Medical Sciences

Therapies for Primary Sclerosing Cholangitis: Vancomycin as a Novel Treatment Option

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Primary sclerosing cholangitis (PSC) is a disease of the bile ducts that causes inflammation and destruction of the intra- and/or extra-hepatic bile ducts. It is also a progressive disorder that leads to fibrosis and liver failure, and increases the risk of malignancy. PSC is a heterogeneous disease that is often associated with inflammatory bowel disease (IBD), mainly ulcerative colitis (UC). As of now, there is no established medical therapy for PSC and many patients will eventually require liver transplantation. PSC is the fifth leading cause for liver transplantation, but transplantation does not guarantee a cure since there is a 20% chance of disease recurrence in the graft. At present the mainstay of therapy is Ursodeoxycholic acid (UDCA) which has largely been studied in various randomised control trials but has failed to alter the long-term outcome and natural course of the disease. Pathogenesis of PSC is still not clearly understood but recent advances have paved way for trial of new therapeutic agents. Here in this review article, we present information gathered from published case reports/series and randomised control trials on the relationship between the microbiota and PSC pathogenesis with a purpose of understanding whether vancomycin is a potential effective pharmacotherapy for patients with this disease.

Vancomycin | Primary sclerosing cholangitis | Pathogenesis | Future therapeutic options

Introduction

Primary sclerosing cholangitis (PSC) is a chronic progressive disease of the bile ducts of unknown pathogenesis, causing biliary tract inflammation and fibrosis of intra- and/or extra-hepatic bile ducts that can ultimately lead to cirrhosis and liver failure (1). It also increases the incidence of malignancy. More than 40% of deaths in PSC patients are cancer related, with 10% of the patients developing cholangiocarcinoma, largely due to the chronic inflammation of the biliary tract (2, 3). Furthermore, PSC is a heterogeneous disorder with genetic, immunologic, or environmental factors influencing its pathogenesis and often coexistence with inflammatory bowel disease (IBD). Fifty to 75% of IBD patients have PSC which increases the risk of colorectal carcinoma in these patients by 10-fold (2, 7). PSC can affect both children and adults, with a slight male predominance. Also 25% of patients with PSC have concurrent autoimmune diseases (8). Many patients will undergo liver transplantation in a median period of 10 years from the onset of the disease, thus making it the fifth leading indication for liver transplantation (4). But transplantation is not a guaranteed solution because 20% of the PSC patients can have a recurrence of the disease post-transplantation (5, 6).

There have been various clinical trials of different pharmacologic agents, including immunosuppressant and antifibrotics. Ursodeoxycholic acid (UDCA) treatment that produces a favourable outcome for another cholestatic disease, primary biliary cirrhosis (PBC), does not alter the natural progression of PSC (11). Thus, as of now, there is no effective pharmacotherapy for PSC (9). Considering the morbidity and mortality of PSC and recurrence of the disease in post-transplant patients, safe and effective pharmacotherapy agents are critically needed. Recent published studies suggest that microbiota, mainly the enteric bacteria, could be important and modifiable pathogenic factor in the PSC pathogenesis and progression. In this review, we discuss the possible role of the microbiota in the etiopathogenesis of PSC and examine the role of vancomycin as a potential effective pharmacotherapy for patients with this disease.

Pathogenesis of PSC

The exact pathogenesis of PSC remains unclear, which can be ascribed to: 1) a lack of appropriate clinical test which can detect early PSC or its progression; and 2) a lack of an ideal animal model.

PSC is recognized as a heterogeneous disorder, having associations with various HLA and non-HLA haplotypes, autoimmune diseases, IBD, especially UC, and various environmental and genetic factors. However, the interactions between these various factors are still not clearly understood (12). We present prevailing notions of the factors contributing to the pathogenesis of PSC.

1) Role of autoimmunity and cellular immunity in PSC

a) Innate Immunity: Histological findings in PSC include mixed inflammatory cells (i.e. lymphocytes, plasma cells and neutrophils), and as the disease advances, portal fibrosis and degeneration and atrophy of biliary epithelial cells (13). Thus, such findings point towards hepatic innate immunity as one of the inciting events in the pathogenesis of PSC. This innate immunity could be initiated by exogenous factors such as the gut luminal antigens including bacteria or its derivatives lipopolysaccharides (LPS, a.k.a. endotoxin) and peptidoglycan (PG), which enter the portal circulation via disrupted intestinal barrier. The consequent activation of inflammatory cells like macrophages, dendritic cells and NK cells by these gut luminal antigens causes the secretion of various cytokines and recruitment of lymphocytes (14). This, in turn, causes biliary epithelial cells (BECs) to initiate pro-inflammatory and pro-fibrotic responses. Normally, BECs express only HLA class I and not class II molecules. But in PSC these cells have an aberrant expression of HLA class II molecules (13, 15) and over express adhesion molecules, thus accelerating the fibrotic inflammatory process (16).

b) Cellular Immunity: An autoimmune etiology for PSC is supported by several factors: 1) concurrent presence of other auto-

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immune disease in up to 25% of patients; 2) strong linkage to HLA complex; 3) tissue infiltration by various immune cells; and 4) presence of various autoantibodies like perinuclear anti-neutrophil cytoplasmic antibodies or nuclear antibodies in the serum of such patients (17). It is believed that the generation of autoantibodies, triggered by some exogenous factor or infection, inhibits normal cell function, mainly that of cholangiocytes (18). Binding of these autoantibodies to BEC antigens causes the up regulation of ERK1/2 signaling and toll like receptors, which in turn, increase the secretion of various cytokines/chemokines, initiating an inflammatory process (19).

2) Role of a leaky Gut in PSC

a) The leaky gut hypothesis: The PSC-IBD association is thought to occur because of increased intestinal permeability in patients with IBD (20) and due to a direct anatomic connection between the gut and the liver through the portal tract via enterohepatic circulation (21). As discussed above, the activation of the hepatic cellular innate immunity often occurs in response to bacteria or its derivatives. The increased intestinal permeability resulting from an inflamed gut in IBD facilitates the translocation of these luminal antigens to reach the liver and bile duct system. This hypothesis is often referred as the "leaky gut hypothesis" which suggests a connection between the gut inflammation and PSC (12). Evidence from various animal models reveals that gut microbial flora dysbiosis and/or exogenous bacterial antigen(s) administration often lead to hepatobiliary inflammation with features like PSC. Indeed, jejunal ligated rats with small bowel bacterial overgrowth develops liver lesions akin to PSC (22). However, treatment of these rats with antibiotics like metronidazole and tetracycline, improve the lesions suggesting that gut microbiota modification may be important in PSC resolution (23). Further, intrahepatic bile duct irregularities with focal areas of narrowing and evidence of bile duct destruction develop upon intraperitoneal injection of PG in rats (24). Moreover, a rectal administration of a chemotactic peptide produced by E. coli resulted in a mixed inflammatory hepatobiliary infiltrate with small duct cholangitis (25). In a recent animal model study, Balb/c mice repeatedly inoculated with S. intermedius developed cholangitis and produced antinuclear antibodies and antibiliary epithelial cell, just as seen in PSC patients (19, 26). All together, these studies indicate that bacteria and their derivatives and metabolites function as molecular mimics to trigger an innate immune response and cause PSC development. However, direct evidence for bacteremia in the portal vein in patients with PSC-IBD are lacking (27, 28). It is possible that the translocation of intestinal bacteria and it products may be episodic and therefore hard to detect but could still play a critical role in accelerating PSC pathogenesis and progression. Future studies are needed to validate this hypothesis.

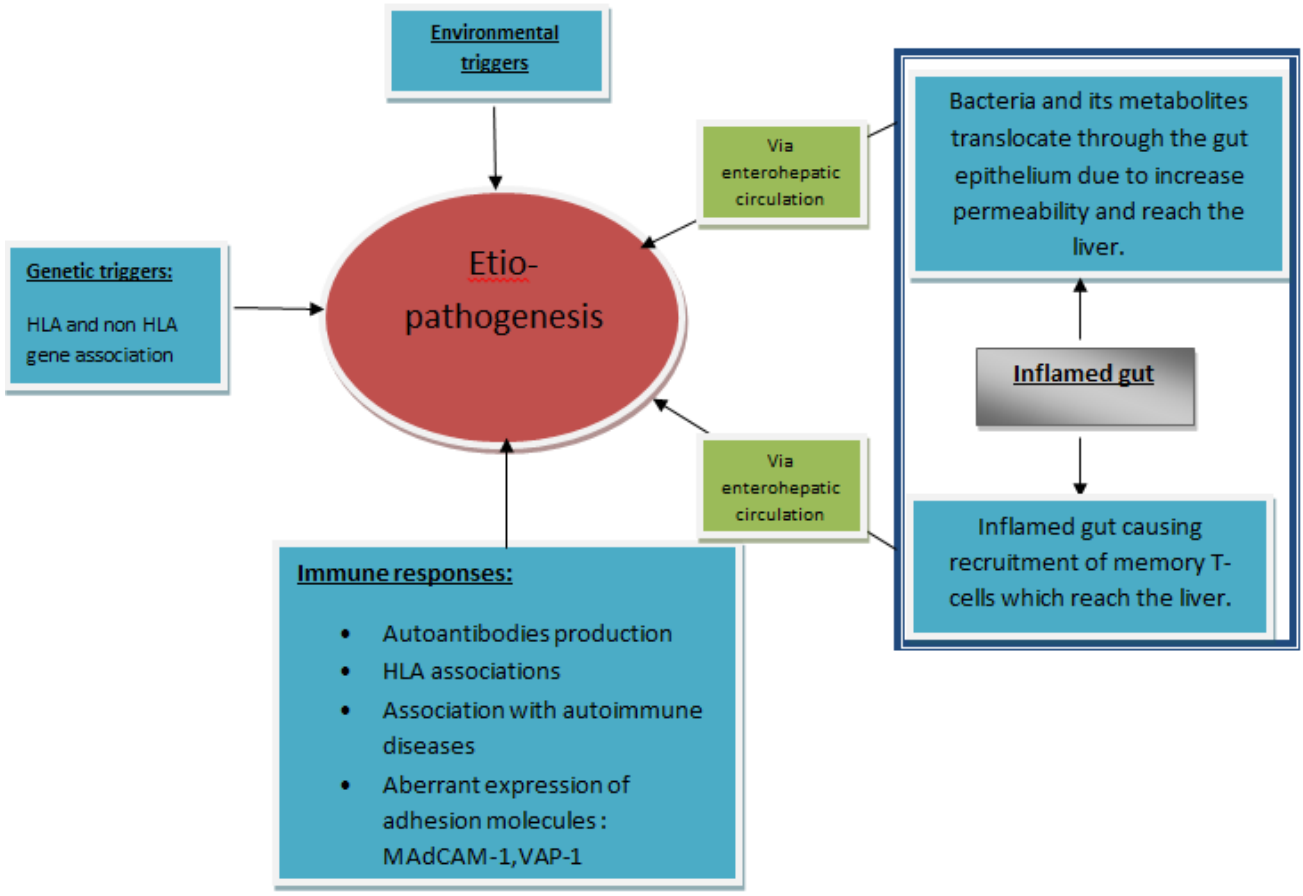

Figure 1. Etio-pathogenesis of PSC.

b) The Gut lymphocyte homing hypothesis: CCR9+ α4β7+ memory T lymphocytes in the inflamed gut may persist as long lived memory cells. These cells can enter the biliary duct system through enterohepatic circulation, thus causing inflammation in PSC via aberrantly expressed adhesion molecules in liver and gut. This hypothesis is referred as "gut lymphocyte homing hypothesis" (29). Mucosal addressin cell adhesion molecule-1 (MAdCAM-1) is usually expressed in the gut of normal healthy people. But, there is an aberrant expression of these adhesion molecules in the liver of PSC patients. In addition, the vascular adhesion protein-1 (VAP-1) expression is abnormally increased in IBD patients (30), and the mucosal plasmocytoid dendritic cells fail to function in PSC patients resulting in altered production of T-regulatory cells. All these factors result in the infiltration of T-helper 17 cells in the liver that accelerates inflammation leading to PSC development.

These models and hypothesis for PSC compiled together still does not completely clarify the pathogenesis of PSC. However, every step of increase in the understanding of this devastating disease brings us closer to an effective therapy in the future. Since PSC is a heterogeneous disorder, it is possible that a combination of different drug treatments would have to be used to target the different aspects of this disease rather than relying on a monotherapy approach.

The etiopathogenesis for PSC is summarized in figure.1.

Treatment Options for PSC

Vancomycin

Vancomycin is a bactericidal glycopeptides which blocks the synthesis of the cell wall in Gram-positive bacteria. It binds to the D-Ala-D-Ala area (31) thus preventing cell wall synthesis of the long polymers of N-acetylmuramic acid and N-acetylglucosamine which are the backbone strands of the bacterial cell wall (32). Gram-negative micro-organisms are not affected because of the porin channels in their cell walls which cannot accommodate the large vancomycin molecule.

As reviewed above, there are various animal studies and hypotheses which have revealed a link between the gut microbiota and PSC. Significant improvement with daily treatment with antibiotics suggests that gut microbiota modification could be an effective form of therapy for PSC patients. Various studies in the form of case reports, case series, and RCT have been conducted using vancomycin as the antibiotic that have shown effective results, as discussed below.

A case report of a 15-year-old female child diagnosed with chronic active UC and PSC demonstrated normalization of the liver enzymes and bile ducts and resolution of her UC symptoms with colonic mucosal healing with a single drug therapy with oral vancomycin (33). In addition, there has been a case series comprising of three pediatric patients with PSC and IBD who have also achieved normalization of their liver tests and resolution of symptoms with oral vancomycin treatment (34). The same investigators recently conducted a study on 14 pediatric patients with PSC and IBD who were treated with oral vancomycin for 54 ± 43 months (35). Nearly all patients showed normalization or significant improvement in serum liver tests, erythrocyte sedimentation rate and symptoms (35). Conversely, when vancomycin was discontinued, there was a recurrence of disease symptoms and an increase in liver enzymes, and retreatment again resulted in normalization of liver enzymes.

There has been a recent double blind randomized clinical trial (36) of vancomycin and metronidazole which included 35 patients with PSC who were randomly distributed into 4 groups: low- and high-dose of metronidazole and vancomycin, respectively. The primary endpoint of the study was a measure of a decrease in alkaline phosphatase at 12 weeks compared to baseline. The secondary endpoints were to determine the decrease in (i) AST, total bilirubin, Mayo PSC risk score and CRP at 12 weeks compared to the baseline value, (ii) decrease in fatigue severity and pruritus VAS score at 12 weeks compared with baseline and (iii) adverse events anytime during the 12 weeks of treatment. Individual responses were variable, but a significant decrease in alkaline phosphatase at 12 weeks (the primary endpoint in the study) was noticed in the low (-46%, P = 0.03) and high dose (-40%, P = 0.02) vancomycin group. In addition, 2 patients in the low-dose vancomycin group had normalization of alkaline phosphatase compared to the other three treatment groups. Moreover, the low dose vancomycin group also reached some of the secondary endpoints exhibiting a significant decrease in Mayo PSC risk score (−0.55, $P = 0.02$) and a trend towards a significant decrease in total bilirubin (−33%, $P = 0.06$) and CRP (−69%, $P = 0.06$). While the high dose vancomycin group experienced significant improvement in pruritus compared to the low dose group, none of the other primary or secondary endpoints were different between the low or the high vancomycin dose groups. Notably, the adverse events in both vancomycin groups were generally mild and infrequent but its chronic use may result in emergence of more vancomycin resistant enterococci. While no serious adverse events were noted in the study, minor adverse events were reported. One patient in the low dose vancomycin group stopped treatment due to migraine headaches and increased diarrhea while another patient in the high dose vancomycin group stopped treatment due to diarrhea and increased fatigue. The possibility of whether these minor adverse events were related to the study medication or they were coincidental was not ruled out. Although not reaching the primary endpoint, the metronidazole groups also experienced some therapeutic effects. A decrease in total bilirubin (-20%, P = 0.03), Mayo PSC risk score (-0.16, P = 0.03) and CRP (-49%, P = 0.03) in the low-dose metronidazole group and a significant decrease in pruritus in the high dose metronidazole group was reported. However, adverse effects were more often encountered in the metronidazole groups like burning in the eyes, nausea, diarrhea, anorexia, fatigue and/or metallic taste (especially in the high dose metronidazole group). Thus, with these encouraging results and safety profile of vancomycin, it was recommended that vancomycin (mainly the low dose) should be further investigated in a bigger, long-term placebo controlled trial (36). Currently a larger clinical trial is ongoing to examine vancomycin efficacy in improving liver biochemistry by studying its antimicrobial and immunomodulatory effects. [ClinicalTrials.gov identifier: NCT01802073].

As discussed, liver transplantation remains the treatment of choice for end stage PSC but disease recurrence in the graft remains an important issue. There was a case report where vancomycin (500 mg vancomycin orally three times a day) was given along with immunosuppressant medications for recurrent PSC post-transplant. Vancomycin treatment resulted in the liver enzymes, erythrocyte sedimentation rate and C-reactive protein levels to return to normal. The patient continues to be on vancomycin and is doing well to date. Her liver tests are normal and the repeat liver biopsy after 3 years from the recurrence reveals no inflammation or fibrosis of the bile ducts (37).

UDCA

Given its proven usage in PBC, UDCA has been extensively studied as a therapy for PSC. Various studies have shown improvement in the level of abnormal liver enzymes, but none

showed survival benefit or delay in the need for liver transplant (38). A double blind RCT using a high dose of UDCA (28–30 mg/kg/day) was conducted which had to be terminated early because of serious adverse effects (39). Also, recent meta-analysis of nine RCTs concluded on UDCA with different doses revealed no significant improvement in mortality and disease progression (40). These results have led the American Association for the Study of Liver Diseases to recommend not using UDCA for patients with PSC (41).

Immunosuppressants

Various immunosuppressants' have been tried for managing PSC. However, most have serious adverse effects and none have been found to be effective (10). This precludes their use for PSC treatment except in patients with overlapping PSC/autoimmune hepatitis and those with PSC and high immunoglobulin 4 serum levels.

Tacrolimus

This drug was studied in an open label, phase II trial of 16 patients with PSC but most of the patients withdrew from the trial due to its adverse events (42). Newer drugs, like Sirolimus and everolimus, which are mechanistic targets of rapamycin (mTOR) inhibitors, have been found to reduce liver fibrosis and inflammation in bile duct ligated rats (43). Thus, this can be a potential therapeutic therapy in PSC. However, further studies are needed before advocating the use of these drugs for PSC therapy.

Anti-fibrotic agents

As PSC progresses, fibrosis is a predominant feature with an eventual replacement of the bile duct by a solid fibrous cord. Hence, various anti-fibrotic agents such aspirfenidone and colchicine have been tried but have failed to show efficacy in the treatment of PSC (44,45). Fibrates which are peroxisome proliferator activated receptor alpha (PPAR-α) agonists that decrease IL-1-induced C-reactive protein expression have also been examined in a case series. PSC patients were treated with bezafibrate for 6 months, but showed no benefits (46).

Future therapeutic options

Vedolizumab

Vedolizumab is a monoclonal antibody and VAP-1 blocker that binds to MadCAM-1, thus preventing activation of α4β7 T cells. The lamina propria and intraepithelial T cells of the small and large intestine have a chemokine receptor CCR9 (47, 48) that bind to CCL25 and results in the activation of α4β7 T cells. These activated cells then bind to MadCAM-1 in the gut endothelium, thereby causing the homing of T cells to the bowel in the healthy state. This process is affected in IBD thus altering the recruitment of pathogenic T cells. After undergoing various trials, vedolizumab is now licensed for IBD therapy (49). Also, an aberrant expression of CCR9 and its ligand CCL25 is observed in the liver of PSC patients (50)**.** Thus, targeting this pathway by vedolizumab can be a therapeutic development in PSC. One such trial is ongoing [ClinicalTrials.gov identifier: NCT02239211].

Simtuzumab

Lysyloxidase 2 (LOXL2) is found to promote fibrogenesis by facilitating the cross linking of type I collagen in various experimental models (51). Thus, Simtuzumab, a monoclonal antibody against LOXL2, can be an effective therapeutic agent, which is now being studied in a phase II trial in patients with PSC (ClinicalTrials.gov Identifier: NCT01672853).

All-trans retinoic acid (ATRA)

ATRA is a potent inhibitor of bile acids in humans (52). Treatment with a combination of UDCA and ATRA has been found to significantly reduce liver fibrosis/necrosis, proliferation of bile duct, and bile salt pool size in bile duct ligated rats compared to ATRA or UDCA alone (53)**.** In a recent study of PSC, 15 patients were given a combination therapy of ATRA and UDCA for 12 weeks. While a significant decrease in abnormal liver enzymes and bile acids was observed, there were frequent adverse events like headache and tinnitus. Thus, additional studies with a lower dose of ATRA are needed to establish the efficacy of this agent (54).

NorUDCA

Removal of one side chain of a methylene group from UDCA produces 24-norUDCA. As discussed above, usage of high dose of UDCA in patients with PSC generates various adverse effects due to the markedly increased levels of hepatotoxic bile acid, lithocholic acid (LCA) (55). In contrast, norUDCA is secreted in an unconjugated, glucoronidated form in the bile (56) and its metabolite does not accumulate or cause hepatotoxicity in animal models (57). In addition, norUDCA administered to Mdr2 knockout mice stimulated canalicular flow and increased the hydrophilicity of bile (58). Thus, norUDCA can also be an effective therapeutic agent in PSC, for which phase II trials in humans are in progress [Clinical Trials. gov identifier: NCT01755507].

Farnesoid X receptor/FXR agonists

FXRs play a key role in bile acid homeostasis by down regulating cytochrome P450 7A1, a rate limiting enzyme in bile salt production (59,60). In addition, it has also been shown to promote liver regeneration (61,62)**.** Obeticholic acid (OCA), a derivative of the natural human bile acid, is a selective FXR agonist with very high FXR affinity (63-65). In an animal model of cholestasis where LCA was administered, it was found that OCA therapy was protective for hepatocytes (66). In another study, OCA therapy reduced liver fibrosis in bile duct ligated rats (67). These results show that FXR agonists could be of therapeutic benefit in PSC, for which a phase II clinical study is ongoing [Clinical Trials. gov identifier: NCT02177136].

Apical sodium-dependent bile acid transporter (ASBT) inhibitors

Abnormal bile acid levels have been found to affect the progression of PSC (68). ASBT is expressed mainly in the distal ileum and helps in the reabsorption of bile acids from the small intestine, thereby maintaining the enterohepatic circulation of the bile acids (69). Thus, the use of ASBT inhibitors to interrupt this pathway could be beneficial in decreasing the bile acid liver load. Currently a phase II clinical trial is ongoing to evaluate the safety and efficacy of LUM001, an ASBT inhibitor, in patients with PSC (ClinicalTrials.gov Identifier: NCT02061540).

Conclusions

Treatment of UC-PSC patients with vancomycin has been shown to improve both UC symptoms and liver enzyme levels suggesting that it could be of therapeutic importance in such patients. Vancomycin may be working both as an antibiotic and immunomodulator. Mechanistically, vancomycin efficacy could be via modifying the gut microbiome to normalize liver enzymes and decrease PSC symptoms. Also, since vancomycin given orally exhibits minimal systemic absorption, it may be playing an immunomodulatory role in the intestine. While clinical trials with vancomycin are few and small, the

results with this antibiotic are encouraging. Further, while vancomycin appears to be a safe and effective therapy in PSC, the concern of emerging antibiotic resistance remains. Fecal and bile transplantation which can alter the microbe flora could also be an important consideration for future therapies in these patients. By focusing on enteric microbe flora as a target in PSC, further trials should identify the mechanisms by which vancomycin works in PSC and then move towards developing more specific antimicrobial agents which have a lower risk of producing resistant microorganisms. The data supporting the PSC microbiota hypothesis continues to grow and with rapid evolvements in molecular and genetic techniques, it is likely that in near future we will have a better understanding of this disease and an effective treatment modality.

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Abbreviations
Primary biliary cirrhosis (PBC); Primary sclerosing cholangitis (PSC), Inflammatory bowel disease (IBD), Ulcerative Colitis (UC), Ursodeoxycholic acid (UDCA), Randomized control trial (RCT), Biliary epithelial cells (BECs), Pathogen associated molecular patterns (PAMPs), lipopolysaccharides (LPS), Peptidoglycan (PG), Mucosal addressin cell adhesion molecule-1 (MAdCAM-1), Vascular adhesion protein-1 (VAP-1), Peroxisome proliferator activated receptor alpha (PPAR-α), Lysyl oxidase 2 (LOXL2), All-trans retinoic acid (ATRA), Lithocholic acid (LCA), Farnesoid X receptor (FXR), Obeticholic acid (OCA), Apical sodium dependent bile acid transporter (ASBT), mechanistic target of rapamycin (mTOR).

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