

Genetics in PSC: What Do the “Risk Genes” Teach Us?

Trine Folseraas · Evaggelia Liaskou · Carl A. Anderson ·
Tom H. Karlsen

© Springer Science+Business Media New York 2014

Abstract A role of genetics in primary sclerosing cholangitis (PSC) development is now firmly established. A total of 16 risk genes have been reported at highly robust (“genome-wide”) significance levels, and ongoing efforts suggest that the list will ultimately be considerably longer. Importantly, this genetic risk pool so far accounts for less than 10 % of an estimated overall PSC susceptibility. The relative importance of genetic versus environmental factors (including gene-gene and gene-environment interactions) in remaining aspects of PSC pathogenesis is unknown, and other study designs than genome-wide association studies are needed to explore these aspects. For some of the loci, e.g. HLA and *FUT2*, distinct interacting environmental factors may exist, and working from the genetic associations may prove one valid path for

determining the specific nature of environmental triggers. So far the biological implications for PSC risk genes are typically merely hypothesized based on previously published literature, and there is therefore a strong need for dedicated translational studies to determine their roles within the specific disease context of PSC. Apparently, most risk loci seem to involve in a subset of biological pathways for which genetic associations exist in a multitude of immune-mediated diseases, accounting for both inflammatory bowel disease as well as prototypical autoimmunity. In the present article, we will survey the current knowledge on PSC genetics with a particular emphasis on the pathophysiological insight potentially gained from genetic risk loci involving in this profound immunogenetic pleiotropy.

Keywords Primary sclerosing cholangitis · Genome-wide association study

T. Folseraas · T. H. Karlsen (✉)
Norwegian PSC Research Center, Department of Transplantation
Medicine, Division of Cancer Medicine, Surgery and
Transplantation, Oslo University Hospital Rikshospitalet, 4950
Nydalen, 0424 Oslo, Norway
e-mail: t.h.karlsen@medisin.uio.no

T. Folseraas · T. H. Karlsen
K.G. Jebsen Inflammation Research Centre, Research Institute of
Internal Medicine, Division of Cancer Medicine, Surgery and
Transplantation, Oslo University Hospital Rikshospitalet, Oslo,
Norway

E. Liaskou
Centre for Liver Research and NIHR Biomedical Research Unit,
Institute of Biomedical Research, University of Birmingham,
Birmingham, UK

C. A. Anderson
Wellcome Trust Genome Campus, Wellcome Trust Sanger Institute,
Hinxton, Cambridge, UK

T. H. Karlsen
Department of Clinical Medicine, University of Bergen, Bergen,
Norway

Introduction

In clinical terms, primary sclerosing cholangitis (PSC) is represented by widespread fibrotic strictures of the intra- and the extra-hepatic biliary tree. The diagnosis is made by imaging, either magnetic resonance or endoscopic retrograde cholangiography [1]. In most patients, the disease is intrinsically progressive, and a lack of effective medical treatment ultimately leads to liver cirrhosis and the need for liver transplantation. Almost identical biliary affections can be observed under the influence of a wide range of toxic and infectious etiologies [2], meaning the stage at which diagnosis is presently being made is the representative of a “final common pathway” for a multitude of etiologies. Little is known of the pre-fibrotic biliary phenotype in PSC, but early biopsy features are often that of subtle lymphocytic infiltration and cholangiocellular reaction. Co-morbidities are common, most

often in the form of inflammatory bowel disease (IBD; 80 %) and autoimmune diseases (25 %), but a considerable fraction of the patients are also at risk of developing biliary cancer (10–15 %).

The aetiology of PSC is unknown. The present molecular understanding of what is principally a radiological phenotype derives from the concepts of bile toxicity in monogenic cholestatic liver diseases as well as those of the associated immunological co-morbidities, IBD in particular (Fig. 1) [3, 4]. The concepts are not mutually exclusive, and both the bile-related and immune-related research avenues are presently effectively translating into novel therapeutic opportunities already entering trials (e.g. *nor*-ursodeoxycholic acid and vedolizumab). Based on the recent discoveries of molecular subentities (i.e. adult *ABCB4* cholangiopathy [5] and IgG4-associated cholangitis [6]), it has been debated whether PSC is a mixed bag of diseases for which not a simple explanatory factor should be sought. However, in Western countries, these are likely marginal phenocopies of what is otherwise predominantly represented by sclerosing cholangitis in the context of IBD (i.e. IBD-associated sclerosing cholangitis).

Risk of PSC in first-degree family members is increased at least 10 times that of the overall population [7, 8], still accounting for a relatively low absolute risk of disease given the generally low incidence (approximately 1–2/100,000 per year [9]). The heritable risk has nevertheless motivated a series of genetic studies [10–15], predominantly along the design of case-control association analysis of genetic markers [16]. Deriving from these studies, a total of 16 statistically robust PSC associations have been identified. In addition, several suggestive PSC associations have been discovered by nominal significant replication of variants of intermediate significance from previous studies [10–12]. In the following, broad themes as to what these findings may teach us about PSC pathogenesis will be outlined.

What Do the Shared Genetics Teach Us?

The majority of the hitherto identified genome-wide significant ($P < 10^{-8}$) associations in PSC are also implicated in other immune-mediated diseases (Table 1). This sharing of risk genes across seemingly distinct disease entities is called pleiotropy and, in the case of PSC, likely affects core aspects of immune function. Only 3 (2q13, 11q23 and 18q21) out of a total of 16 established PSC risk loci demonstrate no overlap to other prototypical immune-mediated diseases based on current reports. The comparisons are confounded by the differing powers of the various studies in other immune-mediated diseases, but apparently, PSC shares the highest number of associated loci with ulcerative colitis (8 out of 16 PSC associated loci), Crohn's disease (8/16), type 1 diabetes (8/16), celiac disease (6/16) and rheumatoid arthritis (6/16), but

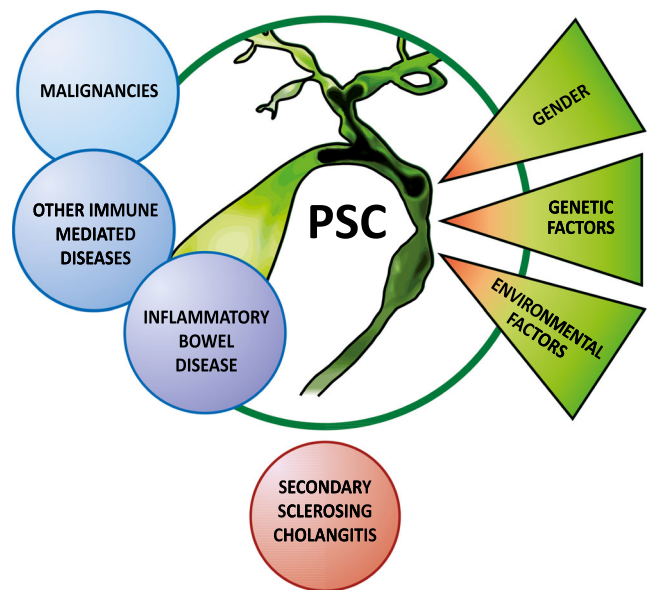


Fig. 1 Primary sclerosing cholangitis (PSC) is a complex phenotype where additive and/or interacting effects from multiple genetic and environmental risk factors are needed for disease development. PSC occurs predominantly in males (70 % of the patients), meaning gender likely modifies disease pathogenesis. An etiologic model of PSC needs to explain the extensive occurrence of co-morbidities, inflammatory bowel disease, cancer and autoimmunity in particular. While similar mechanisms are likely involved in the final presentation of fibrotic bile duct strictures in PSC and secondary sclerosing cholangitis, primary events should by definition be considered distinct. Modified with permission from reference [3]

overlap implicating more than 2 loci is also observed for ankylosing spondylitis, multiple sclerosis, primary biliary cirrhosis, alopecia areata and vitiligo. By assessing the statistics from the genetic analysis of PSC in light of that of genetic studies in seven of these related conditions, the genetic sharing becomes even more apparent, the analysis suggesting the presence of at least 33 additional pleiotropic loci [13].

Inflammatory Bowel Disease

Given a frequency of IBD in PSC of up to 80 %, one would anticipate that a large fraction of the 163 IBD susceptibility loci identified to date would show in the PSC analysis as well. Comparing ulcerative colitis with Crohn's disease, at least 110 of these 163 genes are shared between these two major forms of IBD [17]. However, only half of the 16 established risk loci in PSC can be found among these 163 IBD loci (Table 1). Given the number of cases in the latest IBD analyses is much greater (approximately 10 times) than that in the equivalent PSC study, this number is surprisingly low [13, 17]. Altogether, it may indicate that PSC is more genetically distinct from ulcerative colitis and Crohn's disease, than these two disease entities are from each other, even though ulcerative colitis and Crohn's disease rarely co-occur in the same patient [17]. One recurring theme of dispute is whether PSC

Table 1 Heat map showing non-HLA genetic susceptibility loci shared by primary sclerosing cholangitis and other immune-mediated diseases

Locus	Candidate gene	PSC	UC	CD	T1D	CeD	RA	AS	MS	VIT	AA	PBC	AITD	SLE
1p36	<i>MMEL1</i>	1	1	0	0	1	1	0	1	0	0	1	0	0
2q13	<i>BCL2L11</i>	1	0	0	0	0	0	0	0	0	0	0	0	0
2q33	<i>CD28</i>	1	0	0	1	1	1	0	0	0	1	0	1	0
2q37	<i>GPR35</i>	1	1	1	0	0	0	1	0	0	0	0	0	0
3p21	<i>MST1</i>	1	1	1	0	0	0	0	0	0	0	0	0	0
4q27	<i>IL2, IL21</i>	1	1	1	1	1	1	0	0	0	1	0	0	0
6q15	<i>BACH2</i>	1	0	1	1	1	0	0	1	1	0	0	0	0
10p15	<i>IL2RA</i>	1	0	1	1	1	1	0	1	1	1	0	0	0
11q23	<i>SIK2</i>	1	0	0	0	0	0	0	0	0	0	0	0	0
12q13	<i>HDAC7</i>	1	1	0	0	0	0	0	0	0	0	0	0	0
12q24	<i>SH2B3</i>	1	0	0	1	1	0	1	0	1	0	1	0	0
18q21	<i>TCF4</i>	1	0	0	1	0	0	0	0	0	0	0	0	0
18q22	<i>CD226</i>	1	1	1	1	0	0	0	0	0	0	0	0	0
19q13	<i>PRKD2</i>	1	0	0	1	0	0	0	0	0	0	0	0	0
21q22	<i>PSMG1</i>	1	1	1	1	0	0	1	0	0	0	0	0	0
No. of loci overlapping with PSC (incl HLA)		NA	8	8	8	6	6	4	4	4	4	3	1	1

Shared loci are defined according to genome-wide (GW) significance thresholds ($P < 5 \times 10^{-8}$). Data were obtained from the Immunobase (<http://www.immunobase.org/>), the Catalog of Published Genome-Wide Association Studies (<http://www.genome.gov/26525384>) and supplementing literature searches in PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>). Green colour denotes that shared association occurs for the same genetic variant in primary sclerosing cholangitis (PSC) and the other respective disease. Yellow colour denotes that shared association occurs for the same locus, but for different genetic variants than in PSC

AA alopecia areata, AITD autoimmune thyroid disease, AS ankylosing spondylitis, CD Crohn's disease, CeD celiac disease, MS multiple sclerosis, PBC primary biliary cirrhosis, RA rheumatoid arthritis, SLE systemic lupus erythematosus, T1D type 1 diabetes, VIT vitiligo

should be considered a complication to IBD or a distinct disease entity involving IBD-like features. The moderate genetic overlap between PSC and IBD delineated by the comparisons of genetic findings from well-powered studies in each phenotype may favour a scenario where PSC should be considered a disease distinct from regular IBD. Ultimately, this possibility also relates to the concept of “disease” and by what means “diseases” should be defined and discriminated. Indeed, the present scenario drawn by genetics shows us that present means of disease phenotyping only partially correlate with genotypic and molecular phenotype strata.

The genetic dissimilarities between PSC and IBD are compatible with the clinical observations that IBD in PSC shows features distinct from IBD without PSC, e.g. right-sided dominance, mild inflammation and rectal sparing [18–20]. At the molecular level, there are multiple scenarios in which the genetic differences may align with these observations: (1) PSC and IBD show co-occurrence because they have some genetic risk factors in common, i.e. the moderate observed overlap includes key genetic risk factors still sufficient to cause conventional IBD, yet with some aberrant features (2) PSC and IBD co-occur because the genetic risk factors present in PSC give rise to a distinct disease involving both a biliary and a colonic phenotype, in which the latter is distinct from conventional IBD in the forms of ulcerative colitis or Crohn's disease, and that the degree of genetic risk sharing is of less

importance in comparison to an undetermined, shared environmental impact. The presence of cholestatic liver disease in patients with IBD might serve as clinical modifiers for the bowel affection (e.g. by means of an impact from cholestasis on the gut microbiota) and thus bias our ability to appropriately phenotype the IBD in PSC under any of these scenarios.

Autoimmune Diseases

The presence and pattern of sharing of PSC-associated loci with multiple immune-mediated diseases other than IBD explains the clinical and epidemiological observations that a subset of PSC patients tend to suffer from different immune-mediated diseases [21]. The presence and pattern of sharing also underlines that genetic susceptibility in PSC extends beyond the genetic risk factors involved in IBD and into broader autoimmune pathophysiology. Derived from the pervasive sharing of genetic associations between PSC and prototypical autoimmunity, initiating events in PSC pathogenesis are likely to share characteristics with those known to be involved in these genetically related diseases. Thus, concerning the two main hypotheses as to whether a primary immune disturbance or a toxic bile duct insult serves as the initiating event in PSC, the actual pleiotropic findings are in favour of the autoimmune hypothesis on PSC pathogenesis. Interaction between these bile acid-related aspects and

immune-regulatory mechanisms nevertheless exist [22, 23] and likely involve throughout the disease process.

Some PSC loci are highly pleiotropic, e.g. the 4q27 locus including *IL2/IL21* is associated with PSC, ulcerative colitis, Crohn's disease, type 1 diabetes, celiac disease, rheumatoid arthritis and alopecia areata; and the 6p15 locus including *BACH2* is associated with PSC, Crohn's disease, type 1 diabetes, celiac disease, vitiligo and multiple sclerosis. The highly pleiotropic nature of these loci may point to an essential role for these loci in pathogenic mechanisms common across the majority of immune-mediated diseases [24]. Other PSC-associated loci show more moderate overlap with other immune-mediated diseases, e.g. the 3p21 locus including *MST1* is associated to PSC, ulcerative colitis and Crohn's disease, and the 18q22 locus harbouring *CD226* is associated to PSC, ulcerative colitis and celiac disease. These patterns of associations to different disease groups may be interpreted in the direction that there in addition to more common mechanisms also are distinct mechanistic subsets guiding the development of specific immune-mediated phenotypes in hitherto poorly understood manners [24, 25]. Furthermore, as likely to be shown more clearly by enhanced statistical power in future genetics studies, there is also certainly heterogeneity of effect across the different diseases even when a particular locus is genome-wide significant.

The major histocompatibility complex (MHC) on chromosome 6p21, representing the strongest genetic risk factor in PSC, is of particular interest. The causative risk genes within the MHC in PSC are unknown. The region spans 7.6 million base-pairs (Mb) and a total of 252 expressed genes of which approximately one third relate to immunological functions [26]. The high density of genes under strong selective pressure in the extended MHC has resulted in a complex pattern of linkage disequilibrium (LD) [27], and disease-associated haplotypes extending all of the 7.6 Mb exist [28–30]. For this reason, the mapping of the MHC-associated susceptibility in most multifactorial diseases has not resulted in the unambiguous detection of causative risk genes [31]. The definition of a primary disease locus has been possible for only a few of the MHC-associated phenotypes, and for the autoimmune diseases where this has succeeded the HLA class II (e.g. celiac disease, type I diabetes, multiple sclerosis) and class I (e.g. ankylosing spondylitis, psoriasis), genes are invariably implicated. These loci are also likely to determine PSC susceptibility [32–34]. The application of dense single nucleotide polymorphism (SNP) maps has only minimally refined the MHC association signal [35], but has helped establish a paradigm where variable additional loci in the MHC may add to the predominating associations with HLA class I and class II variants. On the basis of this complexity, the MHC could be considered an “immunologic mini-genome” well worth exploring in depth for a conceptual understanding of autoimmune pleiotropy.

The observed sharing of the PSC-associated risk loci across multiple immune-mediated diseases could imply that the pleiotropic associations will not necessarily be capable to explain the bile duct-specific manifestations in PSC. The PSC associations identified at 11q23 (*SIK2*) and 18q21 (*TCF4*) and the previous identified association at 2q13 (*BCL2L1*) that at time of writing are not reported associated to any other prototypical autoimmune diseases, still hold the potential of representing pathophysiological mechanisms that are specific for PSC, but functional data supporting such a role for these loci are lacking. Importantly, a shared locus association does not preclude disease-specific genetic and biological effects. Disease-specific roles could be exerted by tissue (i.e. bile ducts) and cell (i.e. cholangiocytes) specific effects of otherwise pleiotropic genes. Furthermore, as evident from Table 1, a shared locus association may not necessarily mean a shared variant association [36]. As an example, the top-associated variant at the 16q15 (*BACH2*) locus in PSC have not been robustly associated to any other immune-mediated disease, and other variants in the region account for the *BACH2* associations with other phenotypes. The pervasive sharing of risk genes across PSC and several other traits, together with the clinical occurrence of other traits (IBD, autoimmunity, cancer) in the majority of patients, also raises the hypothesis that exogenous factors rather than a combination of genetic factors have the decisive role in determining the bile duct-specific manifestations in a host genetically susceptible to develop any immune-mediated disease [24]. This hypothesis radically could imply that no PSC-specific risk gene or genetic effect exists.

What Do the Risk Genes Teach Us?

As reviewed elsewhere [37, 38], the exact gene and genetic variant causing the statistical association in case-control genetic association studies is often not possible to determine. I.e. in most cases, there are many plausible candidates (e.g. *GPXI* and *MST1* at 3p21) at the susceptibility locus. If there is only one candidate susceptibility gene, the causative genetic variant is in turn often not known, and in most cases, does not alter protein structure. Furthermore, upon speculating biological implications of risk genes, there is also the inherent risk of “literature bias” [39]; the published experimental works performed prior to the knowledge of an association with PSC (or other immune-mediated diseases) may provide misleading information about gene functions in a disease-specific context. Finally, and importantly, risk genes in PSC cannot be understood in the context of Mendelian genetics. Rather than representatives of disease, they should be considered representatives of biological mechanisms exerting a so far small, yet likely important, role in a complex orchestra also populated by environmental risks (Fig. 2). Within these restraints, a brief

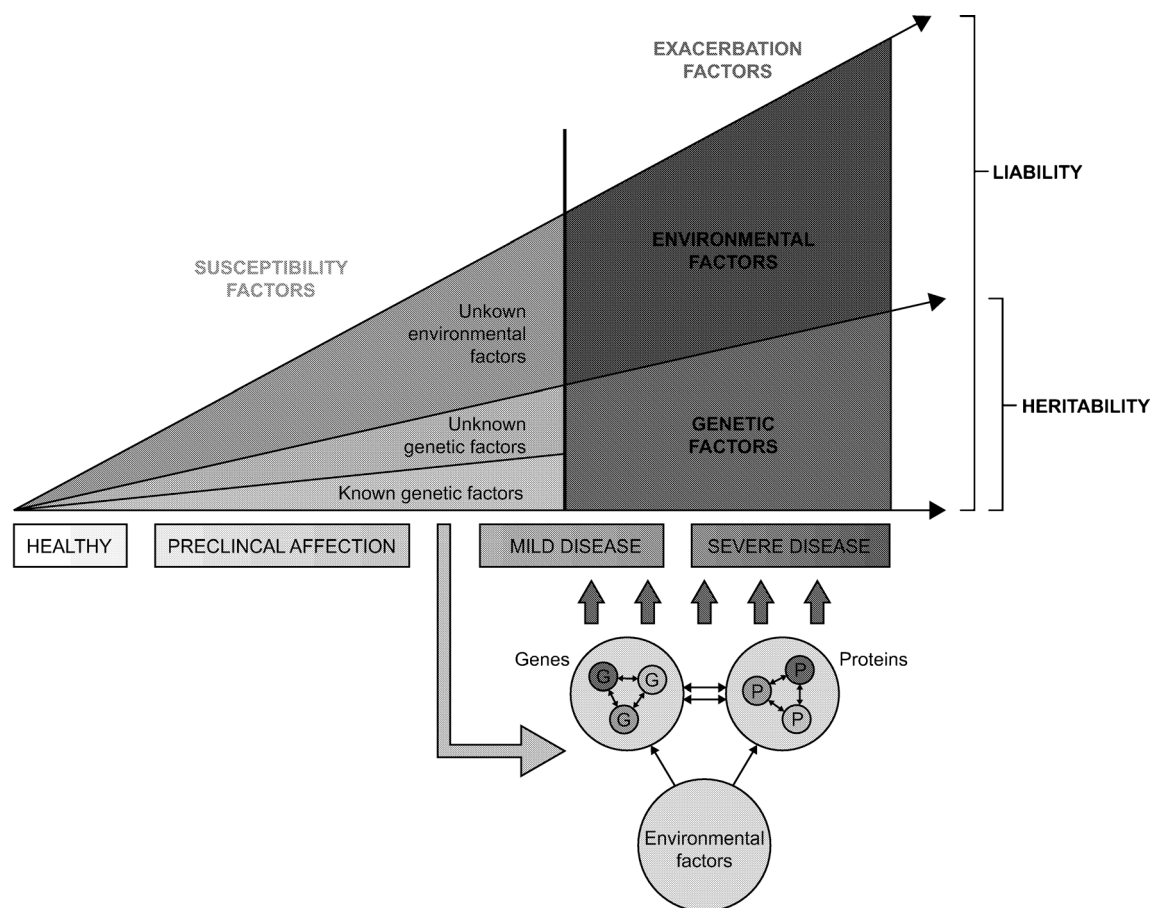


Fig. 2 The overall liability to primary sclerosing cholangitis (PSC) is caused by environmental (*upper part of triangle*) and genetic (*lower part of triangle*) factors. On most instances, the susceptibility factors are non-pathogenic and representatives of common biological affections that also occur in healthy individuals (*left; green part of triangle*). In the context of an unfortunate high additive load of genetic and/or environmental factors, gene-gene, gene-environment and environment-environment interactions (*bottom interaction panel*) [89], clinical disease may emerge (*right; red part of triangle*). The vertical line demarcates clinical emergence of the phenotype. Modifier effects (“exacerbation factors”), e.g. variable degree

of bile acid induced bile duct injury, influence severity of the affection (i.e. *severity running from left to right as the overall height of the diagram*). A clear demarcation between susceptibility factors and exacerbation factors is difficult to make. At present, a total of 16 genome-wide significant ($P < 10^{-8}$) and 33 additional pleiotropic PSC risk genes have been identified (*known genetic factors*), and ongoing efforts will likely make this list considerably longer (*unknown genetic factors*), potentially explaining an even larger proportion of the overall contribution from genetic heritability to PSC susceptibility (*heritability*)

description of annotated functions of what could be considered the main candidate risk genes at the robustly PSC-associated non-MHC loci identified in the current studies is given in Table 2.

T Cells and IL2 Signalling

The large majority of PSC-associated loci involve genes encoding molecules that serve essential functions in immune-related pathways. Loci that harbour genes providing well-established and fundamental functions in the immune-system include 1p36 (*TNFRSF14*), 2q33 (*CD28*, *CTLA4*), 4q27 (*IL2/IL21*), 6p16 (*BACH2*), 10p15 (*IL2RA*), 11q23 (*SIK32*), 12q24 (*SH2B3*), 12q13 (*HDAC7*), 18q22 (*CD226*) and 19q13 (*PRKD2*) (Table 2). Across these loci, there is a

convergence of candidate genes whose products directly or indirectly regulate T cell biology, either through regulating T cell proliferation, differentiation and/or activity (*TNFRSF14*, *CD28*, *IL2/IL21*, *IL2RA*, *SIK2*, *HCDAC7*, *CD226* and potentially also *TCF4*) or apoptosis of T cells (*PRKD2*).

The enrichment of T cell-related associations, together with the strong associations to the MHC in PSC, places adaptive immune mechanisms into the limelight of the likely causal basis of PSC. Broadly, adaptive immune responses depend on HLA-dependent antigen activation of B and T cells resulting in the generation of plasma and T effector cells. Responses are in turn limited in part by suppressive T regulatory cells (Tregs). In addition, regulation of Tregs represents a major constitutive mechanism to keep potential responses to autoantigens from

Table 2 Annotated function of notable candidate genes within non-HLA, genome-wide significant ($P < 5 \times 10^{-8}$) primary sclerosing cholangitis susceptibility (PSC) risk loci ($n = 15$) as determined by available literature

Risk gene	Functional annotation
<i>MMEL1</i> , <i>TNFRSF14</i> (1p36)	MMEL1 is a member of the membrane metallo-endopeptidase family. Little is known about its function. The TNFRSF14 protein is expressed on CD4 and CD8 T lymphocytes, B cells, monocytes, neutrophils, dendritic cells and mucosal epithelium. It acts as a molecular switch modulating T cell activation via binding to its ligand LIGHT [73], but can also propagate inhibitory signals through the immunoglobulin superfamily member BTLA (B and T lymphocyte attenuator). This dual role of TNFRSF14 is thought to regulate immune tolerance [74]
<i>CD28</i> (2q33)	CD28 is expressed on T lymphocytes and upon binding to B7 target ligands provides co-stimulatory signals that are essential for T cell activation, survival and proliferation [75]
<i>GPR35</i> (2q37)	Expressed in epithelial cells in the intestine and in multiple types of immune cells. Specific activation of GPR35 has been demonstrated to significantly reduce IL-4 release from natural killer T cells [56]. Associated with early onset IBD [57]
<i>MST1</i> (3p21)	The <i>MST1</i> encoded protein MSP has an inhibitory function towards macrophages during inflammation [76]
<i>IL2</i> , <i>IL21</i> (4q27)	IL-2 is a cytokine essential for proliferation of T cells following activation of naïve T cells. Upon antigen binding to the T cell receptor, IL-2 production stimulates differentiation and proliferation of antigen-selected T cells. Liver-derived T cells from PSC patients have been demonstrated to produce significantly lower levels of IL-2 compared to patients with primary biliary cirrhosis, autoimmune hepatitis and healthy controls [77]. IL-21 plays a role in both the innate and adaptive immune responses by inducing the differentiation, proliferation and activity of multiple target cells including macrophages, natural killer cells, B cells and cytotoxic T cells
<i>BACH2</i> (6q15)	<i>BACH2</i> encodes a transcription factor, which regulates B cell differentiation [78]. Downregulation of <i>BACH2</i> has been demonstrated to attenuate activation of genes involved in antiviral innate immune response after nucleic-acid triggering [79]
<i>IL2RA</i> (10p15)	<i>IL2RA</i> encodes the alpha chain of the IL-2 receptor (consisted of α , β and γ chains), which is responsible for capturing IL-2 cytokine and mediating its signalling effects. PSC-associated variants within <i>IL2RA</i> [12] lead to reduced expression of IL2RA, which have implications for induction of immune tolerance [80]. <i>Il2ra</i> ^{-/-} mice spontaneously develop intestinal and biliary inflammation [81]
<i>SIK2</i> (11q23)	<i>SIK2</i> encodes a Rho-GTPase activating protein, which influences the expression of interleukin-10 in macrophages and Nur77 an important transcription factor in leukocytes [82]
<i>HDAC7</i> (12q13)	<i>HDAC7</i> encodes a histone deacetylase highly expressed in cytotoxic lymphocytes (CTL). Its phosphorylation favours high IL-2R α expression, increased CTL effector function and IFN γ production. HDAC7 regulates cell survival and T cell receptor (TCR) signalling in CD4/CD8 double positive (DP) thymocytes by upon stimulation altering activity of multiple effectors of TCR signalling, inducing apoptosis of DP thymocytes, producing defects in both T cell numbers and the T cell repertoire [83]
<i>SH2B3</i> (12q24)	SH2B3 is a key regulator of cytokine signalling, and genetic variation within <i>SH2B3</i> has been demonstrated to affect the production of pro-inflammatory cytokines in response to stimulation with bacterial cell wall peptidoglycans, pointing to a role for SH2B3 in innate immunity and in the protection against bacterial infection [84]
<i>TCF4</i> (18q21)	<i>TCF4</i> encodes a transcription factor involved in cell differentiation and growth. <i>Tcf4</i> ^{-/-} knockout mice have revealed that <i>TCF4</i> deficiency leads to a partial block in early B and T cell development [63, 64] and also results in blocked development of plasmacytoid dendritic cells (PDCs) and impaired type 1 interferon secretion from PDCs upon stimulation with “virus resembling” un-methylated DNA [65]
<i>CD226</i> (18q22)	The gene <i>CD226</i> encodes the intracellular adhesion molecule DNAM-1, which is involved in co-stimulation and activation of effector function of T-helper type 1 cells. DNAM-1 is a member of the immunoglobulin superfamily and is constitutively expressed on the majority of CD4 and CD8 T cells, natural killer T cells, monocytes and platelets and a subset of B cells [85]
<i>PRKD2</i> (19q13)	<i>PRKD2</i> encodes a serine-threonine protein kinase. When T cell receptors of thymocytes are engaged, PRKD2 phosphorylates HDAC7 (see above), leading to nuclear exclusion of HDAC7 and loss of its gene regulatory functions, ultimately resulting in apoptosis and negative selection of immature T cells [45, 86]. This negative selection takes place due to a loss of HDAC7-mediated repression of Nur77, which is regulated by SIK2 (see above) [87], linking three PSC loci to this pathway
<i>PSMG1</i> (21q22)	<i>PSMG1</i> encodes a chaperone protein. Little is known about the function of PSMD1, but the protein has been hypothesized to be involved in microbial processing in Crohn's disease [88]

Caution must be made in assessing previously published literature (i.e. “literature bias”), since studies performed have not been designed with the prior knowledge of genetic associations in PSC. For information on additional non-HLA pleiotropic loci ($n = 33$), please see reference [13]. For gene name abbreviations, see <http://www.ncbi.nlm.nih.gov/gene/>

IBD inflammatory bowel disease, IL interleukin

autoreactive T cells in check. In this context, the independent associations found at 4q27 (*IL2/IL21*), 10p15 (*IL2RA*), 11q23 (*SIK32*), 12q13 (*HDAC7*) and 19q13 (*PRKD2*) are worth a special mention.

The associations at the 4q27 region harbouring *IL2/IL21* in PSC largely overlap with that observed in other PSC-related immune-mediated diseases (Table 1). Despite extensive fine-mapping and re-sequencing of this region in multiple diseases,

it has not been possible to establish which of the two candidate genes at 4q27 (*IL2* or *IL21*) is the most important for disease risk. There is also the possibility that they both involve in causing the genetic association signal. The association at 10p15 harbouring *IL2RA*, which encodes IL-2 receptor subunit (IL-2R α), suggests an important role for *IL2* and IL-2-signalling in PSC, but does not rule out additional effects from *IL21* also being at play. The IL-2R α (also known as CD25) chain is one of the three subunits that make up the high affinity receptor for IL-2 (IL-2R) composed of IL-2R α , IL-2R β and IL-2R γ chains. IL-2R α functions only for IL-2 capturing, whereas the β and γ subunits augment ligand binding and induce cellular signalling. Thus, IL-2 via IL-2R α induces T effector cell proliferation, differentiation and survival, and increased IL-2R α /CD25 expression has been associated with increased T cell activation. On the other hand, IL-2 can contribute to termination of inflammatory immune responses, by promoting the development, survival and function of regulatory T cells, by enhancing activation-induced cell death (AICD) and inhibiting the development of inflammatory Th17 cells [40]. Therefore, IL-2 and IL-2R α are required for mounting and sustaining the adaptive immune response, but are also critical for downregulating the immune response via Tregs. Based on these dual effects, the presence, absence and gradient of the IL-2 and IL-2R α signalling may have profoundly different and potential paradox outcomes, as exemplified by IL-2- and IL-2R α -deficient mice developing a lethal lymphoproliferative autoimmune syndrome despite absence of IL-2-dependent immune activation, because concomitant IL-2-dependent Treg immune regulation fails to occur, causing uncontrollable autoimmunity [41, 42].

As the exact causal variants within *IL2* and *IL2RA* in PSC have not yet been determined and characterized, there is dispute whether there is a loss or gain of IL-2 and IL-2R α signalling resulting from the associations in PSC. Most speculations with regard to the associations to *IL2* in immune-mediated diseases in general argue in favour of reduced IL-2 signalling; moreover, most of the *IL2RA* variants associated to PSC result in reduced *IL2RA* expression, putatively favouring loss of or low grade of IL-2 and IL-2R α signalling [12]. The dual roles in immune activation and regulation of IL-2 and IL-2R α make it difficult to conclude whether exaggerated or diminished immune responses, or elements of both, play the main role in disease risk. In summary, the *IL2* and *IL2RA* associations in PSC harbour the potential to influence both T cell-mediated primary immune responses, memory recall responses and further Treg-mediated fine-tuned regulation. A decrease in the frequency and suppressive capacity of Tregs from PSC patients has been recently reported. Notably, the reduced Treg numbers seemed to be associated with homozygosity for the risk allele of the IL-2R α gene SNP (rs10905718) [43]. Additional studies on the effect of this major allele in the IL-2R α signalling pathway will be of high importance.

A potential link between IL-2 and IL-2R α and the association at 12q13 (*HDAC7*) exists. *HDAC7* encodes a histone deacetylase that is highly expressed in cytotoxic T cells (CTLs) and also shows expression in double positive thymocytes during negative selection [44, 45]. In a dephosphorylated resting state, HDAC7 forms nuclear complexes that inhibit expression of several genes encoding key cytokines, cytokine receptors and adhesion molecules important for the effector function of CTLs. Global transcriptome analyses in CTLs have revealed that keeping HDAC7 in resting state in the nucleus represses in particular IL-2R α . Conversely, export of HDAC7 from the nucleus via phosphorylation favours high and prolonged expression of IL-2R α , increased CTL effector function and production of interferon- γ after initial T cell antigen receptor stimulation [44]. Whether HDAC7 represses *IL2RA* directly or whether the action is indirect is not known. There is also evidence that HDAC7 participates in transcriptional repression of Tregs [46].

Phosphorylation of HDAC7 leads to increased expression of Nur77, an orphan nuclear receptor involved in T cell antigen receptor-stimulated apoptosis of thymocytes, which further stimulates the deletion of autoreactive thymocytes [45]. Interestingly, the PSC association at 11q23 is located in *SIK2*, a salt inducible kinase that is also known to influence the expression of Nur77 [47]. Furthermore, the independent association at 19q13 harbours *PRKD2*, which participates in the phosphorylation of HDAC7 after T cell antigen receptor stimulation [48]. Previous studies had shown *prkd2*-null T cells do not make IL-2 and IFN- γ in response to T cell receptor engagement [49]. Later studies revealed that *prkd2*-null mice produce and export T cells from the thymus with the transcriptional program of a normal naïve CD8 T cell, but they are unable to respond to antigen and switch their transcriptional program to an effector T cell state in response to T cell antigen receptor stimulation [50].

Overall, five independent PSC associations on different chromosomes (4q27, 10p15, 11q23, 12q13 and 19q13) harbour genes that fine-tune T cell development in the thymus and program CTLs via effector cells to perform appropriate immune responses. The associations harbouring *HDAC7*, *PRKD2* and *SIK2* are of special interest, as they do not overlap as broadly to other immune-mediated diseases as the other PSC-associated loci (*HDAC7*; ulcerative colitis, *PRKD2*; type 1 diabetes and *SIK2*; none), potentially pointing to more specific roles of these loci in governing PSC-specific aspects of T cell regulation. The question still remains whether the immune-mediated mechanisms in PSC relate to an inappropriate destruction of the bile ducts owing to an immune reaction towards an autoantigen (autoimmune mechanism) or a dysregulated immune response to an exogenous antigen (inflammatory mechanism). The genetic findings do not give a clear answer, as several of the identified susceptibility loci

implicate pathogenic effector pathways that operate in both primary autoimmune and inflammatory mechanisms [51]. The relative importance of adaptive immune-mediated effects on pathogenesis relative to other potential non-immune related mechanisms involving, e.g. bile acid toxicity and fibrosis development also remains unclarified.

Interpreting Non-IL2 Immunobiology

Additional PSC-associated loci could also disrupt immune-related pathways, but genetic complexities at these loci and a lack of knowledge regarding candidate genes function make deciphering their role in pathogenesis more difficult (e.g. 2q13 (*BCL2L1*), 2q37 (*GPR35*), 2p21 (*MST1*), 18q21 (*TCF4*) and 21q22 (*PSMG1*) and 2q33 (*CD28*)).

At 2q27, *GPR35* is the most plausible candidate gene. The variants identified within this locus demonstrate the strongest effect sizes (odds ratios 1.38 and 1.39 for the two most associated SNPs) outside the MHC. *GPR35* encodes a G protein coupled receptor, for which several endogenous and synthetic ligands have been proposed (e.g. kynurenic acid, lysophosphatidic acid and zaprinast), but it is debated whether these ligands are relevant for *GPR35* function in vivo [52–55]. Little is known about the biological function of *GPR35*. The receptor is expressed in epithelial cells in the intestine and in multiple immune cell types (monocytes, T cells, dendritic cells) [52, 56]. The association at *GPR35* is interesting in terms of not being as broadly shared with other immune-mediated diseases; at time of writing, the locus have only been robustly associated with ulcerative colitis, Crohn's disease and ankylosing spondylitis. The *GPR35* association in ulcerative colitis is interesting by also being present in a subgroup of patients with early onset (childhood) disease [57].

The association at 3p21 is one of the most significant associations outside the HLA ($P=2.45 \times 10^{-26}$ in the Immunochip study) and among the associations with the strongest effect sizes (odds ratio 1.33). The association signal in this region spans more than 600 kB and covers more than 10 genes, which complicate the identification of the susceptibility gene in this region. The locus is also implicated in ulcerative colitis and Crohn's disease, in which most authors argue in favour of the *MST1* gene being the main candidate in the region [58, 59]. *MST1* encodes a macrophage-stimulating factor-1 protein (MSP), which is a circulating pre-protein secreted from the liver [60]. MSP has been shown to inhibit IL-12 release, as well as iNOS and COX2 induction of activated macrophages, implicating a negative regulation of macrophage activation exerted by this protein [61]. IL-12, produced by macrophages and APCs in general, during the initial stages of an immune response, is essential for induction of IFN γ by NK and $\gamma\delta$ T cells and driving the differentiation of Th1 cells. Thus, IL-12 provides a

critical link between innate and acquired immunity, which can be impaired by MSP.

The association at 18q21 harbours *TCF4*, which encodes a transcription factor for which genetic associations hitherto have mainly been reported in neuropsychiatric disorders, such as schizophrenia and bipolar disorders. This is nevertheless interesting in light of these diseases potentially having an aetiology influenced by prenatal infections and/or autoimmunity [62]. Analysis of *Tcf4*^{-/-} knockout mice has revealed that *TCF4* deficiency leads to a partial block in early B and T cell development and also results in blocked development of plasmacytoid dendritic cells and impaired type 1 interferon secretion from these upon stimulation with “virus resembling” unmethylated DNA [63–65]. In these aspects, *TCF4* functions thus align with the above-mentioned genetic findings in T- and B-cell biology and in particular T cell development [38]. At 21q22, the *PSMG1*-region is potentially also associated with microbial triggers, since *PSMG1* have been associated to pseudomonas infection in children with early onset CD [66]. Otherwise, little is known about *PSMG1* function.

The association at 2q33 harbours *CD28*, which encodes a T cell co-stimulatory molecule necessary for T cell activation, survival and proliferation. Genetic associations for *CD28* have been also reported for celiac disease, type 1 diabetes and rheumatoid arthritis. Early studies have demonstrated that T cells lacking *CD28* are pathogenic cells that accumulate in various autoimmune diseases as a result of continuous antigenic stimulation [67, 68]. In PSC liver tissue, a significant proportion of CD4 T cells have lost *CD28* expression, at much higher levels compared to PBC. Such *CD28*⁻ T cells localize close to bile ducts, release pro-inflammatory cytokines, TNF α and IFN γ , and induce biliary cell activation. Notably, TNF α is able to induce loss of *CD28* and accumulation of *CD28*⁻ T cells in vitro, and a TNF α -rich microenvironment is evident in PSC liver tissue (Liaskou et al., manuscript submitted). It should be noted that *CD28* is required for IL-2 production, which in turn is required for both the induction (activation of T effector cells) and termination of inflammatory immune responses (induction of T regulatory cells).

Interpreting Suggestive Associations

In addition to the 16 genome-wide significant loci, somewhere between 30 and 40 nominally significant findings have been identified in PSC. The majority of these harmonize well with the overall susceptibility to PSC, being pleiotropic (*REL*, e.g. in Crohn's disease, celiac disease and rheumatoid arthritis; *FUT2*, e.g. in Crohn's disease and psoriasis; *UBASH3A*, e.g. in celiac disease, vitiligo, type 1 diabetes; *ICOSLG*, e.g. in Crohn's disease, ulcerative colitis and celiac disease) and executing important functions in adaptive immunity and T cell-related functions

(e.g. *REL*, *UBASH3A* and *ICOSLG*). This is not surprising, since largely (for 33 of the loci), the detection has been facilitated by taking associations in PSC-associated immune-mediated diseases into account.

The association of *FUT2* is of special interest, as analysis of the bacterial community composition in colonized bile of PSC patients demonstrated that *FUT2* genotype in PSC patients was associated with differences in gut microbial compositions, shown by increased *Firmicutes* and diminished *Proteobacteria*. This interdependence of host genetics with disease-associated gut and bile microbiota is interesting in light of the emerging evidence that alterations of the gut microbiota may provide an important contribution to the pathogenesis of inflammatory conditions [69, 70]. The *FUT2* locus is also interesting for another reason, as variants within the locus have been demonstrated to affect normal variation in serum of alkaline phosphatase (ALP) levels [71, 72]. The *FUT2*-related biological implications contributing to cholestatic ALP elevation may therefore also be at play in the healthy population (reflected by ALP variability, but within the normal range and without presence of disease symptoms). This reflects that within the general population, there are individuals with “susceptible” *FUT2* variants that have not developed disease, presumably because other co-factors are not present (genetic and non-genetic co-factors). This is also the case for all other PSC-associated variants; they are common (frequencies >10 % in the general population) and their biological consequences are common—and mostly not pathogenic.

Conclusions

The combined output of genetic studies in PSC so far explains 7.3 % of the overall PSC susceptibility. More has become evident on general immunobiology and immunogenetics than on the specific genetics of biliary disease. This includes a lack of robust genetic risk factors for the development of cholangiocarcinoma in PSC, alluding to either their absence or lack of statistical power in studies hitherto performed. Still, despite the limited fraction of overall susceptibility so far accounted for, since methods for surveying and precisely determining environmental risk factors are largely lacking, there is a pragmatic upside for which we should consider the application of the genetic association study design as a success that will continue along the models of other diseases (meta-analysis of existing and new data, search for additional causal variants at identified loci, etc.). For the first time, we have a large number of robust biological determinants of PSC pathogenesis. Moreover, as alluded to by the MHC and *FUT2* associations, these genetic determinants are likely to pinpoint the biological contexts in which the undetermined environment operates and may even guide in the detection of environmental factors (e.g. antigenic triggers, features of the gut microbiota, etc.).

Never before have specific opportunities for working into primary events in PSC pathogenesis existed; rather, the research has been guided by findings in “similar” diseases (mostly familial cholestatic syndromes and IBD). These opportunities must now be perused and experimentally explored by the research community. As underscored above, no single PSC susceptibility gene should be expected to account for the full phenotype. Rather, the biological manifestations of disease-associated variants, which also frequently occur in the healthy population, represent pieces of a puzzle that needs to be laid taking the specific disease context and environmental co-factors in consideration (Fig. 2). Along this avenue of thinking, novel therapeutic opportunities are likely to emerge, potentially not only slowing PSC progression, but on the target of the primary disturbances at the gene-environment interface, may even hope to cure.

Acknowledgements We thank Tor Halland (TorDesign) and Øystein Horgmo (University of Oslo) for assistance in preparing Fig. 1 and Fig. 2, respectively.

References

1. Hirschfield GM, Karlsen TH, Lindor KD, Adams DH (2013) Primary sclerosing cholangitis. *Lancet* 382:1587–1599
2. Abdalian R, Heathcote EJ (2006) Sclerosing cholangitis: a focus on secondary causes. *Hepatology* 44:1063–1074
3. Karlsen TH, Boberg KM (2013) Update on primary sclerosing cholangitis. *J Hepatol* 59:571–582
4. Karlsen TH, Schrumpf E, Boberg KM (2010) Update on primary sclerosing cholangitis. *Dig Liver Dis* 42:390–400
5. Gotthardt D, Runz H, Keitel V et al (2008) A mutation in the canalicular phospholipid transporter gene, *ABCB4*, is associated with cholestasis, ductopenia, and cirrhosis in adults. *Hepatology* 48:1157–1166
6. Mendes FD, Jorgensen R, Keach J et al (2006) Elevated serum IgG4 concentration in patients with primary sclerosing cholangitis. *Am J Gastroenterol* 101:2070–2075
7. Bergquist A, Montgomery SM, Bahmanyar S et al (2008) Increased risk of primary sclerosing cholangitis and ulcerative colitis in first-degree relatives of patients with primary sclerosing cholangitis. *Clin Gastroenterol Hepatol* 6:939–943
8. Bergquist A, Lindberg G, Saarinen S, Broome U (2005) Increased prevalence of primary sclerosing cholangitis among first-degree relatives. *J Hepatol* 42:252–256
9. Boberg KM, Aadland E, Jahnsen J, Raknerud N, Stiris M, Bell H (1998) Incidence and prevalence of primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis in a Norwegian population. *Scand J Gastroenterol* 33:99–103
10. Ellinghaus D, Folseraas T, Holm K et al (2013) Genome-wide association analysis in primary sclerosing cholangitis and ulcerative colitis identifies risk loci at GPR35 and TCF4. *Hepatology* 58:1074–1083
11. Folseraas T, Melum E, Rausch P et al (2012) Extended analysis of a genome-wide association study in primary sclerosing cholangitis detects multiple novel risk loci. *J Hepatol* 57:366–375
12. Srivastava B, Mells GF, Cordell HJ et al (2012) Fine mapping and replication of genetic risk loci in primary sclerosing cholangitis. *Scand J Gastroenterol* 47:820–826

13. Liu JZ, Hov JR, Folseraas T et al (2013) Dense genotyping of immune-related disease regions identifies nine new risk loci for primary sclerosing cholangitis. *Nat Genet* 45:670–675
14. Melum E, Franke A, Schramm C et al (2011) Genome-wide association analysis in primary sclerosing cholangitis identifies two non-HLA susceptibility loci. *Nat Genet* 43:17–19
15. Karlsen TH, Franke A, Melum E et al (2010) Genome-wide association analysis in primary sclerosing cholangitis. *Gastroenterology* 138:1102–1111
16. Karlsen TH, Melum E, Franke A (2010) The utility of genome-wide association studies in hepatology. *Hepatology* 51:1833–1842
17. Jostins L, Ripke S, Weersma RK et al (2012) Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 491:119–124
18. Fausa O, Schrumpf E, Elgjo K (1991) Relationship of inflammatory bowel disease and primary sclerosing cholangitis. *Semin Liver Dis* 11:31–39
19. Loftus EV Jr, Harewood GC, Loftus CG et al (2005) PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. *Gut* 54:91–96
20. Jorgensen KK, Grzyb K, Lundin KE et al (2012) Inflammatory bowel disease in patients with primary sclerosing cholangitis: clinical characterization in liver transplanted and nontransplanted patients. *Inflamm Bowel Dis* 18:536–545
21. Saarinen S, Olerup O, Broome U (2000) Increased frequency of autoimmune diseases in patients with primary sclerosing cholangitis. *Am J Gastroenterol* 95:3195–3199
22. Yoneno K, Hisamatsu T, Shimamura K et al (2013) TGR5 signalling inhibits the production of pro-inflammatory cytokines by in vitro differentiated inflammatory and intestinal macrophages in Crohn's disease. *Immunology* 139:19–29
23. McMahan RH, Wang XX, Cheng LL et al (2013) Bile acid receptor activation modulates hepatic monocyte activity and improves nonalcoholic fatty liver disease. *J Biol Chem* 288:11761–11770
24. Mells GF, Kaser A, Karlsen TH (2013) Novel insights into autoimmune liver diseases provided by genome-wide association studies. *J Autoimmun* 46:41–54
25. Cotsapas C, Voight BF, Rossin E et al (2011) Pervasive sharing of genetic effects in autoimmune disease. *PLoS Genet* 7:e1002254
26. Horton R, Wilming L, Rand V et al (2004) Gene map of the extended human MHC. *Nat Rev Genet* 5:889–899
27. Ahmad T, Neville M, Marshall SE et al (2003) Haplotype-specific linkage disequilibrium patterns define the genetic topography of the human MHC. *Hum Mol Genet* 12:647–656
28. Degli-Esposti MA, Leaver AL, Christiansen FT, Witt CS, Abraham LJ, Dawkins RL (1992) Ancestral haplotypes: conserved population MHC haplotypes. *Hum Immunol* 34:242–252
29. Aly TA, Eller E, Ide A et al (2006) Multi-SNP analysis of MHC region: remarkable conservation of HLA-A1-B8-DR3 haplotype. *Diabetes* 55:1265–1269
30. Donaldson PT, Norris S (2001) Immunogenetics in PSC. *Best Pract Res Clin Gastroenterol* 15:611–627
31. Traherne JA (2008) Human MHC architecture and evolution: implications for disease association studies. *Int J Immunogenet* 35:179–192
32. Hov JR, Kosmoliaptsis V, Traherne JA et al (2011) Electrostatic modifications of the human leukocyte antigen-DR P9 peptide-binding pocket and susceptibility to primary sclerosing cholangitis. *Hepatology* 53:1967–1976
33. Hov JR, Lleo A, Selmi C et al (2010) Genetic associations in Italian primary sclerosing cholangitis: heterogeneity across Europe defines a critical role for HLA-C. *J Hepatol* 52:712–717
34. Karlsen TH, Boberg KM, Olsson M et al (2007) Particular genetic variants of ligands for natural killer cell receptors may contribute to the HLA associated risk of primary sclerosing cholangitis. *J Hepatol* 46:899–906
35. Rioux JD, Goyette P, Vyse TJ et al (2009) Mapping of multiple susceptibility variants within the MHC region for 7 immune-mediated diseases. *Proc Natl Acad Sci U S A* 106:18680–18685
36. Parkes M, Cortes A, van Heel DA, Brown MA (2013) Genetic insights into common pathways and complex relationships among immune-mediated diseases. *Nat Rev Genet* 14:661–673
37. Karlsen TH (2012) A lecture on the genetics of primary sclerosing cholangitis. *Dig Dis* 30(Suppl 1):32–38
38. Karlsen TH, Kaser A (2011) Deciphering the genetic predisposition to primary sclerosing cholangitis. *Semin Liver Dis* 31:188–207
39. Henriksen, E.K.K., Melum, E., and Karlsen, T.H. (2014), Update on primary sclerosing cholangitis genetics. *Curr Opin Gastroenterol* In press
40. Hoyer KK, Dooms H, Barron L, Abbas AK (2008) Interleukin-2 in the development and control of inflammatory disease. *Immunol Rev* 226:19–28
41. Malek TR (2008) The biology of interleukin-2. *Annu Rev Immunol* 26:453–479
42. Willerford DM, Chen J, Ferry JA, Davidson L, Ma A, Alt FW (1995) Interleukin-2 receptor alpha chain regulates the size and content of the peripheral lymphoid compartment. *Immunity* 3:521–530
43. Sebode M, Peiseler M, Franke B et al (2014) Reduced FOXP3 regulatory T cells in patients with primary sclerosing cholangitis are associated with IL2RA gene polymorphisms. *J Hepatol* 53(3):311–319
44. Navarro MN, Goebel J, Feijoo-Carnero C, Morrice N, Cantrell DA (2011) Phosphoproteomic analysis reveals an intrinsic pathway for the regulation of histone deacetylase 7 that controls the function of cytotoxic T lymphocytes. *Nat Immunol* 12:352–361
45. Dequiedt F, Kasler H, Fischle W et al (2003) HDAC7, a thymus-specific class II histone deacetylase, regulates Nur77 transcription and TCR-mediated apoptosis. *Immunity* 18:687–698
46. Li B, Samanta A, Song X et al (2007) FOXP3 interactions with histone acetyltransferase and class II histone deacetylases are required for repression. *Proc Natl Acad Sci U S A* 104:4571–4576
47. Hanna RN, Shaked I, Hubbeling HG et al (2012) NR4A1 (Nur77) deletion polarizes macrophages toward an inflammatory phenotype and increases atherosclerosis. *Circ Res* 110:416–427
48. Parra M, Kasler H, McKinsey TA, Olson EN, Verdin E (2005) Protein kinase D1 phosphorylates HDAC7 and induces its nuclear export after T-cell receptor activation. *J Biol Chem* 280:13762–13770
49. Matthews SA, Navarro MN, Sinclair LV, Emslie E, Feijoo-Carnero C, Cantrell DA (2010) Unique functions for protein kinase D1 and protein kinase D2 in mammalian cells. *Biochem J* 432:153–163
50. Navarro MN, Sinclair LV, Feijoo-Carnero C, Clarke R, Matthews SA, Cantrell DA (2012) Protein kinase D2 has a restricted but critical role in T-cell antigen receptor signalling in mature T-cells. *Biochem J* 442:649–659
51. Zhernakova A, van Diemen CC, Wijmenga C (2009) Detecting shared pathogenesis from the shared genetics of immune-related diseases. *Nat Rev Genet* 10:43–55
52. Wang J, Simonavicius N, Wu X et al (2006) Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. *J Biol Chem* 281:22021–22028
53. Oka S, Ota R, Shima M, Yamashita A, Sugiura T (2010) GPR35 is a novel lysophosphatidic acid receptor. *Biochem Biophys Res Commun* 395:232–237
54. Taniguchi Y, Tonai-Kachi H, Shinjo K (2006) Zaprinast, a well-known cyclic guanosine monophosphate-specific phosphodiesterase inhibitor, is an agonist for GPR35. *FEBS Lett* 580:5003–5008
55. Southern C, Cook JM, Neetoo-Isseljee Z et al (2013) Screening beta-arrestin recruitment for the identification of natural ligands for orphan G-protein-coupled receptors. *J Biomol Screen* 18:599–609
56. Fallarini S, Magliulo L, Paoletti T, de Lalla C, Lombardi G (2010) Expression of functional GPR35 in human iNKT cells. *Biochem Biophys Res Commun* 398:420–425

57. Imielinski M, Baldassano RN, Griffiths A et al (2009) Common variants at five new loci associated with early-onset inflammatory bowel disease. *Nat Genet* 41:1335–1340
58. Raelson JV, Little RD, Ruether A et al (2007) Genome-wide association study for Crohn's disease in the Quebec Founder Population identifies multiple validated disease loci. *Proc Natl Acad Sci U S A* 104:14747–14752
59. Goyette P, Lefebvre C, Ng A et al (2008) Gene-centric association mapping of chromosome 3p implicates MST1 in IBD pathogenesis. *Mucosal Immunol* 1:131–138
60. Yoshimura T, Yuhki N, Wang MH, Skeel A, Leonard EJ (1993) Cloning, sequencing, and expression of human macrophage stimulating protein (MSP, MST1) confirms MSP as a member of the family of kringle proteins and locates the MSP gene on chromosome 3. *J Biol Chem* 268:15461–15468
61. Liu QP, Fruit K, Ward J, Correll PH (1999) Negative regulation of macrophage activation in response to IFN-gamma and lipopolysaccharide by the STK/RON receptor tyrosine kinase. *J Immunol* 163:6606–6613
62. Cross-Disorder Group of the Psychiatric Genomics, C., Lee, S.H., Ripke, S. et al (2013) Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. *Nat Genet* 45:984–994
63. Zhuang Y, Cheng P, Weintraub H (1996) B-lymphocyte development is regulated by the combined dosage of three basic helix-loop-helix genes, E2A, E2-2, and HEB. *Mol Cell Biol* 16:2898–2905
64. Bergqvist I, Eriksson M, Saarikettu J et al (2000) The basic helix-loop-helix transcription factor E2-2 is involved in T lymphocyte development. *Eur J Immunol* 30:2857–2863
65. Cisse B, Caton ML, Lehner M et al (2008) Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development. *Cell* 135:37–48
66. Wagner J, Catto-Smith AG, Cameron DJ, Kirkwood CD (2013) *Pseudomonas* infection in children with early-onset Crohn's disease: an association with a mutation close to PSMG1. *Inflamm Bowel Dis* 19:E58–59
67. Fasth AE, Cao D, van Vollenhoven R, Trollmo C, Malmstrom V (2004) CD28nullCD4+ T cells—characterization of an effector memory T-cell population in patients with rheumatoid arthritis. *Scand J Immunol* 60:199–208
68. Markovic-Plese S, Cortese I, Wandinger KP, McFarland HF, Martin R (2001) CD4+CD28- costimulation-independent T cells in multiple sclerosis. *J Clin Invest* 108:1185–1194
69. Markle JG, Frank DN, Mortin-Toth S et al (2013) Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science* 339:1084–1088
70. Spor A, Koren O, Ley R (2011) Unravelling the effects of the environment and host genotype on the gut microbiome. *Nat Rev Microbiol* 9:279–290
71. Yuan X, Waterworth D, Perry JR et al (2008) Population-based genome-wide association studies reveal six loci influencing plasma levels of liver enzymes. *Am J Hum Genet* 83:520–528
72. Chambers JC, Zhang W, Sehmi J et al (2011) Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma. *Nat Genet* 43:1131–1138
73. Steinberg MW, Shui JW, Ware CF, Kronenberg M (2009) Regulating the mucosal immune system: the contrasting roles of LIGHT, HVEM, and their various partners. *Semin Immunopathol* 31:207–221
74. Sedy JR, Gavrieli M, Potter KG et al (2005) B and T lymphocyte attenuator regulates T cell activation through interaction with herpesvirus entry mediator. *Nat Immunol* 6:90–98
75. Smeets RL, Fleuren WW, He X et al (2012) Molecular pathway profiling of T lymphocyte signal transduction pathways; Th1 and Th2 genomic fingerprints are defined by TCR and CD28-mediated signaling. *BMC Immunol* 13:12
76. Hauser F, Deyle C, Berard D et al (2012) Macrophage-stimulating protein polymorphism rs3197999 is associated with a gain of function: implications for inflammatory bowel disease. *Genes Immun* 13:321–327
77. Bo X, Broome U, Remberger M, Sumitran-Holgersson S (2001) Tumour necrosis factor alpha impairs function of liver derived T lymphocytes and natural killer cells in patients with primary sclerosing cholangitis. *Gut* 49:131–141
78. Muto A, Ochiai K, Kimura Y et al (2010) Bach2 represses plasma cell gene regulatory network in B cells to promote antibody class switch. *EMBO J* 29:4048–4061
79. Hong SW, Kim S, Lee DK (2008) The role of Bach2 in nucleic acid-triggered antiviral innate immune responses. *Biochem Biophys Res Commun* 365:426–432
80. Dendrou CA, Plagnol V, Fung E et al (2009) Cell-specific protein phenotypes for the autoimmune locus IL2RA using a genotype-selectable human bioresource. *Nat Genet* 41:1011–1015
81. Hsu W, Zhang W, Tsuneyama K et al (2009) Differential mechanisms in the pathogenesis of autoimmune cholangitis versus inflammatory bowel disease in interleukin-2Ralpha(-/-) mice. *Hepatology* 49:133–140
82. Hanna RN, Carlin LM, Hubbeling HG et al (2011) The transcription factor NR4A1 (Nur77) controls bone marrow differentiation and the survival of Ly6C- monocytes. *Nat Immunol* 12:778–785
83. Kasler HG, Young BD, Mottet D et al (2011) Histone deacetylase 7 regulates cell survival and TCR signaling in CD4/CD8 double-positive thymocytes. *J Immunol* 186:4782–4793
84. Zhemakova A, Elbers CC, Ferwerda B et al (2010) Evolutionary and functional analysis of celiac risk loci reveals SH2B3 as a protective factor against bacterial infection. *Am J Hum Genet* 86:970–977
85. Maier LM, Hafler DA (2009) Autoimmunity risk alleles in costimulation pathways. *Immunol Rev* 229:322–336
86. Dequiedt F, Van Lint J, Lecomte E et al (2005) Phosphorylation of histone deacetylase 7 by protein kinase D mediates T cell receptor-induced Nur77 expression and apoptosis. *J Exp Med* 201:793–804
87. Clark K, MacKenzie KF, Petkevicius K et al (2012) Phosphorylation of CRTC3 by the salt-inducible kinases controls the interconversion of classically activated and regulatory macrophages. *Proc Natl Acad Sci U S A* 109:16986–16991
88. Wagner J, Sim WH, Ellis JA et al (2010) Interaction of Crohn's disease susceptibility genes in an Australian paediatric cohort. *PLoS One* 5:e15376
89. Civelek M, Lusis AJ (2014) Systems genetics approaches to understand complex traits. *Nat Rev Genet* 15:34–48