Dense genotyping of immune-related disease regions identifies nine new risk loci for primary sclerosing cholangitis

Primary sclerosing cholangitis (PSC) is a severe liver disease of unknown etiology leading to fibrotic destruction of the bile ducts and ultimately to the need for liver transplantation. We compared 3,789 PSC cases of European ancestry to 25,079 population controls across 130,422 SNPs genotyped using the Immunochip. We identified 12 genome-wide significant associations outside the human leukocyte antigen (HLA) complex, of which nine were novel, increasing the number of known PSC risk loci to 16. Despite comorbidity with inflammatory bowel disease (IBD) in 72% of the cases, six of the 12 loci showed significantly stronger association with PSC than with IBD, suggesting overlapping yet distinct genetic architectures for these two diseases. We incorporated association statistics from 7 diseases clinically occurring with PSC in the analysis and found suggestive evidence for 33 additional pleiotropic PSC risk loci. Together with network analyses, these findings add to the genetic risk map of PSC and expand on the relationship between PSC and other immune-mediated diseases.

The pathogenesis of PSC is poorly understood, and, owing to the lack of effective medical therapy, PSC remains a leading indication for liver transplantation in northern Europe and the United States, despite its relatively low prevalence (1 in 10,000). Affected individuals are diagnosed at a median age of 30–40 years and suffer from an increased frequency of IBD (60–80%) and autoimmune diseases (25%). Conversely, approximately only 5% of individuals with IBD develop PSC. Sibling relative risk of 9- to 39-fold indicates a strong genetic component to PSC risk. In addition to multiple strong associations within the HLA complex, recent association studies have identified genome-wide significant loci at 1p36 (MMEL1-TNFRSF14), 2q13 (BCL2L11), 2q37 (GPR35), 3p21 (MST1), 10p15 (IL2RA) and 18q21 (TCF4). Several theories have been proposed to explain the development of PSC. The strong HLA associations and the clinical occurrence of PSC with immune-mediated diseases suggest that autoimmunity has a role in pathogenesis. To further characterize the genetic etiology of PSC, we recruited individuals with PSC throughout Europe and North America, more than doubling the number of ascertained cases included in previous genetic studies. We genotyped 196,524 SNPs in 4,228 PSC cases and 27,077 population controls (Online Methods and Supplementary Note) using the Immunochip, a targeted genotyping array with dense marker coverage across 186 known disease loci from 12 immune-mediated diseases. Outside these 186 loci, the Immunochip also assays thousands of SNPs of intermediate significance from multiple meta-analyses of immune-mediated diseases.

Table 1 Association results of 12 non-HLA genome-wide significant risk loci for PSC

<table>
<thead>
<tr>
<th>Chr.</th>
<th>SNP a</th>
<th>Risk allele</th>
<th>RAFT cases</th>
<th>RAFT controls</th>
<th>P-value</th>
<th>OR (95% CI)</th>
<th>LD region b</th>
<th>RefSeq genes in LD region</th>
<th>Notable nearby genes c</th>
<th>Functional annotation d</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p36</td>
<td>rs3748816</td>
<td>A</td>
<td>0.698</td>
<td>0.656</td>
<td>7.41 x 10 -12</td>
<td>1.21 (1.14–1.27)</td>
<td>2.398–2.775</td>
<td>9</td>
<td>MMEL1-TNFRSF14</td>
<td>eQTL, MS, OC, PB, HM</td>
</tr>
<tr>
<td>2q33</td>
<td>rs7426056</td>
<td>C</td>
<td>0.277</td>
<td>0.229</td>
<td>1.89 x 10 -20</td>
<td>1.30 (1.23–1.37)</td>
<td>204,155–204,397</td>
<td>1</td>
<td>C02B</td>
<td>HM, OC</td>
</tr>
<tr>
<td>3p21</td>
<td>rs3197999</td>
<td>A</td>
<td>0.352</td>
<td>0.285</td>
<td>2.45 x 10 -26</td>
<td>1.33 (1.26–1.40)</td>
<td>48,388–51,358</td>
<td>89</td>
<td>MST1</td>
<td>eQTL, MS, OC, PB, HM</td>
</tr>
<tr>
<td>4q27</td>
<td>rs13140464</td>
<td>A</td>
<td>0.871</td>
<td>0.836</td>
<td>8.87 x 10 -13</td>
<td>1.30 (1.21–1.40)</td>
<td>123,204–123,784</td>
<td>4</td>
<td>IL2-IL21</td>
<td>OC, PB</td>
</tr>
<tr>
<td>6q15</td>
<td>rs56258221</td>
<td>G</td>
<td>0.213</td>
<td>0.183</td>
<td>8.36 x 10 -12</td>
<td>1.23 (1.16–1.31)</td>
<td>90,967–91,150</td>
<td>1</td>
<td>BACH2</td>
<td>OC, PB</td>
</tr>
<tr>
<td>10q15</td>
<td>rs4147359</td>
<td>A</td>
<td>0.401</td>
<td>0.349</td>
<td>8.19 x 10 -17</td>
<td>1.24 (1.18–1.30)</td>
<td>6,070–6,206</td>
<td>2</td>
<td>IL2RA</td>
<td>PB</td>
</tr>
<tr>
<td>11q23</td>
<td>rs7937682</td>
<td>G</td>
<td>0.298</td>
<td>0.265</td>
<td>3.17 x 10 -09</td>
<td>1.17 (1.11–1.24)</td>
<td>110,824–111,492</td>
<td>19</td>
<td>SI2K</td>
<td>OC, PB, HM</td>
</tr>
<tr>
<td>12q13</td>
<td>rs11168249</td>
<td>G</td>
<td>0.506</td>
<td>0.466</td>
<td>5.49 x 10 -09</td>
<td>1.15 (1.10–1.21)</td>
<td>46,442–46,534</td>
<td>3</td>
<td>HDAC7</td>
<td>OC, PB, HM</td>
</tr>
<tr>
<td>12q24</td>
<td>rs3184504</td>
<td>A</td>
<td>0.527</td>
<td>0.488</td>
<td>5.91 x 10 -11</td>
<td>1.18 (1.12–1.24)</td>
<td>110,186–111,512</td>
<td>16</td>
<td>SH2B3-AXTN2</td>
<td>MS, OC, HM</td>
</tr>
<tr>
<td>18q22</td>
<td>rs1788097</td>
<td>A</td>
<td>0.518</td>
<td>0.483</td>
<td>3.06 x 10 -08</td>
<td>1.15 (1.10–1.21)</td>
<td>65,633–65,721</td>
<td>2</td>
<td>C02B</td>
<td>MS, OC, PB, HM</td>
</tr>
<tr>
<td>19q13</td>
<td>rs60652743</td>
<td>A</td>
<td>0.864</td>
<td>0.836</td>
<td>6.51 x 10 -10</td>
<td>1.25 (1.16–1.34)</td>
<td>51,850–51,998</td>
<td>6</td>
<td>PRK2-STRN4</td>
<td>OC, PB, HM</td>
</tr>
<tr>
<td>21q22</td>
<td>rs2836883</td>
<td>G</td>
<td>0.777</td>
<td>0.728</td>
<td>3.19 x 10 -17</td>
<td>1.28 (1.21–1.36)</td>
<td>39,374–39,404</td>
<td>1</td>
<td>PSMG1</td>
<td>OC, PB, HM</td>
</tr>
</tbody>
</table>

Chr., chromosome; RAFT, risk allele frequency; OR, odds ratio; CI, confidence interval; HM, overlaps a region of histone modification; MS, missense mutation; OC, overlaps a known region of open chromatin; PB, overlaps a region of protein binding. SNPs from new PSC-associated loci are shown in bold. LD regions around lead SNPs were calculated by extending a distance of 0.1 cM in both directions as defined by the HapMap recombination map. Candidate genes within the same LD region as the associated SNPs. "Denotes whether there are SNPs with r2 > 0.8 with the top SNP that have functional annotations (Supplementary Tables 4–7).
After quality control (Online Methods), 130,422 SNPs from 3,789 PSC cases and 25,079 population controls were available for analysis (Supplementary Figs. 1 and 2 and Supplementary Tables 1 and 2). We imputed a further 80,183 SNPs located in the Immunochip fine-mapping regions using the 1000 Genomes Project reference panel (Online Methods). We performed case-control association tests using a linear mixed model as implemented in MMM (Online Methods). We identified 12 non-HLA genome-wide significant ($P < 5 \times 10^{-8}$) susceptibility loci (Table 1), 9 of which were new (Fig. 1). The most associated SNP within each locus was a common variant (all risk allele frequencies > 0.18) of moderate effect (odds ratios (ORs) between 1.15 and 1.33) (Table 1). Genotype imputation and stepwise conditional regression analyses within each locus did not identify additional independent genome-wide significant signals, nor did genotype-genotype or sex-genotype interaction analyses (Online Methods).

For seven of the nine new loci, the most significantly associated SNP in the locus was the same SNP or was in strong linkage...
dis-equilibrium (LD; $r^2 > 0.8$) with the original association reported for another disease (Supplementary Table 3). The two exceptions were 1q123, where only independent disease associations ($r^2 < 0.01$) have so far been reported, and 6q15, where the most significantly associated PSC variant, rs56258221 (OR = 1.23; $P = 8.36 \times 10^{-12}$), was in low-to-moderate LD with the previously reported BACH2 variants in Crohn’s disease ($r^2 = 0.23$) and type 1 diabetes ($r^2 = 0.12$). Three out of four known non-HLA PSC risk loci present on the ImmunoChip passed genotyping quality control and were confirmed in our analysis (1p36, 3p21 and 10p15; Supplementary Fig. 3 and Supplementary Note).

To prioritize candidate genes within the non-HLA genome-wide significant loci, we searched for the functional consequences of the most associated SNPs or the SNPs in high LD with these ($r^2 > 0.8$), focusing on missense SNPs (eQTLs) (Supplementary Fig. 4 and Supplementary Table 4) and expression quantitative trait loci (eQTLs) (Supplementary Table 5), and we functionally annotated risk loci using data from the Encyclopedia of DNA Elements (ENCODE) Project (Supplementary Table 6 and Supplementary Note)18. We also constructed networks on the basis of functional similarity measures (Online Methods and Supplementary Fig. 5), known protein-protein interactions (DAPPLE19, Supplementary Table 7 and Supplementary Note) and the published literature (GRAIL20, Supplementary Fig. 6 and Supplementary Note) to identify disease-relevant genes. For 6 of the 12 genome-wide significant loci, the same gene (MMEL1, CD28, MST1, SH2B3, CD226 and SIK2) was annotated by more than 1 method (Supplementary Table 7), suggesting these as candidates for further investigation at these loci.

Two newly associated loci are located outside of the ImmunoChip fine-mapping regions (Fig. 1d,e). At 1q23, the most strongly associated SNP, rs7937682 (OR = 1.17; $P = 3.17 \times 10^{-7}$), is located in an intron of SIK2 (encoding salt-inducible kinase 2), which influences the expression of both interleukin (IL)-10 in macrophages and Nur77, an important transcription factor in leukocytes21. The association at 1q123 was with an intronic SNP, rs111668249 (OR = 1.15; $P = 5.49 \times 10^{-9}$), within the HDAC7 gene (encoding histone deacetylase 7), which has also been associated with IBD22. HDAC7 has been implicated in the negative selection of T cells in the thymus23, a key process in the development of immune tolerance. A role for HDAC7 in PSC etiology is supported by the new association at 19q13, where the most associated SNP, rs60652743 (OR = 1.25; $P = 6.51 \times 10^{-10}$), is located within an intron of PRKD2 (encoding serine-threonine protein kinase D2). When T cell receptors of thymocytes are engaged, PRKD2 phosphorylates HDAC7, leading to nuclear exclusion of HDAC7 and loss of its gene regulatory functions, ultimately resulting in apoptosis and negative selection of immature T cells24,25. Notably, this negative selection takes place owing to a loss of HDAC7-mediated repression of Nur77 (regulated by SIK2)26, linking three new PSC loci to this pathway.

The associations at the HLA complex at 6p21 were refined by imputing alleles at HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1, HLA-DQA1 and HLA-DPB1 (Supplementary Note)27. The top associated SNP (rs4143332) was in almost perfect LD ($r^2 = 0.996$) with HLA-B*08:01 (Supplementary Note). In stepwise conditional analysis including both SNP and HLA allele genotypes, rs4143332 (tagging HLA-B*08:01) and a complex HLA class II association signal determined by HLA-DQA1*01:03 and SNPs rs532098, rs1794282 and rs9263964 (Supplementary Fig. 7) explained most of the association signal in PSC. When performing stepwise regression of the HLA alleles only, the class II associations were consistent with previous reports, apart from a new association with HLA-DQA1*01:01 (Supplementary Tables 8–10 and Supplementary Note)28,29. The HLA-DRB1*15:01 association overlapped with the allele for ulcerative colitis (risk increasing) and Crohn’s disease (risk decreasing)30,31. Because imputed genotypes in the class II region were only available for 4 (HLA-DRB1, HLA-DQB1, HLA-DQA1 and HLA-DPB1) out of 20 loci32, further studies involving direct sequencing of all HLA class II loci along with assessments of protein structure and peptide binding are required to causally resolve the link between this HLA subregion and PSC development33,34.

Although 72% of the PSC cases in this study have a diagnosis of concomitant IBD (Supplementary Table 11), only half of our genome-wide significant loci were associated with IBD in the recent International IBD Genetics Consortium (IIBDGC) ImmunoChip analysis (Fig. 2a, Supplementary Fig. 8 and Supplementary Table 3)22, despite the greater sample size of that study (25,683 cases and 15,977 controls). Across the 12 non-HLA PSC-associated loci, we observed greater similarity between the OR estimates for PSC and ulcerative colitis than for those of PSC and Crohn’s disease. We used the Crohn’s disease and ulcerative colitis OR estimates for the 163 IBD-associated n
### Supplementary Figs. 10–12

SNPs in red represent genome-wide significant findings from the main association analysis (Table 1), and SNPs in black are significantly associated with PSC conditional on their pleiotropic effects across the related immune-mediated diseases. The horizontal red line represents a threshold of FDR < 0.001 (Supplementary Table 13), and the blue horizontal line represents a threshold of FDR < 0.01 (see Supplementary Table 14 for a full listing of identified loci).

loci to predict PSC case-control status in our sample (Online Methods) and found a significantly greater area under the receiver operating characteristic (ROC) curve (AUC) when applying ulcerative colitis ORs compared to Crohn’s disease ORs (ulcerative colitis AUC = 0.62, Crohn’s disease AUC = 0.56; $P = 1.2 \times 10^{-5}$; Fig. 2b). This suggests that PSC is genetically more similar to ulcerative colitis than to Crohn’s disease and is consistent with clinical observations of greater comorbidity of PSC with ulcerative colitis than with Crohn’s disease. To further compare the genetic profiles of PSC and IBD, we combined our genome-wide significant PSC-associated loci with the 163 confirmed IBD-associated loci in a functional similarity network (Supplementary Fig. 9 and Supplementary Table 12). We found that the PSC loci were distributed throughout the IBD loci (Supplementary Fig. 9), suggesting that there is no particular functional subcluster of IBD susceptibility genes associated with PSC and vice versa.

Although we consider only those loci reaching a stringent significance threshold ($P < 5 \times 10^{-8}$) to be conclusively associated with PSC, it is likely that additional true associations lie among SNPs with weaker associations. An alternative approach for controlling for multiple hypothesis testing is false discovery rate (FDR) control, which regulates the expected proportion of incorrectly rejected null hypotheses. FDR control is well suited to focused genotyping platforms such as the MetaboChip and the Immunochip because it implicitly accounts for the expected enrichment in association. To further increase this enrichment, we exploited the known pleiotropy between related immune-mediated traits and calculated the FDR for association with PSC conditional on previously published summary statistics from each of the related phenotypes (yielding a per-SNP conditional FDR) (Online Methods). We identified 33 non-HLA loci with conditional FDR < 0.001 in this analysis (Fig. 3), all of which showed suggestive levels of significance ($5 \times 10^{-6} < P < 5 \times 10^{-5}$) in the standard association analysis (Supplementary Figs. 10–12 and Supplementary Tables 13 and 14). These loci were integrated into the functional similarity network analysis (Supplementary Fig. 13 and Supplementary Table 15), highlighting potential candidate susceptibility genes.

In conclusion, the present study increases the number of genome-wide significant loci in PSC from 7 to 16 (including the HLA complex). The 9 new variants together explain 0.9% of variance in PSC liability, increasing the total amount of variance explained by the 16 known loci to 7.3% (Online Methods). The data convincingly show that genetic susceptibility to PSC extends considerably beyond risk factors involved in the closely related IBD phenotype and into autoimmune pathophysiology. Furthermore, analysis of pleiotropic immune-related genetic variants highlights 33 additional suggestive loci in PSC, overall representing major new avenues for research into pathogenesis.


**METHODS**

Methods and any associated references are available in the online version of the paper.

**Note:** Supplementary information is available in the online version of the paper.

**ACKNOWLEDGMENTS**

We thank all individuals with PSC and healthy controls for their participation, and we are indebted to all physicians and nursing staff who recruited subjects. We thank T. Wesse, T. Henke, S. Sedghpour Sabet, R. Vogler, G. Jacobs, I. Urbach, W. Abelrecht, V. Polkonen, V. Barbu, K. Holm, H. Dahlen Sollid, B. Woldseth, I.A. Anmarkrud and L.W. Torbjørnsen for expert help. U. Beuers, F. Braun, E. Kreisel, T. Berg and R. Günther are acknowledged for contributing German individuals with PSC. B.A. Lie and The Norwegian Bone Marrow Donor Registry at Oslo University Hospital, Rikshospitalet (Oslo, Norway) and the Nord-Trøndelag Health Study (HUNT) are acknowledged for sharing healthy Norwegian controls. Banco Nacional de ADN (Salamanca, Spain) is acknowledged for providing Spanish control samples. This study makes use of genotyping data generated by the Dietary, Life style and Genetic determinants of Obesity and Metabolic syndrome (DILGOM) consortium (see URLs), the Cooperative Research in the Region of Augsburg (KORA) study and the Heinz Nixdorf Recall (Risk Factors, Evaluation of Coronary Calciumification, and Lifestyle) study. We acknowledge the members of the International PSC Study Group, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Inflammatory Bowel Disease Genetics Consortium (IBDGC), the UK-PSC Consortium and the Alberta IBD Consortium for their participation. J. Barrett is acknowledged for contributions to the design of the
Immunochip experiment. Individuals who have shared summary statistics and statistical software are acknowledged in the Supplementary Note.

The study was supported by The Norwegian PSC Research Center (see URLs), by the German Ministry of Education and Research through the National Genome Research Network (01GS0809–GP7), by the Deutsche Forschungsgemeinschaft (FR 2821/2-1), by the EU Seventh Framework Programme FP7-2007-2013 (262055) ESGI, by the Integrated Research and Treatment Center–Transplantation (01EO0802) and by the PopGen Biobank (see URLs). J.Z.L., T.S. and C.A.A. are supported by a grant from the Wellcome Trust (098051). Additional financial support for the study and the coauthors is listed in the Supplementary Note.

AUTHOR CONTRIBUTIONS

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

Reprints and permissions information is available online at [www.nature.com/reprints/index.html](http://www.nature.com/reprints/index.html).

7. Jimmilt L. Liu1,2, Johannes Rokshund Hov1,2,5,6, Trine Folseraa2,4,6, Eva Ellingshaus6,8, Simon M Rushbrook2, Nadezhda Toncheva4, Ole A Andreassen4,5, Rinse K Weersma10, Tobias J Weismüller11,12,8, Bertus Eekste13, Pietro Invernizzi14, Gideon M Hirschfield15,16, Daniel Nils Gotthardt17, Albert Pares18, David Ellingshaus6, Tejas Shah1, Brian D Junar19, Piotr Milkiewicz20, Christian Rust21, Christoph Schramm22, Tobias Müller23, Bijesh Srivastava24, Georgios Dalekos25,26, Markus M Nöthen27,28, Stefan Herrm27,28, Juliane Winkelmann29–31, Mitja Mitrovic32, Felix Braun33, Cyriel Y Ponsioen34, Peter J P Croucher35, Martina Sternek36, Andreas Teufe37, Andrew L Mason38, Janna Saarela39, Virpi Leppa40, Ruslan Dorfman41, Domenico Alvaro42, Annarosa Floreni43, Suna Onengut-Gumuscu44,45, Stephen R Rich46,47, Wesley K Thompson48,

1Welcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK. 2Norwegian PSC Research Center, Department of Transplantation Medicine, Division of Cancer Medicine, Surgery and Transplantation, Oslo University Hospital, Rikshospitalet, Oslo, Norway. 3K.G. Jebsen Inflammation Research Centre, Research Institute of Internal Medicine, Oslo University Hospital, Rikshospitalet, Oslo, Norway. 4Institute of Clinical Medicine, University of Oslo, Oslo, Norway. 5Section of Gastroenterology, Department of Transplantation Medicine, Division of Cancer Medicine, Surgery and Transplantation, Oslo University Hospital, Rikshospitalet, Oslo, Norway. 6Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, Kiel, Germany. 7Department of Gastroenterology and Hepatology, Norfolk and Norwich, University Hospitals National Health Service (NHS) Trust, Norwich, UK. 8Max Planck Institute for Informatics, Saarbrücken, Germany. 9K.G. Jebsen Centre for Psychosis Research, Division of Mental Health and Addiction, Oslo University Hospital, Ulleval, Oslo, Norway. 10Department of Gastroenterology and Hepatology, Universitair Medisch Centrum (UMC) Utrecht, The Netherlands. 11Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany. 12Integrated Research and Treatment Center–Transplantation (IFB-bx), Hannover Medical School, Hannover, Germany. 13Snyder Institute of Chronic Diseases, Department of Medicine, University of Calgary, Calgary, Alberta, Canada. 14Center for Autoimmune Liver Diseases, Humanitas Clinical and Research Center, Rozzano, Italy. 15Division of Gastroenterology, Department of Medicine, University of Toronto, Toronto, Ontario, Canada. 16Centre for Liver Research, National Institute for Health Research (NIHR) Biomedical Research Unit, Birmingham, UK. 17Department of Medicine, University Hospital of Heidelberg, Heidelberg, Germany. 18Liver Unit, Hospital Clinic, Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Centro de Investigación Biomédica en Red en el Área Temática de Enfermedades Hepáticas y Digestivas (CIBERehd), University of Barcelona, Barcelona, Spain. 19The International IB Study Group, Centre for Basic Research in Digestive Diseases, Division of Gastroenterology and Hepatology, Mayo Clinic, College of Medicine, Rochester, Minnesota, USA. 20Liver Unit and Liver Research Laboratories, Pomeranian Medical University, Szczecin, Poland. 21Department of Medicine 2, Grosshadern, University of Munich, Munich, Germany. 221st Department of Medicine, Christian Albrechts University of Kiel, Kiel, Germany. 23Department of Gastroenterology and Hepatology, Norwegian PSC Research Center, Department of Transplantation Medicine, Sapienza University of Rome, Rome, Italy. 24Department of Surgical, Oncological and Gastroenterological Sciences, University of Padova, Padova, Italy. 25Institute of Human Genetics, Charité Universitätsmedizin Berlin, Berlin, Germany. 26Institute of Medical Biometry and Statistics, University of Lübeck, Lübeck, Germany. 27Division of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany. 28Department of Medical Genetics, Technische Universität München, Munich, Germany. 29Institute of Human Genetics, Helmholtz Zentrum München–German Research Center for Environmental Health, Neuherberg, Germany. 30Department of Genetics, University of Groningen and University Medical Centre Groningen, the Netherlands. 31Department of General, Visceral, Thoracic, Transplantation, and Pediatric Surgery, University Medical Centre Schleswig-Holstein, Campus Kiel, Kiel, Germany. 32Department of Gastroenterology and Hepatology, Academic Medical Center, Amsterdam, the Netherlands. 33Department of Environmental Science, Policy, and Management, University of California, Berkeley, Berkeley, California, USA. 34Department of Hepatobiliary Surgery and Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. 351st Department of Medicine, University of Mainz, Mainz, Germany. 36Division of Gastroenterology and Hepatology, University of Alberta, Edmonton, Alberta, Canada. 37Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland. 38Public Health Genomics Unit, FIMM, University of Helsinki and National Institute for Health and Welfare, Helsinki, Finland. 39Department of Genetics, Institute for Medical Biometry and Statistics, University of Tübingen, Tübingen, Germany. 40Division of Gastroenterology, National Institute for Health and Welfare, Helsinki, Finland. 41Division of Gastroenterology, Hepatology and Endocrinology, University of Cambridge, Cambridge, UK. 42Department of Medical Genetics, Northwell Health, Manhasset, New York, USA. 43Department of General, Visceral, Thoracic, Transplantation, and Pediatric Surgery, University Medical Center Schleswig-Holstein, Campus Kiel, Kiel, Germany. 44Department of Gastroenterology and Hepatology, Academic Medical Center, Amsterdam, the Netherlands. 45Department of Medicine, University of Mainz, Mainz, Germany. 46Division of Gastroenterology and Hepatology, University of Michigan, Ann Arbor, Michigan, USA. 47Department of Medical Genetics, University of Bonn, Bonn, Germany. 48Department of Internal Medicine, University of Virginia, Charlottesville, Virginia, USA. 49Division of Endocrinology & Metabolism, University of Virginia, Charlottesville, Virginia, USA. 50Department of Internal Medicine, University of Virginia, Charlottesville, Virginia, USA. 51Department of Public Health, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway. 52Department of Clinical and Experimental Medicine, KU Leuven, Leuven, Belgium. 53Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK. 54Division of Gastroenterology and Hepatology, Department of Internal Medicine, Landskommers Hospital University, Reykjavik, Iceland. 55Physiology and Experimental Medicine, Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada. 56Epigen, Campus Akershus University Hospital (AHUS), Akershus University Hospital, Nordbyhagen, Norway. 57Inflammatory Bowel Disease (IBD) Group, Zane Cohen Centre for Digestive Diseases, Mount Sinai Hospital, Toronto, Ontario, Canada. 58Department of Gastroenterology, Hepatology, and Nutrition, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA. 59Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA. 60Rheumatology Unit, Department of Medicine, Karolinska Institutet and Karolinska University Hospital Solna, Stockholm, Sweden. 61Department of Medicine II, University Hospital Munich-Grosshadern, Ludwig-Maximilians-University Munich, Germany. 62Division of Digestive Diseases, Centro Médico Teknon, Barcelona, Spain. 63Division of Gastroenterology, Istituto di Ricovero e Cura a Carattere Scientifico, Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, Italy. 64Unit of Gastroenterology SOD2, Azienda Ospedaliero Universitaria Careggi, Florence, Italy. 65Department of Gastroenterology and Hepatology, Digestive Disease Institute, Cleveland Clinic, Cleveland, Ohio, USA. 66Department of Pathology, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio, USA. 67Department of Internal Medicine, Institute of Medicine, Sahlgrenska Academy and University Hospital, Gothenburg, Sweden. 68Assistance Publique–Hôpitaux de Paris (AP-HP), Hôpital Saint Antoine, Department of Hepatology, Assistance Publique–Hôpitaux de Paris (AP-HP), Hôpital Saint Antoine, Department of Hepatology, University Pierre et Marie Curie (UPMC) Université de Paris 6, Paris, France. 69Division of Gastroenterology and Hepatology, University of California, Davis, Davis, California, USA. 70Department of Gastroenterology, University Hospitals Leuven, Leuven, Belgium. 71Department of Bioinformatics, Institute of Biometrics and Medical Informatics, University Medicine Greifswald, Greifswald, Germany. 72Further details appear in the Supplementary Note. 73Université de Montréal, Research Center, Montreal, Quebec, Canada. 74Montreal Heart Institute, Research Center, Montreal, Quebec, Canada. 75Department of Hepatology, Department of Medicine, University of Cambridge, Cambridge, UK. 76Department of Gastroenterology and Hepatology, Karolinska University Hospital Huddinge, Karolinska Institutet, Stockholm, Sweden. 77Section of Digestive Diseases, Department of Medicine, Yale University, New Haven, Connecticut, USA. 78Department of General Internal Medicine, University at Buffalo, Buffalo, New York. 79Department of Gastroenterology, Department of Medicine, Helsinki University Hospital, Helsinki, Finland. 80Division of Gastroenterology, Department of Medicine, Hennepin County Medical Center, Minneapolis, Minnesota, USA. 81Department of Radiology, University of California, San Diego, La Jolla, California, USA. 82Department of Neuroscience, University of California, San Diego, La Jolla, California, USA. 83Department of Hepatology, John Radcliffe University Hospitals NHS Trust, Oxford, UK. 84Division of Gastroenterology, Department of Clinical Medicine, University of Bergen, Bergen, Norway. 85Present address: Department of Internal Medicine 1, University Hospital of Bonn, Bonn, Germany. 86These authors contributed equally to this work. 87These authors jointly directed this work. Correspondence should be addressed to T.H.K. (t.h.karslen@medisin.uio.no) or C.A.A. (c.a.anderson@sanger.ac.uk).
ONLINE METHODS

Study subjects. The study participants are described in Supplementary Table 16 and the Supplementary Note.

Ethical approval. Subject recruitment was approved by the ethics committees or institutional review boards of all participating centers. Written informed consent was obtained from all participants.

Quality control. SNPs with call rate < 80% were removed before commencing sample quality control (n = 235). Per-individual genotype call rate and heterozygosity rate were calculated using PLINK42, and outlying samples were identified using Aberrant44, which automatically identifies outliers in otherwise Gaussian distributions (Supplementary Fig. 1). A set of 20,837 LD-pruned (r² < 0.1) SNPs with MAF > 10% present on both the Immunochip and the Illumina HumanOmni2.5-8 array used in the 1000 Genomes Project (see URLs) were used to estimate identity by descent and ancestry. For each pair of individuals with estimated identity by descent 20.9, the sample with the lower call rate was removed (unless case-control status was discordant between the pair, in which case, both samples were removed, n = 92). Related individuals (0.1875 < identity by descent <0.9) remained in the analysis to maximize power because mixed model association analysis can correctly account for relatedness. Principal-components analysis, implemented in SMARTPCA (Eigenstrat)44, was used to identify samples of non-European ancestry. Principal components were defined using population samples from the 1000 Genomes Project45 genotyped using the Illumina HumanOmni2.5-8 genotyping array (see URLs) and then projected into cases and controls (Supplementary Fig. 2)14,22,46. After sample quality control, 3,789 PSC cases and 25,079 controls remained. SNPs with MAF of less than 0.1%, Hardy-Weinberg equilibrium P<1 × 10⁻⁵ or call rate lower than 98% and those that failed the PLINK v1.07 non-random differential missing data rate test between cases and controls (P<1 × 10⁻⁵) were excluded. After the completion of marker quality control (Supplementary Table 2), 131,220 SNPs were available for analysis, further reduced to 130,422 after cluster plot inspection.

Statistical methods. Genomic inflation factor. The Immunochip contains 3,120 SNPs that were part of a bipolar disease replication effort and other studies that were not immune related. After quality control, 2,544 of these were used as null markers to estimate the overall inflation of the distribution of association test statistics.

Imputation. Using 85,747 SNPs after quality control located in the Immunochip fine-mapping regions, additional genotypes were imputed using IMPUTE2 with the 1000 Genomes Project Phase 1 (March 2012) reference panel of 1,092 individuals47 and 744,740 SNPs. Imputation was performed separately in ten batches, with the case:control and country of origin ratios constant across batches. SNPs with a posterior probability less than 0.9 and those with differential missingness (P<1 × 10⁻⁵) in the ten batches were removed, as were SNPs that did not pass the exclusion thresholds used for genotyped SNP quality control. After imputation, a total of 163,379 SNPs in the Immunochip fine-mapping regions, including 153,857 SNPs from the reference panel, were available for analysis.

Association analysis. Case-control association tests were performed using a linear mixed model as implemented in MIMM15. A covariance matrix, R, of a random-effects component was included in the model to explicitly account for confounding due to population stratification and cryptic relatedness between individuals. This method has been shown to better control for population stratification than correction for principal components or meta-analyses of matched subsets of cases and controls48-50. R is a symmetric n × n matrix with each entry representing the relative sharing of alleles between two individuals compared to the average in the sample, and it is typically estimated using genome-wide SNP data15. To avoid biases in the estimation of R due to the design of the Immunochip, SNPs were first pruned for LD (r² < 0.1). Of the remaining SNPs, we then removed those that were in the HLA region or had MAF < 10%. Finally, we excluded SNPs that showed modest association (P<0.005) with PSC in a linear regression model fitting the first ten principal components as covariates. A total of 17,260 SNPs were used to estimate R.

Owing to computational limitations, we estimated the R matrix and performed all association analyses applying R separately for UK (n = 9,696) and non-UK (n = 19,172) samples and then combined the results using a fixed-effects (inverse variance-weighted) meta-analysis, as carried out previously46. This reduced the AUC inflammation factor, estimated using the 2,544 null SNPs, from 1.24 to 1.02 (Supplementary Fig. 14), showing excellent control for population stratification. Stepwise conditional regression was used to identify possible independent associations at genome-wide significant loci. SNP × SNP interactions between all pairs of genome-wide significant SNPs were tested using the PLINK epistasis command. Signal intensity plots of all non-HLA loci with association P value < 5 × 10⁻⁶ were visually inspected using Evoker51, SNPs that clustered poorly were removed (n = 798).

Prediction of PSC using IBD SNPs. OR estimates for Crohn’s disease and ulcerative colitis in 163 IBD-associated SNPs were obtained from Jostins et al.22. We used the R package Mangrove (see URLs) to estimate each individual’s probability of developing PSC in our 3,789 PSC cases and 25,079 controls, assuming additive risk (log-additive OR). The performance of our predictor using either Crohn’s disease or ulcerative colitis ORs was assessed by constructing a ROC curve, showing the proportion of true and false positives at each probability threshold. The AUC was calculated to compare the predictive powers of the ulcerative colitis and Crohn’s disease ORs. The DeLong method was used to test whether the AUC was significantly greater using ulcerative colitis ORs compared to Crohn’s disease ORs52.

Functional similarity networks. In functional similarity networks, each edge represents the strong functional similarity of two genes based on annotated Gene Ontology (GO) terms as determined by the functional similarity measure rfunSim53. rfunSim similarity values above the recommended cutoff of 0.8 were retrieved using the FunSimMat web service44. Resulting networks were visualized and analyzed using Cytoscape55.

To construct PSC-specific networks from functional similarity networks that contained more than one gene per locus (Supplementary Figs. 5 and 13), the connectivity of each gene was assessed by computing different topology measures for the corresponding node: (i) degree (number of direct edges to other nodes), (ii) shortest path closeness (inverted average shortest path distance to other nodes) and (iii) shortest path betweenness (fraction of shortest paths passing through the node). Similarity edges between genes in the same locus and genes nodes that were not contained in the resulting largest connected subnetworks were ignored. Genes were first ranked according to each measure and were then assigned the best of the three ranks. The PSC-specific network was generated from the top ranked genes in their respective loci.

Pleiotropy analysis. We included summary statistics from GWAS of seven PSC-associated diseases (Crohn’s disease, celiac disease, psoriasis, rheumatoid arthritis, sarcoidosis, type 1 diabetes and ulcerative colitis; Supplementary Table 17). For all diseases, we constructed conditional stratified quantile-quantile plots of the empirical quantiles of nominal −log₁₀(P values) for SNP association with PSC for all SNPs (Supplementary Fig. 10) and for different overlapping subsets of SNPs determined by the significance of their association with the PSC-associated autoimmune disorder (SNP subsets defined by P < 1, P <0.1, P <0.01 and P <0.001 in the pleiotropic phenotype). For a given PSC-associated phenotype, enrichment for pleiotropic signals in PSC can be observed as an increasing leftward deflection from the expected null distribution with lower P-value thresholds in the second phenotype (Supplementary Note). Enrichment in the stratified quantile-quantile plots is directly interpretable in terms of the true discovery rate (TDR), equivalent to 1−FDR56. Specifically, it can be shown that a conservative estimate of FDR can be calculated from the horizontal shift of the quantile-quantile curve from the expected line y = x, with a larger shift corresponding to a smaller FDR for a given nominal P value (Supplementary Note). We calculated the conditional TDR as a function of P value in PSC across a series of P-value thresholds in the pleiotropic trait (Supplementary Fig. 10).

To assess the significance of the association with PSC, we assigned a pleiotropic (conditional) FDR value for PSC for each SNP. The pleiotropic FDR value for each SNP was based on the P value of the SNP in PSC relative to the P-value distribution of other SNPs in the same conditioning subset, where
subsets were defined by the pleiotropic association (lowest \( P \) value among associated diseases) of the SNP. Notably, the conditioning procedure was blind to the \( P \) value of the SNP with respect to PSC. The pleiotropic FDR was then interpolated from conditional FDR curves using established stratified FDR methods\(^{41,57} \) (Supplementary Note). The increase in power from using pleiotropic FDR is demonstrated by dividing the total sample in half and observing that empirical replication rates between the training and test halves increase with decreasing \( P \) value in the pleiotropic disease (Supplementary Fig. 15). The SNP with the lowest FDR within each LD block (as defined by 1000 Genomes Project data) was considered the lead SNP of a new pleiotropic PSC locus, if below a threshold of 0.001 (loci defined by FDR < 0.001 and FDR < 0.01 are shown in Supplementary Tables 13 and 14). All test statistics were adjusted for population stratification by genomic control (Supplementary Fig. 16 and Supplementary Note).

**Variance explained and heritability.** The proportion of variance explained by the genome-wide significant loci and HLA alleles was calculated using a liability threshold model\(^{46} \) assuming a disease prevalence of 10 in 100,000 and multiplicative risk.