1 **Crosstalk between the gut and the liver via susceptibility loci: novel advances in**
2 **inflammatory bowel disease and autoimmune liver disease**

3 **Short title: Susceptibility loci for IBD and AILD**

4
5 Xinyang Li, Jun Shen, Zhihua Ran

6 State Key Laboratory for Oncogenes and Related Genes, Key Laboratory of
7 Gastroenterology & Hepatology, Ministry of Health, Division of Gastroenterology and
8 Hepatology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University,
9 Shanghai Cancer Institute, Shanghai Institute of Digestive Disease, 160# Pu Jian Ave,
10 Shanghai 200127, China

11 Correspondence: Shen Jun (shenjun79@sina.cn)

Abstract

Inflammatory bowel disease (IBD) is an autoimmune disorder characterized by chronic, relapsing intestinal inflammation. Autoimmune liver disease (AILD) may be involved in IBD as an extra-intestinal manifestation (EIM). Epidemiologic and anatomic evidence have demonstrated an intimate crosstalk between the gut and the liver. In this review, we briefly introduced nine groups of susceptibility loci shared by inflammatory bowel and autoimmune liver disease for the first time. The genome-wide association studies (GWAS) evidence of pathways involving crosstalk between the gut and the liver is clarified and explained. It has been found that *HNF4- α* , *GPR35*, *MST1R*, *CARD9*, *IL2/IL21/IL2R*, *BACH2*, *TNFRSF14*, *MAdCAM-1*, and *FUT2* are the genes involved in tight junction formation, macrophage function, T helper cell or T_{reg} cell cycle and function, TNF secretion, lymphocyte homing or intestinal dysbiosis, respectively. The intimate crosstalk between the gut and liver in immunity is also highlighted and discussed in this review.

Keywords: inflammatory bowel disease; autoimmune liver disease; pathogenesis; susceptibility loci

Introduction

Inflammatory bowel diseases (IBD) include ulcerative colitis (UC) and Crohn's disease (CD). Complex, multifactorial disorders of the bowel characterized by chronic relapsing of intestinal inflammation are the core pathologic manifestation of IBD. Recent studies convincingly demonstrate that various genetic factors, the environment, and immune responses to external factors (e.g., microbiota) are linked to the onset of IBD, although the precise mechanisms remain unknown [1]. Accumulating evidence suggests that an overactivation of the immune response in the intestine to specific microbes will facilitate the development of IBD in hosts with a genetic predisposition [2]. Therefore, a trend of genome-wide association studies (GWAS) to identify IBD susceptibility loci have emerged. A total of 163 IBD loci have been found that meet the genome-wide significance thresholds. Moreover, a total of 110 out of 163 loci are associated with both disease phenotypes. Of the remaining 53 loci, 30 are classified as CD-specific and 23 as UC-specific [3].

Approximately 30% of IBD patients develop abnormal liver function associated with the disease. The abnormal liver function can be caused by primary extra-intestinal manifestations (EIM), or secondary extra-intestinal complications [4]. Primary EIM includes musculoskeletal, mucocutaneous, eyes, and hepatobiliary systems that are directly associated with IBD. However, there are also complications secondary to IBD pathophysiologic pathways, including gallstones, anemia, malnutrition, and osteoporosis [5-8]. In addition, the incidence of a particular EIM differs between UC or CD, and some EIMs occur in parallel to the activity of IBD [9]. As the most frequently encountered

1 hepatobiliary EIM of IBD, primary sclerotic cholangitis (PSC) has been studied
2 comprehensively. However, the consistency of PSC and IBD activity has not been
3 completely confirmed [10]. Autoimmune hepatitis (AIH) and overlap syndrome (primarily
4 AIH/PSC-overlap) has been found to be linked to IBD as well [11]. Moreover, molecular
5 mimicry is also evident from studies involving the etiology of primary biliary cirrhosis
6 (PBC). Similarities in both the bacterial antigens and pyruvate dehydrogenase E2 molecules
7 can be recognized by anti-mitochondrial antibodies (AMA). In addition, the intestinal
8 microbiota may also be involved in the pathogenesis of PBC [12].

9 Recent studies demonstrate an intimate link between IBD and autoimmune liver
10 disease, with epidemiologic evidence linked to several underlying pathways [4]. However,
11 the mechanism of the corresponding susceptibility has not been clearly defined, despite the
12 evidence provided by the GWAS. Since both IBD and autoimmune liver disease are both
13 autoimmune conditions, separate steps involved in the immunologic pathogenesis of IBD
14 and autoimmune liver disease share a certain degree of similarity. *HNF4- α* , *GPR35*,
15 *MST1R*, *CARD9*, *IL2/IL21/IL2R*, *BACH2*, *TNFRSF14*, *MAdCAM-1*, and *FUT2* have
16 verified loci shared by both PSC and IBD (Table.1). The loci can also be shared by PBC,
17 AIH, or either IBD-type individually [13, 14]. *HNF4- α* regulates innate immunity, and the
18 first line defense mediated by the tight junctions of the mucosa, is instructed by *HNF4- α*
19 [15]. As a pivotal component of innate immunity, macrophages are instructed by *GPR35*,
20 *MST1R*, *p53*, *IL2/IL21/IL2R*, and *BACH2*; the genes that are involved in T cell
21 functionality. T_{reg} cells play roles in adaptive immunity, and *MAdCAM-1* serves as a
22 lymphocyte homing molecule. Bacteria that have been linked to the onset of IBD are

coordinated by *FUT2*. Any abnormalities in *FUT2* leads to dysbiosis, which triggers inflammation in the liver .

Correlation between IBD and autoimmune liver disease

Clinical manifestations and the pathophysiologic mechanisms of IBD and autoimmune liver disease

As many as 80% of PSC patients have IBD [9]. However, only 2% - 7.5% of all IBD patients suffer from PSC. Additionally, 85% - 90% of PSC patients with IBD present as UC, and the remaining patients suffer from CD [16]. For the extremely high incidence of IBD and higher colon carcinoma in the PSC group, all PSC patients are required to perform a colonoscopy regularly. Close, long-term surveillance is necessary even after a liver transplantation [17]. The chronological relationship between the diagnosis of IBD and PSC remains unknown. The unparalleled chronological onset or relapse of IBD and PSC indicates a shared underlying pathologic pathway or primary hepatobiliary molecular abnormality, rather than merely circulating immunologic molecules or cells [18].

Several studies have been conducted regarding the phenotype of IBD, with or without PSC. IBD patients with PSC could be considered as a different phenotype or distinct clinical entity of IBD. This specific IBD phenotype with concomitant PSC is denoted PSC-IBD syndrome. The uniqueness of PSC-IBD syndrome is characterized by rectal sparing and backwash ileitis in the PSC-IBD group (52% and 51%, respectively) compared with the control groups (6% and 7%, respectively). The overall survival from the initial clinic visit was significantly worse among PSC-IBD group than chronic ulcerative colitis group (79% vs. 97%) at five years [19]. Compared with females, males are not as

1 frequently affected by the distinct PSC-IBD syndrome. In UC patients without PSC, the
2 distal or sigmoid colon-rectum is primarily affected, while few patients suffer
3 from backwash ileitis. A right-sided predominant pattern is described in PSC-UC syndrome
4 [20]. In CD patients with concomitant PSC, the left colon is more frequently involved,
5 compared with isolated CD patients. Unlike PSC-UC syndrome, PSC-CD affects females
6 more than males, frequently manifesting as small duct PSC. The incidence of cancer, liver
7 transplantation, or death is lower compared with isolated CD patients [21].

8 A higher rate of pancolitis and a less aggressive course of AIH with IBD have been
9 described. However, a younger age at onset, refractory to treatment, a higher death rate, or
10 the need for a liver transplantation are characteristics of AIH-IBD. The prevalence of IBD
11 in patients with an overlapping PSC–AIH has been reported to be slightly higher than in
12 patients with isolated AIH [22]. Moreover, the high rate of concomitant IBD indicates the
13 dominant role of PSC in PSC-AIH with IBD. The incidence of overlapping syndromes,
14 including AIH–PBC or AIH–PSC in adult patients with both IBD and AIH is higher than in
15 patients with only IBD and AIH [11]. More studies will be included after the determination
16 of diagnosis criteria for AIH-PSC, or AIH-PBC.

17 The epidemiologic and chronologic characteristics of IBD and autoimmune liver
18 disease indicate an intimate but complex relationship. The restoration of T cells after liver
19 transplantation to treat cholestasis is postulated to be responsible for the exacerbation of
20 IBD. However, under the interference of immunosuppression drugs, the precise effect of T
21 cells is uncertain [23].

22 Oral immune tolerance develops in the intestine and liver. The nature of high
23 exposure to foreign material in the intestine requires an effective oral tolerance. Therefore,

1 the pathogenesis of IBD and autoimmune liver disease indicates the loss of immune
2 tolerance [24, 25]. In addition, genetic factors have also been studied. Genes related to T
3 cell apoptosis and the JAK-STAT signaling pathway are shared between PSC and IBD [4].
4 Moreover, both colon cancer and cholangiocellular carcinoma are more common in PSC-
5 IBD patients [13].

6 Leakage of microbiota into the liver caused by an impairment in the gut
7 mucosal barrier, and disrupted physiological liver function may elicit gut dysfunction [26].
8 Studies and clinical trials indicate that one aspect of the underlying pathogenesis of IBD is
9 highly likely to be an overactive immune response to physiologic resident flora [27].
10 Microbiota also contributes to the pathogenesis of impaired intestinal barrier-associated bile
11 duct injury in the clinic.

12 **Shared susceptibility loci and IBD pathways of and autoimmune liver disease**

13 All clinical manifestations are a demonstration of the response to exogenous stimuli,
14 of which the response is determined by corresponding pathways. The pathway is
15 directed by gene expression that are responsible for relevant stimulation. Therefore, the
16 study of susceptibility loci is required to facilitate the treatment and prognosis of patients.

17 There are 110 shared susceptibility loci in UC and CD out of a total of 163
18 susceptibility loci. Compared with the high rate of shared susceptibility loci between UC
19 and CD, only 8 out of 16 PSC genes are shared by both PSC and IBD. The inconsistency of
20 clinical findings and genetic analysis require further effort into the underlying pathogenesis
21 [13]. There is a hypothesis that has been proposed, indicating that exogenous factors also
22 play a crucial role in PSC pathogenesis [13].

Innate immunity

Physical barriers and humeral molecules

Hepatocyte nuclear factor 4-alpha (*HNF4- α*) is a member of the superfamily of nuclear receptors. The expression of *HNF4- α* can be detected in hepatocytes, as well as the epithelia of the pancreas, kidneys, stomach, and intestine [15]. *HNF4- α* knock-out mice exhibited IBD-like colitis [28]. Moreover, *HNF4- α* is drastically reduced in CD or UC patients compared to the healthy controls [28]. *HNF4- α* was reported to be important in maintaining physiologic intestinal epithelial cell differentiation, lipid metabolism, and epithelial junctions [29]. The structural or functional integrity is established by a single layer of the epithelium and a mucus layer [30]. Claudin-15 is a direct gene target of *HNF4- α* in the intestinal epithelia, which belongs to a group of molecules that constitutes the tight junctions. Differentiation and the formation of tight junctions in the bowels are instructed by Claudin-15 [31]. A reduction in the expression of *HNF4- α* makes the intestinal epithelium vulnerable to stimuli (rather than automatic inflammation). The downstream effect of *HNF4- α* also includes Paneth or stem cell differentiation, as well as goblet cell mucin expression [15].

HNF4- α represents the central regulator of gene transcription in hepatic physiology. On one hand, *HNF4- α* is known as the master regulator of hepatic differentiation, which regulates over 60% of the hepatocyte-specific genes [32]. *HNF4- α* knock-out mice have shown that *HNF4- α* is necessary for the early embryonic development and function of the adult liver [33]. On the other hand, several studies suggest that pro-inflammatory cytokines dramatically induce *HNF4- α* [15]. Cytokines regulate the *HNF4- α* downstream pathway

1 through the control of proteasomal degradation, DNA binding affinity, transcriptional
2 activity, and cofactor interaction [15].

3 Once a stimulus interferes with the expression of *HNF4-α*, both bowel and liver
4 function will be disturbed. Few studies have been completed that specify the exact role of
5 *HNF4-α* to date. However, the pivotal role of *HNF4-α* in both the liver and the gut indicate
6 a clear crosstalk in the pathologic pathway for the two organs (Figure.1).

7 ***GPR35***

8 2q37.3 and at 18q21.2 are two identified GWAS susceptibility loci with statistical
9 significance in PSC patients. The related allele at 2q37.3 has proven to be in PSC and UC,
10 whereas 18q21.2 is more likely to act as a non-UC and PSC specific risk locus. 2q37.3
11 resides within the *GPR35* gene, which testifies the role of *GPR35* in PSC and UC. *GPR35*
12 is expressed at high levels in both the intestinal crypt enterocytes, and several
13 subpopulations of immune cells [34, 35]. KYNA is the typical ligand of *GPR35*, and is
14 highly expressed in liver excretions of the intestine, and can be dramatically induced by
15 inflammation [36]. However, there are no previous studies on the function of *GPR35* in
16 PSC patients. Available data only reveal the influence of *GPR35* on the inflammatory
17 process in the gastrointestinal and biliary tract [37].

18 *GPR35*-expressing human peripheral blood mononuclear cells elicit an anti-
19 inflammatory response due to a decrease in TNF secretion [35]. *GPR35*-deficient
20 macrophages grow in clusters. The aggregation of the macrophages can be explained by the
21 downstream $\beta 1$ integrin and $\beta 2$ integrin-mediated pathways. The close adhesion of
22 monocytes to fibronectin and ICAM-1 can be noted subsequently [38] (Figure.1). The
23 *GPR35* agonist also induces a strong macrophage recruitment in the blood *in vivo* [39].

1 Since known orphan GPCRs have offered tremendous promise, they may serve as
2 unprecedented potent, selective therapeutic targets, providing the possibly of fewer side
3 effects [40]. GPR35 is a typical orphan GPCR, which has recently been identified as a
4 novel chemokine-CXCL17 receptor [39]. Encouragingly, efforts have been made to study
5 the clinical effects of the known pathogenesis of the GPR35/CXCR8 pathway. For
6 example, bowel inflammation is alleviated by the GPR35/CXCR8 agonist, 1,4-dihydroxy-
7 2-naphthoicacid (DHNA) [41]. Therefore, more study should focus on the clinical effect of
8 DHNA on PSC as well as PSC-IBD patients.

9 ***MST1R***

10 The human macrophage stimulating 1 receptor (*MST1R*) gene product, recepteur
11 d'origine nantais (RON), is a membranous receptor for MSP. RON functions during the
12 inflammatory process mediated by MSP under conditions of cellular stress and elicit innate
13 immune responses induced by bacteria [42]. By maintaining a lower affinity to RON,
14 missense SNP disturbs the effectiveness of MSP, which leads to a poor efficacy of the
15 MSP/RON signaling pathway [43]. Mice with a deficiency in RON kinase activity are
16 highly vulnerable to a key ligand for TLR4 (i.e., lipopolysaccharide), which suggests that
17 RON can shape the innate immune response via the TLR4 pathway [44].

18 The intestine is the largest immune organ in the human body, and is constantly
19 exposed to foreign antigens [45]. Intestinal mucosal macrophages differ from other
20 macrophages. Intestinal mucosal macrophages do not express co-stimulatory molecules,
21 such as CD80, CD86, and CD40. Consistent with this phenotype, intestinal mucosal
22 macrophages do not elicit an inflammatory response to normally pro-inflammatory
23 components, such as TLR ligands. Conversely, they produce anti-inflammatory cytokines, -

1 such as IL-10 [45]. The IL-10 functions to maintain the activity of local regulatory T cells
2 [46]. TLR4 is significantly elevated in primary intraepithelial cells, as well as the lamina
3 propria mononuclear cells within the lower intestinal tract in the active phase of IBD [47].
4 The total number of macrophages entering the mucosa through the broken intestinal barrier
5 is increased in UC, as well as in CD [48].

6 Due to the anatomical relationship to the bowel, the liver serves as a sentry of
7 portal blood. The resident macrophages in the liver are called Kupffer cells. Following LPS
8 stimulation, the expression levels of TNF- α and IL-1 β genes were significantly upregulated
9 in KCs [49]. As TLR3 is involved primarily in PBC, whereas TLR4 and 9 is correlated
10 with the pathogenesis of PSC, there is a shocking increase of TNF secretion from TLR⁺
11 cells in both PSC and PBC patients [50]. Activation of downstream TLR4 transcription
12 factors induces the transcription of proinflammatory cytokines, including TNF- α , IL-6, and
13 IL-1 β [51] (Figure.1).

14 Alpinetin as well as maqian fruits essential oil (MQEO) exhibited protective effect
15 against DSS-induced intestinal inflammation through TLR4 mediated pathway [52, 53],
16 which provide an potential option for further exploration of IBD and PSC therapy.

17 **Adaptive (cell-specific) immunity**

18 PSC is characterized by T cell infiltration of the portal region [18]. Th1 cells in
19 PBC, characterized by secreting IFN- γ in the response to IL-12 are involved in the
20 hyperresponsiveness to intracellular pathogens [54]. Meanwhile, many susceptibility loci in
21 autoimmune liver disease and IBD direct the proliferation, differentiation, activity, or
22 apoptosis of T cells.

CARD9

CARD9 is a pivotal adaptor of innate immune signaling pathways. It is primarily expressed on the cell surface of myeloid dendritic cells or macrophages, and only at low levels in non-activated T and B lymphocytes [55]. CARD9 expression in the intestine tract is low under conditions of low inflammation. However, when myeloid cells are stationed in the mucosa, CARD9 is likely to increase due to mucosal inflammation [56]. CARD9 is also a downstream molecule that mediates the effect of a considerable number of innate cell surface receptors, including TLRs, NLRs, and immune receptor tyrosine-based activation motifs (ITAM)-associated receptors. Following bacterial stimulation to these receptors, CARD9 interacts with BCL10/CLAP, and activates the NF- κ B pathway [57]. In addition, CARD9 is required for signaling from most TLRs in dendritic cells, leading to the induction of the inflammatory cytokines, TNF α and IL-6, and stimulates T cells to differentiate into type 17 helper T cells [56, 58] (Figure.1). An overreaction of Th17 cells to microbes in patients with PSC has also been documented both *in vivo* and *in vitro*. Moreover, PSC patients experience the process even without IBD-related mechanisms [59]. Activation of the syk-CARD9 complex via dectin-1 (the C-type lectin molecule) promotes a pro-inflammatory reaction, including the maturation of dendritic cells, production of cytokines (e.g., IL-23), and the differentiation of T cells, which coincides with the known pathogenic pathway of IBD [60].

Moreover, IL-23 is critical for Th17 differentiation, and thus becomes a possible target for PSC treatment [61]. Ustekinumab is a monoclonal antibody directed against the p40 subunit of IL-12 and IL-23 [62]. Several clinical trials strongly indicate the superiority

of ustekinumab on (anti-TNF) refractory CD in both maintenance and remission stage [63]. On the basis of successful CD therapy by ustekinumab, more effort can be made for a more reasonable strategy of PSC.

IL-2/IL-21/IL-2R

The IL-2 family consists of IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. IL-2 is vital for the development of Th1, Th2, T_{reg}, as well as Th17 differentiation [58]. In addition, recombinant IL-2 administration has been shown to promote the cell cycle and activation of T_{reg} cells in both humans and mice [64]. Abnormal signaling through the IL-21R/γc/JAK3/STAT3 pathway interferes with normal humoral immune responses through the disturbance of long-term B-cell memory. Reduced natural killer (NK)-cell cytotoxicity and Th17 cytokine production are involved in the abnormalities of IL-21 signaling, or related pathways [65].

All involved molecules in the IL-2 pathway (e.g., IL-2/IL-21/JAK3/STAT3), are responsible for the susceptibility to IBD [66]. It has been found that the involved region of IBD exhibited an abnormally elevated level of IL-21 expression. This abnormal increase is most marked in CD patients, but UC patients also show a significant increase compared to that found in controls [67]. It is important to note that IL-2Ra is an established CD pathogenic molecule, and the *IL-2* gene is also located within a susceptibility locus for UC [66]. Il-2ra^{-/-} mice spontaneously develop an inflammatory response in both the intestine, as well as the bile ducts. In addition, the Il-2ra^{-/-} model has long been used to study PBC [68] (Figure.1).

An abnormality in the IL-2 pathway provides evidence for the higher susceptibility of IBD and autoimmune liver disease in patients with IL-2 defects. Abnormal signaling

1 through the IL-21R/γc/JAK3/STAT3 pathway, and the IL-2-related activation of the
2 JAK/STAT pathway provides another potential mechanism of IBD. This is because the
3 function of Th17 is closely linked to the JAK/STAT pathway. A pivotal downstream
4 pathway is mediated by JAK-STAT, which balances both the adaptive and innate
5 components of mucosal immunity together with the repair of the intestinal mucosa. In
6 addition, the JAK-STAT pathway serves as a pivotal mediator in many physiologic
7 processes, including epithelial proliferation, differentiation, and apoptosis. The highly
8 pathologic inflammatory status of the intestine (i.e., in cases of UC and CD) is associated
9 with elevated STAT signaling [69].

10 Patients with greater active UC levels treated with oral JAK inhibitor Tofacitinib
11 have a higher effective and remission rate than those receiving the placebo [70]. Moreover,
12 with the theoretical basis, PSC as an atypical autoimmune disease, may benefit from the
13 treatment of Tofacitinib.

14 ***BACH2***

15 *BACH2* is thought to regulate many aspects of B cell function, including class
16 switching, recombination (CSR), and somatic hypermutation [71]. However, recent studies
17 indicate that *BACH2* “locks” the T cells in a naïve state, and subsequently impairs the
18 establishment of T cell memory. Defects of *Bach2* result in the loss of the naïve T-cell
19 population, and promote T cell memory primarily via Th2 genes. *BACH2* has a common
20 pathogenic pathway in both T and B cells. Moreover, *BACH2*^{-/-} mice exhibit a tendency to
21 rapidly differentiate into mature Th2 and plasma cells, simultaneously resulting in the loss
22 of naïve cells. [72].

BACH2 controls the balance between tolerance and immunity. Therefore, many autoimmune diseases can be related to an abnormal differentiation instruction to CD4⁺ T-cells. In particular, the function of T_{reg} cells is impacted significantly by *BACH2*. Moreover, the effectiveness of T_{reg} cells must be ensured by normal expression of *BACH2* genes [66]. Abnormally reduced Bcl-2 and Mcl-1 levels, and an elevated Bim/Bcl-2 ratio eventually leads to the loss of T_{reg} cells in *BACH2*-deficient individuals [73] (Figure.1). The most well-characterized PSC variant of *BACH2*, rs56258221 (OR = 1.23; P = 8.36 × 10⁻¹²), is in a low-to-moderate LD with the previously reported *BACH2* variants in Crohn's disease [74]. Therefore, as immune tolerance and function of T_{reg} cells are emphasized in both IBD and autoimmune liver disease, *BACH2* may be a potential candidate for therapy of these gastrointestinal diseases.

TNFRSF14

TNFRSF14 has been found to be a susceptibility loci for both autoimmune liver disease and IBD [13]. *TNFRSF14* encodes a protein known as herpes virus entry mediator (i.e., HVEM or TNFRSF14). LIGHT (shows inducible expression) is a receptor expressed by T lymphocytes, and competes with herpes simplex virus glycoprotein D (HSV1gD) for HVEM. In addition, LIGHT also elicits a pro-inflammatory response as it functions as a potent T cell co-stimulatory molecule after binding HVEM. Expression of B and T-lymphocyte attenuator (BTLA) on T cells can be stimulated by T cell receptor (TCR) activation, and it is selectively expressed by cells programmed to produce Th1 inclined cytokines [75]. Interestingly, activated T cells in the mesenteric lymph nodes (MLNs) of LIGHT transgenic mice express the α4β7 integrin at a higher level, which is involved in lymphocyte homing. In many autoimmune diseases, the abnormal T cell activation disturbs

1 the balance between tolerance and inflammation caused by the absence of HVEM-BTLA
2 signaling (Figure.1). While IBD is certainly one of the most typical examples [76], PSC, is
3 only linked to TNFRSF by the GWAS [74]. As a new susceptibility locus shared by IBD
4 and autoimmune liver disease, *TNFRSF14* is highly likely to be involved in a pathogenic
5 pathway, as well as a therapy target.

6 *TNFRSF14* encoding HVEM have the TNF-related ligands: LIGHT and LT α . The
7 LIGHT and LT α binding sites have a similar molecular structure to TNF receptors and
8 matching TNF-related ligands [76]. Although a TNF antagonist has no effect on the clinical
9 presentation of PSC, the exploration of a linked pathway, and the study of newly artificially
10 synthesized TNF monoclonal antibodies should be continued.

11 **Lymphocyte homing**

12 The recirculation of lymphocytes orchestrates the IBD and IBD-induced liver
13 pathological manifestation. Lymphocyte homing to normal and inflammatory tissue sites is
14 instructed partly by molecules, such as chemokines, cytokines and adhesive molecules [77].
15 T cell maturation is induced by professional antigen presenting cells (i.e., dendritic cells),
16 non-professional antigen presenting cells (e.g., epithelial cells) or other cells. Mature
17 leukocytes migrate through the vessels and are attracted to inflamed tissue, and an accurate
18 adhesive system completes the attraction. The primarily step of adhesion is assisted by the
19 integrin family that are anchored on the leukocyte membrane. One family of integrin is the
20 $\alpha 4\beta 7$ integrin. This family of integrins are predominately expressed in the gut and gut-
21 associated lymphoid tissues, and the ligand is mucosal addressin cell adhesion molecule 1
22 (*MAdCAM-1*) [78]. Compared to Th1 cells that rely on *MAdCAM-1*, Th2 use the vascular

1 adhesion protein (VAP)-1 [79]. Both *MAdCAM-1* and VAP-1 are induced by IFN- α , and
2 one *in vitro* experiment has shown that the VAP-1-induced functional *MAdCAM-1*
3 expression on the surface of endothelial cells [77]. However, the previously considered gut-
4 specific ligand, *MAdCAM-1* later proved to be induced in the portal inflamed liver [14].
5 Potential agents have been studied, and a clinical trial is currently ongoing [80].

6 Integrin antagonists are a novel, highly selective anti-inflammatory drugs.
7 Vedolizumab (MNL-02) is a rhIgG1 monoclonal antibody that terminates leukocyte
8 homing through blocking the $\alpha 4\beta 7$ integrin [81]. A meta-analysis that included three RCTs
9 found that Vedolizumab administered UC patients had a significant improvement in the
10 clinical response and remission rates compared to the control group [82]. Since *MAdCAM-*
11 *1* has been shown to be involved in both PSC and UC, there is great anticipation that
12 Vedolizumab is a likely potential agent for PSC.

13 **Bacterial interaction**

14 The liver is exposed to gut-derived toxic factors when the intestinal barrier is
15 disturbed. The pathologic, disrupted liver status subsequently results in intestinal
16 dysfunction [26]. Substantial evidence exists regarding the opinion that an overwhelmed
17 immune response in response to the resident flora explains part of the pathogenesis of IBD
18 [27]. Additionally, the defective innate immune defense (i.e., reduced luminal immune
19 molecules, and impaired phagocytosis) can deteriorate the inflammation in the intestine,
20 which amplifies the leakage of bacteria into the portal vein [83]. The dysfunction of the
21 innate immune response to bacteria in the gut and the liver eventually initiate a cascade of
22 immune responses [84].

1 An abnormal accumulation of portal-derived lipopolysaccharides in the biliary
2 epithelium has been shown in a rat model, and the involvement of lipopolysaccharide to
3 intestinal mediated bile duct injury has been suspected [85]. Microbes are involved in the
4 initial stages of autoimmune and intestinal inflammation [86]. Thus, the effectiveness of
5 antibiotics has been shown in several clinical trials [87, 88]. However, the standard use of
6 antibiotics in PSC remains to be elucidated.

7 **FUT2**

8 The FUT2 gene encodes α -1,2-fucosyltransferase. In addition, the ABO histo-blood
9 group antigens expressed on the GI mucosa and in the body fluid is determined by the
10 secretion of α -1,2-fucosyltransferase [89]. Moreover, FUT2 is responsible for the synthesis
11 of the H antigen, which serves as both a receptor for adhesive molecules, as well as an
12 energy source for the intestinal flora [90]. The colonic microbiota of non-secretors (i.e.,
13 individuals lacking a functional FUT allele) changes in both composition and function,
14 thereby disturbing the normal mucosal immunity, and potentially why FUT2 is one of the
15 CD susceptibility loci in the GWAS [91]. Bacteria belonging to *Blautia*, *Dorea*
16 *formicigenerans*, *Ruminococcus gnavus*, and *Clostridium sphenoides* were significantly
17 abundant in the non-secretors compared with the secretors [90]. All of these bacterial
18 species are linked to IBD pathogenesis. Therefore, this may indicate that non-secretors have
19 greater tendency to maintain an abnormal flora [92, 93] (Figure.1). Intestinal bacterial
20 species are covered with various glycan-degrading enzymes and the ability to access mucus
21 glycan. All of the respective structures guarantee the bacteria will benefit from complex,
22 intestinal glycan structures, including the ABO histo-blood group antigens.[94].

1 The PSC-associated FUT2 variant was found to significantly change the
2 components of the bile flora. Interestingly, the relative amount of different bacteria is
3 coincident with the colon bacteria components of different FUT2 genotypes and secretor
4 status. The differences in non-secretors include a significant increase of *Firmicutes* with a
5 decrease in *Proteobacteria*. Parallel changes in the bile and colon have also been identified
6 in other phyla (*Bacteroidetes*, *Actinobacteria*, and *Tenericutes*) [95]. This can be
7 explained by the alteration in the binding between the ligand and the receptor, as mucosal
8 carbohydrate receptors can be recognized, and the pathogen adhesions bind to these
9 receptors.

10 With regards to the microbial treatment of PSC, typical antibiotics for UC (e.g.,
11 rifampin) are ineffective [96]. However, since the initial step of both PSC and IBD is
12 exposure to microbiota, a related study is needed.

13 **Conclusion and Future Areas of Study**

14 In this review, detailed and comprehensive explanation at several specific
15 susceptibility loci is provided based on previous study or research. Moreover, the drug
16 efficacy of DHNA, alpinetin or maqian, ustelimumab, tofacitinib, vedolizumab via *GPR35*,
17 *MST1R*, *CARD9*, *IL-2/IL-21/IL-2R*, *MAdCAM-1* mediated pathway are emphasized in
18 corresponding part [41, 52, 53, 63, 70, 82]. However, all mentioned drugs are merely in
19 animal experiment or clinical trial for the therapy for PSC or PSC-IBD. In addition, it is
20 proposed that study into new artificial TNF monoclonal antibody or potent antibiotics
21 should be put on the schedule, with the theoretical basis of *TNFRSF14* and *FUT2* mediated
22 pathway in both disease. In particular, few research has been conducted on the therapy
23 aiming at *HNF4-α* or *BACH2* pathway.

1 The instructive principle in IBD therapy has progressed from the administration of
2 nonselective immunosuppressives to a pathogenesis-based prescription [80]. Deep
3 remission is the new goal of IBD patients, which can only be achieved by having an
4 increasing understanding of the shared pathways, and a close follow-up of patients under a
5 pathway-based treatment approach. Therefore, more close observation into the
6 susceptibility loci of IBD and PSC expose a potential target for therapy.

1 **Acknowledgments**

2 This work was supported by grants from the National Key Technology Research and
3 Development Program of China (No. 2012BA106B03), and the National Science
4 Foundation of China (No. 81370508 and No. 81470820)

[Insert Addendum to Cover Letter here.]

NOTE: Answer the following questions on this page:

- (1) what is the scientific question being asked by this study, (2) what are the principal new findings that answer this question, and (3) what is the significance of the results for the field?

[Insert Abstract here <120 words]

[Insert Running Title <50 characters]

[Insert Introduction text here (no subheadings)]

[Insert Results and Discussion here (may include subheadings)]

[Insert Experimental Procedures here]

References

- [1] I. Loddo, C. Romano, Inflammatory Bowel Disease: Genetics, Epigenetics, and Pathogenesis, *Frontiers in immunology*, 6 (2015) 551.
- [2] C. Abraham, J.H. Cho, Inflammatory bowel disease, *The New England journal of medicine*, 361 (2009) 2066-2078.
- [3] L. Jostins, S. Ripke, R.K. Weersma, R.H. Duerr, D.P. McGovern, K.Y. Hui, J.C. Lee, L.P. Schumm, Y. Sharma, C.A. Anderson, J. Essers, M. Mitrovic, K. Ning, I. Cleynen, E. Theatre, S.L. Spain, S. Raychaudhuri, P. Goyette, Z. Wei, C. Abraham, J.P. Achkar, T. Ahmad, L. Amininejad, A.N. Ananthakrishnan, V. Andersen, J.M. Andrews, L. Baidoo, T. Balschun, P.A. Bampton, A. Bitton, G. Boucher, S. Brand, C. Buning, A. Cohain, S. Cichon, M. D'Amato, D. De Jong, K.L. Devaney, M. Dubinsky, C. Edwards, D. Ellinghaus, L.R. Ferguson, D. Franchimont, K. Fransen, R. Gearry, M. Georges, C. Gieger, J. Glas, T. Haritunians, A. Hart, C. Hawkey, M. Hedl, X. Hu, T.H. Karlsen, L. Kupcinskis, S. Kugathasan, A. Latiano, D. Laukens, I.C. Lawrance, C.W. Lees, E. Louis, G. Mahy, J. Mansfield, A.R. Morgan, C. Mowat, W. Newman, O. Palmieri, C.Y. Ponsioen, U. Potocnik, N.J. Prescott, M. Regueiro, J.I. Rotter, R.K. Russell, J.D. Sanderson, M. Sans, J. Satsangi, S. Schreiber, L.A. Simms, J. Sventoraityte, S.R. Targan, K.D. Taylor, M. Tremelling, H.W. Verspaget, M. De Vos, C. Wijmenga, D.C. Wilson, J. Winkelmann, R.J. Xavier, S. Zeissig, B. Zhang, C.K. Zhang, H. Zhao, I.B.D.G.C. International, M.S. Silverberg, V. Annese, H. Hakonarson, S.R. Brant, G. Radford-Smith, C.G. Mathew, J.D. Rioux, E.E. Schadt, M.J. Daly, A. Franke, M. Parkes, S. Vermeire, J.C. Barrett, J.H. Cho, Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease, *Nature*, 491 (2012) 119-124.

- [4] S. van Sommeren, M. Janse, J. Karjalainen, R. Fehrmann, L. Franke, J. Fu, R.K. Weersma, Extraintestinal manifestations and complications in inflammatory bowel disease: from shared genetics to shared biological pathways, *Inflammatory bowel diseases*, 20 (2014) 987-994.
- [5] G. Son, M. Kremer, I.N. Hines, Contribution of gut bacteria to liver pathobiology, *Gastroenterology research and practice*, 2010 (2010).
- [6] O.H. Nielsen, M. Ainsworth, M. Coskun, G. Weiss, Management of Iron-Deficiency Anemia in Inflammatory Bowel Disease: A Systematic Review, *Medicine*, 94 (2015) e963.
- [7] D.D. Mijac, G.L. Jankovic, J. Jorga, M.N. Krstic, Nutritional status in patients with active inflammatory bowel disease: prevalence of malnutrition and methods for routine nutritional assessment, *European journal of internal medicine*, 21 (2010) 315-319.
- [8] C.A. Lima, A.C. Lyra, R. Rocha, G.O. Santana, Risk factors for osteoporosis in inflammatory bowel disease patients, *World journal of gastrointestinal pathophysiology*, 6 (2015) 210-218.
- [9] G. Trikudanathan, P.G. Venkatesh, U. Navaneethan, Diagnosis and therapeutic management of extra-intestinal manifestations of inflammatory bowel disease, *Drugs*, 72 (2012) 2333-2349.
- [10] S.R. Vavricka, A. Schoepfer, M. Scharl, P.L. Lakatos, A. Navarini, G. Rogler, Extraintestinal Manifestations of Inflammatory Bowel Disease, *Inflammatory bowel diseases*, 21 (2015) 1982-1992.
- [11] P.J. Trivedi, R.W. Chapman, PSC, AIH and overlap syndrome in inflammatory bowel disease, *Clinics and research in hepatology and gastroenterology*, 36 (2012) 420-436.
- [12] M. Kummen, E. Schrumpf, K.M. Boberg, Liver abnormalities in bowel diseases, *Best practice & research. Clinical gastroenterology*, 27 (2013) 531-542.
- [13] T. Folseraas, E. Liaskou, C.A. Anderson, T.H. Karlsen, Genetics in PSC: what do the "risk genes" teach us?, *Clinical reviews in allergy & immunology*, 48 (2015) 154-164.

- [14] A.J. Grant, P.F. Lalor, S.G. Hubscher, M. Briskin, D.H. Adams, MAdCAM-1 expressed in chronic inflammatory liver disease supports mucosal lymphocyte adhesion to hepatic endothelium (MAdCAM-1 in chronic inflammatory liver disease), *Hepatology*, 33 (2001) 1065-1072.
- [15] J.P. Babeu, F. Boudreau, Hepatocyte nuclear factor 4-alpha involvement in liver and intestinal inflammatory networks, *World journal of gastroenterology : WJG*, 20 (2014) 22-30.
- [16] U. Navaneethan, Hepatobiliary manifestations of ulcerative colitis: an example of gut-liver crosstalk, *Gastroenterology report*, 2 (2014) 193-200.
- [17] S.R. Vavricka, G. Rogler, C. Gantenbein, M. Spoerri, M. Prinz Vavricka, A.A. Navarini, L.E. French, E. Safroneeva, N. Fournier, A. Straumann, F. Froehlich, M. Fried, P. Michetti, F. Seibold, P.L. Lakatos, L. Peyrin-Biroulet, A.M. Schoepfer, Chronological Order of Appearance of Extraintestinal Manifestations Relative to the Time of IBD Diagnosis in the Swiss Inflammatory Bowel Disease Cohort, *Inflammatory bowel diseases*, 21 (2015) 1794-1800.
- [18] R. Chapman, Etiopathogenesis of primary sclerosing cholangitis, *World Journal of Gastroenterology*, 14 (2008) 3350.
- [19] E.V. Loftus, Jr., G.C. Harewood, C.G. Loftus, W.J. Tremaine, W.S. Harmsen, A.R. Zinsmeister, D.A. Jewell, W.J. Sandborn, PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis, *Gut*, 54 (2005) 91-96.
- [20] A. O'Toole, A. Alakkari, D. Keegan, G. Doherty, H. Mulcahy, D. O'Donoghue, Primary sclerosing cholangitis and disease distribution in inflammatory bowel disease, *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association*, 10 (2012) 439-441.

- [21] J.S. Halliday, J. Djordjevic, M. Lust, E.L. Culver, B. Braden, S.P. Travis, R.W. Chapman, A unique clinical phenotype of primary sclerosing cholangitis associated with Crohn's disease, *Journal of Crohn's & colitis*, 6 (2012) 174-181.
- [22] E.M. DeFilippis, S. Kumar, Clinical Presentation and Outcomes of Autoimmune Hepatitis in Inflammatory Bowel Disease, *Digestive diseases and sciences*, 60 (2015) 2873-2880.
- [23] J. Neuberger, B. Gunson, S. Hubscher, P. Nightingale, Immunosuppression affects the rate of recurrent primary biliary cirrhosis after liver transplantation, *Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society*, 10 (2004) 488-491.
- [24] C.C. Hsieh, C.H. Hung, L. Lu, S. Qian, Hepatic immune tolerance induced by hepatic stellate cells, *World journal of gastroenterology : WJG*, 21 (2015) 11887-11892.
- [25] J. Levine, The impact of immune dysregulation on the development of autoimmune gastrointestinal and liver disease, *Current problems in pediatric and adolescent health care*, 44 (2014) 322-323.
- [26] D. Compare, P. Coccoli, A. Rocco, O.M. Nardone, S. De Maria, M. Carteni, G. Nardone, Gut--liver axis: the impact of gut microbiota on non alcoholic fatty liver disease, *Nutrition, metabolism, and cardiovascular diseases : NMCD*, 22 (2012) 471-476.
- [27] W. Strober, I. Fuss, P. Mannon, The fundamental basis of inflammatory bowel disease, *The Journal of clinical investigation*, 117 (2007) 514-521.
- [28] M. Darsigny, J.P. Babeu, A.A. Dupuis, E.E. Furth, E.G. Seidman, E. Levy, E.F. Verdu, F.P. Gendron, F. Boudreau, Loss of hepatocyte-nuclear-factor-4alpha affects colonic ion transport and causes chronic inflammation resembling inflammatory bowel disease in mice, *PloS one*, 4 (2009) e7609.

- [29] A.L. Cattin, J. Le Beyec, F. Barreau, S. Saint-Just, A. Houllier, F.J. Gonzalez, S. Robine, M. Pincon-Raymond, P. Cardot, M. Lacasa, A. Ribeiro, Hepatocyte nuclear factor 4alpha, a key factor for homeostasis, cell architecture, and barrier function of the adult intestinal epithelium, *Molecular and cellular biology*, 29 (2009) 6294-6308.
- [30] R. Okamoto, M. Watanabe, Role of epithelial cells in the pathogenesis and treatment of inflammatory bowel disease, *Journal of gastroenterology*, (2015).
- [31] N. Khan, A.R. Asif, Transcriptional regulators of claudins in epithelial tight junctions, *Mediators of inflammation*, 2015 (2015) 219843.
- [32] S. Gunewardena, C. Walesky, U. Apte, Global Gene Expression Changes in Liver Following Hepatocyte Nuclear Factor 4 alpha deletion in Adult Mice, *Genomics data*, 5 (2015) 126-128.
- [33] F. Parviz, C. Matullo, W.D. Garrison, L. Savatski, J.W. Adamson, G. Ning, K.H. Kaestner, J.M. Rossi, K.S. Zaret, S.A. Duncan, Hepatocyte nuclear factor 4alpha controls the development of a hepatic epithelium and liver morphogenesis, *Nature genetics*, 34 (2003) 292-296.
- [34] S. Fallarini, L. Magliulo, T. Paoletti, C. de Lalla, G. Lombardi, Expression of functional GPR35 in human iNKT cells, *Biochemical and biophysical research communications*, 398 (2010) 420-425.
- [35] J. Wang, N. Simonavicius, X. Wu, G. Swaminath, J. Reagan, H. Tian, L. Ling, Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35, *The Journal of biological chemistry*, 281 (2006) 22021-22028.
- [36] P. Paluszkiwicz, W. Zgrajka, T. Saran, J. Schabowski, J.L. Piedra, O. Fedkiv, S. Rengman, S.G. Pierzynowski, W.A. Turski, High concentration of kynurenic acid in bile and pancreatic juice, *Amino acids*, 37 (2009) 637-641.

- [37] D. Ellinghaus, T. Folseraas, K. Holm, E. Ellinghaus, E. Melum, T. Balschun, J.K. Laerdahl, A. Shiryayev, D.N. Gotthardt, T.J. Weismuller, C. Schramm, M. Wittig, A. Bergquist, E. Bjornsson, H.U. Marschall, M. Vatn, A. Teufel, C. Rust, C. Gieger, H.E. Wichmann, H. Runz, M. Sterneck, C. Rupp, F. Braun, R.K. Weersma, C. Wijmenga, C.Y. Ponsioen, C.G. Mathew, P. Rutgeerts, S. Vermeire, E. Schrumpf, J.R. Hov, M.P. Manns, K.M. Boberg, S. Schreiber, A. Franke, T.H. Karlsen, Genome-wide association analysis in primary sclerosing cholangitis and ulcerative colitis identifies risk loci at GPR35 and TCF4, *Hepatology*, 58 (2013) 1074-1083.
- [38] M.C. Barth, N. Ahluwalia, T.J. Anderson, G.J. Hardy, S. Sinha, J.A. Alvarez-Cardona, I.E. Pruitt, E.P. Rhee, R.A. Colvin, R.E. Gerszten, Kynurenic acid triggers firm arrest of leukocytes to vascular endothelium under flow conditions, *The Journal of biological chemistry*, 284 (2009) 19189-19195.
- [39] J.L. Maravillas-Montero, A.M. Burkhardt, P.A. Hevezi, C.D. Carnevale, M.J. Smit, A. Zlotnik, Cutting edge: GPR35/CXCR8 is the receptor of the mucosal chemokine CXCL17, *Journal of immunology*, 194 (2015) 29-33.
- [40] D.M. Shore, P.H. Reggio, The therapeutic potential of orphan GPCRs, GPR35 and GPR55, *Frontiers in pharmacology*, 6 (2015) 69.
- [41] Y. Okada, Y. Tsuzuki, J. Miyazaki, K. Matsuzaki, R. Hokari, S. Komoto, S. Kato, A. Kawaguchi, S. Nagao, K. Itoh, T. Watanabe, S. Miura, *Propionibacterium freudenreichii* component 1,4-dihydroxy-2-naphthoic acid (DHNA) attenuates dextran sodium sulphate induced colitis by modulation of bacterial flora and lymphocyte homing, *Gut*, 55 (2006) 681-688.
- [42] P.K. Wagh, G.M. Zinser, J.K. Gray, A. Shrestha, S.E. Waltz, Conditional deletion of beta-catenin in mammary epithelial cells of Ron receptor, Mst1r, overexpressing mice alters mammary tumorigenesis, *Endocrinology*, 153 (2012) 2735-2746.

- [43] N. Gorlatova, K. Chao, L.R. Pal, R.H. Araj, A. Galkin, I. Turko, J. Moulton, O. Herzberg, Protein characterization of a candidate mechanism SNP for Crohn's disease: the macrophage stimulating protein R689C substitution, *PloS one*, 6 (2011) e27269.
- [44] S.E. Waltz, L. Eaton, K. Toney-Earley, K.A. Hess, B.E. Peace, J.R. Ihlendorf, M.H. Wang, K.H. Kaestner, S.J. Degen, Ron-mediated cytoplasmic signaling is dispensable for viability but is required to limit inflammatory responses, *The Journal of clinical investigation*, 108 (2001) 567-576.
- [45] E.R. Mann, Intestinal antigen-presenting cells in mucosal immune homeostasis: Crosstalk between dendritic cells, macrophages and B-cells, *World Journal of Gastroenterology*, 20 (2014) 9653.
- [46] A.M. Mowat, C.C. Bain, Mucosal macrophages in intestinal homeostasis and inflammation, *Journal of innate immunity*, 3 (2011) 550-564.
- [47] E. Cario, Toll-like receptors in inflammatory bowel diseases: a decade later, *Inflammatory bowel diseases*, 16 (2010) 1583-1597.
- [48] A.A. Kuhl, U. Erben, L.I. Kredel, B. Siegmund, Diversity of Intestinal Macrophages in Inflammatory Bowel Diseases, *Frontiers in immunology*, 6 (2015) 613.
- [49] L.M. Liu, D.Y. Liang, C.G. Ye, W.J. Tu, T. Zhu, The U1/U2 system mediates upregulation of proinflammatory cytokines through p38 MAPK and NF-kappaB pathways in LPS-stimulated Kupffer cells, *PloS one*, 10 (2015) e0121383.
- [50] A. Mencin, J. Kluwe, R.F. Schwabe, Toll-like receptors as targets in chronic liver diseases, *Gut*, 58 (2009) 704-720.
- [51] E. Seki, B. Schnabl, Role of innate immunity and the microbiota in liver fibrosis: crosstalk between the liver and gut, *The Journal of physiology*, 590 (2012) 447-458.

- [52] X. He, Z. Wei, J. Wang, J. Kou, W. Liu, Y. Fu, Z. Yang, Alpinetin attenuates inflammatory responses by suppressing TLR4 and NLRP3 signaling pathways in DSS-induced acute colitis, *Scientific reports*, 6 (2016) 28370.
- [53] K.L. Ji, X.Q. Gan, Y.K. Xu, X.F. Li, J. Guo, M.M. Dahab, P. Zhang, Protective effect of the essential oil of *Zanthoxylum myriacanthum* var. *pubescens* against dextran sulfate sodium-induced intestinal inflammation in mice, *Phytomedicine : international journal of phytotherapy and phytopharmacology*, 23 (2016) 883-890.
- [54] G. Rong, Y. Zhou, Y. Xiong, L. Zhou, H. Geng, T. Jiang, Y. Zhu, H. Lu, S. Zhang, P. Wang, B. Zhang, R. Zhong, Imbalance between T helper type 17 and T regulatory cells in patients with primary biliary cirrhosis: the serum cytokine profile and peripheral cell population, *Clinical and experimental immunology*, 156 (2009) 217-225.
- [55] Y.M. Hsu, Y. Zhang, Y. You, D. Wang, H. Li, O. Duramad, X.F. Qin, C. Dong, X. Lin, The adaptor protein CARD9 is required for innate immune responses to intracellular pathogens, *Nature immunology*, 8 (2007) 198-205.
- [56] J. Bertin, Y. Guo, L. Wang, S.M. Srinivasula, M.D. Jacobson, J.L. Poyet, S. Merriam, M.Q. Du, M.J. Dyer, K.E. Robison, P.S. DiStefano, E.S. Alnemri, CARD9 is a novel caspase recruitment domain-containing protein that interacts with BCL10/CLAP and activates NF-kappa B, *The Journal of biological chemistry*, 275 (2000) 41082-41086.
- [57] H.S. Goodridge, T. Shimada, A.J. Wolf, Y.M. Hsu, C.A. Becker, X. Lin, D.M. Underhill, Differential use of CARD9 by dectin-1 in macrophages and dendritic cells, *Journal of immunology*, 182 (2009) 1146-1154.
- [58] M. Janse, L.E. Lamberts, L. Franke, S. Raychaudhuri, E. Ellinghaus, K. Muri Boberg, E. Melum, T. Folseraas, E. Schrumpf, A. Bergquist, E. Bjornsson, J. Fu, H. Jan Westra, H.J. Groen,

R.S. Fehrmann, J. Smolonska, L.H. van den Berg, R.A. Ophoff, R.J. Porte, T.J. Weismuller, J. Wedemeyer, C. Schramm, M. Sterneck, R. Gunther, F. Braun, S. Vermeire, L. Henckaerts, C. Wijmenga, C.Y. Ponsioen, S. Schreiber, T.H. Karlsen, A. Franke, R.K. Weersma, Three ulcerative colitis susceptibility loci are associated with primary sclerosing cholangitis and indicate a role for IL2, REL, and CARD9, *Hepatology*, 53 (2011) 1977-1985.

[59] J. Katt, D. Schwinge, T. Schoknecht, A. Quaas, I. Sobottka, E. Burandt, C. Becker, M.F. Neurath, A.W. Lohse, J. Herkel, C. Schramm, Increased T helper type 17 response to pathogen stimulation in patients with primary sclerosing cholangitis, *Hepatology*, 58 (2013) 1084-1093.

[60] A. Zhernakova, E.M. Festen, L. Franke, G. Trynka, C.C. van Diemen, A.J. Monsuur, M. Bevova, R.M. Nijmeijer, R. van 't Slot, R. Heijmans, H.M. Boezen, D.A. van Heel, A.A. van Bodegraven, P.C. Stokkers, C. Wijmenga, J.B. Crusius, R.K. Weersma, Genetic analysis of innate immunity in Crohn's disease and ulcerative colitis identifies two susceptibility loci harboring CARD9 and IL18RAP, *American journal of human genetics*, 82 (2008) 1202-1210.

[61] J.M. Benson, D. Peritt, B.J. Scallon, G.A. Heavner, D.J. Shealy, J.M. Giles-Komar, M.A. Mascelli, Discovery and mechanism of ustekinumab: a human monoclonal antibody targeting interleukin-12 and interleukin-23 for treatment of immune-mediated disorders, *mAbs*, 3 (2011) 535-545.

[62] E.G. Simon, S. Samuel, S. Ghosh, G.W. Moran, Ustekinumab: a novel therapeutic option in Crohn's disease, *Expert opinion on biological therapy*, 16 (2016) 1065-1074.

[63] P. Wils, Y. Bouhnik, P. Michetti, B. Flourie, H. Brix, A. Bourrier, M. Allez, B. Duclos, J.C. Grimaud, A. Buisson, A. Amiot, M. Fumery, X. Roblin, L. Peyrin-Biroulet, J. Filippi, G. Bouguen, V. Abitbol, B. Coffin, M. Simon, D. Laharie, B. Pariente, D. Groupe d'Etude Therapeutique des Affections Inflammatoires du Tube, Subcutaneous Ustekinumab Provides

- Clinical Benefit for Two-Thirds of Patients With Crohn's Disease Refractory to Anti-Tumor Necrosis Factor Agents, *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association*, 14 (2016) 242-250 e241-242.
- [64] J. Yamanouchi, D. Rainbow, P. Serra, S. Howlett, K. Hunter, V.E. Garner, A. Gonzalez-Munoz, J. Clark, R. Veijola, R. Cubbon, S.L. Chen, R. Rosa, A.M. Cumiskey, D.V. Serreze, S. Gregory, J. Rogers, P.A. Lyons, B. Healy, L.J. Smink, J.A. Todd, L.B. Peterson, L.S. Wicker, P. Santamaria, Interleukin-2 gene variation impairs regulatory T cell function and causes autoimmunity, *Nature genetics*, 39 (2007) 329-337.
- [65] M. Desjardins, B.D. Mazer, B-cell memory and primary immune deficiencies: interleukin-21 related defects, *Current opinion in allergy and clinical immunology*, 13 (2013) 639-645.
- [66] A. Mizoguchi, T. Takeuchi, H. Himuro, T. Okada, E. Mizoguchi, Genetically Engineered Mouse Models for Studying Inflammatory Bowel Disease, *The Journal of pathology*, (2015).
- [67] G. Monteleone, I. Monteleone, D. Fina, P. Vavassori, G. Del Vecchio Blanco, R. Caruso, R. Tersigni, L. Alessandroni, L. Biancone, G.C. Naccari, T.T. MacDonald, F. Pallone, Interleukin-21 enhances T-helper cell type I signaling and interferon-gamma production in Crohn's disease, *Gastroenterology*, 128 (2005) 687-694.
- [68] W. Hsu, W. Zhang, K. Tsuneyama, Y. Moritoki, W.M. Ridgway, A.A. Ansari, R.L. Coppel, Z.X. Lian, I. Mackay, M.E. Gershwin, Differential mechanisms in the pathogenesis of autoimmune cholangitis versus inflammatory bowel disease in interleukin-2 α (-/-) mice, *Hepatology*, 49 (2009) 133-140.
- [69] A.F. Heneghan, J.F. Pierre, K.A. Kudsk, JAK-STAT and intestinal mucosal immunology, *Jak-Stat*, 2 (2013) e25530.

- [70] B.E. Sands, New drugs on the horizon for IBD, *Digestive diseases*, 32 Suppl 1 (2014) 74-81.
- [71] A. Muto, K. Ochiai, Y. Kimura, A. Itoh-Nakadai, K.L. Calame, D. Ikebe, S. Tashiro, K. Igarashi, Bach2 represses plasma cell gene regulatory network in B cells to promote antibody class switch, *The EMBO journal*, 29 (2010) 4048-4061.
- [72] S. Tsukumo, M. Unno, A. Muto, A. Takeuchi, K. Kometani, T. Kurosaki, K. Igarashi, T. Saito, Bach2 maintains T cells in a naive state by suppressing effector memory-related genes, *Proceedings of the National Academy of Sciences of the United States of America*, 110 (2013) 10735-10740.
- [73] E.H. Kim, D.J. Gasper, S.H. Lee, E.H. Plisch, J. Svaren, M. Suresh, Bach2 regulates homeostasis of Foxp3⁺ regulatory T cells and protects against fatal lung disease in mice, *Journal of immunology*, 192 (2014) 985-995.
- [74] J.Z. Liu, J.R. Hov, T. Folseraas, E. Ellinghaus, S.M. Rushbrook, N.T. Doncheva, O.A. Andreassen, R.K. Weersma, T.J. Weismuller, B. Eksteen, P. Invernizzi, G.M. Hirschfield, D.N. Gotthardt, A. Pares, D. Ellinghaus, T. Shah, B.D. Juran, P. Milkiewicz, C. Rust, C. Schramm, T. Muller, B. Srivastava, G. Dalekos, M.M. Nothen, S. Herms, J. Winkelmann, M. Mitrovic, F. Braun, C.Y. Ponsioen, P.J. Croucher, M. Sterneck, A. Teufel, A.L. Mason, J. Saarela, V. Leppa, R. Dorfman, D. Alvaro, A. Floreani, S. Onengut-Gumuscu, S.S. Rich, W.K. Thompson, A.J. Schork, S. Naess, I. Thomsen, G. Mayr, I.R. Konig, K. Hveem, I. Cleynen, J. Gutierrez-Achury, I. Ricano-Ponce, D. van Heel, E. Bjornsson, R.N. Sandford, P.R. Durie, E. Melum, M.H. Vatn, M.S. Silverberg, R.H. Duerr, L. Padyukov, S. Brand, M. Sans, V. Annese, J.P. Achkar, K.M. Boberg, H.U. Marschall, O. Chazouilleres, C.L. Bowlus, C. Wijmenga, E. Schrumpf, S. Vermeire, M. Albrecht, U.-P. Consortium, I.B.D.G.C. International, J.D. Rioux, G. Alexander,

- A. Bergquist, J. Cho, S. Schreiber, M.P. Manns, M. Farkkila, A.M. Dale, R.W. Chapman, K.N. Lazaridis, P.S.C.S.G. International, A. Franke, C.A. Anderson, T.H. Karlsen, Dense genotyping of immune-related disease regions identifies nine new risk loci for primary sclerosing cholangitis, *Nature genetics*, 45 (2013) 670-675.
- [75] P. Han, O.D. Goularte, K. Rufner, B. Wilkinson, J. Kaye, An inhibitory Ig superfamily protein expressed by lymphocytes and APCs is also an early marker of thymocyte positive selection, *Journal of immunology*, 172 (2004) 5931-5939.
- [76] M.W. Steinberg, T.C. Cheung, C.F. Ware, The signaling networks of the herpesvirus entry mediator (TNFRSF14) in immune regulation, *Immunological reviews*, 244 (2011) 169-187.
- [77] E. Liaskou, M. Karikoski, G.M. Reynolds, P.F. Lalor, C.J. Weston, N. Pullen, M. Salmi, S. Jalkanen, D.H. Adams, Regulation of mucosal addressin cell adhesion molecule 1 expression in human and mice by vascular adhesion protein 1 amine oxidase activity, *Hepatology*, 53 (2011) 661-672.
- [78] T. Lobaton, S. Vermeire, G. Van Assche, P. Rutgeerts, Review article: anti-adhesion therapies for inflammatory bowel disease, *Alimentary pharmacology & therapeutics*, 39 (2014) 579-594.
- [79] C.S. Bonder, M.U. Norman, M.G. Swain, L.D. Zbytnuik, J. Yamanouchi, P. Santamaria, M. Ajuebor, M. Salmi, S. Jalkanen, P. Kubes, Rules of recruitment for Th1 and Th2 lymphocytes in inflamed liver: a role for alpha-4 integrin and vascular adhesion protein-1, *Immunity*, 23 (2005) 153-163.
- [80] G. Bamias, T.T. Pizarro, F. Cominelli, Pathway-based approaches to the treatment of inflammatory bowel disease, *Translational research : the journal of laboratory and clinical medicine*, 167 (2016) 104-115.

- [81] S. Danese, New therapies for inflammatory bowel disease: from the bench to the bedside, *Gut*, 61 (2012) 918-932.
- [82] Y. Jin, Y. Lin, L.J. Lin, C.Q. Zheng, Meta-analysis of the effectiveness and safety of vedolizumab for ulcerative colitis, *World journal of gastroenterology : WJG*, 21 (2015) 6352-6360.
- [83] F. Fava, S. Danese, Intestinal microbiota in inflammatory bowel disease: friend of foe?, *World journal of gastroenterology : WJG*, 17 (2011) 557-566.
- [84] H.D. Ma, Y.H. Wang, C. Chang, M.E. Gershwin, Z.X. Lian, The intestinal microbiota and microenvironment in liver, *Autoimmunity reviews*, 14 (2015) 183-191.
- [85] H. Koga, S. Sakisaka, M. Yoshitake, M. Harada, H. Kumemura, S. Hanada, E. Taniguchi, T. Kawaguchi, R. Kumashiro, M. Sata, Abnormal accumulation in lipopolysaccharide in biliary epithelial cells of rats with self-filling blind loop, *International journal of molecular medicine*, 9 (2002) 621-626.
- [86] J.H. Tabibian, J.A. Talwalkar, K.D. Lindor, Role of the microbiota and antibiotics in primary sclerosing cholangitis, *BioMed research international*, 2013 (2013) 389537.
- [87] M. Farkkila, A.L. Karvonen, H. Nurmi, H. Nuutinen, M. Taavitsainen, P. Pikkarainen, P. Karkkainen, Metronidazole and ursodeoxycholic acid for primary sclerosing cholangitis: a randomized placebo-controlled trial, *Hepatology*, 40 (2004) 1379-1386.
- [88] M.G. Silveira, N.J. Torok, A.A. Gossard, J.C. Keach, R.A. Jorgensen, J.L. Petz, K.D. Lindor, Minocycline in the treatment of patients with primary sclerosing cholangitis: results of a pilot study, *The American journal of gastroenterology*, 104 (2009) 83-88.

- [89] I. Franks, Gut microbiota: FUT2 genotype influences the gut microbiota in patients with Crohn's disease and healthy individuals, *Nature reviews. Gastroenterology & hepatology*, 9 (2012) 2.
- [90] P. Wacklin, J. Tuimala, J. Nikkila, T. Sebastian, H. Makivuokko, N. Alakulppi, P. Laine, M. Rajilic-Stojanovic, L. Paulin, W.M. de Vos, J. Matto, Faecal microbiota composition in adults is associated with the FUT2 gene determining the secretor status, *PloS one*, 9 (2014) e94863.
- [91] M. Tong, I. McHardy, P. Ruegger, M. Goudarzi, P.C. Kashyap, T. Haritunians, X. Li, T.G. Graeber, E. Schwager, C. Huttenhower, A.J. Fornace, Jr., J.L. Sonnenburg, D.P. McGovern, J. Borneman, J. Braun, Reprograming of gut microbiome energy metabolism by the FUT2 Crohn's disease risk polymorphism, *The ISME journal*, 8 (2014) 2193-2206.
- [92] M. Rajilic-Stojanovic, E. Biagi, H.G. Heilig, K. Kajander, R.A. Kekkonen, S. Tims, W.M. de Vos, Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome, *Gastroenterology*, 141 (2011) 1792-1801.
- [93] D.N. Frank, A.L. St Amand, R.A. Feldman, E.C. Boedeker, N. Harpaz, N.R. Pace, Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases, *Proceedings of the National Academy of Sciences of the United States of America*, 104 (2007) 13780-13785.
- [94] N.M. Koropatkin, E.A. Cameron, E.C. Martens, How glycan metabolism shapes the human gut microbiota, *Nature reviews. Microbiology*, 10 (2012) 323-335.
- [95] T. Folseraas, E. Melum, P. Rausch, B.D. Juran, E. Ellinghaus, A. Shiryaev, J.K. Laerdahl, D. Ellinghaus, C. Schramm, T.J. Weismuller, D.N. Gotthardt, J.R. Hov, O.P. Clausen, R.K. Weersma, M. Janse, K.M. Boberg, E. Bjornsson, H.U. Marschall, I. Cleynen, P. Rosenstiel, K. Holm, A. Teufel, C. Rust, C. Gieger, H.E. Wichmann, A. Bergquist, E. Ryu, C.Y. Ponsioen, H.

Runz, M. Sterneck, S. Vermeire, U. Beuers, C. Wijmenga, E. Schrumpf, M.P. Manns, K.N.

Lazaridis, S. Schreiber, J.F. Baines, A. Franke, T.H. Karlsen, Extended analysis of a genome-wide association study in primary sclerosing cholangitis detects multiple novel risk loci, *Journal of hepatology*, 57 (2012) 366-375.

[96] J.H. Tabibian, A. Gossard, M. El-Youssef, J.E. Eaton, J. Petz, R. Jorgensen, F.B. Enders, A. Tabibian, K.D. Lindor, Prospective Clinical Trial of Rifaximin Therapy for Patients With Primary Sclerosing Cholangitis, *American journal of therapeutics*, (2014).

[97] B. Ardesjo, C.M. Hansson, C.E. Bruder, F. Rorsman, C. Betterle, J.P. Dumanski, O. Kampe, O. Ekwall, Autoantibodies to glutathione S-transferase theta 1 in patients with primary sclerosing cholangitis and other autoimmune diseases, *Journal of autoimmunity*, 30 (2008) 273-282.

1

2 **Figure Legends**

3 **Figure 1.** An overview of the shared loci and pathogenesis between PSC and IBD, for both UC
4 and CD.

5 In macrophages, CARD9 molecules can be synthesized and induce the production of IL-6 as
6 well as other pro-inflammatory cytokines. *HNF4- α* is a gene that can be activated by IL-1 β via
7 the p38 pathway in hepatocytes, leading to the production of pro-inflammatory molecules,
8 including iNOS. IL-1 β can also act on the intestinal epithelial cells to induce the synthesis of
9 Claudin-15, a component of the tight junctions between epithelial cells. *GPR35* acts as a receptor
10 for several endogenous ligands. The downstream effect is to recruit inflammatory cells. The
11 RON-mediated pathway inhibits the pro-inflammatory activity of the intestinal epithelium.
12 Beneficial flora in the intestinal cavity can be “burst” by *FUT2*-induced fucose production. In
13 addition, HVEM on T lymphocytes promotes the function of effector T cells, while the IL-2R is
14 affiliated with the cell cycle of T_{reg} cells. Humoral immunity is promoted by the *BACH2* gene by
15 regulating the differentiation of B lymphocytes.

1 **Figure1**

2

Tables

Table 1. Susceptibility loci and points of interest shared by PSC and IBD, including UC and CD

Loci	Gene	Points of interest (PSC)	Points of interest (UC)	Points of interest (CD)
1p36	MMEL/TNFRSF14	rs3748816	rs10797432	rs10797432
2q37	GPR35	rs3749171	rs3749171	rs3749171
3p21	MST1	rs3197999	rs3197999	rs3197999
4q27	IL2/IL21	rs13140464	rs7657746	rs7657746
6q15	BACH2	rs56258221	rs1847472	rs1847472
9q34	CARD9	rs4077515	rs4077515	rs4077515
10p15	IL2RA	rs4147359		rs12722489
17q21	VAP-1		rs12942547	rs12942547
19q13	FUT2	rs516246,rs601338	rs516246	rs516246
20q13	HNF4A		rs6017342	rs6017342

The same points shared by PSC and IBD are shown in red.

UC: ulcerative colitis; CD: Crohn's disease; PSC: Primary sclerosing cholangitis; IBD: inflammatory bowel disease