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Dissecting the genetic basis and mechanisms underlying the associations between multiple extrahepatic factors and autoimmune liver diseases

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ABSTRACT

Background: Autoimmune liver diseases (AILDs) encompass autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), and primary sclerosing cholangitis (PSC). The onset of these diseases is fundamentally influenced by genetic susceptibility. Although various extrahepatic factors are potentially linked to AILDs, the genetic underpinnings and mechanisms of these associations remain unclear.

Methods: Utilizing large-scale genome-wide association study (GWAS) data, this study systematically investigated the relationships between extrahepatic autoimmune diseases (EHAIDs), immune cells, and various triggering factors with AILDs. Mendelian randomization (MR) was employed to assess the causal effects of these extrahepatic factors on AILDs, complemented by linkage disequilibrium score (LDSC) regression to uncover shared genetic architecture and causal effects underlying the associations between autoimmune diseases. We employed colocalization, enrichment analysis, and protein-protein interaction (PPI) network to identify the functions of shared loci. Additionally, we proposed that activated immune cells in the circulation may contribute to liver and biliary tract inflammation via migration, mediating the impact of extrahepatic factors on AILDs. This hypothesis was tested using two mediation analysis methods: two-step MR (TSMR) and multivariable MR (MVMR).

Results: Causal associations between multiple extrahepatic factors and AILDs were identified. Notably, $CD27^{+}$ B cells were found to be a risk factor for PBC, while PSC progression was associated with $CD28^{+}$ $CD8^{+}$ T cells exhaustion and increased levels of $CD28^{-}$ $CD8^{+}$ T cells. Mediation analyses revealed 64 pathways via TSMR and 15 pathways via MVMR, indicating that the effects of extrahepatic factors on AILDs may be mediated by circulating immune cells. The shared genetic architecture also contributed to these associations. Analysis of shared loci and gene functions identified ATXN2 as being shared between PBC and 9 EHAIDs, while SH2B3 and PSMG1 were shared with 6 and 5 EHAIDs, respectively, in PSC.

Conclusions: Our research compared three distinct AILDs, enhancing the understanding of their etiology and providing new evidence on risk factors, diagnostic markers, and potential therapeutic targets.

1. Introduction

Autoimmune liver diseases (AILDs) are a group of immune-mediated chronic inflammatory liver conditions that include three main types: autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), and primary sclerosing cholangitis (PSC). Although AILDs have a relatively low incidence, uncontrolled inflammation often progresses to end-stage liver disease, necessitating liver transplantation and imposing a significant clinical burden [1]. The immune injury mechanisms and pathological features of these three AILDs differ. AIH primarily presents with interface hepatitis histologically [2], while PBC and PSC primarily affect bile duct cells. PBC is characterized by chronic granulomatous

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lymphocytic cholangitis centered around the intrahepatic small bile ducts [3]. PSC involves both intra- and/or extrahepatic bile ducts, with bile duct damage and fibrosis leading to multifocal bile duct stricturing, cholestasis, cirrhosis, and progressive liver dysfunction [4].

The etiology of AILDs remains unclear. Genetic susceptibility provides a foundation for the disease, but factors such as infections, the commensal microbiome, drugs, smoking, and alcohol consumption also play crucial triggering roles [5–7]. Approximately one-third of AILD patients have extrahepatic autoimmune diseases (EHAIDs), suggesting a shared genetic background may underlie this phenomenon. For instance, genome-wide association studies (GWAS) have demonstrated that about half of the susceptibility genes for PSC overlap with the genetic structures of other autoimmune diseases, including type 1 diabetes, rheumatoid arthritis, and multiple sclerosis [8]. Additionally, around 70 % of PSC patients have inflammatory bowel disease (IBD). Some researchers propose that PSC could be an extraintestinal manifestation of IBD, suggesting that they may be considered as a single disease entity. However, clinical observations indicate that PSC-related IBD presents differently from ulcerative colitis and Crohn's disease. Furthermore, the two conditions can influence each other through the gut-liver axis, including gut microbiota and bile acid metabolism [9]. Therefore, it remains unclear whether the co-occurrence of these diseases is due to a shared genetic architecture or a causal relationship.

AILDs are characterized by lymphocyte-mediated immune dysregulation, loss of tolerance, and cell destruction, resulting in chronic liver inflammation driven by the accumulation of various immune cells [10]. Activated immune cells in circulation may induce liver and biliary inflammation through migration, potentially mediating the impact of extrahepatic factors on AILDs. This hypothesis provides an intriguing perspective for understanding the pathogenesis of AILDs. Jang et al. used mass cytometry to report abnormalities in the abundance of specific immune cell subsets in the peripheral blood of PBC patients [11]. However, while circulating immune cells reflect the immune status of the liver, they may also be related to other organs, complicating observational studies with confounding factors. Additionally, reverse causality poses a challenge. Abnormal levels of circulating immune cells in AILD patients do not necessarily indicate whether these cells are risk/protective factors for AILDs, biomarkers for disease progression, or compensatory mechanisms. Observational studies can identify associations, but determining causality remains difficult. Although prospective randomized controlled trials (RCTs) are the gold standard for causal inference, the low incidence of AILDs in the general population makes large-scale epidemiological surveys costly.

Genetic evidence can offer valuable insights. Researchers have developed the linkage disequilibrium score (LDSC) regression method to identify genetic correlations between complex traits and diseases [12], which is widely used to assess the impact of genetic overlap. Mendelian randomization (MR) can help investigate causal relationships within these associations. MR infers causality by using appropriate genetic variants as instrumental variables (IVs). According to Mendelian inheritance laws, the random allocation of genetic factors during gamete formation ensures that the separation and combination of genetic variants controlling different traits occur independently before disease onset, effectively simulating the RCT process and minimizing confounding factors and reverse causality [13]. Using publicly available GWAS summary data, this study includes 14 EHAIDs, 731 circulating immune cell traits, and various suspected triggering factors (including 23 drug uses, 4 smoking behaviors, 1 drinking behavior, 211 gut microbiota, and 19 special pathogen infections). MR was employed to evaluate causal associations between these traits and the three AILDs, while LDSC was used to assess genetic correlations between EHAIDs and AILDs. Subsequent colocalization analysis identified pleiotropic loci shared with AILDs [14]. The functions of these shared loci were further elucidated through Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analyses, as well as protein-protein interaction (PPI) network analysis. Finally, we hypothesize that circulating immune cells mediate the effects of multiple EHAIDs and triggering factors on AILDs, and use mediation analysis to explore the role of immune cells in this process. The analysis workflow for this study is illustrated in Fig. 1.

2. Materials and methods

2.1. Data source

The GWAS summary data utilized in this study were all derived from populations of European ancestry. Detailed information regarding sample sizes, publication institutions and years, and data download websites is provided in Supplementary Table S1. The data for AIH (N_{case} = 821, $N_{control}$ = 484,413) were obtained from a GWAS meta-analysis including cases from the UK Biobank and the FinnGen Consortium [15]. For PBC (N_{case} = 8,021, $N_{control}$ = 16,489), the data were derived from a GWAS encompassing five cohorts of European ancestry [16]. PSC data (N_{case} = 2,871, $N_{control}$ = 12,019) were sourced from the International PSC Study Group (IPSCSG) [17]. This study incorporated a total of 731 circulating immune cell traits, obtained from an independent GWAS involving 3757 Sardinians. Flow cytometry was employed to analyze genetic characteristics of immune cells, including 118 absolute cell counts, 389 surface antigens, 32 morphological parameters, and 192 relative counts (ratios between cell levels) [18]. The study also included data on 14 EHAIDs, which are: inflammatory bowel disease [19], Crohn's disease [19], ulcerative colitis [19], rheumatoid arthritis [20], ankylosing spondylitis [21], systemic lupus erythematosus [22], multiple sclerosis [23], type 1 diabetes [24], celiac disease [25], psoriasis [26], asthma [27], myasthenia gravis [28], Graves' disease [29], and Hashimoto thyroiditis [15]. Data for these conditions were sourced from the largest GWASs or disease alliances. Additionally, the study examined multiple suspected triggering factors related to AILDs. Genetic variation data associated with 23 drug use categories were collected from approximately 320,000 individuals in the UK Biobank. Wu et al. conducted a GWAS on self-reported drug use across these 23 medication categories to uncover new insights related to drug use and to predict associated risks [30]. Data on smoking and drinking behaviors were obtained from the GWAS and Sequencing Consortium of Alcohol and Nicotine Use (GSCAN) [31], which included four smoking phenotypes (age of initiation of regular smoking, smoking initiation, cigarettes per day, and smoking cessation) and one drinking phenotype (drinks per week). Smoking initiation denotes whether the individual had ever smoked regularly, while cigarettes per day and drinks per week reflect the severity of smoking and drinking, respectively. Human gut microbiome data were acquired from the international consortium MiBioGen [32]. This study included 24 cohorts and 18,340 individuals, predominantly of European descent (16 cohorts, 13,266 individuals). The researchers analyzed the composition of 211 taxa (131 genera, 35 families, 20 orders, 16 classes, and 9 phyla) of gut microorganisms in fecal samples through 16S rRNA gene sequencing and investigated associations between human genetic variation and these microbial compositions. The study also included GWAS data on 19 common special pathogen infections. Data for human papillomavirus (HPV), Epstein-Barr virus, herpes simplex virus types 1 and 2, human herpesvirus types 6 and 7, varicella-zoster virus, human cytomegalovirus, polyomavirus, influenza A virus, *Helicobacter pylori*, *Chlamydia trachomatis*, and Toxoplasma gondii were derived from serum-specific antigen or antibody detection [33–35]. Data for COVID-19, mumps, typhoid fever, scarlet fever, rheumatic fever, and tuberculosis were obtained from clinically diagnosed cases [15,29,36].

2.2. Instrumental variables (IVs) selection

For screening IVs, we initially aimed for a significance threshold of $P < 5 \times 10^{-8}$. Due to limitations in sample size, not all exposures yielded sufficient SNPs (single nucleotide polymorphisms) at this threshold.

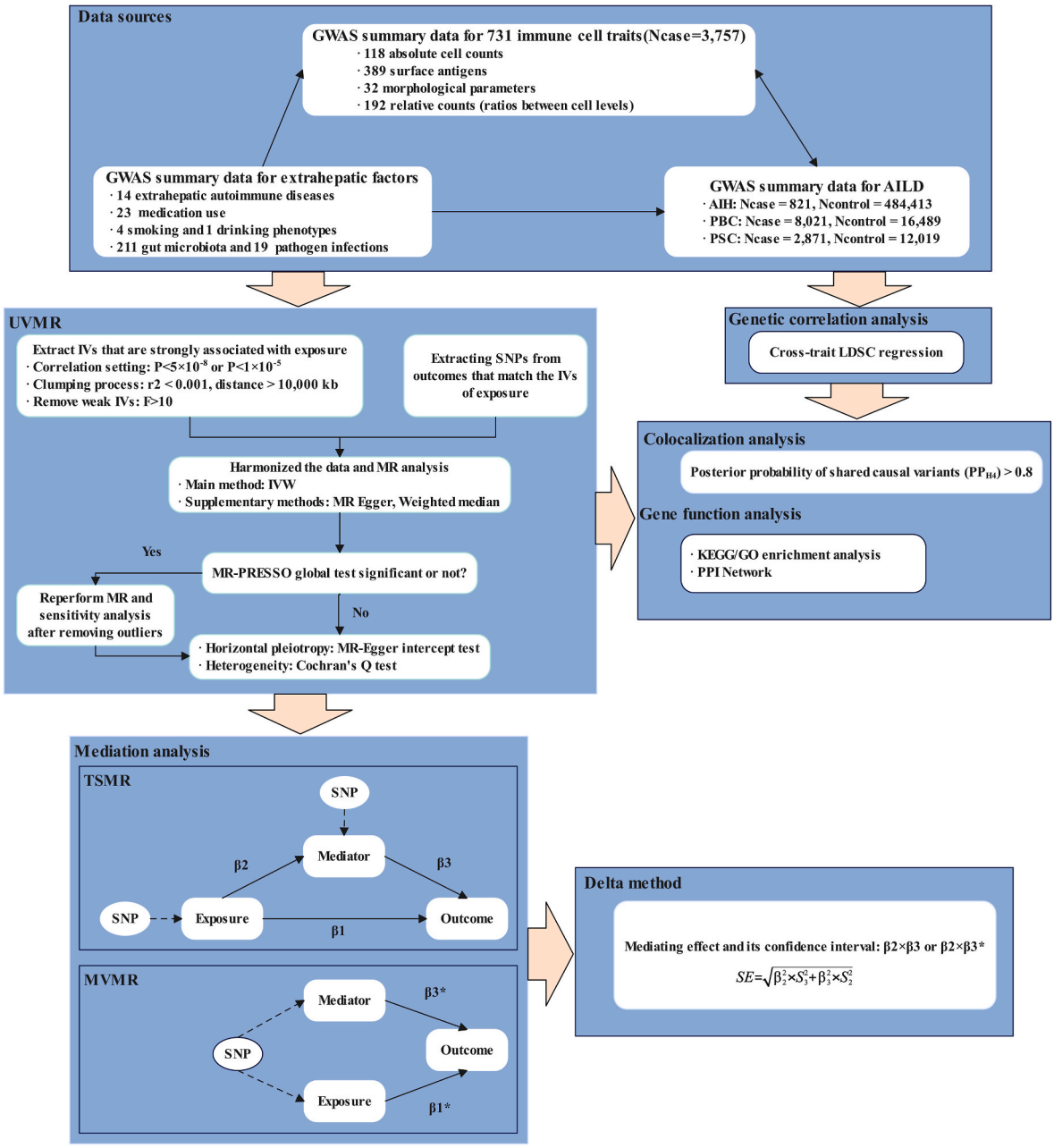

Fig. 1. Overview of the study design.

Thus, we applied varying thresholds based on exposure type. For PBC, PSC, EHAIDs, drug use, COVID-19, smoking, and drinking behaviors, a threshold of $P < 5 \times 10^{-8}$ was used. For AIH, immune cell traits, gut microbiota, and other pathogenic infections, a more lenient threshold of $P < 1 \times 10^{-5}$ was applied. To ensure that selected SNPs were independent, we performed clumping procedure ($r^2 < 0.001$, distance>10,000 kb) based on the European 1000 Genomes Project reference panel [37]. To minimize the impact of weak IVs, we calculated the strength of each IV ($F = beta^2/se^2$) and included only those with F > 10 in subsequent analyses [38].

2.3. Univariate MR (UVMR) analysis

We used UVMR to assess the effects of EHAIDs and potential triggers on AILDs, and bidirectional UVMR to explore the causal relationships between circulating immune cells and AILDs. Causal estimates were obtained using three methods: inverse variance weighted (IVW) [13], MR-Egger [39], and weighted median [40], with IVW as the main method. When only one IV was available, the Wald ratio method was applied. The effect size in MR analysis was expressed as odds ratio (OR) or beta (β). Multiple sensitivity analyses were conducted to ensure the reliability of the MR results. The Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) test was used to identify horizontal pleiotropic outliers [41], and MR analysis was repeated after excluding these outliers to obtain adjusted estimates. The MR-Egger intercept test and Cochran's Q test were used to detect pleiotropy and heterogeneity, respectively. To address multiple hypothesis testing in the analysis of 731 immune cell traits and AILDs, we corrected the false discovery rate (FDR) of IVW results using the Benjamini-Hochberg method to obtain significance values (q). Values of $q < 0.05$ indicated statistical significance, while $P < 0.05$ but $q > 0.05$ suggested potential genetic association [42]. Due to the complex linkage disequilibrium structure of the human leukocyte antigen (HLA) region, which makes it susceptible to horizontal pleiotropy and violations of the MR

assumptions. We removed the SNPs in this region on the short arm of chromosome 6 and re-performed the MR analysis to further examine the impact of HLA region SNPs on causal associations. To avoid sample overlap, we excluded a few phenotypes derived from the UK Biobank and FinnGen Consortium in the AIH analysis. This study adhered to the Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization (STROBE-MR) statement [43] (**Supplementary file**).

2.4. Cross-trait LDSC regression

We employed cross-trait LDSC regression to assess the genetic correlation between EHAIDs and AILDs. SNPs from the GWAS data were filtered using HapMap 3, and those with a minor allele frequency below 0.01 were excluded. The chi-square statistic for a single trait [44] was substituted with the product of the z-scores for the two traits, with genetic covariance estimated by the slope of the LDSC regression. After SNP-heritability standardization, this genetic covariance reflects the genetic correlation (r_g). It is important to note that this method is not influenced by sample overlap; however, if one or both traits have very low heritability, LDSC analysis results may be unobtainable [12].

2.5. Colocalization analysis

Colocalization analysis was conducted using the coloc package to identify loci shared by the two traits in the genome. Genetic variants involved in the MR analysis were used as lead SNPs, and SNPs within a 200 kb surrounding region were extracted [45]. For each locus, the Bayesian method assessed the support for five exclusive hypotheses: H0 (no association with either trait); H1 (only associated with trait 1); H2 (only associated with trait 2); H3 (associated with both traits, but with different causal variants); and H4 (associated with both traits, sharing the same causal variant). The analysis yields a posterior probability (PP) for each hypothesis. A posterior probability of shared causal variants (PP_{H4}) greater than 0.8 indicates strong evidence of colocalization [14].

2.6. KEGG/GO enrichment analysis and PPI network

To elucidate the role of the shared loci identified in the colocalization analysis, we investigated the functions of these SNPs. KEGG and GO enrichment analyses were conducted using the DAVID bioinformatics resource (https://david.ncifcrf.gov/) [46]. KEGG analysis primarily identifies regulatory pathways associated with genes, while GO analysis encompasses biological processes (BP), molecular functions (MF), and cellular components (CC). Subsequently, the STRING database (https://string-db.org/) was utilized to construct a PPI network to explore potential interactions among the identified genes [47].

2.7. Mediation analysis

We employed two mediation methods, two-step MR (TSMR) [48] and multivariate MR (MVMR) [49,50], to investigate the mediating role of circulating immune cells in the relationship between extrahepatic factors and AILDs. In this analysis, EHAIDs and trigger factors served as exposures, immune cells as mediators, and AILDs as outcomes. In the previous UVMR analysis, we calculated the total effect of extrahepatic factors on AILDs (β1) and the effect of immune cells on AILDs (β3). The same UVMR method was used to determine the effect of extrahepatic factors on immune cells (β2). The mediating effect of immune cells (β2 × β3) was computed using the Delta method [51]. The proportion mediated, representing the ratio of the mediating effect to the total effect, was given by (β2 × β3)/β1. The TSMR result was considered significant if the P values for β1, β2, and β3 (P1, P2, and P3) were all significant and if the mediating effect (β2 × β3) and the total effect (β1) were in the same direction. MVMR was used to validate the TSMR findings and to address potential biases from genetic overlap. After adjusting for immune cells, the effect of extrahepatic factors on AILDs was β1*, and after adjusting for extrahepatic factors, the effect of immune cells on AILDs was β3*. At this stage, the mediated effect of immune cells was β2 × β3*, and the proportion mediated was β2 × β3*/β1 [52].

3. Results

We calculated the F values for all IVs (Supplementary Table S2), and all were greater than 10, suggesting that this study was unlikely to be influenced by weak instrumental variable bias.

3.1. Associations between EHAIDs and AILDs

UVMR and LDSC regression were utilized to evaluate the causative roles and genetic correlations in the associations between 14 EHAIDs and 3 AILDs (Fig. 2). The UVMR analysis identified 14 significant causal associations, with EHAIDs serving as risk factors for AILDs in all cases. The LDSC regression analysis revealed 16 associations with genetic correlation. Except for asthma and PBC, the remaining 15 correlations were positive. Additionally, significant results were observed in both UVMR and LDSC regression for 7 associations. Notably, in the MR analysis, we employed the MR-PRESSO global test to exclude the influence of pleiotropic SNPs, indicating a combined effect of causation and genetic overlap in these associations.

We employed colocalization analysis to investigate the shared genetic variation between EHAIDs and AILDs, with the results detailed in Supplementary Table S3. To determine the functional roles of these loci, we identified the nearest genes by querying the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) database. Our analysis revealed that 9 loci were shared with AIH, 83 loci were shared with PBC, and 48 loci were shared with PSC. In particular, rs10774625 (ATXN2) was found in both PBC and 9 EHAIDs. For PSC, rs3184504 (SH2B3) and rs4817988 (PSMG1) were shared with 6 and 5 EHAIDs, respectively. To elucidate the regulatory pathways and interactions of these shared loci, we conducted GO/KEGG enrichment analysis and constructed a PPI network. The findings indicated that these loci were involved in various immune responses and disease processes, with notable differences among the three AILDs (Fig. 3). In AIH, the shared loci were primarily associated with HLA-related antigen presentation, T cell activation and cytotoxic response, and the differentiation of Th1, Th2, and Th17 cells. For PBC, the shared loci were related to a range of cytokines and surface antigens and participated in multiple signaling pathways, including interleukins, chemokines, interferons, JAK-STAT, NF-κB, Toll-like receptor signaling, and immunoglobulin-mediated immune responses. These loci also impacted the proliferation and differentiation of B and T cells, as well as inflammatory processes such as autophagy and apoptosis. The PPI network analysis showed that loci associated with PSC predominantly included CTLA4, IL2RA, BACH2, SH2B3, and CLEC16A, with CTLA4 occupying a central position in the network. Enrichment analysis indicated that CTLA4 was mainly involved in the negative regulation of T cell proliferation and associated with various immune diseases.

3.2. Associations between immune cell traits and AILDs

We conducted bidirectional MR analysis to explore the causal relationships between 731 immune cell traits and AILDs. Significant associations ($P < 0.05$) identified using the IVW method are detailed in Supplementary Table S4. The associations that remained significant after FDR correction ($q < 0.05$) are illustrated in Fig. 4.

Among the effects of immune cells on AILDs, 35 immune phenotypes were causally associated with AIH, 49 with PBC, and 33 with PSC. After FDR correction, seven immune phenotypes showed strong associations with AILDs. Specifically, CD25 on IgD- $CD27^-$ B cells had a protective effect in PBC, whereas CD27 on $CD24^+$ $CD27^+$ B cells, CD27 on IgD +

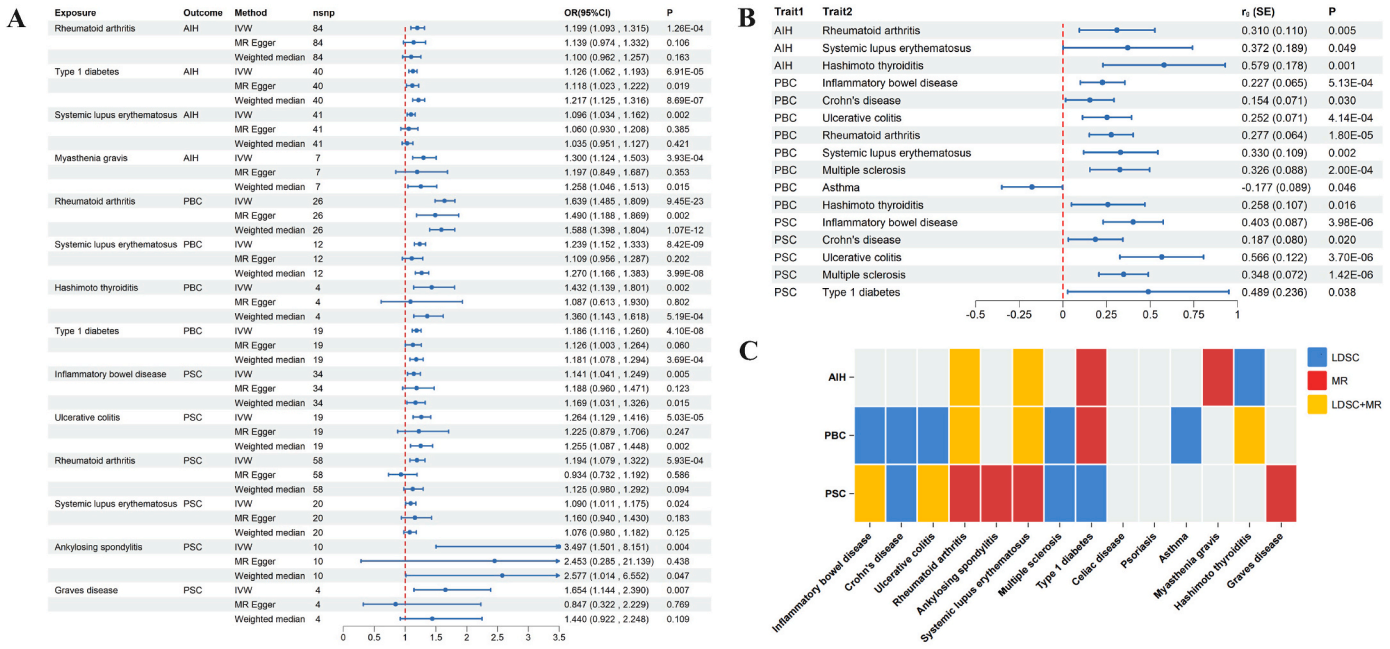

Fig. 2. UVMR and LDSC regression elucidate the causal effects and shared genetic architecture underlying the association of 14 EHAIDs and 3 AILDs. (A) Associations with significant causal effects discovered through UVMR. (B) Associations with significant genetic correlation discovered through LDSC regression. (C) Integration of UVMR and LDSC regression results: red indicates significant MR results, blue indicates significant LDSC results, and yellow indicates significant results for both methods.

$CD24^+$ B cells, CD27 on IgD- CD38dim B cells, CD27 on unswitched memory B cells, and CD27 on switched memory B cells were risk factors for PBC. Additionally, CD28 on resting CD4 regulatory T cells played a protective role in PSC.

Among the effects of AILDs on immune cells, AIH was causally associated with 30 immune phenotypes, PBC with 63 immune phenotypes, and PSC with 92 immune phenotypes. After FDR correction, AILDs were strongly associated with 12 immune phenotypes. Specifically, PBC onset was associated with decreased levels of CD4 regulatory T cell %CD4+ T cell, resting CD4 regulatory T cell %CD4+ T cell, $CD33^-$ HLA DR-absolute count, $CD3^-$lymphocyte %leukocyte, natural killer absolute count, and natural killer %lymphocyte, but an increased level of T cell %lymphocyte. On the other hand, PSC onset was associated with increased levels of effector memory $CD8^+$ T cell absolute count, $CD28^-$ CD127- CD25++ $CD8^+$ T cell absolute count, $CD28^-$ CD25++ $CD8^+$ T cell absolute count, $CD28^-$ $CD8^+$ T cell %CD8+ T cell, and $CD28^-$ $CD8^+$ T cell absolute count.

Colocalization analysis identified shared causal variation in two of these significant associations. In the association between PBC and CD4 regulatory T cell %CD4+ T cell, the PP_{H4} of rs60600003 (ELMO1) = 0.96. In the association between PBC and natural killer absolute count, the PP_{H4} of rs10774625 (ATXN2) = 0.98.

3.3. Associations between triggering factors and AILDs

In the UVMR analysis of potential triggers for AILDs, we identified a total of 41 statistically significant associations: 8 related to AIH, 15 to PBC, and 18 to PSC. Among these, thyroid preparations were found to be a risk factor for PBC, while drugs affecting bone structure and mineralization, as well as calcium channel blockers and antihypertensives, were associated with an increased risk of PSC. Regular smoking in the past was associated with an increased risk of AIH and PBC but a decreased risk of PSC. Additionally, certain gut microbiota and pathogen infections were found to have a causal relationship with AILDs. For detailed information, refer to Fig. 5.

3.4. The mediating role of immune cells in the associations between extrahepatic factors and AILDs

TSMR was employed to investigate the mediating role of circulating immune cells in the effects of EHAIDs and triggering factors on AILDs. Initially, we assessed the causal effects of the aforementioned extrahepatic factors related to AILDs on immune cells, identifying a total of 89 significant associations (Supplementary Table S5). After excluding associations with conflicting directions of mediating and total effects, 64 mediating pathways were ultimately confirmed using the TSMR method (Supplementary Table S6). The mediating effects and their 95 % confidence intervals (CIs) were calculated using the Delta method, revealing 5 significant mediating effects. Notably, CD27 on IgD + $CD24^+$ B cells, CD27 on IgD- $CD38^-$ B cells, and CD27 on switched memory B cells mediated the association between smoking and PBC, with proportions mediated of 14.5 % (P = 0.046), 17.4 % (P = 0.026), and 11.1 % (P = 0.028), respectively. $CD28^-$ $CD8^+$ T cell %T cell mediated the association between gut microbiota (class Deltaproteobacteria) and PBC, with a proportion mediated of 12.8 % (P = 0.033). Additionally, CD25 on IgD- $CD27^-$ B cells mediated the association between gut microbiota (genus Blautia) and PBC, with a proportion mediated of 20.0 % (P = 0.044).

We further validated the 64 mediating pathways identified in TSMR using MVMR. Among these, 15 mediating pathways were significant in the MVMR analysis (Table 1). The Delta method was again applied to calculate the mediating effects and their 95 % CIs, with two immune cell mediators showing significant effects. Specifically, CD27 on switched memory B cells mediated the promoting effect of smoking on PBC, with a proportion mediated of 11.8 % (P = 0.032). Additionally, $CD28^-$ $CD8^+$ T cell %T cell mediated the protective effect of gut microbiota (class Deltaproteobacteria) on PBC, with a proportion mediated of 8.5 % (P = 0.049).

3.5. Sensitivity analysis

Outliers in the associations were detected using the MR-PRESSO global test, and MR analysis along with sensitivity tests were repeated after excluding these outliers. Among the 446 significant associations

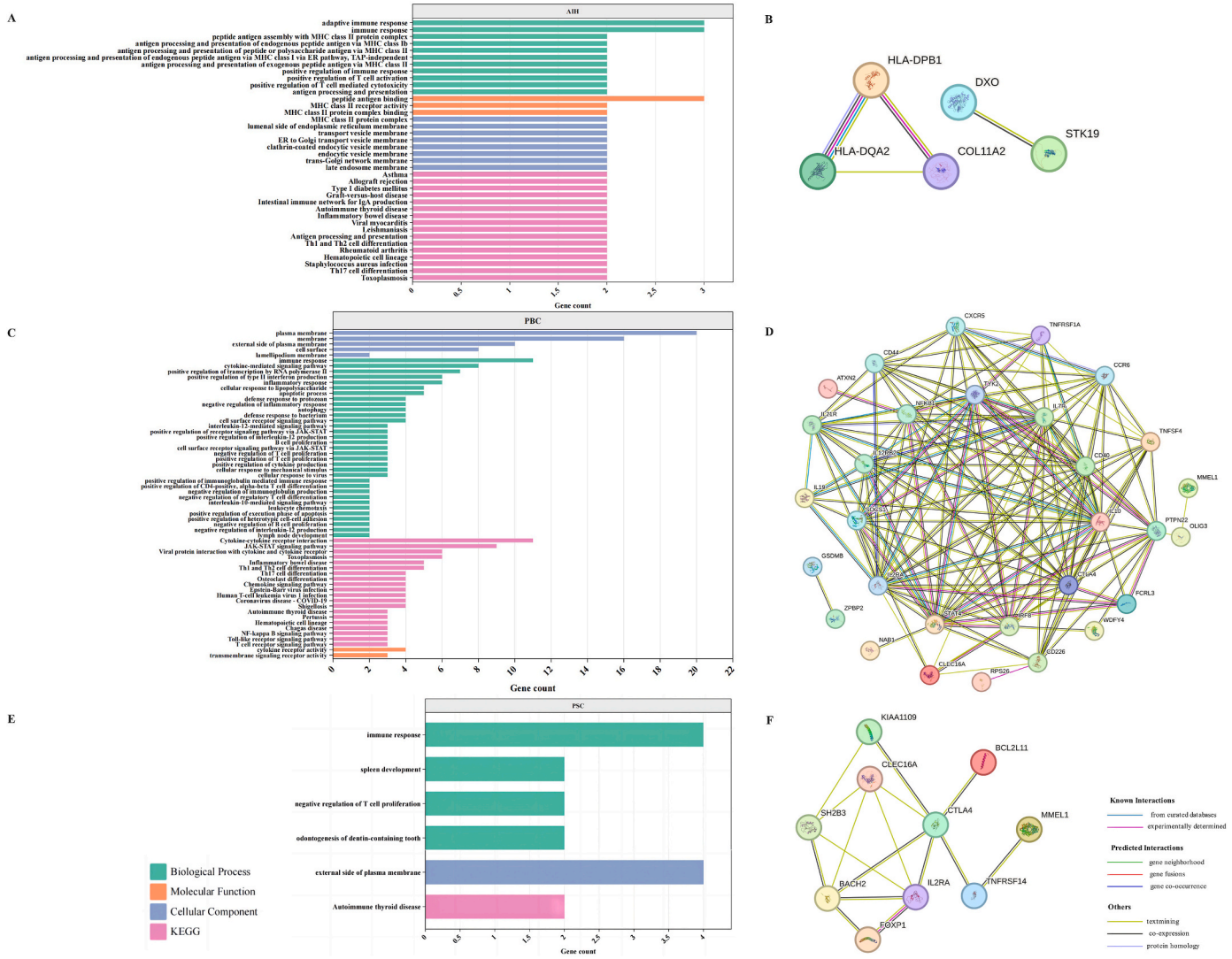

Fig. 3. Enrichment analysis and PPI network results show differences in the functions of shared loci among the three AILDs. (A) Enrichment analysis of shared loci related to AIH. (B) PPI network of shared loci related to AIH. (C) Enrichment analysis of shared loci related to PBC. (D) PPI network of shared loci related to PBC. (E) Enrichment analysis of shared loci related to PSC. (F) PPI network of shared loci related to PSC.

identified in this study (Supplementary Table S7), the Cochran's Q test revealed heterogeneity in 24 associations (5.4 %), while the MR-Egger intercept test indicated horizontal pleiotropy in 14 associations (3.1 %). A total of 24,933 SNPs were selected as instrumental variables in this study, of which 733 were in the HLA region (accounting for approximately 2.9 %). The MR results after removing these SNPs showed that among the significant results we reported after FDR correction, these associations were basically consistent with the results of previous analyses, indicating that this study was less affected by bias related to HLA genetic factors (Supplementary Table S8).

4. Discussion

Based on a substantial amount of publicly available genetic data, this study systematically examined the complex relationships between extrahepatic factors (14 EHAIDs, immune cells, and suspected triggers) and AILDs using MR, LDSC regression, colocalization, enrichment analysis, PPI network, and mediation analysis. We investigated the potential impact of various extrahepatic factors on AILDs and distinguished the shared genetic architecture and causal effects underlying the associations between autoimmune diseases, while also visualizing the functions of these shared loci. Additionally, we employed mediation analysis to explore the pathways through which extrahepatic factors contribute to liver inflammation, indicating that some circulating immune cells play a mediating role in the relationship between extrahepatic factors and AILDs.

Although epidemiological surveys have established some associations between EHAIDs and AILDs, the mechanisms underlying these associations remain unclear. In our study, we identified 14 significant causal associations via UVMR and observed significant genetic overlap in 16 associations through LDSC regression. We noted both causal effects and genetic overlap in seven associations; for instance, significant causal effects and genetic correlations were found in the analysis of rheumatoid arthritis and PBC. Mediation analysis indicated that the effect of rheumatoid arthritis on PBC may be mediated by CD127 on $CD28^{+}$ CD45RA $+$ $CD8^{+}$ T cells and CD45 on immature myeloid-derived suppressor cells. Colocalization analysis identified 20 loci shared between the two diseases, among which IL-12 and STAT4 have been elaborated in previous studies. In rheumatoid arthritis, IL-12 plays a key role in promoting the polarization of naive $CD4^{+}$ T cells into mature Th1 effector cells. IL-12 can also induce the production of IFN-γ by Th1 cells, NK cells, and $CD8^{+}$ T cells [53]. In PBC, IL-12 exerts a pro-inflammatory effect by activating Th1 cells, stimulate the production of IFN-γ and can also inhibit the production of IL-17 by pro-inflammatory Th17 cells through

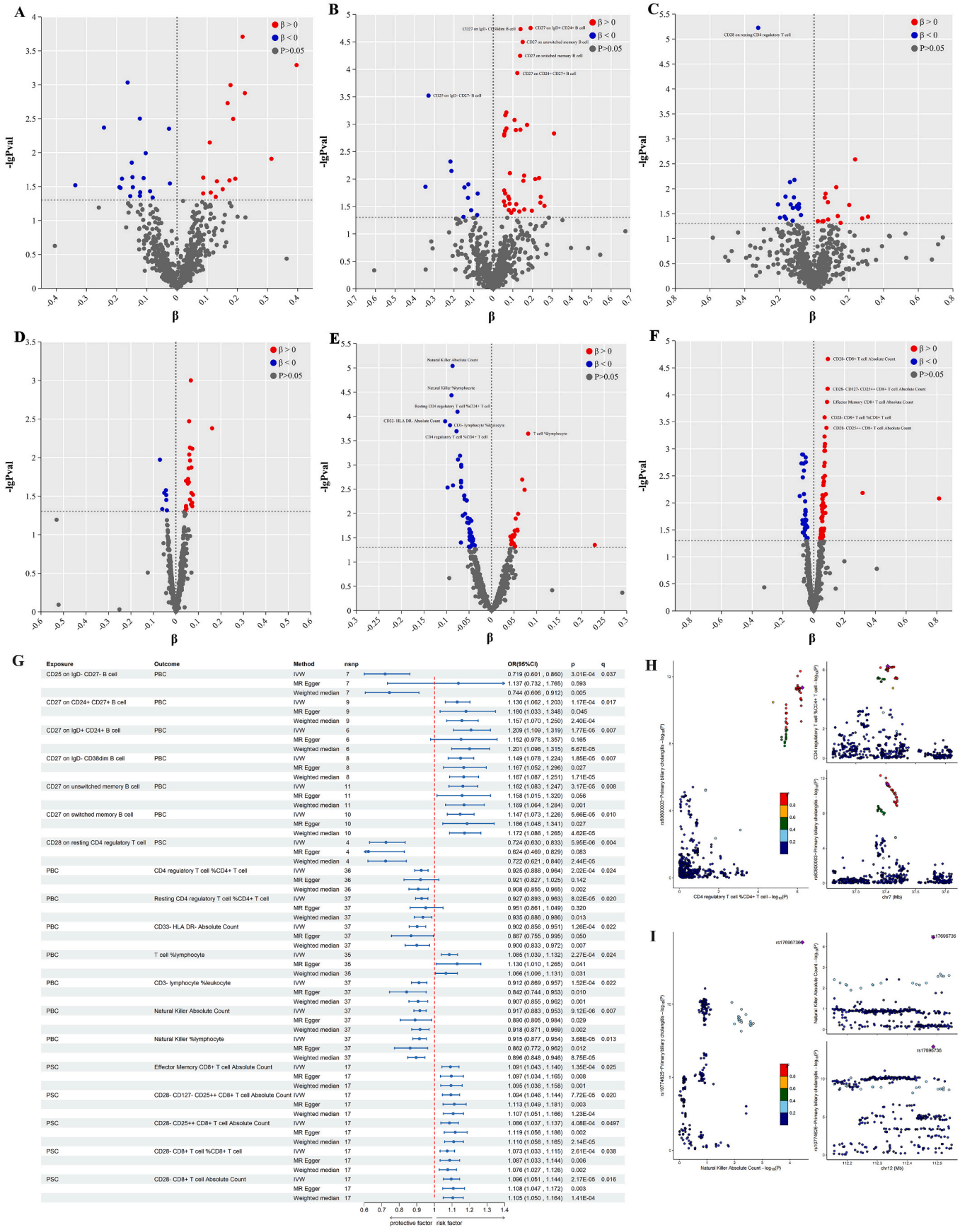

Fig. 4. Causal associations between circulating immune cells and AILDs. (A) (B) (C) depict the MR results of 731 immune cell traits on AIH, PBC, and PSC, respectively. (D) (E) (F) depict the reverse MR results of AIH, PBC, and PSC on 731 immune cell traits, respectively. (G) Significant associations remaining after FDR correction. (H) (I) Colocalization analysis results of these significant MR associations.

Exposure	Outcome	Method	nsnp	OR(95%CI)	P
Smoking initiation (ever regular vs never regular)	AIH	IVW	83	3.237 (1.109 , 9.451)	0.032
Gut microbiota abundance (family Victivallaceae)	AIH	IVW	12	1.420 (1.112 , 1.814)	0.005
Gut microbiota abundance (genus Anaerostipes)	AIH	IVW	12	0.261 (0.149 , 0.457)	2.50E-06
Gut microbiota abundance (genus Clostridium innocuum group)	AIH	IVW	9	0.673 (0.486 , 0.932)	0.017
Gut microbiota abundance (genus Family XIII UCG001)	AIH	IVW	7	0.547 (0.305 , 0.978)	0.042
Gut microbiota abundance (genus Holdemania)	AIH	IVW	15	0.660 (0.460 , 0.948)	0.025
Gut microbiota abundance (genus Ruminiclostridium9)	AIH	IVW	9	0.394 (0.203 , 0.762)	0.006
Gut microbiota abundance (genus Ruminococcaceae NK4A214 group)	AIH	IVW	13	1.644 (1.025 , 2.637)	0.039
Medication use (thyroid preparations)	PBC	IVW	46	1.256 (1.161 , 1.358)	1.13E-08
Smoking initiation (ever regular vs never regular)	PBC	IVW	39	2.437 (1.224 , 4.854)	0.011
Gut microbiota abundance (class Coriobacteriia)	PBC	IVW	3	2.178 (1.295 , 3.661)	0.003
Gut microbiota abundance (class Deltaproteobacteria)	PBC	IVW	4	0.518 (0.362 , 0.742)	3.25E-04
Gut microbiota abundance (class Negativicutes)	PBC	IVW	3	1.972 (1.164 , 3.338)	0.012
Gut microbiota abundance (family Coriobacteriaceae)	PBC	IVW	3	2.178 (1.295 , 3.661)	0.003
Gut microbiota abundance (family Desulfovibrionaceae)	PBC	IVW	3	0.528 (0.345 , 0.810)	0.003
Gut microbiota abundance (genus Adlercreutzia)	PBC	IVW	4	0.706 (0.525 , 0.950)	0.022
Gut microbiota abundance (genus Bifidobacterium)	PBC	IVW	5	1.494 (1.003 , 2.226)	0.049
Gut microbiota abundance (genus Blautia)	PBC	Wald ratio	1	0.246 (0.093 , 0.651)	0.005
Gut microbiota abundance (genus Flavonifractor)	PBC	IVW	2	1.612 (1.014 , 2.564)	0.044
Gut microbiota abundance (genus Ruminiclostridium5)	PBC	IVW	5	1.419 (1.017 , 1.980)	0.040
Gut microbiota abundance (order Coriobacteriales)	PBC	IVW	3	2.178 (1.295 , 3.661)	0.003
Gut microbiota abundance (order Selenomonadales)	PBC	IVW	3	1.972 (1.164 , 3.338)	0.012
Varicella zoster virus glycoproteins E and I antibody levels	PBC	IVW	4	0.678 (0.518 , 0.886)	0.004
Medication use (drugs affecting bone structure and mineralization)	PSC	IVW	7	1.542 (1.096 , 2.169)	0.013
Medication use (calcium channel blockers)	PSC	IVW	86	1.230 (1.068 , 1.417)	0.004
Medication use (antihypertensives)	PSC	IVW	6	1.459 (1.029 , 2.068)	0.034
Smoking initiation (ever regular vs never regular)	PSC	IVW	71	0.326 (0.148 , 0.717)	0.005
Gut microbiota abundance (class Negativicutes)	PSC	IVW	6	1.716 (1.004 , 2.933)	0.048
Gut microbiota abundance (family Actinomycetaceae)	PSC	IVW	3	0.595 (0.361 , 0.982)	0.042
Gut microbiota abundance (family Rhodospirillaceae)	PSC	IVW	12	1.301 (1.009 , 1.676)	0.042
Gut microbiota abundance (genus Actinomyces)	PSC	IVW	4	0.618 (0.423 , 0.901)	0.012
Gut microbiota abundance (genus Alloprevotella)	PSC	IVW	3	0.685 (0.500 , 0.938)	0.018
Gut microbiota abundance (genus Barnesiella)	PSC	IVW	9	0.617 (0.398 , 0.954)	0.030
Gut microbiota abundance (genus Peptococcus)	PSC	IVW	8	0.769 (0.608 , 0.973)	0.029
Gut microbiota abundance (genus Ruminococcaceae UCG013)	PSC	IVW	8	1.632 (1.039 , 2.565)	0.034
Gut microbiota abundance (order Actinomycetales)	PSC	IVW	3	0.594 (0.360 , 0.981)	0.042
Gut microbiota abundance (order Selenomonadales)	PSC	IVW	6	1.716 (1.004 , 2.933)	0.048
Chlamydia trachomatis pGP3 antibody levels	PSC	IVW	11	1.219 (1.073 , 1.386)	0.002
Human herpes virus 6 p101k antibody levels	PSC	IVW	16	0.879 (0.792 , 0.975)	0.014
Anti-herpes simplex virus 2 IgG seropositivity	PSC	IVW	15	3.235 (1.717 , 6.097)	2.82E-04
Toxoplasma gondii p22 antibody levels	PSC	IVW	17	0.898 (0.823 , 0.980)	0.015

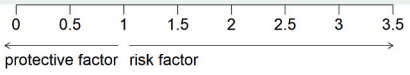

Fig. 5. Triggering factors associated with the onset of AILDs.

IL-23 [54]. The specific cellular effects of IL-12 are mainly manifested in inducing the activation of the transcription factor STAT4, and GWAS have identified at least three IL-12-related genes that are closely related to PBC: IL12A, IL12RB2, and STAT4 [55]. We found that these genes are also related to rheumatoid arthritis and systemic lupus erythematosus.

We compared the shared loci across the three AILDs and found that, although PBC and PSC may overlap genetically with some of the same EHAIDs, the specific shared loci are quite different. In PBC, rs10774625 (ATXN2) is shared with 9 EHAIDs. Ataxin-2, the protein encoded by this gene, is well-documented for its role in neurodegenerative diseases [56]. Additionally, research has linked ataxin-2 to insulin resistance and dyslipidemia [57]. For PSC, rs3184504 (SH2B3) and rs4817988 (PSMG1) are shared with 6 and 5 EHAIDs, respectively. SH2B3 was initially identified as a regulator of hematopoiesis and lymphocyte differentiation, involved in various signaling pathways of growth factor and cytokine receptors, and is closely related to autoimmunity and inflammation. Although GWAS have shown associations between SH2B3 and type 1 diabetes, celiac disease, rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis, the specific mechanisms in these diseases are not fully understood [58]. The association between PSMG1 and autoimmune diseases has been observed only in pediatric-onset inflammatory bowel disease [59], and its role remains unclear.

We investigated the causal relationships between 731 immune cell traits and three AILDs, with several exhibiting strong associations after FDR correction. Our analysis identified five CD27 B cell traits as risk factors for PBC and revealed that CD27 B cells mediate the enhancing effect of smoking on PBC. CD27 (TNFRSF7), a member of the tumor necrosis factor receptor family, has CD70 (TNFSF7) as its sole known ligand, and the $CD27^{-}CD70$ pathway plays a crucial role in stimulating T and B cell activation. In B cells, CD27 is predominantly expressed on memory B cells within the germinal center. CD27 is not only a marker of memory B cells but may also be essential for their formation. Additionally, in vitro studies indicate that the $CD27^{-}CD70$ interaction promotes plasma cell differentiation and immunoglobulin production. CD27's involvement has been documented in systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, psoriasis, and neuroimmune diseases [60,61]. Li X et al. reported the accumulation of CD27 memory B and plasma cells in the hepatic portal tracts of PBC patients using spatial transcriptomics and multiplex immunofluorescence [62]. Targeted therapies against CD27/CD70 have demonstrated significant potential in cancer treatment [63], highlighting CD27 as an attractive target for PBC treatment in our study. In reverse MR analysis, we observed that the onset of PSC was strongly associated with increased levels of four $CD28^{-}$ $CD8^{+}$ T cells ($q < 0.05$). Among the associations noted before FDR correction ($P < 0.05$), these findings were

Table 1
Mediating pathways verified by MVMR analysis.

Exposure	Mediator	Outcome	Direct effect (β1*±SE)	P1*	Direct effect (β3*±SE)	P3*	Mediating effect (β2 × β3*±SE)	*Pm*	Proportion mediated (β2 × β3*/β1)
Myasthenia gravis	Plasma Blast-Plasma Cell %B cell	AIH	0.218 ± 0.068	0.001	−0.119 ± 0.046	0.009	0.012 ± 0.007	0.094	4.4 %
Gut microbiota (family Victivallaceae)	$CD14^+$ $CD16^+$ monocyte %monocyte	AIH	0.266 ± 0.115	0.021	−0.267 ± 0.086	0.002	0.051 ± 0.028	0.062	14.6 %
Gut microbiota (genus Clostridium innocuum group)	CD62L- $CD86^+$ myeloid Dendritic Cell %Dendritic Cell	AIH	−0.380 ± 0.144	0.008	0.237 ± 0.061	9.75E-05	−0.043 ± 0.023	0.059	11.0 %
Gut microbiota (genus Clostridium innocuum group)	HLA DR + Natural Killer Absolute Count	AIH	−0.380 ± 0.161	0.019	−0.231 ± 0.086	0.007	−0.042 ± 0.024	0.086	10.5 %
Gut microbiota (genus Clostridium innocuum group)	HLA DR + Natural Killer %Natural Killer	AIH	−0.340 ± 0.168	0.043	−0.185 ± 0.084	0.028	−0.032 ± 0.021	0.130	8.0 %
Gut microbiota (genus Ruminiclostridium 9)	$CD14^+$ $CD16^+$ monocyte %monocyte	AIH	−0.863 ± 0.297	0.004	−0.210 ± 0.078	0.007	−0.078 ± 0.043	0.069	8.3 %
Smoking initiation	CD27 on $CD24^+$ $CD27^+$ B cell	PBC	1.009 ± 0.444	0.023	0.114 ± 0.037	0.002	0.071 ± 0.040	0.075	8.0 %
Smoking initiation	CD27 on memory B cell	PBC	0.966 ± 0.477	0.043	0.128 ± 0.040	0.001	0.082 ± 0.047	0.080	9.2 %
Smoking initiation	CD27 on switched memory B cell	PBC	0.902 ± 0.424	0.034	0.146 ± 0.039	2.03E-04	0.105 ± 0.049	0.032	11.8 %
Smoking initiation	CD127 on $CD28^+$ CD45RA + $CD8^+$ T cell	PBC	0.999 ± 0.416	0.016	0.129 ± 0.043	0.003	0.087 ± 0.048	0.071	9.8 %
Gut microbiota (class Deltaproteobacteria)	Terminally Differentiated $CD8^+$ T cell Absolute Count	PBC	−0.550 ± 0.217	0.011	−0.242 ± 0.095	0.011	−0.075 ± 0.042	0.076	11.4 %
Gut microbiota (class Deltaproteobacteria)	$CD28^-$ $CD8^+$ T cell %T cell	PBC	−0.613 ± 0.122	5.55E-07	−0.144 ± 0.058	0.013	−0.056 ± 0.028	0.049	8.5 %
Gut microbiota (family Desulfovibrionaceae)	Terminally Differentiated $CD8^+$ T cell Absolute Count	PBC	−0.508 ± 0.255	0.047	−0.242 ± 0.103	0.019	−0.078 ± 0.047	0.097	12.3 %
Gut microbiota (family Desulfovibrionaceae)	$CD28^-$ $CD8^+$ T cell %T cell	PBC	−0.605 ± 0.142	1.97E-05	−0.139 ± 0.062	0.026	−0.050 ± 0.030	0.094	7.8 %
Rheumatoid arthritis	CD45 on CD33dim HLA DR + CD11b-	PSC	0.328 ± 0.085	1.08E-04	−0.295 ± 0.120	0.014	0.018 ± 0.012	0.124	10.0 %

Pm, the significance value of the mediating effect.

particularly intriguing. PSC occurrence was linked to reduced levels of four $CD28^+$ $CD8^+$ T cells and increased levels of 13 $CD28^-$ $CD8^+$ T cells. Previous research has shown that CD28 is a primary co-stimulatory molecule for T cell activation, and its loss during activation results in $CD28^-$ T cells, which are typically antigen-specific and highly differentiated. Although $CD28^-CD8^+$ T cells lose proliferative capacity during replicative senescence due to repeated antigenic stimulation, they can transiently upregulate telomerase activity and proliferate under certain conditions. $CD28^-CD8^+$ T cells are functionally heterogeneous, often exhibiting enhanced cytotoxic or immunomodulatory capabilities [64,65]. In PSC research, Liaskou E et al. observed that $CD28^-$ T cells accumulate in the liver of PSC patients, releasing high levels of TNF-α and IFN-γ, and are localized around the bile ducts [66]. Consequently, our study suggests that the depletion of circulating $CD28^+$ $CD8^+$ T cells and the increase in $CD28^-$ $CD8^+$ T cells are promising markers for PSC occurrence and inflammatory activity.

Despite rigorous quality control procedures, this study has several inherent limitations. First, our research utilized a substantial amount of publicly available GWAS data, and the reliability of our findings is contingent upon the quality of these data. Due to the lack of detailed information on these cohorts, we were unable to perform further stratified analyses based on variables such as age, gender, and other demographic factors. Second, Although bioinformatics research has uncovered many new insights, these associations cannot be definitively concluded and ultimately need to be validated in patients. Therefore, follow-up case-control studies and foundational experiments are necessary. Third, developmental compensation might introduce bias, and the widespread use of certain immune modulators and anti-inflammatory drugs could alter the natural associations between genetic variation and immune traits. Fourth, the study was limited to European populations due to the lack of relevant data on other racial groups, necessitating caution when generalizing these findings to other populations.

CRediT authorship contribution statement

Zheng Zhang: Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jiayi Zhang:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Xinyang Yan:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jiachen Wang:** Writing – review & editing, Validation, Software, Methodology, Formal analysis. **Haoxiang Huang:** Writing – review & editing, Validation, Methodology. **Menghao Teng:** Writing – review & editing, Validation, Software. **Qingguang Liu:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization. **Shaoshan Han:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Ethics statement

This study was based on publicly available GWAS databases and no new participants were included, so ethical review and informed consent were not applicable.

Data availability

Supplementary Table S1Data will be made available on request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtauto.2024.100260.

Data availability

Data will be made available on request.

References

[1] P.J. Trivedi, G.M. Hirschfield, Recent advances in clinical practice: epidemiology of autoimmune liver diseases, Gut 70 (2021) 1989–2003.
[2] G. Mieli-Vergani, D. Vergani, A.J. Czaja, M.P. Manns, E.L. Krawitt, J.M. Vierling, A. W. Lohse, A.J. Montano-Loza, Autoimmune hepatitis, Nat. Rev. Dis. Prim. 4 (2018) 1–21.
[3] A. Lleo, G.-Q. Wang, M.E. Gershwin, G.M. Hirschfield, Primary biliary cholangitis, Lancet 396 (2020) 1915–1926.
[4] J.K. Dyson, U. Beuers, D.E. Jones, A.W. Lohse, M. Hudson, Primary sclerosing cholangitis, Lancet 391 (2018) 2547–2559.
[5] E. Liaskou, G.M. Hirschfield, M.E. Gershwin, Mechanisms of tissue injury in autoimmune liver diseases, Semin. Immunopathol. (2014) 553–568. Springer.
[6] K. Arndtz, G.M. Hirschfield, The pathogenesis of autoimmune liver disease, Dig. Dis. 34 (2016) 327–333.
[7] T. Liwinski, M. Heinemann, C. Schramm, The intestinal and biliary microbiome in autoimmune liver disease—current evidence and concepts, Semin. Immunopathol. (2022) 485–507. Springer.
[8] A. Floreani, S. De Martin, M.F. Secchi, N. Cazzagon, Extrahepatic autoimmunity in autoimmune liver disease, Eur. J. Intern. Med. 59 (2019) 1–7.
[9] K.N. van Munster, A. Bergquist, C.Y. Ponsioen, Inflammatory bowel disease and primary sclerosing cholangitis: one disease or two? J. Hepatol. 80 (1) (2024) 155–168.
[10] A. Floreani, P. Restrepo-Jiménez, M.F. Secchi, S. De Martin, P.S. Leung, E. Krawitt, C.L. Bowlus, M.E. Gershwin, J.-M. Anaya, Etiopathogenesis of autoimmune hepatitis, J. Autoimmun. 95 (2018) 133–143.
[11] J.S. Jang, B.D. Juran, K.Y. Cunningham, V.K. Gupta, Y.M. Son, J.D. Yang, A.H. Ali, E.A.L. Enninga, J. Sung, K.N. Lazaridis, Single-cell mass cytometry on peripheral blood identifies immune cell subsets associated with primary biliary cholangitis, Sci. Rep. 10 (2020) 12584.
[12] B. Bulik-Sullivan, H.K. Finucane, V. Anttila, A. Gusev, F.R. Day, P.-R. Loh, L. Duncan, An atlas of genetic correlations across human diseases and traits, Nat. Genet. 47 (2015) 1236–1241.
[13] C.A. Emdin, A.V. Khera, S. Kathiresan, Mendelian randomization, JAMA 318 (2017) 1925–1926.
[14] C. Giambartolomei, D. Vukcevic, E.E. Schadt, L. Franke, A.D. Hingorani, C. Wallace, V. Plagnol, Bayesian test for colocalisation between pairs of genetic association studies using summary statistics, PLoS Genet. 10 (2014) e1004383.
[15] S. Sakaue, M. Kanai, Y. Tanigawa, J. Karjalainen, M. Kurki, S. Koshiba, A. Narita, T. Konuma, K. Yamamoto, M. Akiyama, A cross-population atlas of genetic associations for 220 human phenotypes, Nat. Genet. 53 (2021) 1415–1424.
[16] H.J. Cordell, J.J. Fryett, K. Ueno, R. Darlay, Y. Aiba, Y. Hitomi, M. Kawashima, N. Nishida, S.-S. Khor, O. Gervais, An international genome-wide meta-analysis of primary biliary cholangitis: novel risk loci and candidate drugs, J. Hepatol. 75 (2021) 572–581.
[17] S.-G. Ji, B.D. Juran, S. Mucha, T. Folseraas, L. Jostins, E. Melum, N. Kumasaka, E. J. Atkinson, E.M. Schlicht, J.Z. Liu, Genome-wide association study of primary sclerosing cholangitis identifies new risk loci and quantifies the genetic relationship with inflammatory bowel disease, Nat. Genet. 49 (2017) 269–273.
[18] V. Orrù, M. Steri, C. Sidore, M. Marongiu, V. Serra, S. Olla, G. Sole, S. Lai, M. Dei, A. Mulas, Complex genetic signatures in immune cells underlie autoimmunity and inform therapy, Nat. Genet. 52 (2020) 1036–1045.
[19] J.Z. Liu, S. Van Sommeren, H. Huang, S.C. Ng, R. Alberts, A. Takahashi, S. Ripke, J. C. Lee, L. Jostins, T. Shah, Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations, Nat. Genet. 47 (2015) 979–986.
[20] E. Ha, S.-C. Bae, K. Kim, Large-scale meta-analysis across East Asian and European populations updated genetic architecture and variant-driven biology of rheumatoid arthritis, identifying 11 novel susceptibility loci, Ann. Rheum. Dis. 80 (2021) 558–565.
[21] P. Bowness, K. Gafney, H. Gaston, D.D. Gladman, P. Rahman, Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci, Nat. Genet. 45 (2013) 730–738.
[22] J. Bentham, D.L. Morris, D.S. Cunninghame Graham, C.L. Pinder, P. Tombleson, T. W. Behrens, J. Martín, B.P. Fairfax, J.C. Knight, L. Chen, Genetic association analyses implicate aberrant regulation of innate and adaptive immunity genes in the pathogenesis of systemic lupus erythematosus, Nat. Genet. 47 (2015) 1457–1464.
[23] I.M.S.G. Consortium, ANZgene, Multiple sclerosis genomic map implicates peripheral immune cells and microglia in susceptibility, Science 365 (2019) eaav7188.
[24] V. Forgetta, D. Manousaki, R. Istomine, S. Ross, M.-C. Tessier, L. Marchand, M. Li, H.-Q. Qu, J.P. Bradfield, S.F. Grant, Rare genetic variants of large effect influence risk of type 1 diabetes, Diabetes 69 (2020) 784–795.
[25] G. Trynka, K.A. Hunt, N.A. Bockett, J. Romanos, V. Mistry, A. Szperl, S.F. Bakker, M.T. Bardella, L. Bhaw-Rosun, G. Castillejo, Dense genotyping identifies and localizes multiple common and rare variant association signals in celiac disease, Nat. Genet. 43 (2011) 1193–1201.
[26] L.C. Tsoi, S.L. Spain, J. Knight, E. Ellinghaus, P.E. Stuart, F. Capon, J. Ding, Y. Li, T. Tejasvi, J.E. Gudjonsson, Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity, Nat. Genet. 44 (2012) 1341–1348.
[27] F. Demenais, P. Margaritte-Jeannin, K.C. Barnes, W.O. Cookson, J. Altmüller, W. Ang, R.G. Barr, T.H. Beaty, A.B. Becker, J. Beilby, Multiancestry association study identifies new asthma risk loci that colocalize with immune-cell enhancer marks, Nat. Genet. 50 (2018) 42–53.
[28] R. Chia, S. Saez-Atienzar, N. Murphy, A. Chiò, C. Blauwendraat, R.H. Roda, P. J. Tienari, H.J. Kaminski, R. Ricciardi, Identification of genetic risk loci and prioritization of genes and pathways for myasthenia gravis: a genome-wide association study, Proc. Natl. Acad. Sci. USA 119 (2022) e2108672119.
[29] M.I. Kurki, J. Karjalainen, P. Palta, T.P. Sipilä, K. Kristiansson, K.M. Donner, M. P. Reeve, H. Laivuori, M. Aavikko, M.A. Kaunisto, FinnGen provides genetic insights from a well-phenotyped isolated population, Nature 613 (2023) 508–518.
[30] Y. Wu, E.M. Byrne, Z. Zheng, K.E. Kemper, L. Yengo, A.J. Mallett, J. Yang, P. M. Visscher, N.R. Wray, Genome-wide association study of medication-use and associated disease in the UK Biobank, Nat. Commun. 10 (2019) 1891.
[31] M. Liu, Y. Jiang, R. Wedow, Y. Li, D.M. Brazel, F. Chen, G. Datta, J. Davila-Velderrain, D. McGuire, C. Tian, Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use, Nat. Genet. 51 (2019) 237–244.
[32] A. Kurilshikov, C. Medina-Gomez, R. Bacigalupe, D. Radjabzadeh, J. Wang, A. Demirkan, C.I. Le Roy, J.A. Raygoza Garay, C.T. Finnicum, X. Liu, Large-scale association analyses identify host factors influencing human gut microbiome composition, Nat. Genet. 53 (2021) 156–165.
[33] K. Suhre, M. Arnold, A.M. Bhagwat, R.J. Cotton, R. Engelke, J. Raffler, H. Sarwath, G. Thareja, A. Wahl, R.K. DeLisle, Connecting genetic risk to disease end points through the human blood plasma proteome, Nat. Commun. 8 (2017) 14357.
[34] G. Butler-Laporte, D. Kreuzer, T. Nakanishi, A. Harroud, V. Forgetta, J.B. Richards, Genetic determinants of antibody-mediated immune responses to infectious diseases agents: a genome-wide and HLA association study, Open Forum Infect. Dis. (2020) ofaa450. Oxford University Press US.
[35] P. Scepanovic, C. Alanio, C. Hammer, F. Hodel, J. Bergstedt, E. Patin, C. W. Thorball, N. Chaturvedi, B. Charbit, L. Abel, Human genetic variants and age are the strongest predictors of humoral immune responses to common pathogens and vaccines, Genome Med. 10 (2018) 1–13.
[36] F. Wang, S. Huang, R. Gao, Y. Zhou, C. Lai, Z. Li, W. Xian, X. Qian, Z. Li, Y. Huang, COVID-19 Host Genetics Initiative the COVID-19 Host Genetics Initiative, a global initiative to elucidate the role of host genetic factors in susceptibility and severity of the SARS-CoV-2 virus pandemic, Eur. J. Hum. Genet. 28 (2020) 715–718.
[37] L. Clarke, X. Zheng-Bradley, R. Smith, E. Kulesha, C. Xiao, I. Toneva, B. Vaughan, D. Preuss, R. Leinonen, M. Shumway, The 1000 Genomes Project: data management and community access, Nat. Methods 9 (2012) 459–462.
[38] J. Xie, H. Huang, Z. Liu, Y. Li, C. Yu, L. Xu, C. Xu, The associations between modifiable risk factors and nonalcoholic fatty liver disease: a comprehensive Mendelian randomization study, Hepatology 77 (2023) 949–964.
[39] J. Bowden, G. Davey Smith, S. Burgess, Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression, Int. J. Epidemiol. 44 (2015) 512–525.
[40] J. Bowden, G. Davey Smith, P.C. Haycock, S. Burgess, Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator, Genet. Epidemiol. 40 (2016) 304–314.

[41] M. Verbanck, C.-Y. Chen, B. Neale, R. Do, Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases, Nat. Genet. 50 (2018) 693–698.
[42] J. Chen, F. Xu, X. Ruan, J. Sun, Y. Zhang, H. Zhang, J. Zhao, J. Zheng, S.C. Larsson, X. Wang, Therapeutic targets for inflammatory bowel disease: proteome-wide Mendelian randomization and colocalization analyses, EBioMedicine 89 (2023).
[43] V.W. Skrivankova, R.C. Richmond, B.A. Woolf, J. Yarmolinsky, N.M. Davies, S. A. Swanson, T.J. VanderWeele, J.P. Higgins, N.J. Timpson, N. Dimou, Strengthening the reporting of observational studies in epidemiology using Mendelian randomization: the STROBE-MR statement, JAMA 326 (2021) 1614–1621.
[44] B.K. Bulik-Sullivan, P.-R. Loh, H.K. Finucane, S. Ripke, J. Yang, N. Patterson, M. J. Daly, A.L. Price, B.M. Neale, LD Score regression distinguishes confounding from polygenicity in genome-wide association studies, Nat. Genet. 47 (2015) 291–295.
[45] Y. Cai, S. Xie, X. Jia, D. Chen, D. Wu, W. Bao, J. Cai, J. Mao, J. Ye, Integrated analysis of Mendelian Randomization and Bayesian colocalization reveals bidirectional causal association between inflammatory bowel disease and psoriasis, Ann. Med. 55 (2023) 2281658.
[46] B.T. Sherman, M. Hao, J. Qiu, X. Jiao, M.W. Baseler, H.C. Lane, T. Imamichi, W. Chang, DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update), Nucleic Acids Res. 50 (2022) W216–W221.
[47] D. Szklarczyk, R. Kirsch, M. Koutrouli, K. Nastou, F. Mehryary, R. Hachilif, A. L. Gable, T. Fang, N.T. Doncheva, S. Pyysalo, The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest, Nucleic Acids Res. 51 (2023) D638–D646.
[48] S. Burgess, R.M. Daniel, A.S. Butterworth, S.G. Thompson, E.-I. Consortium, Network Mendelian randomization: using genetic variants as instrumental variables to investigate mediation in causal pathways, Int. J. Epidemiol. 44 (2015) 484–495.
[49] S. Burgess, S.G. Thompson, Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects, Am. J. Epidemiol. 181 (2015) 251–260.
[50] E. Sanderson, Multivariable Mendelian randomization and mediation, Cold Spring Harbor perspectives in medicine 11 (2021) a038984.
[51] D.P. MacKinnon, C.M. Lockwood, J.M. Hoffman, S.G. West, V. Sheets, A comparison of methods to test mediation and other intervening variable effects, Psychol. Methods 7 (2002) 83.
[52] Q. Wang, H. Dai, T. Hou, Y. Hou, T. Wang, H. Lin, Z. Zhao, M. Li, R. Zheng, S. Wang, Dissecting causal relationships between gut microbiota, blood metabolites, and stroke: a Mendelian randomization study, Journal of stroke 25 (2023) 350.
[53] R.M. Pope, S. Shahrara, Possible roles of IL-12-family cytokines in rheumatoid arthritis, Nat. Rev. Rheumatol. 9 (2013) 252–256.
[54] A.F. Gulamhusein, G.M. Hirschfield, Primary biliary cholangitis: pathogenesis and therapeutic opportunities, Nat. Rev. Gastroenterol. Hepatol. 17 (2020) 93–110.
[55] A. Lleo, M.E. Gershwin, A. Mantovani, P. Invernizzi, Towards common denominators in primary biliary cirrhosis: the role of IL-12, J. Hepatol. 56 (2012) 731–733.
[56] L.A. Becker, A.D. Gitler, Ataxin-2 is Droppin'some knowledge, Neuron 98 (2018) 673–675.
[57] S. Carmo-Silva, C. Nobrega, L.P. de Almeida, C. Cavadas, Unraveling the role of ataxin-2 in metabolism, Trends Endocrinol. Metabol. 28 (2017) 309–318.
[58] J. Devallière, B. Charreau, The adaptor Lnk (SH2B3): an emerging regulator in vascular cells and a link between immune and inflammatory signaling, Biochem. Pharmacol. 82 (2011) 1391–1402.
[59] S. Kugathasan, R.N. Baldassano, J.P. Bradfield, P.M. Sleiman, M. Imielinski, S. L. Guthery, S. Cucchiara, C.E. Kim, E.C. Frackelton, K. Annaiah, Loci on 20q13 and 21q22 are associated with pediatric-onset inflammatory bowel disease, Nat. Genet. 40 (2008) 1211–1215.
[60] B.K. Han, N.J. Olsen, A. Bottaro, The CD27–CD70 pathway and pathogenesis of autoimmune disease, Semin. Arthritis Rheum. (2016) 496–501. Elsevier.
[61] O. Grimsholm, CD27 on human memory B cells–more than just a surface marker, Clin. Exp. Immunol. 213 (2023) 164–172.
[62] X. Li, Y. Li, J. Xiao, H. Wang, Y. Guo, X. Mao, P. Shi, Y. Hou, X. Zhang, N. Zhao, Unique DUOX2+ ACE2+ small cholangiocytes are pathogenic targets for primary biliary cholangitis, Nat. Commun. 14 (2023) 29.
[63] A.M. Starzer, A.S. Berghoff, New emerging targets in cancer immunotherapy: CD27 (TNFRSF7), ESMO open 4 (2019) e000629.
[64] M. Strioga, V. Pasukoniene, D. Characiejus, CD8+ CD28− and CD8+ CD57+ T cells and their role in health and disease, Immunology 134 (2011) 17–32.
[65] D. Mou, J. Espinosa, D.J. Lo, A.D. Kirk, CD28 negative T cells: is their loss our gain? Am. J. Transplant. 14 (2014) 2460–2466.
[66] E. Liaskou, L.E. Jeffery, P.J. Trivedi, G.M. Reynolds, S. Suresh, T. Bruns, D. H. Adams, D.M. Sansom, G.M. Hirschfield, Loss of CD28 expression by liver-infiltrating T cells contributes to pathogenesis of primary sclerosing cholangitis, Gastroenterology 147 (2014) 221–232, e227.