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Genetic insight into dissecting the immunophenotypes and inflammatory profiles in the pathogenesis of Sjogren syndrome

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Abstract

Background Sjogren syndrome (SS) is a chronic systemic autoimmune disease and its pathogenesis often involves the participation of numerous immune cells and inflammatory factors. Despite increased researches and studies recently focusing on this area, it remains to be fully elucidated. We decide to incorporate genetic insight into investigation of the causal link between various immune cells, inflammatory factors and pathogenesis of Sjogren syndrome (SS).

Methods Our study leveraged the genetic variants of multi-omics statistics extracted from genome-wide association study (GWAS), the University of Bristol and the FinnGen study. We performed a bidirectional Mendelian randomization and mediation study based on randomly allocated instrumental variables to infer causality, followed by external validation with UK Biobank data and Bayesian colocalization.

Results We demonstrated that an elevated level of CD27 on IgD + CD24 + B cell, a subset of B cells expressing both IgD and CD24, was associated with a higher risk of SS (OR = 1.119, 95% CI: 1.061–1.179, $P < 0.001$), while CD3 on CD45RA + CD4 + Treg was a protective factor (OR = 0.917, 95%CI: 0.877–0.959, $P < 0.001$). Results of meta-analysis and colocalization further supported the significant results identified in the primary analysis. A total of 4 inflammatory cytokines and 7 circulating proteins exhibited potential causal relationships with SS despite no significant result achieved after FDR correction. Finally, results of mediation analysis indicated that CD40L receptor levels had significant mediating effects (β = 0.0314, 95% CI: 0.0004–0.0624, $P = 0.0471$) at a mediation proportion of 28% (95% CI: 0.364%-55.6%) in causal relationship between CD27 on IgD + CD24 + B cell and SS.

Conclusions By providing a novel genetic insight into unveiling the roles of autoimmunity and inflammation in Sjogren syndrome, our findings may potentially lead to identifying new clinical biomarkers for disease monitoring and

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therapeutic targets that offer more effective alternatives for treating this condition. Therefore, our study may provide valuable evidence for future clinical intervention and targeted immunotherapy.

Keywords Sjogren syndrome, Sjogren's syndrome, Sicca syndrome, Immunophenotype, Mendelian randomization

Background

Sjogren syndrome (SS) is a common systemic autoimmune disorder associated with lymphocytic infiltration of exocrine organs, notably the lacrimal and salivary glands. This usually leads to significant dryness of the eyes and mouth and parotid enlargement [1]. Meanwhile, it may also involve various systemic manifestations affecting multiple organ systems, resulting in diverse characteristics and severity. Sjogren syndrome can occur alone or secondary to other systemic autoimmune disorders [2]. Until now, its pathogenesis remains unclear, with potential involvement of both genetic and environmental factors. Current studies indicated that the generation of autoantibodies and B cell activation may be linked to the onset of this illness [3]. For diagnosis of Sjogren syndrome, tests of salivary gland biopsy and antibodies are essential complements to typical clinical signs and symptoms. Treatment usually includes symptomatic therapies, disease-modifying agents, and biological drugs for systemic management [4].

Multiple environmental factors, such as viral infection and smoking, are related to SS development [5]. It is implied that they can trigger abnormal immunological reactions, activating T and B cells and increasing cytokine and chemokine levels [6]. Recent studies also examined how immune cells interplay with epithelial cells of the gland, suggesting their critical function in mediating and perpetuating aberrant autoimmune reactions [7]. Multiple cytokines have been shown to actively participate in mediating the abnormal T cell and B cell response, contributing to SS development. However, the exact causal relationships between immune cells, inflammatory factors and SS remain unclear. Additionally, novel therapeutic targets for SS patients based on various immune cells and relative cytokines are also an area of dynamic research. Recent trials of biological therapies targeting specific immune cells, their co-stimulation and IFN pathway have shown promising outcomes in SS cases [8]. These findings shed light on the association between immunological traits and SS progression, meanwhile indicating that circulating cytokines may be a potential mediating factor. However, studies on confirming the causality and discovering the exact immunophenotypes and inflammatory profiles are quite scarce, possibly due to the fact that large-sample observational studies and rigorous experiments are usually costly, complicated and difficult to control. By contrast, novel biomedical approaches with the abundance of genetic variants exhibit huge benefits in supporting the connections and the underlying mechanisms.

Mendelian randomization is a useful strategy for causal inference of exposure and outcome utilizing genotypes as pivotal instrument variables [9]. This method can replicate the framework of randomized controlled trials (RCTs) since diversity of SNPs is randomly distributed by nature. Given that RCT implementation can be impeded by small-scale and ethical issues, new MR algorithm offers a substitute for causal inference with appealing edges of lower cost and greater feasibility [10]. Meanwhile, compared to observational studies, MR overcomes the interference of potential confounding factors and reverse causality as genetic instruments are independent of environmental influences [11]. Here, we intend to identify the immunophenotypes and inflammatory profiles with causal implications for SS via Mendelian randomization analysis and provide insight into clarifying the potential genetic connections and searching for effective novel therapeutic targets.

Methods

Study design

Muiti-omics data for investigation were derived from genome-wide association studies (GWAS), a large-scale dataset accessible to the general public. All GWAS studies included in this paper were ethically approved by their institutions. Secondly, bidirectional two-sample Mendelian randomization analyses were performed to evaluate the causality of immunophenotypes, inflammatory factors and SS. Furthermore, we conducted mediation studies to explore how inflammatory variables mediate the causality between immunophenotypes and SS. The study design of our MR analysis is described in Fig. 1. Three key presumptions need to be met when screening instrumental variables (IVs) for our study: First, there needs to be a clear correlation between exposure and the genetic instruments used; Secondly, genetic instruments should be independent of potential confounders between exposure and outcome; Thirdly, genetic variants can only affect outcome through exposure [9]. All analyses were performed based on the guidance of STROBE-MR [12].

Multi-omics statistics from genome-wide association study

Statistics we used as exposure variables were extracted from the GWAS Catalog (https://www.ebi.ac.uk/gwas/), which has been the largest peripheral blood immunophenotype published so far. Therefore, it provides more comprehensive and reliable conclusions for causality

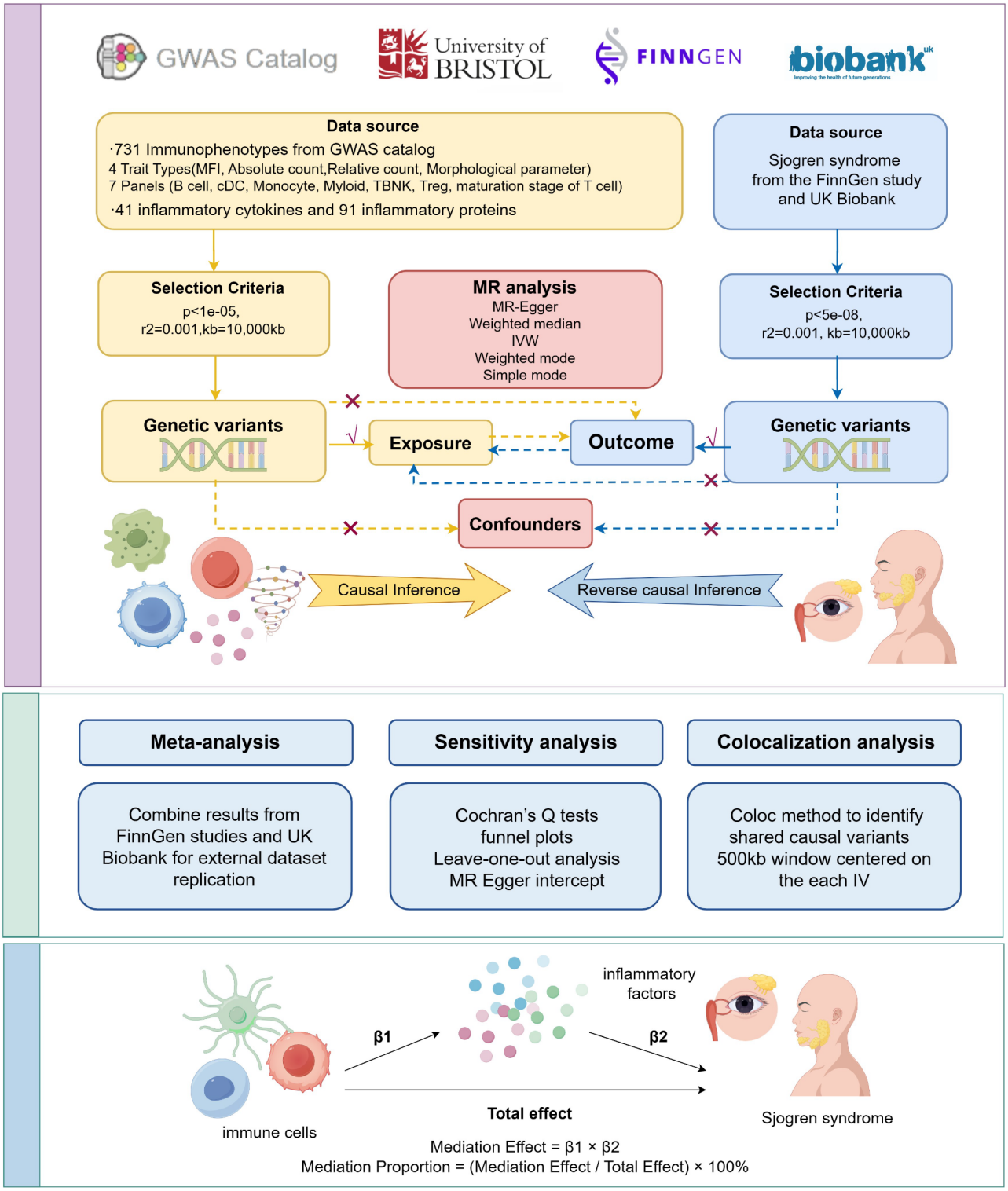

Fig. 1 Overview of the study design

estimation. The entry numbers range from GCST0001391 to GCST0002121 [13]. The summary of the dataset contains information of 731 immunophenotypes and around 22 million genetic variations from 3,757 individuals of European population adjusted for sex and age. It includes 4 trait types, namely 118 absolute cell counts (AC), 192 relative cell counts (RC), 32 morphological characteristics (MP) and 389 median fluorescence intensities (MFI)

representing surface antigen level. B cells, conventional dendritic cells (cDC), monocytes, myeloid cells, TBNK (T cells, B cells, natural killer cells), mature T cell stage, and Treg panels are all 7 panels included.

Additionally, the dataset for inflammatory cytokines was the meta-analysis summary statistics obtained from a GWAS study of the University of Bristol (https://data.bris.ac.uk/data/dataset), which involves 41 traits of circulating inflammatory cytokines from 8,293 Finnish individuals within three cohorts [14, 15]. Meanwhile, information on 91 inflammatory proteins was collected from another recently published study designed to explore the underlying inflammatory responses contributing to tissue damage as well as the pathogenesis of multiple autoimmune diseases [16]. A summary of this data can be extracted from the GWAS Catalog database (ID: GCST90274758-GCST90274848). Genetic adjustment for age, sex, body mass index, and other fundamental components, as well as population stratification, was undertaken in data collection (Table S1).

SS data from FinnGen studies and UK Biobank

The summarized data of SS (finngen_R10_M13_SJOGREN) was derived from a dataset contained in the latest documentation of the FinnGen study, recently updated on December 18, 2023, which can be accessed through the publicly available website of FinnGen consortiums (https://finngen.gitbook.io/documentation/). Diagnostic codes of M35.0 (ICD-10), 7102 (ICD-9), and 73,490 (ICD-8) were all included for the diagnosis of patients with SS, but the ICD-10 code was the major criterion. The participants were exclusively drawn from the European population, comprising 2,735 patient cases and 399,355 controls respectively with both males and females included [17]. We also used the dataset of ieu open gwas project (ebi-a-GCST90013879) obtained from UK Biobank as an external replication cohort for identified immunophenotype validation, which contains the sample size of 407,746 European individuals and 11,039,117 SNPs [18].

Screening for genetic instrument

When selecting instrument variants, we screened out single nucleotide polymorphisms (SNPs) that were strongly correlated with any immunological phenotype or inflammatory component. Differently, we adjusted the statistically significant threshold of strong association to 1×10^{-5} instead to avoid a limited number of SNPs when following a common threshold at $p<5\times10^{-8}$, as threshold relaxation was often adopted in order to incorporate a broader set of genetic instruments. Our SNPs included also satisfied the standard of eliminating linkage disequilibrium (LD), in which we set $R^2<0.001$ with a genetic distance of 10,000 Kb. Meanwhile, we harmonized all genetic variants between exposure and outcome for effect alignment. Last but not least, to ensure the strength of correlation, F-statistic values were calculated for SNP selection so as to prevent the marginal instrumental bias ($F>10$) [19]. The following formulas exhibit how F-statistic values and R were calculated in this study. [$F=R^2\times(N-2)/(1-R^2)$; $R^2=2\times EAF\times(1-EAF)\times\beta^2$](N is the sample size and EAF represents effect allele frequency).

Bidirectional mendelian randomization

Mendelian randomization is an epidemiological approach that utilizes genetic variants as an instrumental variable for the exposure to make causal inferences. To identify the potential connections of immune cells and SS condition, MR can serve as a useful tool to determine the causality. Our study was mainly conducted under R 4.3.2 software with the package TwoSampleMR, with meta, coloc, and ComplexHeatmap packages serving as indispensable complements. We employed multiple statistical approaches for MR analysis, including inverse-variance-weighted (IVW), MR-Egger regression, weighted median (WM), simple mode, and weighted mode [20]. The IVW technique is regarded as the principal approach to analyzing causality between immune characteristics and SS, which usually provides the most precise causal estimation under the condition that horizontal pleiotropy exists in MR analysis [20]. The other four methods offer an essential complement to the main method. The reverse MR analysis used the same methods [21, 22]. When defining positive correlations, we use the Benjamini–Hochberg (BH) approach to reduce the false discovery rate (FDR), enhancing the credibility of positive results.

Sensitivity analysis

In sensitivity analysis, Cochran's Q tests of both IVW and MR-Egger were utilized to examine if there was potential heterogeneity among different genetic variances selected during the analysis [23]. The symmetrical distribution pattern of the funnel plots also visualized the results of no heterogeneity. Leave-one-out analysis was also employed to evaluate the effect of eliminating specific SNP on the overall outcomes [24]. Additionally, MR Egger intercept was performed to determine the existence of horizontal pleiotropy. If $P>0.05$ was detected in these tests, it means no existence of horizontal pleiotropy [25]. All methods employed in sensitivity analysis are conducive to ensuring the robustness of the MR analysis.

External dataset validation and meta-analysis

Additionally, the robustness of significant findings was further confirmed by external dataset validation and Bayesian colocalization. To further validate the significant immunophenotypes identified in the primary analysis, we then repeated our study with the replication cohort in UK Biobank. We make sure both datasets

utilized in this study incorporate an adequate number of SNPs and sample size without population overlap. Based on their MR results, a meta-analysis was finally performed to combine MR estimates, which can be considered as ultimate results of external validation.

Bayesian colocalization analyses

Bayesian colocalization was implemented with the coloc method to identify the potential shared causal variants in immunophenotypes and SS [26]. We performed the colocalization analysis to examine if identified associations of immunophenotypes with SS were driven by linkage disequilibrium. MR outcomes further supported by colocalization provide much stronger evidence on causal inference. For each locus, the Bayesian method analyzed the existing evidence supporting five exclusive hypotheses: H0, there are no causal variants for either of the two traits; H1, causal variants are associated with exposure; H2, causal variants are associated with the outcome; H3, associations driven by distinct variants for exposure and outcome; H4, associations driven by shared causal variants for both two traits. If PP exceeds the threshold of 0.8 in model H4, it will be considered as evidence of colocalization. In our analysis, we detected independent SNPs at the threshold of 1×10^{-5} for the identified phenotype on a 500 kb genetic window centered on each IV to identify the shared variants in the colocalization analysis.

Mediation analysis

Mediation analyses are valuable in assessing the roles of mediators through which exposure exerts causal influence on the outcome [27]. Firstly, a two-sample MR analysis was performed between immunophenotypes significantly associated with SS and inflammatory factors, during which β1 was obtained. Subsequently, β2 was calculated by performing a second step of MR analysis between inflammatory factors and SS by applying the same two-sample MR techniques. We then calculated the mediation effect with the results of two steps MR (mediation effect=β1×β2). The total effect of immunophenotypes on SS was calculated in the previous MR analysis. Therefore, the direct effect can be calculated using the formula (direct effect=total effect - mediation effect). Meanwhile, the mediation proportion [mediation proportion = (mediation effect / total effect) × 100%] and 95% confidence intervals (CI) using the delta method can also be calculated [28]. Potential mediation effects exist when significant results are obtained in both steps, as well as the total causal relationship between exposure and outcome. Evidence is much stronger if mediation effects were detected significantly different from 0 when P value of mediation < 0.05.

Results

Identifying genetically associated immunophenotypes by MR

Five detection techniques were applied to the two-sample MR analysis in order to investigate the causality between immunophenotypes and SS. Our findings suggested that 36 subtypes of immune cells had potential causal relationships with SS, among which 2 phenotypes were significantly correlated with SS after FDR adjustment for P value (P_{FDR}<0.05). Results of IVW method of CD27 on IgD+CD24+B cell indicated a positive correlation between this immune phenotype and SS (OR=1.119, 95% CI: 1.061–1.179, P<0.001, P_{FDR} = 0.023), showing that it might become a risk factor in SS development. The other MR methods show similar results: weighted median (OR=1.121, 95% CI: 1.035–1.214, P=0.005), MR-Egger (OR=1.111, 95% CI: 1.032–1.196, P=0.009), simple mode (OR=1.030, 95%CI: 0.866–1.225, P=0.744), weighted mode (OR=1.142, 95% CI: 1.065–1.223, P=0.035), with consistency in β value direction (β>0). Comparably, results from the other phenotype of CD3 on CD45RA+CD4+Treg in IVW (OR=0.917, 95%CI: 0.877–0.959, P<0.001, P_{FDR} = 0.047) indicated that the immune cell was a protective factor for SS. Outcomes of other methods agreed with IVW: MR-Egger (OR=0.919, 95% CI: 0.863–0.978, P=0.012), weighted mode (OR=0.914, 95% CI: 0.863–0.967, P=0.004), simple mode (OR=0.959, 95%CI: 0.873–1.055, P=0.398) weighted median (OR=0.911, 95% CI: 0.858–0.967, P=0.002). All estimates of β were in the same direction (β<0). Results of our study are shown in Fig. 2 and Table S2.

Further, in sensitivity analysis, Cochran's Q tests of both IVW and MR-Egger were used to evaluate data heterogeneity, and both P>0.05 suggested no existence of heterogeneity in the MR study. The funnel plot and leave-one-out analysis also visualized the results. Meanwhile, since results from the MR-Egger intercept showed P>0.05, it can be concluded that there was no horizontal pleiotropy, and our hypothesis was established. Therefore, we can assume that our MR study was statistically significant and robust. The results of the sensitivity analysis are summarized in Table S2 and TableS3. In the reverse MR analysis, we considered SS as the exposure variable and all 731 immunophenotypes as the outcomes. After FDR adjustment, none of the results were statistically significant in detecting the casual relationship of SS on immune cells, and sensitivity analyses were also undertaken. (Fig. S1, Table S4 and Table S5)

External dataset validation, meta-analysis, and colocalization analyses

Significant phenotypes in the primary study were further validated by a combined analysis of FinnGen studies and UK Biobank. (Table S6, Table S7 and Table S8).

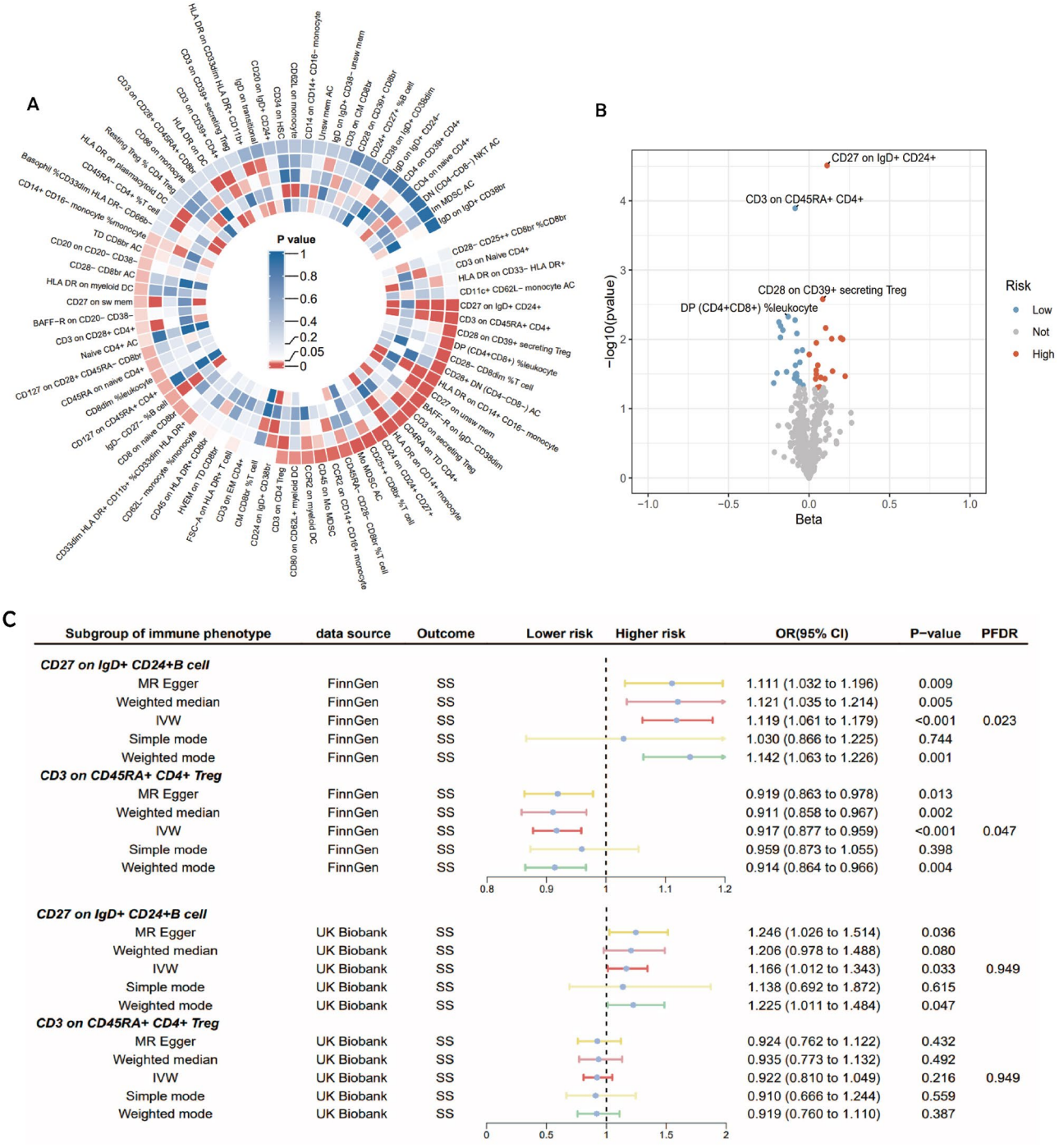

Fig. 2 Causal estimates of significant immunophenotypes identified by MR. In circular heatmap, circles from the outside to the center display the preliminary MR estimates for casual relationships of immunophenotypes and SS with five different methods of MR analysis, which are Inverse variance weighted, Weighted median, MR Egger, Simple mode and Weighted mode, respectively. Seventy-five traits of immune cells with at least one method at nominal significance ($P < 0.05$) are exhibited in the heatmap highlighted in red. The legend in the middle of the graph explains how shades of color represent the P value in all methods. (**A**) The volcano plots exhibit the results of IVW in causal estimates between immunophenotypes and SS (**B**). Forest plot of significant MR results of casual associations between immune phenotypes and SS with FDR adjustment. (**C**)

Their results are supportive of the significant associations of CD27 on IgD+CD24+B cell on SS and CD3 on CD45RA+CD4+Treg on SS. In the meantime, meta-analysis also confirmed 27 other putative genetic causal links between immunophenotypes and SS, as Fig. 3 illustrates. Colocalization has been used to assess the possible genetic relationships between two detected immune cells and SS in order to calculate the probability

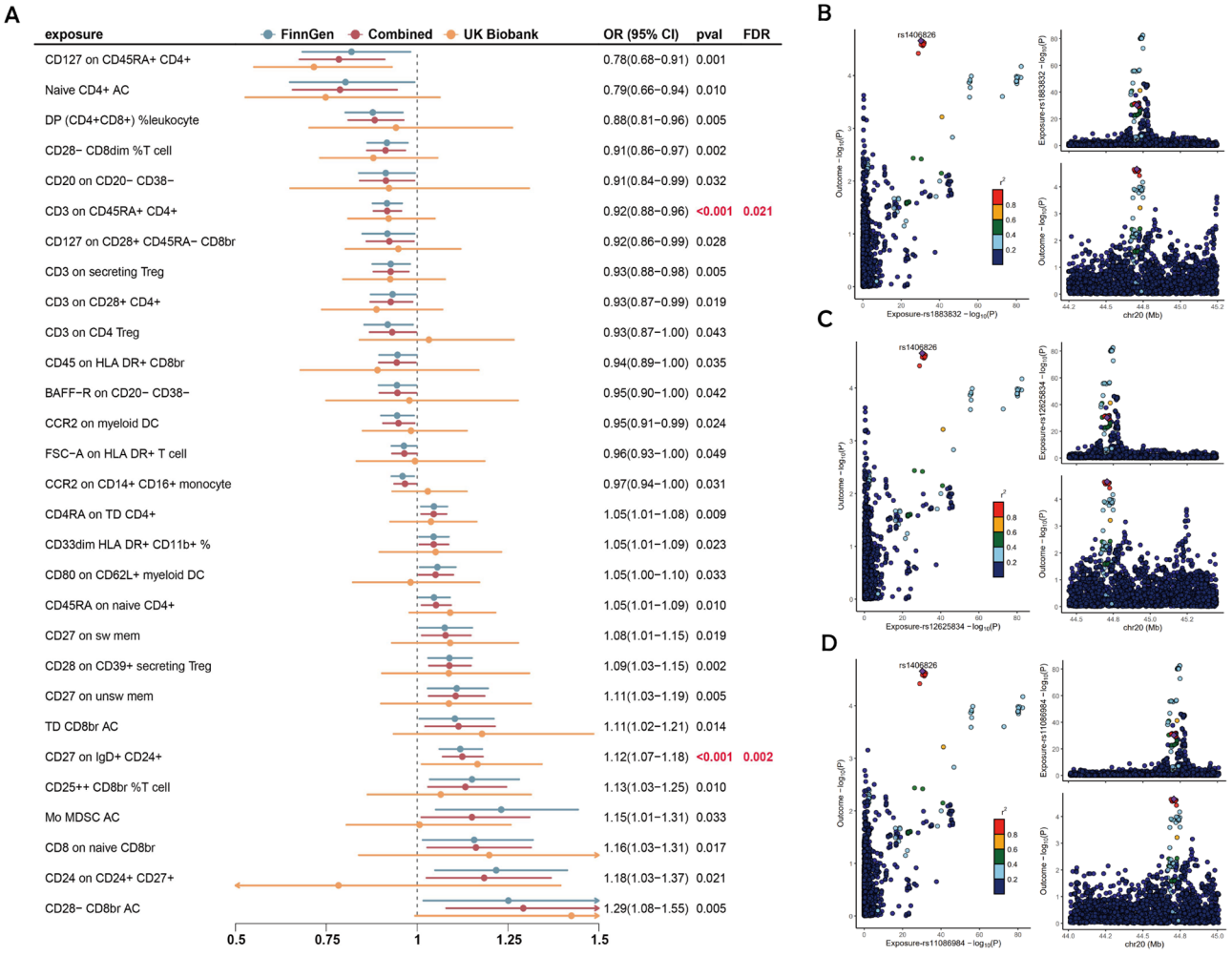

Fig. 3 Meta-analysis of MR IVW results of cohorts from FinnGen studies and UK Biobank and colocalization analyses. The forest plot exhibits meta-analysis of MR IVW results of both cohorts from FinnGen studies and UK Biobank for validation of the identified association between immune cells and the risk of SS. (**A**) And the results of Bayesian colocalization analysis in significantly associated immune cells are also provided. SNP labeled in the graph is identified as the lead SNP, which has the largest PP.H4 value. (**B**-**D**)

of a shared causal genetic variant. Results of our colocalization suggested that three common causal variants were colocalized shared genetic variants, including rs11086984, rs1883832 and rs12625834 between CD27 on IgD+CD24+B cell and SS, given that the largest PP for H4 was 0.924 (rs11086984), 0.923 (rs1883832), 0.922 (rs12625834) respectively within the 500 kb window around top SNP. Therefore, this is supportive of shared causal variants between CD27 on IgD+CD24+B cell and SS, providing strong evidence for the causal inference. Meanwhile, no shared causal variants passing the threshold (HPP4>0.8) were identified in the colocalization analysis between CD3 on CD45RA+CD4+Treg and SS. Results of external validation and colocalization are summarized in Fig. 3 and supplementary tables (Table S9–S12).

Exploring the causal effects between inflammatory factors and SS

To explore the roles of inflammatory factors within the causal relationship, MR analysis between inflammatory factors and SS was performed, and results of five methods are summarised in Fig. 4 and Fig. S2. In primary MR analysis of 41 inflammatory cytokines, 4 traits, including IL-5, G-CSF, MIG, and IL-2RA, were estimated to be causally associated with Sjogren syndrome development. The results of IVW method indicated that an increased level of MIG had a positive correlation with an increased chance of SS (OR=1.170, 95% CI: 1.008–1.358, P=0.038), while IL-5 (OR=0.847, 95% CI: 0.744–0.963, P=0.011), G-CSF (OR=0.839, 95% CI: 0.711–0.990, P=0.038) and IL-2RA (OR=0.895, 95% CI: 0.806–0.995, P=0.040) were reported as protective factors related to a lower risk of SS. However, no significant results were noted between inflammatory cytokine levels and Sjogren syndrome at

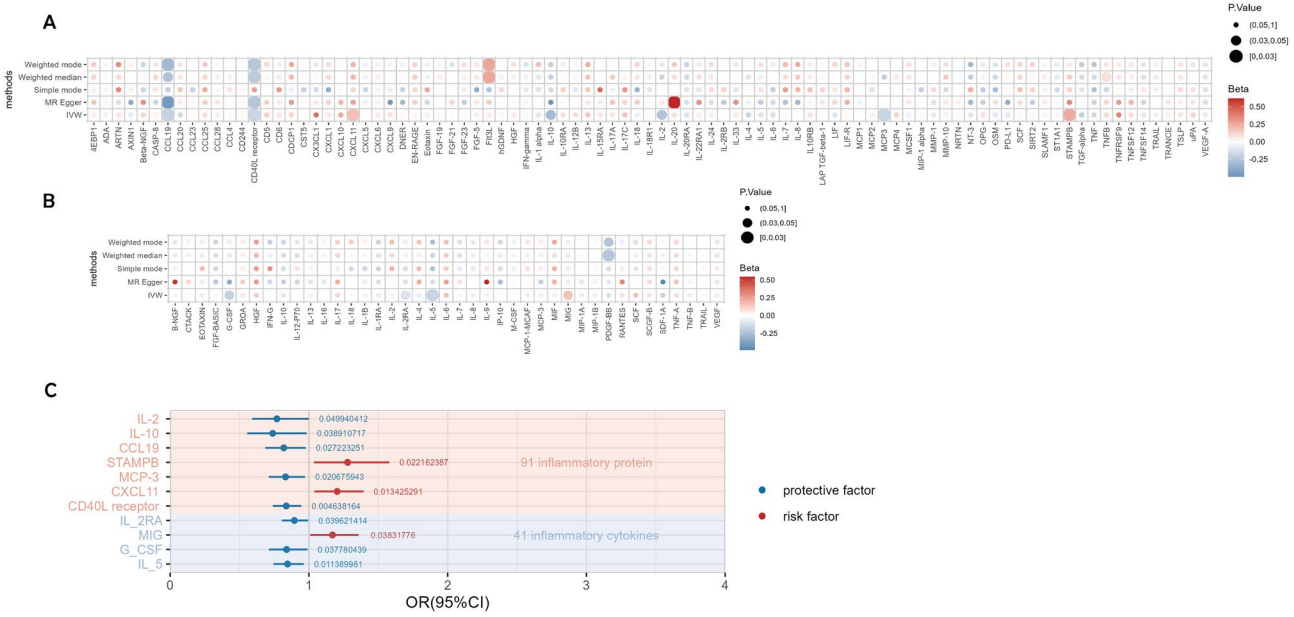

Fig. 4 Heatmaps and forests plots illustrating potential significant inflammatory profiles. Two heatmaps exhibit the preliminary causal estimates for associations between 41 inflammatory cytokines and SS (**A**) and 91 inflammatory proteins and SS (**B**) using five major methods of MR analysis. P values in these plots are represented by shades and the size of circles, as described in the legend. The multi-group forest plot shows the potential positive results in both MR analyses. (**C**)

the threshold of FDR correction (Table S13). In the sensitivity analysis, no heterogeneity or horizontal pleiotropy was found. (Table S14). Results of reverse MR between SS and 41 inflammatory cytokines as well as sensitivity analyses are summarised in supplementary materials (Table S15, Table S16). Reverse MR analysis showed genetic liability of SS with levels of identified inflammatory factors. Among 41 traits included, both HGF (OR=0.919, 95% CI: 0.849–0.993, *P*=0.034) and IP-10 (OR=1.161, 95% CI:1.019–1.322, *P*=0.025) were estimated to have potential associations with SS development.

In analysis of 91 inflammatory proteins, a total of 7 proteins, comprising CD40L receptor levels, C-X-C motif chemokine 11 levels, Monocyte chemoattractant protein-3 levels, STAM binding protein levels, C-C motif chemokine 19 levels and Interleukin-10 levels and Interleukin-2 levels had causal relationships with Sjogren syndrome. Among them, results of IVW suggested that elevated levels of C-X-C motif chemokine 11 (OR=1.204, 95% CI: 1.039–1.394, *P*=0.013) and STAM binding protein levels (OR=1.280, 95% CI: 1.036–1.580, *P*=0.022) can be recognized as risk factors contributing to SS development. On contrary, CD40L receptor levels (OR=0.837, 95% CI: 0.740–0.947, *P*=0.005), Monocyte chemoattractant protein-3 levels (OR=0.831, 95% CI: 0.711–0.972, *P*=0.021), C-C motif chemokine 19 levels (OR=0.819, 95% CI: 0.686–0.978, *P*=0.027), Interleukin-10 levels (OR=0.739, 95% CI: 0.555–0.985, *P*=0.039) were associated with a reduced risk of SS (Table S17). Similarly, the absence of heterogeneity and horizontal pleiotropy in our study was further validated by the sensitivity analysis of the MR- Cochran's Q tests and Egger Intercept (Table S18). Results of reverse MR between SS and 91 inflammatory proteins and related sensitivity analyses were also summarised in supplementary materials (Table S19, Table S20). According to MR results in reverse, SS was causally related to increased levels of multiple factors, C-C motif chemokines such as CCL25, CCL4, interleukins such as IL-15RA, IL-17 A, IL-17 C, IL-1alpha, and PD-1 L, and TNF-related activation-induced cytokine levels.

Mediation effects of immune cells on SS via inflammatory factors

In mediation analysis, we evaluated the mediating effects of 41 inflammatory cytokines and 91 inflammatory proteins to explore their potential roles in mediating the causal effect of the identified immune cells in Sjogren syndrome pathogenesis. Our results are summarized in Table 1 and Fig. S3. As revealed by our mediation analysis, 22 mediating relationships of inflammatory factors have been identified. In the causal relationship between CD27 on IgD+CD24+B cell and SS, we identified that CD40L receptor levels in circulation had significant mediating effects (β=0.0314, 95% CI: 0.0004–0.0624, *P*=0.0471) at a mediation proportion of 28% (95% CI: 0.364-55.6%). Meanwhile, a significant positive mediation influence was also found in the causal association between CD27 on switched memory B cell levels (β=0.0436, 95% CI: 0.0041–0.0831, *P*=0.0305) and SS,

Table 1 Mediation analysis of significant immune cells associated with SS

Exposure	Mediator	Outcome	Total effect	Direct effect	Mediation effect	*P* value	Mediation proportion
CD27 on IgD + CD24 + B cell	CD40L receptor levels	SS	0.1122	0.0808	0.0314 (0.0004,0.0624)	0.0471*	28% (0.364%, 55.6%)
CD80 on CD62L + myeloid DC	CD40L receptor levels	SS	0.0542	0.0480	0.0063 (-0.0131,0.0256)	0.5250	11.6%
IgD- CD27- %B cell	CD40L receptor levels	SS	-0.1396	-0.0691	-0.0705 (-0.196,0.0549)	0.2706	50.5%
CD8dim %leukocyte	CD40L receptor levels	SS	-0.0777	-0.0724	-0.0053 (-0.0247,0.014)	0.5908	6.83%
CD27 on switched memory B cell	CD40L receptor levels	SS	0.0732	0.0296	0.0436 (0.0041,0.0831)	0.0305*	59.6% (5.59%, 114%)
CD27 on unswitched memory B cell	CD40L receptor levels	SS	0.1035	0.0573	0.0462 (-0.0008,0.0933)	0.0541	44.7%
DP (CD4 + CD8+) %leukocyte	C-X-C motif chemokine 11 levels	SS	-0.1301	-0.1222	-0.0078 (-0.032,0.0163)	0.5251	6.02%
HLA DR on myeloid DC	C-C motif chemokine 19 levels	SS	-0.0769	-0.0856	-0.0087(-0.0268, 0.0442)	0.6300	-11.3%
FSC-A on HLA DR + T cell	C-C motif chemokine 19 levels	SS	-0.0371	-0.0422	0.0050(-0.0304,0.0405)	0.7803	-13.6%
Terminally Differentiated CD8 + T cell	Interleukin-10 levels	SS	0.0989	0.0784	0.0205(-0.0662,0.107)	0.6429	20.7%
CD80 on CD62L + myeloid DC	Interleukin-10 levels	SS	0.0542	0.0437	0.0105(-0.0761,0.0972)	0.8118	19.4%
CD45 on HLA DR + CD8 + T cell	Interleukin-10 levels	SS	-0.0554	-0.0436	-0.0118(-0.0984,0.0749)	0.7899	21.3%
Monocytic Myeloid-Derived Suppressor Cells	Interleukin-10 levels	SS	0.2083	0.1959	0.0124(-0.0743,0.099)	0.7794	5.95%
CD27 on unswitched memory B cell	Interleukin-10 levels	SS	0.1035	0.0950	0.0086(-0.0781,0.0952)	0.8465	8.27%
CD3 on CD28 + CD4 + T cell	Monocyte chemoattractant protein-3 levels	SS	-0.0699	-0.0771	0.0072(-0.0127,0.0361)	0.6261	-10.3%
CD3 on secreting Treg	Monocyte chemoattractant protein-3 levels	SS	-0.0755	-0.0821	0.0066(-0.0224,0.0354)	0.6571	-8.67%
CD80 on CD62L + myeloid DC	STAM binding protein levels	SS	0.0542	0.0626	-0.0083(-0.0604,0.0437)	0.7538	-15.4%
CD25 + + CD8br %T cell	STAM binding protein levels	SS	0.1408	0.1299	0.0109(-0.0412,0.063)	0.6825	7.72%
CD3 on CD45RA + CD4 + Treg	MIG	SS	-0.0866	-0.094	0.0074 (-0.016,0.0308)	0.5358	-8.55%
CD33dim HLA DR + CD11b+ %CD-33dim HLA DR + myeloid cell	MIG	SS	0.0443	0.0506	-0.0064 (-0.0298,0.017)	0.5932	-14.4%
CD27 on unswitched memory B cell	G_CSF	SS	0.1035	0.1137	-0.0102(-0.0392,0.0188)	0.4917	-9.84%
CD3 on secreting Treg	G_CSF	SS	-0.0755	-0.0691	-0.0065(-0.0354,0.0225)	0.6624	8.54%

The 95% CI for the mediation proportion will not be calculated if it covers 0. *represents a P value less than 0.05, indicating a significant effect of mediation

with a mediation ratio of 59.6% (95% CI: 5.59-114%). Additional 20 immune cell-SS pathways mediated by inflammatory cytokines also exhibited potential evidence based on our findings. Apart from the two immunophenotypes mentioned above, causal effects of CD80 on CD62L + myeloid DC, IgD- CD27- %B cell, CD8dim %leukocyte, CD27 on unswitched memory B cell on SS can also mediated by CD40L receptor levels at 11.6%, 50.5%, 6.83% and 44.7% respectively. It is also noteworthy that the pathway from DP (CD4 + CD8+) % leukocyte to SS was potentially mediated by C-X-C motif chemokine 11 levels at 6.02%. Moreover, mediating effects of HLA DR on myeloid DC and FSC-A on HLA DR + T cell to SS through C-C motif chemokine 19 levels were −11.3% and −13.6%. Mediating ratios of Terminally Differentiated CD8 + T cell, CD80 on CD62L + myeloid DC, CD45 on HLA DR + CD8 + T cell, Monocytic Myeloid-Derived Suppressor Cell and CD27 on unswitched memory B cell through Interleukin-10 levels were 20.7%, 19.4%, 21.3%, 5.95% and 8.27% respectively. Monocyte chemoattractant protein-3 levels mediated CD3 on CD28 + CD4 + T cell, and CD3 on secreting Treg on SS at ratios of -10.3% and −8.67%. STAM binding protein levels also mediated CD80 on CD62L + myeloid DC and CD25 + + CD8br %T

cell at proportions of -15.4% and 7.72%. The mediating effects of CD3 on CD45RA+CD4+Treg and CD33dim HLA DR+CD11b+ %CD33dim HLA DR+myeloid cell by MIG were at -8.55% and −14.4%, and the mediation effects of CD27 on unswitched memory B cell and CD3 on secreting Treg through G-CSF were −9.84% and 8.54%.

Discussion

Our study performed an MR analysis on the causality between immunophenotypes, inflammatory factors, and SS in a bidirectional way, using publicly available data retrieved from GWAS. With the support of five main methods in statistical analysis, we managed to confirm the significant causal associations between immunophenotypes and SS, providing evidence for further study into the pathogenesis of the disease and potential targets for treatment. According to our findings, CD27 on IgD+CD24+B cells can contribute to an increased risk of SS. On the contrary, an increase of CD3 on CD45RA+CD4+Treg becomes a protective factor against SS development. Interestingly, several inflammatory factors may have potential causal effects on SS, among which CD40L receptor levels may serve as a mediator in causality between CD27 on IgD+CD24+B cells and SS as well as CD27 on switched memory B cells and SS.

Our study observed an association between CD27 on IgD+CD24+B cells and SS, which indicated the role of this immunophenotype in promoting SS development. IgD+CD27+B cell is a subset of unswitched memory B cells and CD24 is a molecule involved in cell signaling and adhesion [29]. CD27 has been observed to be expressed on somatically mutated B cells and is considered as a positive marker for memory B cells in blood circulation. For decades, studies have highlighted the significant pathogenic roles of B cells in the progression of Sjogren syndrome [30, 31]. It is demonstrated that the abnormal autoimmune response and antibody production in SS are associated with dysregulation of B cell subsets in the affected tissue [32]. Earlier a subset within the CD24(hi)CD27(+) B-cell subpopulation that negatively regulates monocyte cytokine production through IL-10-dependent pathways was found to present in patients with Sjögren syndrome and other kinds of autoimmune diseases [33]. One study in 2019 further explored the participation of CD27+B cells in the pathogenesis of the disease by comparing subsets extracted from patients diagnosed with primary Sjogren syndrome with healthy controls. The results indicated that an increased number of CD27+memory B cells as well as long-lived autoantibodies-producing plasma cells in the glandular tissue affected might have associations with etiology of the disease [34]. Furthermore, a recent study confirmed that the quantity and maturity of CD27+circulating antibody-secreting cells are increased and closely associated with Sjogren Syndrome, thus being regarded as promising biomarkers as well as potential targets for treatment [35]. As for progress in targeted therapy, the application of rituximab, a monoclonal antibody targeting CD20, has been initiated in SS patients with severe and refractory systemic diseases [36]. It was also indicated to have an impact on the phenotype of CD24+IgD B cells, while current data attained from clinical trials in large-scale RCTs is controversial and unsatisfying [37]. Based on current study, a deeper understanding of the pathogenic roles of this phenotype, expression of CD27 and their correlations with specific autoantibodies or cytokines might be conducive to a better understanding of the pathogenesis and development of potential therapeutic strategies.

Additionally, our study also provided evidence for a reduced risk of SS caused by increased CD3 on CD45RA+CD4+Treg. Treg cells, particularly CD4+Treg cells, are pivotal regulators against autoreactive and harmful inflammatory responses, thus maintaining immune homeostasis [38]. CD45RA is the high molecular weight form of CD45 typically expressed by non-activated CD4+Treg cells [39]. Various subsets of T cells, including Treg cells, have proved to be essential for the development of SS, although many potential mechanisms remain unknown and require further elucidation [40, 41]. In recent studies, the number of Treg cells was detected to decrease in patients with SS and negatively correlated with disease biomarkers such as IgG antibodies and rheumatoid factor levels [42]. Furthermore, an imbalanced Th17/Treg ratio was suggested to play a role in SS development. Considering that the normal function of Treg cells is vital to maintaining immune tolerance and restricting aberrant autoimmune responses, the impaired Treg function and stability, as well as the over-proliferation of Th17, are closely related to the pathogenesis of the disease [42, 43]. Recently, many novel treatments targeting Treg have been proposed. These treatments promote Treg proliferation and inhibit Th17 polarization, thereby helping to restore the Th17/Treg balance [8, 44]. Therefore, Treg cells are suggested to be involved in underlying mechanisms of impaired immune tolerance in Sjogren syndrome, and its implications for therapy are worthy of further exploration.

An autoimmune response in SS can be directed by specific cytokines production, which may serve as potential biomarkers for monitoring disease activity. IL-4, IL-5 and IL-10 are Th2-related cytokines associated with chronic inflammatory responses in autoimmune diseases [45]. Research indicates that T cell cytokines, including IFN-gamma and IL-2, are expressed in the salivary glands of patients with SS, whereas IL-4 and IL-5 are not detected

[46, 47]. One study using animal models demonstrated significant changes in serum levels of IL-2, IL-5 and GM-CSF, as well as negative correlations between IL-4 and TNF-alpha in saliva and salivary secretion [48]. The results of mediation analysis in this study indicate that the causal association of CD27 on IgD+CD24+B cells and CD27 on switched memory B cells with SS can be mediated by circulating CD40L receptor levels. Actually, CD40 and its ligand CD40L are widely expressed in various cell types, particularly CD40 on B cells and myeloid cells, and CD40L on T cells, which play important roles as the stimulatory immune checkpoints and are related to the survival, proliferation, antibody production and antibody isotype switching of B cells [49]. Previous studies have detected increased expressions of CD40/CD40L on epithelial cells of the salivary gland and infiltrating lymphocytes in samples of SS patients compared with control groups. CD40 and CD40L are suggested to play a role in protecting the infiltrating lymphocytes from apoptosis in SS pathogenesis [50, 51].

In the last decade, great progress has been made in comprehending the pathogenesis of Sjogren syndrome, which offers new opportunities towards targeted and individualized therapeutic strategies [52]. Ongoing clinical trials are evaluating the effectiveness of therapies targeting inflammatory cytokines, surface makers of T and B cells and their pathway activation [53–56]. An important pathway of co-stimulation can be activated by CD40 and CD40L interaction. Therefore, its dysregulation can contribute to progression of various autoimmune diseases, such as SS. Iscalimab is an anti-CD40 monoclonal antibody with the blocking effect of CD40 signaling, which has been confirmed to show initial clinical benefits in a randomized, double-blind, phase 2b clinical trial [57]. Meanwhile, approaches targeting Treg cells for SS treatment, including various IL-7 receptor antagonists, are also under active investigation [58]. In the coming years, continued research should focus on further elucidating the complex mechanisms underlying Sjogren syndrome, and at the same time, more extensive clinical trials will be required to fully assess the long-term effectiveness and safety of emerging targeted therapies. Innovative discoveries made by biomedical analysis provide solid foundation for further exploration of their roles via animal models and promote their translation into therapeutic fields.

This study has strengths in several aspects. First and foremost, it was the first MR study to dissect the causal associations between the immune cells, inflammation and SS, with the largest scale dataset of immunophenotypes and inflammatory traits publicly available to date. Consequently, two immune cell phenotypes identified significantly associated with SS provided novel insights for exploring potential immunotherapeutic targets in SS treatment. Meanwhile, with five methods of MR analysis and various approaches to examine sensitivity, we ensured the accuracy and robustness of our statistical analysis in the absence of potential horizontal pleiotropy. Our study also has limitations. Firstly, our data on immune cell phenotypes were limited to blood samples. Their accumulation and interaction in the affected tissue, and the role of peripheral immunity in disease pathogenesis, were insufficiently illustrated. A multi-omic approach involving tissue-specific data from salivary glands would enhance the clinical relevance and significance of our results. Secondly, in epidemiology, SS often shows a higher prevalence among individuals with a non-European background. Due to current limitations in available data with ethnic diversity, we will seek to collaborate with research teams from different regions and ethnic backgrounds to ensure that our findings are applicable to a wider range of populations. This could involve accessing datasets from other countries or conducting multicenter studies that include participants from diverse ethnic groups. Additionally, in future research, we plan to delve deeper into the combined genetic and proteomic data by incorporating epigenetic and environmental factors into our analysis. By including these factors, we hope to gain a more comprehensive understanding of the complex mechanisms underlying SS pathogenesis and open up new avenues for therapeutic intervention.

Conclusions

Our study proved the causal relationships between immunophenotypes, inflammation and SS through a bidirectional two-sample MR and mediation analysis. This opens avenues for further exploration into the pathogenesis of SS and facilitates the identification of potential immunotherapeutic targets.

Abbreviations

SS	Sjogren syndrome
MR	Mendelian randomization
OR	Odd ratio
IVW	Inverse-variance weighted
WM	weighted median
FDR	False discovery rate
CD	Cluster of Differentiation
IL	Interleukin

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Author contributions
JYX: Conceptualization; Formal analysis; Writing – original draft. SCS: Conceptualization; Methodology; Writing – original draft. YJH: Writing – original draft. LZ: Writing – review & editing. JXZ: Writing – review & editing. All authors read and approved the final manuscript.

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Data availability
All data generated or analysed during this study are included in this published article and its supplementary information files. Data of GWAS catalog can be accessed through https://www.ebi.ac.uk/gwas/. Data of FinnGen studies are available through https://www.finngen.fi/en. Other dataset mentioned in the article can also be accessed as referenced.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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