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ARTICLE

ST2 and CSF-1 as potential druggable targets of inflammatory bowel diseases: Results from two-sample Mendelian randomization study

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Abstract

Novel druggable targets are warranted for inflammatory bowel disease (IBD) treatment. We aimed to identify novel circulating proteins with causal associations with the risk of IBDs and provide potential therapeutic targets for IBD treatment. We performed a two-sample Mendelian randomization (MR) study to explore the associations of 55 circulating biomarkers on the risk of IBD, Crohn's disease (CD), and ulcerative colitis (UC) by leveraging the summary statistics from large genomewide association studies and protein quantitative trait loci studies. The individual estimate was pooled together by meta-analyses to estimate the causal effects of each outcome. In univariable MR, we identified several circulating proteins showed potential correlation with IBD, UC, and CD. Of note, we observed that a genetically proxied increased level of suppression of tumorigenicity 2 (ST2) was associated with an elevated risk of IBD (odds ratios [ORs] 1.133, 95% confidence interval [CI] 1.091–1.176, $p < 0.0001$), CD (ORs 1.188, 95% CI 1.103–1.281, $p < 0.0001$), and UC cohorts (ORs 1.087, 95% CI 1.050–1.125, $p < 0.0001$). Additionally, we observed a consistent positive correlation between the level of CSF-1 and the increased risk of IBD in individual MR, with statistically significant causal associations in the meta-analyses with ORs equal to 1.217 (IBD, 95% CI 1.115–1.328, $p < 0.0001$), 1.223 (CD, 95% CI 1.082–1.382, $p = 0.0013$), and 1.179 (UC, 95% CI 1.055–1.317, $p = 0.0037$). This study provided evidence for potential casual associations between circulating ST2 and CSF-1 levels, and increased risks of IBD, UC, and CD, implicating potential treatment targets for IBD and subtypes.

Jiarui Mi, Xia Wu, and Xiaoyin Bai contributed equally to this study.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Circulating biomarkers like CSF-1 and ST2 have been reported related to inflammatory bowel diseases (IBDs), but whether they are causal factors to IBDs are unknown.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study identified novel circulating proteins which have potential causal associations with IBDs.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Elevated circulating ST2 and CSF-1 levels are potentially causally correlated with increased risks of IBD, ulcerative colitis, and Crohn's disease.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

It provides novel insights into CSF-1 and ST2 as potential therapeutic targets for IBD treatment.

INTRODUCTION

Inflammatory bowel disease (IBD) is a group of diseases characterized by chronic, relapsing inflammation in the gastrointestinal tract with causes and pathogenesis not fully understood. The current treatment strategies for IBD, including ulcerative colitis (UC) and Crohn's disease (CD), can be divided into small molecule drugs and biological immunomodulator therapies. In recent decades, the treatment of IBD has evolved significantly due to the better understanding of the role of TNF-α, IL-12/23, integrins, etc. on the pathological roles of IBD. In the meanwhile, targeted immune-treatment showed promising results in a subset of patients with IBD.[1] Yet, the therapies are still not definitely curative; about 30% of the patients do not respond to primary biological therapies, and another 30% of patients are refractory due to the secondary loss of response.[2,3] As such, it is essential to continue exploring the underlining mechanisms of IBD development and making progress in identifying new drug therapies in promoting disease remission and improving the quality of life for patients with IBD.

With the application of serum proteomic profiling techniques, a wide range of circulating proteins have been studied in patients with IBD, as they can be used as biomarkers to monitor disease activity, predict disease outcomes, or as targets for pharmacological intervention.[4–6] However, most of the studies are cross-sectional or retrospective. Researchers are unable to provide consolidated evidence indicating the potential causal associations of the proteins on the disease risks, due to the fact that conclusions of these studies can be biased by measured or unmeasured confounding factors as well as reverse causations. Meanwhile, prospective cohort studies are laborious and require rigorous quality control and repeated measurements, which is hardly manageable in large cohorts.

In the past 2 decades, with the development of novel genomics technologies, researchers are now able to investigate human genetics information in a very large-scale fashion. In particular, genomewide association studies (GWASs) have been widely performed in different populations to discover genetic loci driving complex phenotypes, especially human diseases. A substantial number of novel methods based on GWAS summary statistics have been introduced for disease risk assessment (polygenic risk scores) and finding shared genetic predispositions by cross-traits analysis. Among these methods, Mendelian randomization (MR) is particularly useful to unravel the underlying causal associations between the exposures and the disease outcome. The underlying rationale of MR is to leverage valid single nucleotide polymorphisms (SNPs) as instrumental variables (IVs) to proxy the exposure of interest, and to investigate the collective effects of these SNPs on the risk of the outcomes. Using valid IVs, we can remove pleiotropic effects to the maximum extent. In addition, because the human genetic information is segregated and reunited during gametes formation in a random fashion, and such genetic information remains consistent throughout the disease progression, the conclusions of MR are resistant to reverse causality. Using MR, recent studies have identified novel circulating protein biomarkers associated with risks of diseases, such as type 1 diabetes, cardiovascular diseases, and pulmonary fibrosis.[5,7–9] More interestingly, MR has recently been applied to identify potential druggable targets with the emergence of large-scale expression quantitative trait loci (QTL), protein QTL (pQTL), and metabolite QTL data.[10] Such application is of

particular clinical importance, as it not only demonstrates the risk factors, but also indicates potential drug targets useful for further interventions.

In this study, we performed comprehensive two-sample MR analyses to identify novel circulating protein biomarkers for IBDs as potential risk factors and therapeutic targets. We used the summary statistics from several well-acknowledged largest GWASs and pQTL studies in European ancestry and identified several circulating proteins that showed therapeutic values for further investigations.

METHODS

Study design

Here, we performed a univariable two-sample MR study by leveraging druggable target pQTL datasets and summary statistics of IBD, CD, and UC GWASs originate from multiple cohorts in European ancestry using inverse-variance weighted (IVW) method. Various sensitivity analyses were also performed to consolidate the findings. Last, we pooled the effects together by performing meta-analyses to give an overall estimate of causal effects. Prior to the analysis, the three major assumptions of MR need to be examined: First, the IVs are supposed to demonstrate associations with the exposures of interest. Second, the IVs are independent of any known confounders. This was done by searching for pleiotropic effects through Phenoscanner (http://www.phenoscanner.medschl.cam.ac.uk/) and assessment of linkage disequilibrium (LD; the default setting: p value $= 1\times10^{-5}$, $r^2 = 0.8$, reference genome build GRCh37). Finally, the effects of the IVs on the outcomes are mediated solely by the exposures of interest.

All the original studies had received written informed consent from the participants and acquired ethical approvals from the local research ethics committees and institutional review boards.

The selection of genetic proxied variants and data source

The circulating proteins and the IVs were selected from the summary statistics of the Olink CVD-I pQTL dataset from 13 cohorts with more than 30,000 participants of European ancestry.[5] In the presence of between-study heterogeneity, the genetic variants need to surpass a Bonferroni-corrected p value threshold ($p<5.6\times10^{-10}$) in discovery studies and demonstrate suggestive significance in the replication studies with the same directional beta coefficients in the presence of between-study heterogeneity. They also need to demonstrate conventional genomewide significance ($p<5\times10^{-8}$) in the meta-analyses of discovery and replication datasets. Next, we selected independent (LD $R^2 = 0$) variants, including cis- and trans-pQTLs, as genetic proxies for the level of circulating proteins. Last, we searched the SNPs on Phenoscanner with p value $= 1\times10^{-5}$ and $R^2 = 0.8$ to rule out measurable potential pleiotropic effects. After clumping and harmonization, circulating proteins with more than one SNP can be used as IVs were studied. Overall, 55 unique circulating biomarkers with 523 IVs were tested for causal associations with IBDs, including UC and CD. The complete information on the selected IVs for individual measurements are shown in Table S1.

We used summary-level statistics from three large-scale IBD, CD, and UC GWAS cohorts (by de Lange et al., International Inflammatory Bowel Disease Genetics Consortium [IIBDGC], and FinnGen R5) for the two-sample MR analyses. The detailed information for each outcome dataset is listed in Table S2. The reference genome built for GWAS data is GRCh37(hg19) across different datasets. The size for the LD block for SNP pruning is 500 kb with step size 50. We used 10,000 kb and $r^2 = 0.001$ for the clumping in the "TwoSampleMR" package.

Statistical methods

The IVW method was used as the major analysis for causal estimation. This method combines the Wald ratio of individual SNPs with a fixed-effect model when IVs less than or equal to three or random-effect model when IVs greater than three for the assessment of the outcome. The heterogeneity of the analyses was assessed with Cochran's Q values, I^2 statistics, and the H statistics based on previous publications.[11] In addition, we performed a meta-analysis to combine the IVW causal estimates using the fixed-effect model based on Cochran's Q value (Cochran's $Q\geq0.1$). We used the default setting (10,000 kb and $r^2 = 0.001$) for clumping.

Besides, sensitivity analyses were performed with MR-Egger, weighted median, and MR-PRESSO (Mendelian Randomization Pleiotropy RESidual Sum and Outlier) methods.

We used the MR-Egger method to identify potential pleiotropy effects based on the p value for the intercept[12] and the weighted median to provide precise causal estimates against invalid IVs.[13] We used the MR-PRESSO method to detect potential outliers and provide corrected causal estimates via outlier removal.[14] The F-statistics was introduced to rule out weak instruments using the approximation method described by Bowden et al.[15] IVs with F-statistic greater than or equal to 10 were considered effective for MR analyses. For each measurement, we performed the power calculation using the web-based tool mRnd (https://shiny.cnsgenomics.com/mRnd/).[16]

The MR results were two-sided, and any p value less than 0.0009 (0.05/55 adjusted with the Bonferroni method) was considered statistically significant. A p value between 0.05 and 0.0009 was considered suggestively significant. For the meta-analysis, p value less than 0.05 was considered statistically significant. All the analyses were performed on the R platform (version 4.0.2). The "TwoSampleMR" (0.5.5), "Mendelian Randomization" (0.5.0), "MR-PRESSO," "meta," "forestplot," and "upsetR" packages were used for statistical analyses and data visualization.[14,17,18]

RESULTS

Screening the circulating proteins for causal factors of IBD

MR analyses revealed possible causal relationships between 14 protein biomarkers and IBDs. The overall analysis results were shown in Table S3.The intersections of biomarkers in IBD subgroups are shown in an upset plot (Figure 1), where the left side bars indicate the total size of protein biomarkers in each subgroup. The top bars indicate the size of each intersecting set. Each black dot represents circulating protein biomarker(s) included in the intersection. After clumping and harmonization, the associations among the selected 55 biomarkers and the outcomes of interest were visualized using heatmap based on the IVW method (Figure 2). We observed positive causal effects of circulating ST2 on CD and IBD in all three cohorts, and on UC in two cohorts (de Lange et al. and IIBDGC; Figure 3); circulating colony-stimulating factor-1 (CSF-1) on CD and IBD in two cohorts (de Lange et al. and IIBDGC), and UC in one cohort (de Lange et al.; Figure 4); and CCL4 on CD (odds ratio [OR] 1.300, 95% confidence interval [CI] 1.074–1.573, $p = 0.007$), UC (OR 1.200, 95% CI 1.068–1.348, $p = 0.002$), and IBD (OR 1.233, 95% CI 1.135–1.340, $p = 7.40\,\text{E-}7$) in only one cohort (FinnGen R5). We observed negative causal effects of VEGF-D on CD in two cohorts, one from de Lange et al. (OR 0.817, 95% CI 0.714–0.936, $p = 0.003$) and the other

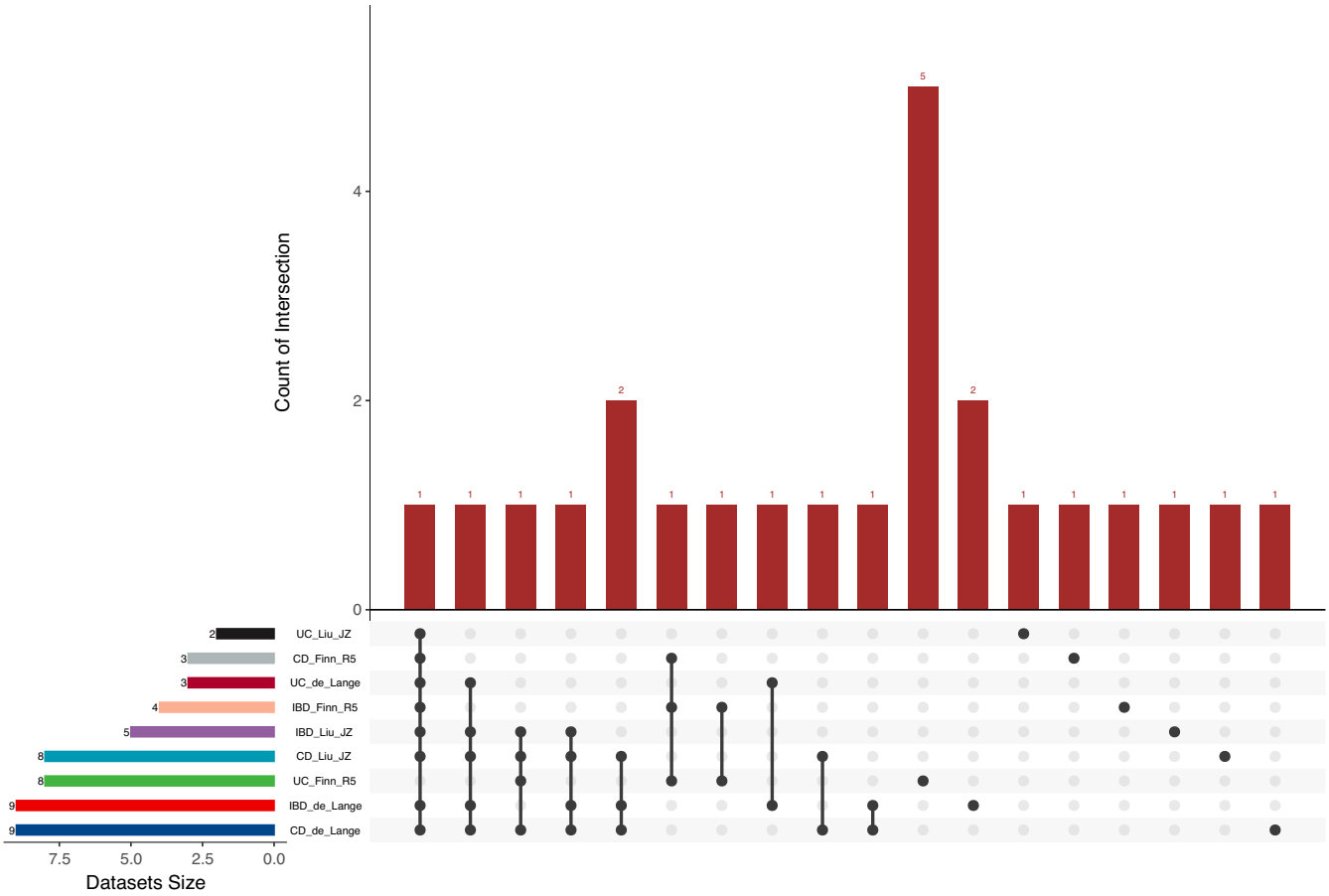

FIGURE 1 Upset plot showing the intersections of circulating proteins biomarkers in different IBD, UC, and CD subgroups. The left side bars indicate the total size of protein biomarkers in each subgroup. The top bars show the size of each intersecting set. Each black dot represents circulating protein biomarker(s) included in the intersection. CD, Crohn's disease; IBD, inflammatory bowel disease; UC, ulcerative colitis

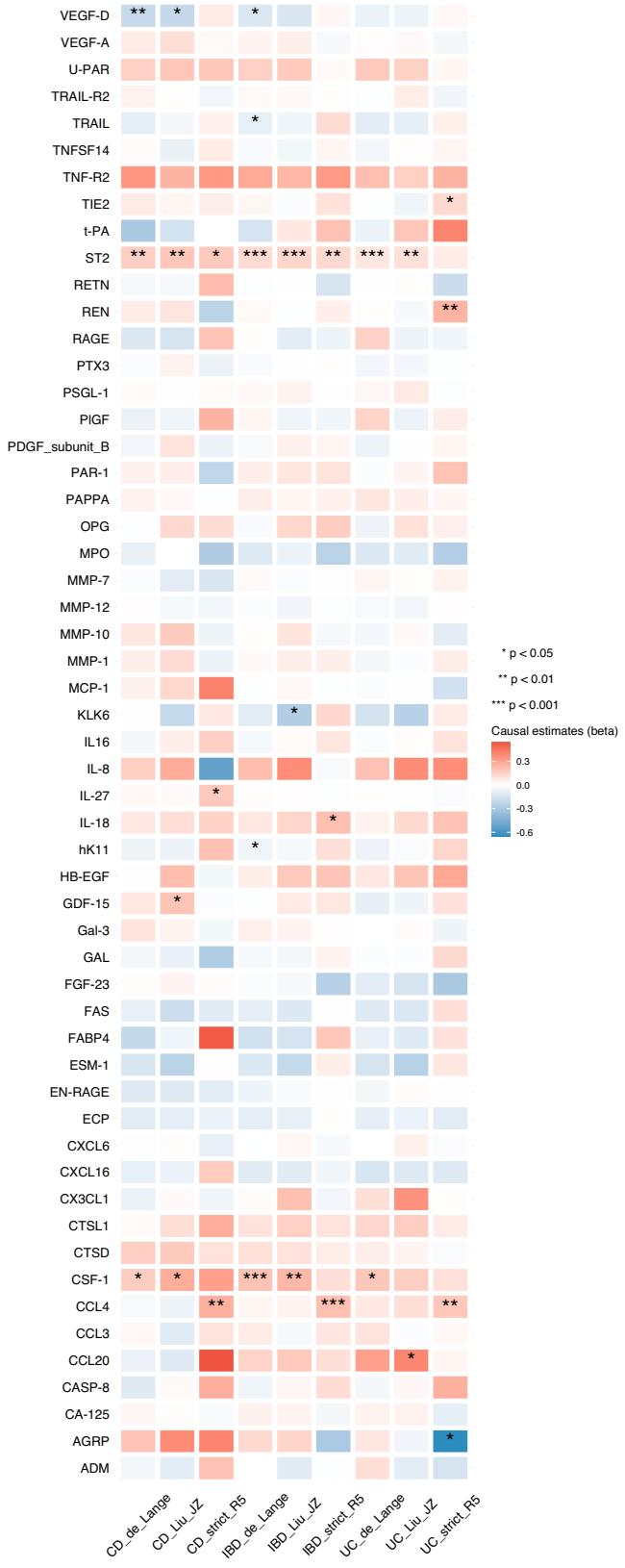

FIGURE 2 Heatmap showing the causal estimates of circulating proteins biomarkers on IBD, UC, and CD. CD, Crohn's disease; IBD, inflammatory bowel disease; UC, ulcerative colitis

from IIBDGC (OR 0.813, 95% CI 0.670–0.987, $p = 0.036$), and on IBD in one cohort from de Lange et al. (OR 0.880, 95% CI 0.793–0.977, $p = 0.017$).

Other statistically significant biomarker-IBD pairs identified in one type of IBDs in one cohort are as follows: negative effects of TRAIL (de Lange et al.), KLK6 (IIBDGC), and hK11 (de Lange et al.) on IBD; positive effect of IL-18 (FinnGen R5) on IBD; positive effects of IL-27 (FinnGen R5) and GDF-15 (de Lange et al.) on CD; positive effects of TIE2 (FinnGen R5), REN (FinnGen R5), and CCL20 (IIBDGC) on UC, and negative effects of AGRP (FinnGen R5) on UC (Figure 2). The complete MR analysis statistics are provided in Table S3.

ST2 and the risk of IBD

We selected nine SNPs (rs11603123, rs13020553, rs186021206, rs2460382, rs35518360, rs4311080, rs635634, rs672806, and rs7604529) for the cohort from de Lange et al. and 10 SNPs each (adding rs672806) for the IIBDGC and FinnGen R5 cohorts. Next, we performed two-sample MR analyses in the three cohorts and observed consistent significant positive associations between circulating ST2 and IBDs (IBD, UC, and CD) in all cohorts except the FinnGen R5 UC cohort. The scattered plots showing the associations between ST2 and IBDs are provided in Figure S1. After combining the causal estimates from the three cohorts, the meta-analyses results indicated that a genetically proxied higher level of circulating ST2 was associated with an increased risk of IBD (ORs 1.133, 95% CI 1.091–1.176, $p < 0.0001$). Specifically, circulating ST2 exhibited a stronger causal effect in the CD cohorts (ORs 1.188, 95% CI 1.103–1.281, $p < 0.0001$) compared with the UC cohorts (ORs 1.087, 95% CI 1.050–1.125, $p < 0.0001$; Figure 3). The effect estimates of each IV for circulating ST2 on the risk of IBDs are listed in Table S4. The power analysis for ST2 is given in Table S5.

MR sensitivity analyses were largely consistent with primary analyses. No statistically significant pleiotropy was identified based on the MR-Egger pleiotropy test (Table S6). Heterogeneity test revealed heterogeneity in the CD cohort from de Lange et al. ($p = 6.336\,\text{E-}9$), the IIBDGC CD cohort ($p = 2.966\,\text{E-}5$), and the IBD cohorts from de Lange et al. and IIBDGC with $p = 0.011$ and $p = 0.030$, respectively. No significant heterogeneity was identified in the rest of the cohorts (Table S6). Weighted median analyses yielded significant, consistent effect estimates in all the IBD, UC, and CD cohorts from de Lange et al. and IIBDGC. Although the significance of causal effects was not detected in all the FinnGen cohorts,

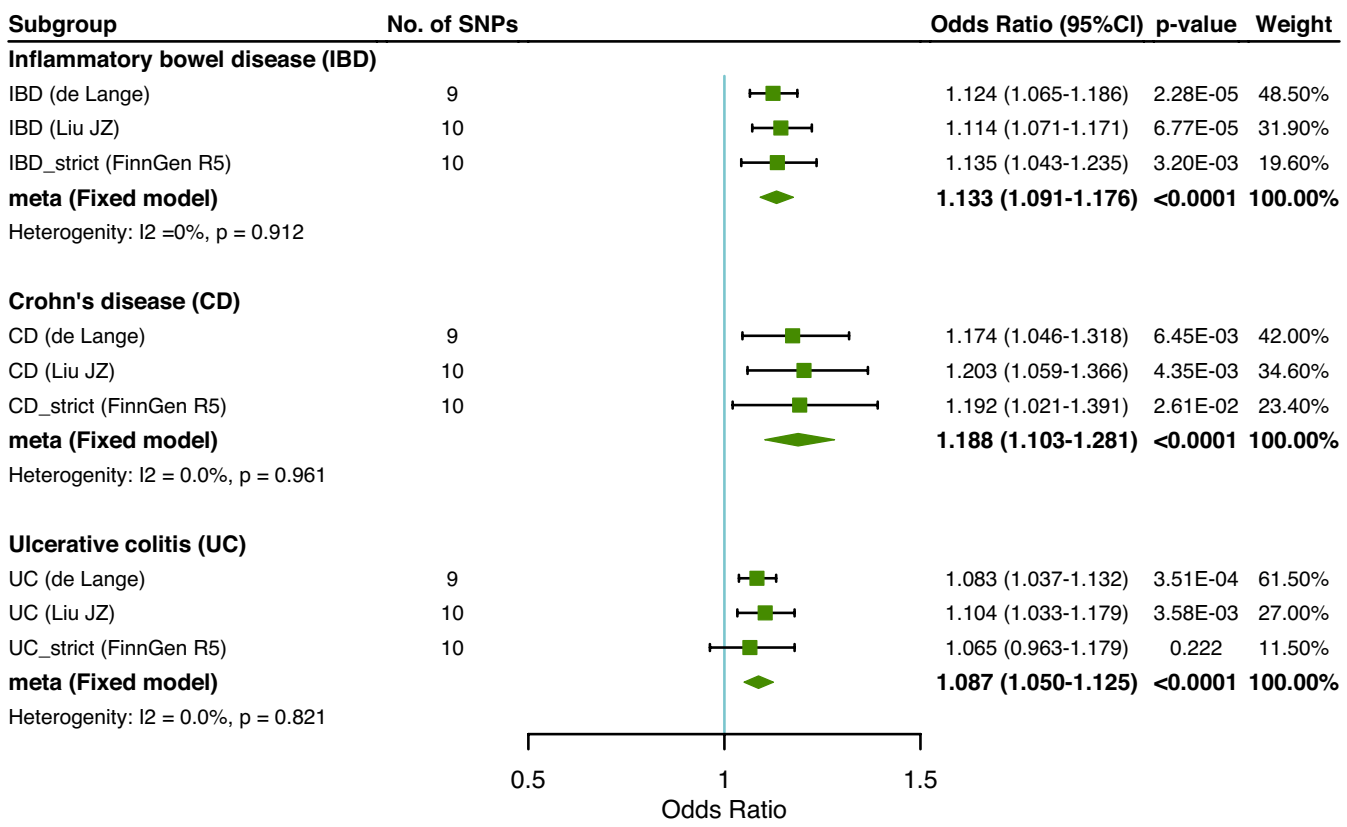

FIGURE 3 Inverse variance weighted results of ST2 levels on the risk of IBD and subtypes with two-sample MR. Estimated odds ratios represent the effect per standard deviation increase of ST2 level. CD, Crohn's disease; CI, confidence interval; IBD, inflammatory bowel disease; MR, Mendelian randomization; SNP, single nucleotide polymorphism; UC, ulcerative colitis

the positive trends still remained (Table S7). After MR-PRESSO outlier adjustment, we also observed significant, consistent effect estimates in all the cohorts except in one UC cohort (FinnGen R5) with consistent trend (Table S7).

CSF-1 and the risk of IBD

We selected two SNPs (rs11579145 and rs2050462) for the three cohorts. The results showed consistent positive correlations of circulating CSF-1 on IBDs, with the CD cohorts from de Lange et al. (OR 1.178, 95% CI 1.013–1.370, $p = 0.034$) and IIBDGC (OR 1.308, 95% CI 1.053–1.625, $p = 0.015$), the UC cohort from de Lange et al. (OR 1.203, 95% CI 1.036–1.397, $p = 0.015$), and the IBD cohorts from de Lange et al. (OR 1.220, 95% CI 1.085–1.372, $p = 8.76\,E\text{-}04$) and IIBDGC (OR 1.265, 95% CI 1.078–1.484, $p = 0.004$) being statistically significant (Figure 4). After combining the causal estimates from the three cohorts, the meta-analyses results indicated that a genetically proxied higher level of circulating CSF-1 was associated with an increased risk of IBD (ORs 1.217, 95% CI 1.115–1.328, $p < 0.0001$). More specifically, CSF-1 exhibited slightly stronger causal effects in the CD cohorts (ORs 1.223, 95% CI 1.082–1.382, $p = 0.0013$) compared with the UC cohorts (ORs 1.179, 95% CI 1.055–1.317, $p = 0.0037$; Figure 4). No obvious heterogeneity was found in the heterogeneity analysis (Table S8). Pleiotropy and MR-PRESSO were not performed due to the limited number of SNPs. The effect estimates of each IV for CSF-1 on the risk of IBDs are listed in Table S9. The power analysis for CSF-1 is given in Table S5.

DISCUSSION

In this study, we performed MR analyses using a well-recognized, large-scale pQTL dataset in three well-acknowledged IBD cohorts and demonstrated that genetically determined ST2 and CSF-1 had positive causal effects on IBD, UC, and CD. We also noticed some yet inconsistent evidence that VEGF-D was inversely correlated with UC, CD, and IBD; meanwhile, CCL4 was positively associated with CD and IBD. To our knowledge, this is one of the largest and most comprehensive MR analyses of the circulating protein determinants for IBDs so far. We used the pQTL dataset from over 30,000 individuals and three large-scale, available IBD GWAS datasets, enabling

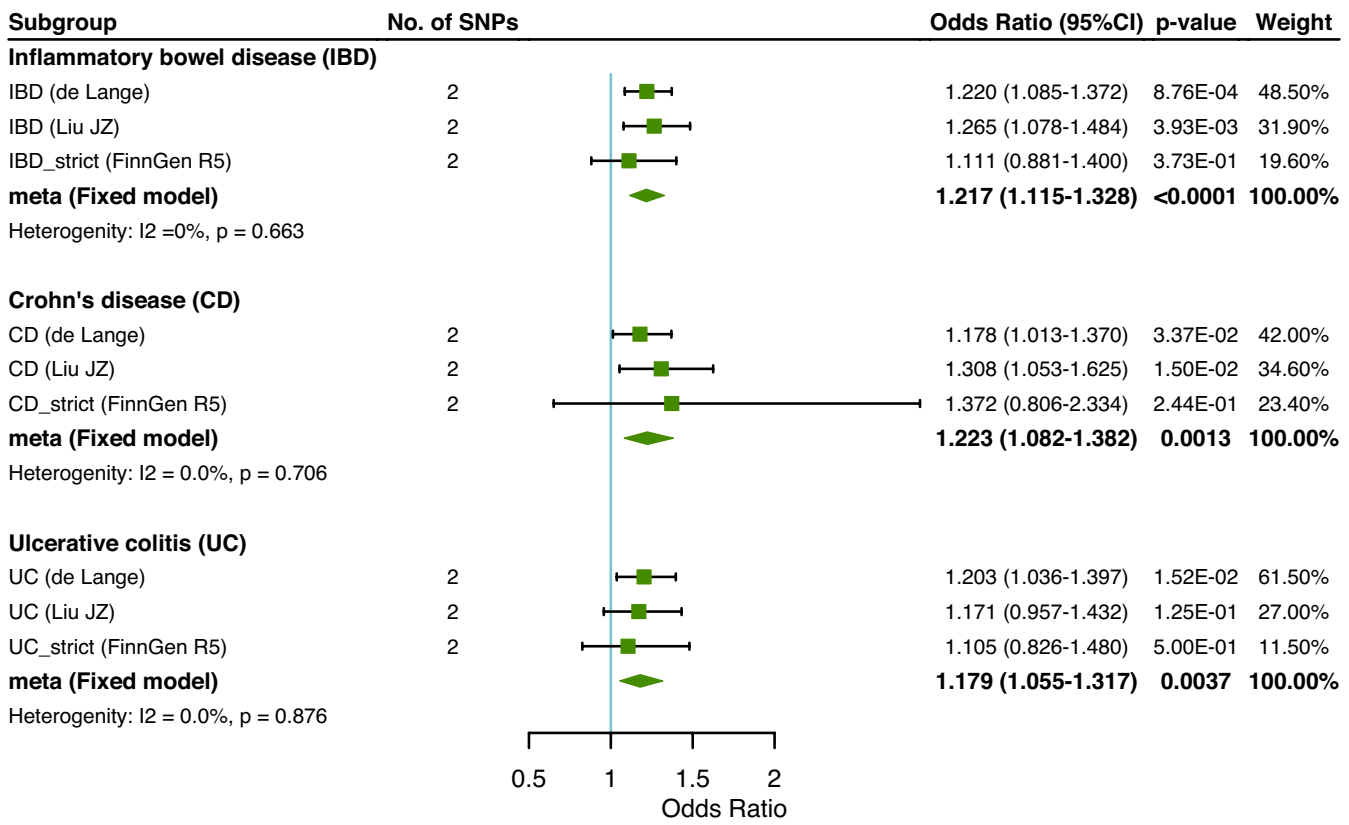

FIGURE 4 Inverse variance weighted results of CSF-1 levels on the risk of IBD and subtypes with two-sample MR. Estimated odds ratios represent the effect per standard deviation increase of CSF-1 level. CD, Crohn's disease; CI, confidence interval; IBD, inflammatory bowel disease; SNP, single nucleotide polymorphism; UC, ulcerative colitis

adequate power to estimate the relationship between genetically determined circulating proteins and IBDs.

ST2, belonging to the IL-1 receptor (IL-1R) family member, is encoded by the IL1RL1 gene and is expressed as both a membrane-anchored receptor (ST2L) and a soluble isoform (sST2) generated by alternative splicing. ST2L is activated by IL-33 and is able to modulate immune cell functions, whereas sST2 lacks transmembrane and intracellular domains and can inhibit IL-33 signaling by acting as a decoy receptor.[19,20] Previous studies have shown that ST2 is an IL-33 receptor and is widely expressed in T helper 2 (Th2) cells and innate lymphoid cells 2 (ILC2) in mediating type 2 immunity for the protection of helminth infection. In a steady-state, IL-33 resides in the nucleus of various cell types in the mucosal, especially in the intestinal epithelial cells (IECs).[21,22] Whereas the IECs undergo apoptosis during helminth infection, IL-33 is released and proto-cleaved by enzymes secreted from mast cells. The truncated IL-33 is bioactive and binds to ST2L on Th2 and ILC2, inducing the release of numerous hallmark cytokines in type 2 immunity, including IL-4, IL-5, and IL-13. These lead to recruitment of B cells, IgE production, and eosinophils infiltration. It also promotes the intestinal stem cells to differentiate toward tuft cells and goblet cells.[22]

The role of type 2 immunity in the pathogenesis of IBD has not been very well studied, although there are several reports suggesting a correlation between ST2 and IBD.[23–25] Besides, it remains unclear whether ST2 has a role in the development of IBD or if it is solely a marker of an IBD phenotype.[19,26,27] Previous studies investigating the role of IL-33/ST2 in intestinal inflammation revealed controversial results. Some studies used various mouse models and found that IL-33/ST2 modulated Th2-associated cytokines IL-5 and IL-13, and promoted mucosa healing, which indicated IL-33/ST2 plays a crucial protective role in colitis.[19,28] In the meanwhile, other studies found that IL-33/ST2 deficiency could protect mice from DSS or TNBS-induced colitis, and application of exogenous IL-33 could exacerbate infectious colitis.[20,29,30] Considering that the sST2 demonstrated sequestration effect by decoying IL-33, the normal mucosal type 2 immunity can be disrupted.[31] This is of great value because in steady-states, the intestinal helminth and other commensals are continuously educating the development of normal intestinal barriers and functional immune-surveillance. In addition, a very recent study also suggested that various other immune cell types express ST2. Particularly, the $ST2^{+}GATA3^{+}$ Treg

cells can be activated, thus inducing the inhibitory effects on Th1 and Th17 cell-mediated pro-inflammatory effects. Blocking such effects by elevating sST2 can attenuate the Treg suppressive effects and aggravate pro-inflammatory microenvironment in intestinal mucosa.[32] A recent study showing that myeloid-derived IL-33 can limit drug-induced colitis in murine models further supports such hypothesis.[33] Besides, IL-33/ST2 axis is also involved in tissue fibrosis.[34] More recently, Imai et al.[35] found that in mice, upregulation of ST2 by adherent-invasive *E. coli* was critical for the induction of intestinal fibrosis, suggesting an important role of ST2 in CD.

In clinical studies, sST2 was found elevated in patients with UC and CD and was thought to be associated with IBD presence, and as a potential biomarker in monitoring disease activity and predicting treatment response.[23,36,37] A study by Díaz-Jiménez et al.[38] analyzing 153 patients with IBD demonstrated that sST2 was a promising biomarker in distinguishing between active and inactive UC. Although it is still unclear if ST2L or sST2 play pathological roles in IBD development in humans, our MR study provide novel evidence suggesting that the disruption of IL-33 signaling could place causal effects on UC and CD development. Recently, astegolimab, a newly developed anti-ST2 monoclonal antibody, has been studied in patients with asthma and chronic obstructive pulmonary disease, and exhibits promising results in clinical trials.[39,40] Whether the drug can also be applied in the treatment of IBD or aggravate the disease is still unclear. Further basic research and clinical studies are warranted to decipher the mechanisms through which it plays a causal role in IBD.

CSF-1 (also named macrophage-colony stimulating factor) by interacting with its receptor, CSF-1R (also known as CD115 or c-fms), triggers a cascade of signaling pathways that promote macrophage and monocyte differentiation, proliferation, and proper functioning.[41] In the gut, CSF-1 plays an essential role in the proliferation of colonic crypt epithelial cells and in maintaining homeostasis.[42–44] In human studies, Zwicker et al.[45] found significantly increased expression of CSF-1 in patients with CD. These results indicate that CSF-1 seems to play a deleterious role in IBDs. Our results add on new information to these previous findings and implicate a potential causal association between CSF-1 and IBD. Of note, currently, several CSF-1 inhibitors are under development for various diseases, including some promising drug candidates at early stages of development for colitis.[46] For example, some studies in mouse colitis models found that blockade of CSF-1 via anti-CSF-1 monoclonal antibody led to alleviated colitis, indicated by decreased macrophages and T cells infiltration, as well as the downregulation of IL-6 expression.[47,48] Besides, a small molecule inhibitor of CSF-1 receptor kinase (JNJ-40346527) showed benefits, leading to the attenuated clinical disease scores in mouse colitis model.[49] Future preclinical and clinical studies are warranted to explore the effects of anti-CSF-1 drugs in IBD treatment.

Additionally, previous studies have shown that IL-18 plays pleiotropic roles in intestinal barrier maintenance and exerting pro-inflammatory effects on the pathogenesis of human IBD and mouse colitis models.[50–53] In this study, we suggested that the upregulation of IL-18 might indicate increased risks of IBD and its subtypes. A comprehensive MR study (involving 2-sample and 3-sample MR design) about IL-18 recently done by our group showed a positive correlation of IL-18 and the susceptibility of IBD with MR meta-analyses.[54] However, we should also admit that the MR study design is unable to recognize a potential U-shape correlation between the IL-18 level and the disease risk. Gain- and loss-of-function studies in mouse models as well as multi-omics analyses in a mouse colitis model and human samples are highly needed to further elucidate the IL-18 function in the initiation, progression, and healing stages of intestinal inflammation.

Our study has several limitations. The two-sample MR approach assumes a linear relationship between the exposure and the outcome. We are not able to evaluate a possible nonlinear relationship between the circulating proteins and IBDs in this study. Future studies examining more druggable circulating proteins and larger samples will be needed to verify our findings and further explore potential drug targets for IBD. Third, we are assuming that all MR assumptions can be fully satisfied in the first stage of screening, However, unmeasured pleiotropic effects cannot be completely ruled out. Fourth, due to different study purposes, the disease diagnosis and definition, as well as other unmeasurable factors, and the heterogeneity of different studies needs to be be aware. Fifth, due to the limited number of participants involved in the pQTL study, for some proteins, only one SNP was identified showing associations. Future updates with larger cohorts and meta-analyses are warranted to further explore the causal associations between druggable circulating protein targets and the susceptibility of IBD, CD, and UC. Last, our results are based on summary statistics in European ancestry, we should be careful to extend our conclusions to other populations. Large-scale GWAS and pQTL studies together with meta-analyses are needed for further exploration in broad spectrum of human populations.

In summary, our study comprehensively evaluated the relationships between circulating proteins and IBDs using the MR approach, highlighting possible causal effects of elevated circulating ST2 and CSF-1 on the risk of IBD, UC, and CD. These findings demonstrated novel insights on the pathogenesis of IBDs and provided new lines of evidence for drug repurposing strategies in future IBD treatment.

However, data from multiple levels, such as single-cell RNA sequencing data from patients with IBD, are warranted to give more consolidated conclusions in the future.

AUTHOR CONTRIBUTIONS

J.M., X.W., and X.B. wrote the manuscript. J.M., X.B., and H.Y. designed the research. J.M., X.W., X.B., and Y.Y. performed the research. J.M. and X.W. analyzed the data.

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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