid biosynthetic reactions in the liver and the decrease in the bile-acid deconjugation reactions in the intestinal tract, indicating a decrease in the gut microbiota carrying the deconjugation gene for BSH, as has been mentioned above.

We previously performed a correlation analysis between the bile-acid profile and gut microbiota in a fatty liver model and a colon cancer model in mice fed a high-fat diet and indicated that this correlation analysis is useful for assessing the functionality of dietary factors [29, 30]. High-fat dietary load increased serum levels of conjugated bile acids (tauro-CA, tauro-DCA) and decreased levels of unconjugated bile acids (such as CA), but the administration of epigallocatechin gallate (EGCG), a functional ingredient in tea, normalized bile-acid profiles. Furthermore, an analysis of gut microbiota revealed a positive correlation between an increase in the abundance of *Akkermansia* and their effects on bile acids [29]. Similarly, in the colon cancer model, we found that agarooligosaccharide suppressed the increase in levels of taurine-conjugated bile acids and suppressed the increase of bacteria classified into *Clostridium* subcluster XIVa that are involved in secondary bile-acid metabolism.

As described above, quantitative bile-acid profile measurement using the LC–MS/MS method has made it possible to obtain integrated bile-acid information that distinguishes between conjugated and unconjugated types; consequently, the roles of bile acids in physiology and pathophysiology are being elucidated. It is important to note that such analysis should be conducted with the understanding that the bile-acid profiles are influenced by dietary factors, including high-fat diet, liver function, lipid metabolism, cholesterol level, and gut microbiota.

4. An inhibitor of ileal bile-acid transporter (IBAT)

In a gnotobiotic experiment wherein feces of patients with chronic constipation, especially those with delayed intestinal transit time, were transplanted into germ-free mice, short chain fatty acids and secondary bile acids, which are metabolites of the gut microbiota, were identified to be factors that promote intestinal peristalsis, and the administration of butyric acid or DCA activated serotonin signals in the gut and restored the decreased intestinal peristalsis of these gnotobiotic mice [31]. In addition, the amount of bile acids in the feces of patients with chronic constipation and irritable bowel syndrome was found to be decreased, suggesting that improving this decrease in the bile-acid concentration in the large intestine may be a suitable therapeutic strategy.

Elobixibat, the first inhibitor of IBAT, expressed in epithelial cells in the terminal ileum and suppresses the reabsorption of bile acids, thereby causing bile acids to flow into the lumen of the large intestine (**Figure 2**). Elobixibat is effective as a therapeutic agent for chronic constipation as it induces a secondary bile-acid signal, causing water to be secreted into the lumen of the large intestine and thus promoting gastrointestinal peristalsis [3, 4]. These physiological effects of bile acids are thought to be mediated by the TGR5 receptor localized in intestinal cells. The analysis of bile acids in feces has indicated an increase in DCA levels after the administration of Elobixibat compared to those before administration [23]. Intestinal neuroendocrine cells (EC cells) are considered to be the main TGR5 receptor-expressing cells, and TGR5 receptor-deficient mice have been demonstrated to have decreased intestinal peristalsis [32]. The binding of bile acids to the TGR5 receptor expressed on the luminal side of EC cells is hypothesized to activate serotonin synthesis signals and enhance secretion, leading to the activation of peristalsis. The reactivity of the TGR5 receptor to different bile acids varies; the TGR5 receptor has high reactivity with LCA and DCA and low reactivity with CDCA and CA [33].

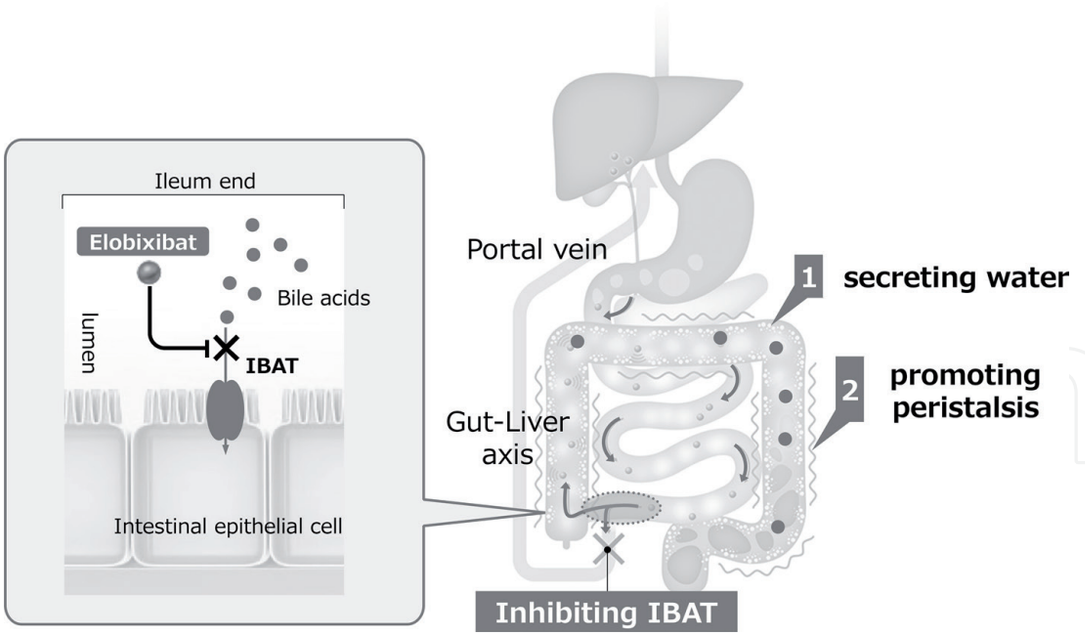

Figure 2.
Ileal bile-acid transporter (IBAT) inhibitor (elobixibat) and its effect on the function of the large intestine. Elobixibat inhibits IBAT expressed in epithelial cells in the terminal ileum and partially suppresses bile-acid reabsorption. The flow of bile acids into the lumen of the large intestine activates TGR5 receptors, causing the secretion of water into the lumen of the large intestine and thus promoting gastrointestinal peristalsis.

TGR5 receptor expression is also reported to be affected by gut microbiota [12]. In other words, the dose dependence of the efficacy of Elobixibat may be strongly influenced by the intestinal bile-acid profile and intestinal flora of each individual. In addition, Elobixibat activates bile-acid metabolism, and in future studies, it is necessary to consider the effects of Elobixibat on the whole body, including effects on cholesterol metabolism and fatty liver.

An observational study that used the Japanese version of the Patient Assessment of.

Constipation-Quality of Life (PAC-QOL) questionnaire has demonstrated that the scores of physical discomfort and psychosocial discomfort significantly decreased in patients with constipation after the treatment with Elobixibat for 4 weeks, indicating the possibility that Elobixibat could affect the gut-brain axis [34].

5. *Akkermansia muciniphila* and bile-acid signals for well-being

Well-being is a key word in the WHO's definition of health: a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity. Thus, medical care aimed at wellbeing is necessary. Recent reports have revealed the involvement of gut microbiota and bile-acid metabolism in extending healthspan and lifespan [35]. The gut microbiota of two different mouse models of progeria was characterized by intestinal dysbiosis with alterations including an increase in the abundance of Proteobacteria and Cyanobacteria and a decrease in the abundance of Verrucomicrobia compared to the gut microbiota of control mice. Fecal microbiota transplantation from wild-type mice enhanced healthspan and lifespan in both progeroid mouse models, and transplantation with the Verrucomicrobia *Akkermansia muciniphila* was sufficient to exert beneficial effects. Furthermore, an analysis of gut microbiota metabolites revealed that various bile acids were reduced in the progeroid mouse models, and this dysregulation in bile acids was improved by transplantation with *Akkermansia muciniphila* [35].

Morita et al. [36] reported that *Akkermansia* was more abundant in individuals from Ogimi and *Lachnospiraceae*, *Collinsella*, *Peptococcus*, and S24–7 were more abundant in individuals not from Ogimi. Ogimi is a village located in the northern region of Okinawa's main island and has a population of approximately 3,000; it is known as the village of longevity in Japan. Grajeda-Iglesias et al. [37] recently demonstrated that *Akkermansia muciniphila*, especially if it is pasteurized, causes major changes in metabolism, elevating the concentrations of several metabolites that have been previously associated with positive effects on health in mouse models. Pasteurized *Akkermansia muciniphila* was more efficient than live *Akkermansia muciniphila* in elevating the intestinal and circulatory concentrations of polyamines, short-chain fatty acids, 2-hydroxybutyrate, and multiple bile acids, all of which may have a positive impact on human health.

Bile-acid analysis by LC–MS/MS has enabled the identification of new metabolites related to health and longevity. Detailed metagenomic analysis and intestinal metabolite analysis of Japanese centenarians recently revealed a novel bile-acid metabolite, isoallo-lithocholic acid, which has a bactericidal effect on specific intestinal bacteria [38]. Although details of the effect of *Akkermansia muciniphila* on bile-acid metabolism are not fully understood, our previous analysis using a high-fat diet load mouse model suggests that the bacterium promotes the deconjugation of conjugated bile acids and suppresses the production of secondary bile acids [29]. A double-blind comparative study in obese individuals has already been conducted to investigate whether *Akkermansia muciniphila* improves the intestinal environment [39]. They demonstrated that Akkermansia muciniphila reduced the levels of the relevant blood markers for liver dysfunction and inflammation while the overall gut microbiome structure was unaffected after three months of supplementation [39].

The neuroprotective effect of tauroursodeoxycholic acid (TUDCA), a hydrophilic bile acid and naturally produced in the liver by conjugation of taurine to UDCA, has been recently elucidated [40]. Several studies have shown that TUDCA has neuroprotective action in several models of neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and Huntington's disease [40]. Currently, there is one registered clinical trial with TUDCA in Alzheimer's disease, in the United States (Clinical Trials registration: NCT03533257).

Additionally, countermeasures against sarcopenia are important clinical issues in research aimed at improving well-being. Maintaining muscle strength with diet and exercise is important to prevent sarcopenia, and recent studies have shown that TGR5 present in the skeletal muscle plays an important role in muscle maintenance [41, 42]. Exercise-induced stimulation enhances TGR5 receptor expression in the skeletal muscle, and the increased after-meal levels of bile acids in the blood act as ligands for the TGR5 receptor, helping maintain muscle and prevent sarcopenia. Furthermore, TGR5 signaling in the muscle activates muscle metabolism and enhances glucose clearance, which also has a positive effect on glucose metabolism [41, 42]. Countermeasures against so-called frail complexes, such as sarcopenia, locomotive syndrome, and frailty, are extremely important clinical issues in supporting medical care aimed at improving well-being. It should be emphasized that the gut microbiota-bile acid-receptor signal is a novel therapeutic and prophylactic target molecule for improving frailty.

Acknowledgements

This work was partly supported by the MAFF Commissioned Project Study on the "Project for the realization of foods and dietary habits to extend healthy life expectancy" (grant number JPJ009842).

Author contributions

All authors were involved in the study design. This review was based on animal and human studies conducted by the group of YN and TT. RI performed metagenome analysis of gut microbiota. YN wrote the manuscript, and all authors reviewed and approved the paper.

Competing interests

YN received scholarship funds from EA Pharma. Co. Ltd., a collaboration research fund from Taiyo Kagaku Co., Ltd., and received lecture fees from Mylan EPD Co., Takeda Pharma. Co. Ltd., Mochida Pharma. Co. Ltd., EA Pharma. Co. Ltd., Otsuka Pharma. Co. Ltd., and Miyarisan Pharma. Co. Ltd. TT received a collaboration research fund from Fujifilm Medical Co., Ltd., and received lecture fees by Mochida Pharma. Co. Ltd., and Yanssen Pharmaceutical K.K. This study was partly supported by these funds. Neither the funding agency nor any outside organization participated in the study design or had any competing interests. These companies approved the final version of the manuscript.

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