Research article

Exploration of the potential causative genes for inflammatory bowel disease: Transcriptome-wide association analysis, Mendelian randomization analysis and Bayesian colocalisation

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ABSTRACT

Background: Inflammatory bowel disease (IBD) poses a complex challenge due to its intricate underlying mechanisms, and curative treatments remain elusive. Consequently, there is an urgent need to identify genes causally associated with IBD.
Methods: We extracted blood eQTL data from the GTExv8.ALL.Whole_Blood database, genome-wide association studies (GWAS) summary statistics of IBD from the IEU GWAS database, and performed a three-fold analysis protocol, including transcriptome-wide association analysis, Mendelian randomisation analysis, Bayesian colocalisation, and subsequent potential therapeutic agents identification.
Results: We identified four pathogenic genes, namely CARD9, RTEL1, STMN3 and ARFRP1, that promote the development of IBD, encompassing both ulcerative colitis (UC) and Crohn's disease (CD). Notably, ARFRP1 exhibited the ability to suppress IBD (encompassing UC and CD) development. Regarding drug prediction, cyclophosphamide emerged as a promising novel therapeutic option for IBD, encompassing UC and CD.
Conclusion: We identified several potential genes related to IBD (UC and CD), including CARD9, RTEL1, STMN3 and ARFRP1, warranting further investigation in functional studies to elucidate underlying disease mechanisms. Additionally, clinical studies exploring the potential of cyclophosphamide as a treatment avenue for IBD are warranted.

1. Introduction

Inflammatory bowel disease (IBD) is a prevalent autoimmune bowel disorder, affecting approximately 1 in 200 individuals, with a rising incidence [1]. IBD manifests primarily in two major forms: Crohn's disease (CD) and ulcerative colitis (UC). CD typically exhibits lesions in the ileum and colon, characterised by intermittent inflammatory changes of the permeable wall, colonoscopic thickening of the bowel wall, narrowing of the lumen, gooseneck-like shape alterations and potential abscesses or fistulae in the perianal area [2]. In contrast, UC predominantly develops on the mucosal surface of the rectum, displaying continuous inflammatory foci, punctate ulcers and haemorrhages upon enteroscopy [3]. Patients with IBD often experience a poor quality of life due to the chronic and recurrent nature of the disease, which also imposes a substantial economic burden [4]. Among the various factors contributing to IBD

susceptibility, genetic predisposition takes centre stage [3]. Currently, the causal genes underlying IBD remain incompletely defined, underscoring the need for active exploration of key molecular features in its pathogenesis, which can offer potential avenues for curative therapies.

Advancements in high-throughput sequencing technologies have enabled genome-wide association studies (GWAS) to identify susceptibility loci for IBD [2]. However, the intricate underlying mechanisms governing IBD present a significant barrier to translating risk loci into clinical treatment.

Recent advancements in mining quantitative trait loci (QTL) data and the development of novel statistical methods have facilitated the bridging of genotype and gene expression (eQTL) and the rational integration of multidimensional data [5,6]. Transcriptome-wide association studies (TWASs) have gained prominence for analysing the association between gene expression and phenotypes, yielding noteworthy insights [7,8]. Additionally, Mendelian randomisation and Bayesian colocalisation analyses offer valuable tools to integrate QTL and disease GWAS data, identifying candidate genes [9,10]. Mendelian randomisation, grounded in the fundamental principle of 'random assignment of parental alleles to offspring', adheres to three core assumptions for causal inference [11]. Bayesian colocalisation analysis assesses the extent to which two traits share causative genetic variation [12]. Thus, the combination of TWAS, Mendelian randomisation and Bayesian colocalisation facilitates the identification of gene-phenotype causality at the transcriptome level.

This study employs a TWAS-based approach, integrating pooled IBD GWAS data with data on expressed quantitative trait loci (eQTLs) in blood to initially identify IBD-causing genes. Subsequently, we conduct SMR and Bayesian colocalisation analysis of the pooled data to determine potential causal associations. Finally, we employ drug target prediction to uncover potential therapeutic drugs based on the identified target genes, potentially advancing the exploration of novel IBD treatments.

2. Methods and materials

2.1. Study design

This study integrates GWAS results for eQTL data associated with IBD, encompassing both UC and CD, with a specific focus on blood-related data. All data utilized herein are publicly available, and the analytic workflow is depicted in Fig. 1.

2.2. Data sources

Using the IEU GWAS database (https://gwas.mrcieu.ac.uk/datasets/), we accessed GWAS summary statistics pertinent to IBD, including CD and UC, from the International IBD Genetics Consortium (IIBDGC). Notably, these IBD studies comprised 89.8% of European ancestry, with the remaining subjects of East Asian, Indian or Iranian ancestry [13]. To ensure homogeneity, we excluded data from non-European ancestry populations, resulting in datasets encompassing 12,882 patients with IBD and 21,770 controls; 5956 patients with CD and 14,927 controls; and 6968 patients with UC and 20,464 controls. The definitive diagnosis of IBD and its subtypes relied on histopathological, endoscopic and radiological criteria, collectively.

For blood tissue eQTL data, information was extracted from the GTExv8 database. The TWAS data format was sourced from http://gusevlab.org/projects/fusion/, while the SMR data format was obtained from https://yanglab.westlake.edu.cn/software/smr/#eQTLsummarydata.
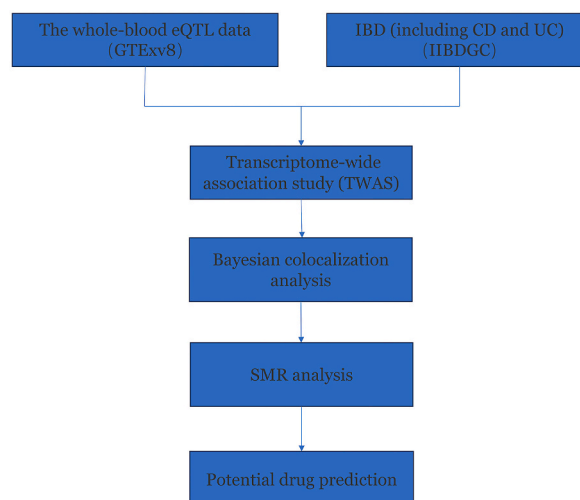

Fig. 1. Flowchart of the study.

2.3. TWAS

To conduct TWAS for IBD (including UC and CD), we employed the FUSION (http://gusevlab.org/projects/fusion/) tool [14]. FUSION excels in constructing correlation prediction models for eQTLs using reference data and simultaneously utilising GWAS summary statistics to evaluate correlations between predicted gene expression and phenotypic traits. In this TWAS analysis, we adhered to default FUSION settings and incorporated three key datasets: (i) GWAS summary statistics for IBD (including UC and CD) and TWAS in LD score format; (ii) FUSION gene expression prediction models for blood tissues, specifically GTExv8.ALL.Whole_Blood data ALL. Whole_Blood data, and *cis*-regulated plasma gene data; (iii) third-stage data from the 1000 Genomes Project.

Several predictive models for TWAS analysis, including top1, blup, lasso, enet and bslmm, were considered, We selected the model demonstrating the highest predictive capacity to implement the TWAS analysis of IBD.

2.4. Bayesian colocalisation analysis

Bayesian colocalisation analysis assesses the consistency of causal variance between exposure and outcome, avoiding false signals in MR caused by cascade disequilibrium or pleiotropy [15]. Accordingly, we employed the 'COLOC' software package for this analysis. The analysis generates five hypotheses, each associated with corresponding posterior probabilities: H0 (no correlation between two traits), H1 (correlation with exposure but not outcome), H2 (opposite correlation to H1), H3 (correlation between both exposure and outcome but with distinct causal variants) and H4 (correlation with shared causal variants). H4 is deemed significant when the posterior probability (PPH4) surpasses 0.8 and is considered to have a shared causal relationship [15].

2.5. SMR analysis

We performed SMR analysis using the SMR software tool for Linux version 1.3.1, specifically targeting *cis*-regions, along with the Heterogeneity in Instrument Dependent Diversity (HEIDI) test. Detailed computational procedures and methodological specifics can be found in the original SMR publication [16]. SMR, a variant of MR, examines single-nucleotide variants, also known as single-nucleotide polymorphisms. In our study, blood summary level eQTL data served as the exposure object, while IBD (including UCs and CDs) summary level GWAS data served as the outcome object. This analysis primarily assesses the presence of causal or pleiotropic associations, which implies shared causal variants, between gene expression and phenotype. Default settings were maintained for SMR, with false discovery rate (FDR) correction set at less than 0.05 for multiple endpoint considerations [17]. For the HEIDI test, a p-value less than 0.05 was considered significant, indicating that the observed association was attributed to chain reaction and necessitated their exclusion from the analysis [16].

2.6. Drug-gene interactions and potential therapeutic targets

The Enrichr online platform (https://amp.pharm.mssm.edu/Enrichr/) houses numerous publicly available drug and gene data resources [18], including the DSigDB database. The DSigDB database serves as a comprehensive drug effect database, which integrates drug action and biological effect correlation information [19]. This resource is instrumental in discovering new drugs, elucidating disease mechanisms and facilitating personalised medical solutions.

To rigorously assess the binding efficacy and interaction patterns between candidate drugs or small molecules and their specific target proteins, this study employed AutoDock Vina 1.2.2, a sophisticated computer-aided protein-ligand docking platform [20]. The molecular structure of cyclophosphamide utilized in this experiment was directly sourced from the PubChem Compound Database (URL:https://pubchem.ncbi.nlm.nih.gov/) [21]. Concurrently, the three-dimensional coordinate information for the RTEL1 protein (PDB ID: 7WU8, resolution of 1.6 Å) was acquired from the Protein Data Bank (PDB, URL: http://www.rcsb.org/). Prior to undertaking the docking experiments, this research meticulously preprocessed the data files of the protein and ligand molecules, which included converting file formats to PDBQT, removing all water molecules, and appending the necessary polar hydrogen atoms to ensure the accuracy and reliability of the docking process. Additionally, this study employed the CB-Dock method, an advanced protein-ligand docking technique characterized by its ability to autonomously identify potential binding sites of the ligand, thereby calculating the site's central position and dimensions [22]. This technique, by customizing the docking box dimensions for the ligand query results and leveraging the powerful algorithms of AutoDock Vina, facilitates precise molecular docking. Through extensive benchmark testing and analysis, it was observed that the docking strategy based on specific cavity centers significantly enhances the success rate and precision of blind docking [23]. Thus, by integrating our uniquely developed curvature-based cavity detection method (CurPocket), CB-Dock not only predicts the potential binding sites of the target protein but also utilizes AutoDock Vina to predict the exact binding position of the queried ligand, thereby optimizing the docking process and augmenting the precision and efficiency of the predictions.

2.7. Building gene interaction networks

To elucidate the intricate web of genetic interactions, this study employed GeneMANIA, a versatile and user-friendly platform designed to generate hypotheses regarding gene functions [24]. This tool facilitated the construction of gene-gene interaction networks, offering an online predictive analysis framework capable of exploring the myriad interactions within a target gene's milieu, including protein and genetic interactions, domain protein similarities, co-expression, co-localization, and functional associations. Through this analysis, we delved into the connections between genes and their mutual influences, thereby advancing our

understanding of genetic interplay.

3. Results

3.1. Analysis between blood tissue eQTL and IBD

The TWAS analysis that linked blood tissue eQTL with IBD successfully identified genes associated with IBD. The QQ plot (Fig. S1 A) reveals significant deviations between the observed p-value and the expected p-value around Expected-log10(p) = 3, signifying the pivotal role of strongly associated gene loci in disease development. Following Bonferroni correction (p < 6.55996E-06, May 0, 7622), we identified 86 genes significantly associated with IBD, which were distributed across loci on 17 chromosomes (excluding 4, 8, 11, 13 and 18) (Fig. 2A). Detailed results of this analysis are provided in Table S1. Additionally, Table S1 presents results from colocalisation analysis, with 36 genes demonstrating strong associations (PP4 > 0.8) in TWAS analysis, suggesting shared causal variants and implicating these genes in key biological processes in IBD.

We conducted conditional and association analyses to assess whether these 86 positive genes were derived from multiple association traits or were conditionally independent. Notably, we observed that within the 2.5–3 MB range of chromosome 1, locus-trait associations may be predominantly explained by LOC100996583 (Fig. 1S B). Corresponding association maps are also available (Fig. 1S C-U).

In the integrated analysis of GWAS summary data for IBD and blood eQTL data using the SMR method, we set the SMR FDR threshold of less than 0.05, with the HEIDI test demonstrating heterogeneity greater than 0.05. Finally, this yielded 62 genes significantly associated with IBD (Table S2).

By merging the results of TWAS and SMR analyses, we identified 21 co-positive genes (Fig. 2B). Among these, 11 genes exhibited a negative correlation with IBD, while 10 genes displayed a positive correlation. Notably, RTEL1 and ARFRP1, emerged as the genes with the most significant positive and negative effects, respectively.

3.2. Analysis of blood tissue eQTL and IBD subtypes

Following the analysis process for IBD, we extended our investigation to TWAS for UC and CD. After applying the Bonferroni correction threshold (p < 6.55996E-06, May 0, 7622), we identified 54 genes with strong causal associations with UC and 56 genes significantly correlated with CD. The Manhattan plots depicting these findings are illustrated in Fig. 3A–B, while detailed results are available in Tables S3–4. In the colocalisation analysis, 22 genes in UC displayed a PP4 greater than 0.8, as did 28 genes in CD
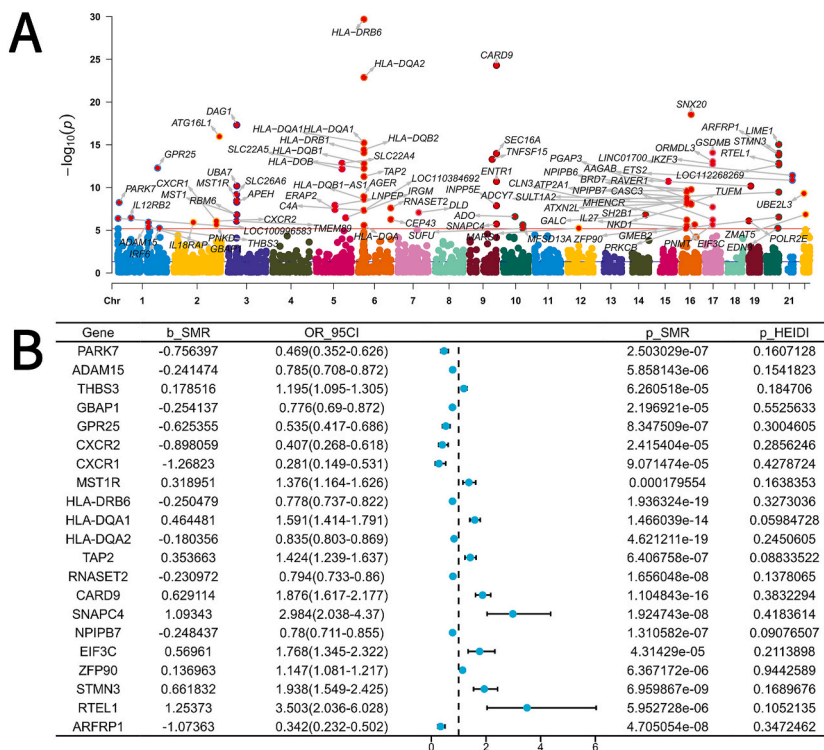

Fig. 2. (A) TWAS analysis, Manhattan map of IBD transcriptome association study, corrected by Bonferroni(P < 6.55996E-06, May 0, 7622). (B) Forest map combining positive results of IBD in TWAS and SMR analysis.
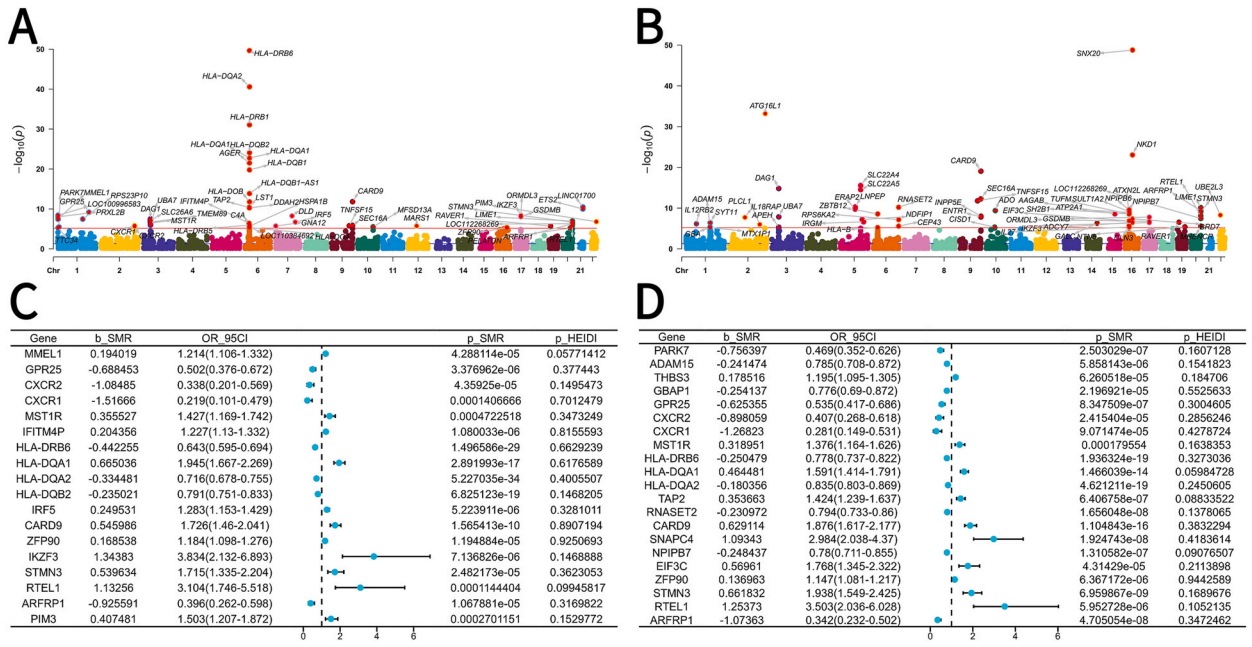
Fig. 3. (A) TWAS analysis, Manhattan map of UC transcriptome association study, corrected by Bonferroni(P < 6.55996E-06, May 0, 7622). (B) TWAS analysis, Manhattan map of CD transcriptome association study, corrected by Bonferroni(P < 6.55996E-06, May 0, 7622). (C) Forest map combining positive results of UC in TWAS and SMR analysis. (D) Forest map combining positive results of CD in TWAS and SMR analysis.
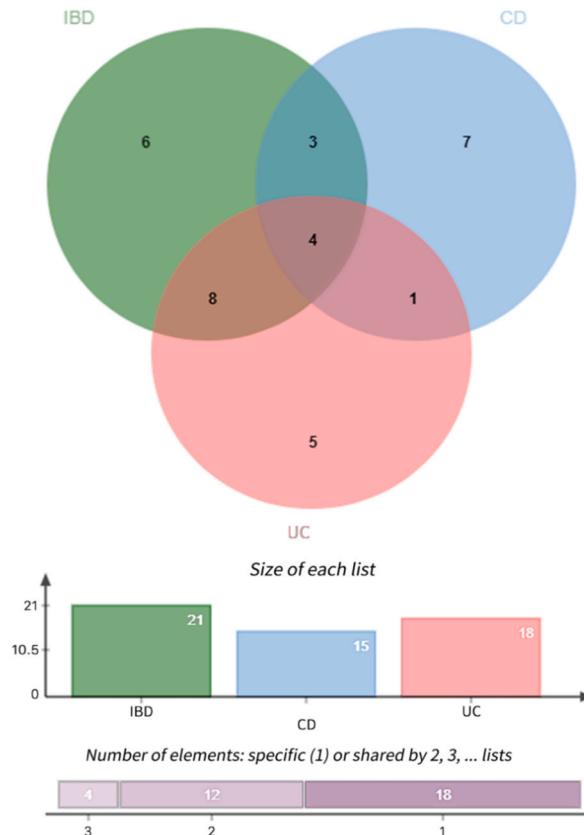

Fig. 4. The intersection genes of IBD and its subtypes (UC and CD).

(Tables S3–4). Additionally, QQ plots and gene association plots for these subtypes are presented in Figs. 2S–3S. In the SMR analysis, adhering to the criteria of 'FDR less than 0.05' and 'HEIDI greater than 0.05', we identified 44 and 38 causal genes in UC and CD, respectively (Tables S5–6). Finally, a forest plot summarising the results of the TWAS and SMR analyses can be found in Fig. 3C–D.

3.3. Intersecting genes between IBD and its subtypes (UC and CD)

After synthesizing eQTL data from blood tissue with associations to inflammatory bowel disease (IBD) and its subtypes, ulcerative colitis (UC), and Crohn's disease (CD), we employed Tissue Expression Weighted Analysis (TWAS) and Summary-data-based Mendelian Randomisation (SMR) to delve into the genetic underpinnings of these conditions. By aggregating and comparing the TWAS and SMR results for IBD and its subtypes, UC and CD, we identified four core genes consistently exhibiting positive associations across all analyses: CARD9, RTEL1, STMN3, and ARFRP1(Fig. 4). The high consistency in the causal associations of these genes across IBD and its subtypes suggests a pivotal role in the disease's onset and progression. Specifically, through intersecting analysis of 21 IBD-positive genes, 18 UC-positive genes, and 15 CD-positive genes, we pinpointed these four common genes, reinforcing their central role in the pathology of IBD and its subtypes and providing a robust genetic foundation for further investigation into their mechanisms within the disease process. Notably, these intersecting genes demonstrated strong causal associations in the TWAS analysis and exhibited high probabilities (PP4>0.8) in subsequent colocalisation analyses, indicating they may harbor shared causal variants playing significant roles in the development and progression of IBD and its subtypes. Furthermore, the application of this integrative analysis approach not only enhances our understanding of the genetic complexity of IBD but also offers valuable clues for future therapeutic target identification and disease mechanism research.

3.4. Targeted drug prediction

In this investigation, we harnessed the DSigDB database within the Enrichr platform to unearth potential pharmacological targets associated with IBD pathogenic genes CARD9, RTEL1, STMN3, and ARFRP1, as illustrated in Fig. 5. A comprehensive analysis of these four pivotal pathogenic genes led to the identification of several candidate drugs that interact with these genes. Notably, cyclophosphamide emerged as a significant contender due to its strong association with RTEL1 and STMN3 (Overlap = 2/280, Adjusted *P*-value = 0.054060583), suggesting these genes as potential targets for cyclophosphamide in the treatment of IBD, as detailed in Table S7. The compound score for cyclophosphamide (Combined Score = 480.0267344) far surpassed that of other drugs, such as Lenalidomide (Combined Score = 641.2429069, albeit only associated with RTEL1, Overlap = 1/49) and DMBA (Combined Score = 383.7782872, solely related to STMN3, Overlap = 1/74), reinforcing the hypothesis of cyclophosphamide's therapeutic potential against RTEL1 and STMN3 in IBD. Moreover, the correlation with diazinon (related to RTEL1 and CARD9, Overlap = 2/1027, Adjusted *P*-value = 0.150745502) was observed, and despite its lower statistical significance and combined score (Combined Score = 78.04455848), this finding still unveils the potential of CARD9 as a prospective drug target, meriting further exploration.

Employing molecular docking techniques, we assessed the interaction between Cyclophosphamide and the RTEL1 protein. The docking results revealed a binding energy of −4.2 kcal/mol between Cyclophosphamide and RTEL1, indicating a moderately strong interaction. Additionally, the volume of the binding pocket was found to be 1663 Å^3, providing ample space for Cyclophosphamide to accommodate its structure and interact with multiple residues of the RTEL1 protein, as depicted in Fig. 6A–C.

These findings unveil the potential interactions between Cyclophosphamide and other candidate drugs with genes associated with IBD, offering valuable insights for further mechanistic studies and exploration in clinical applications. Notably, the potential application of Cyclophosphamide, a known chemotherapeutic agent, in the treatment of IBD warrants further experimental validation to confirm its safety and efficacy.

3.5. Constructing diagnostic gene interaction networks

In our study, we utilized GeneMania to construct a detailed gene-gene interaction network aimed at unraveling the intricate
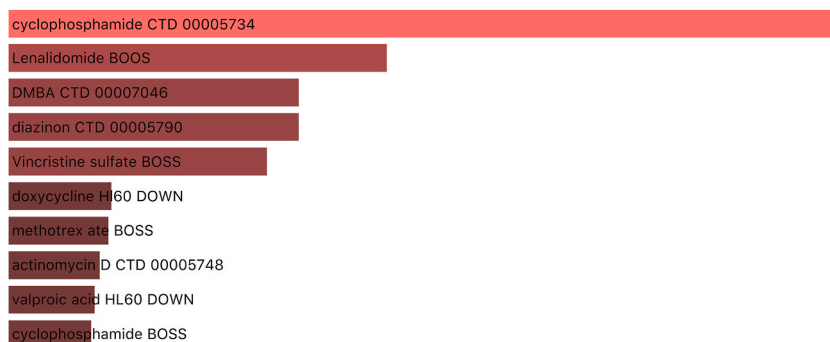

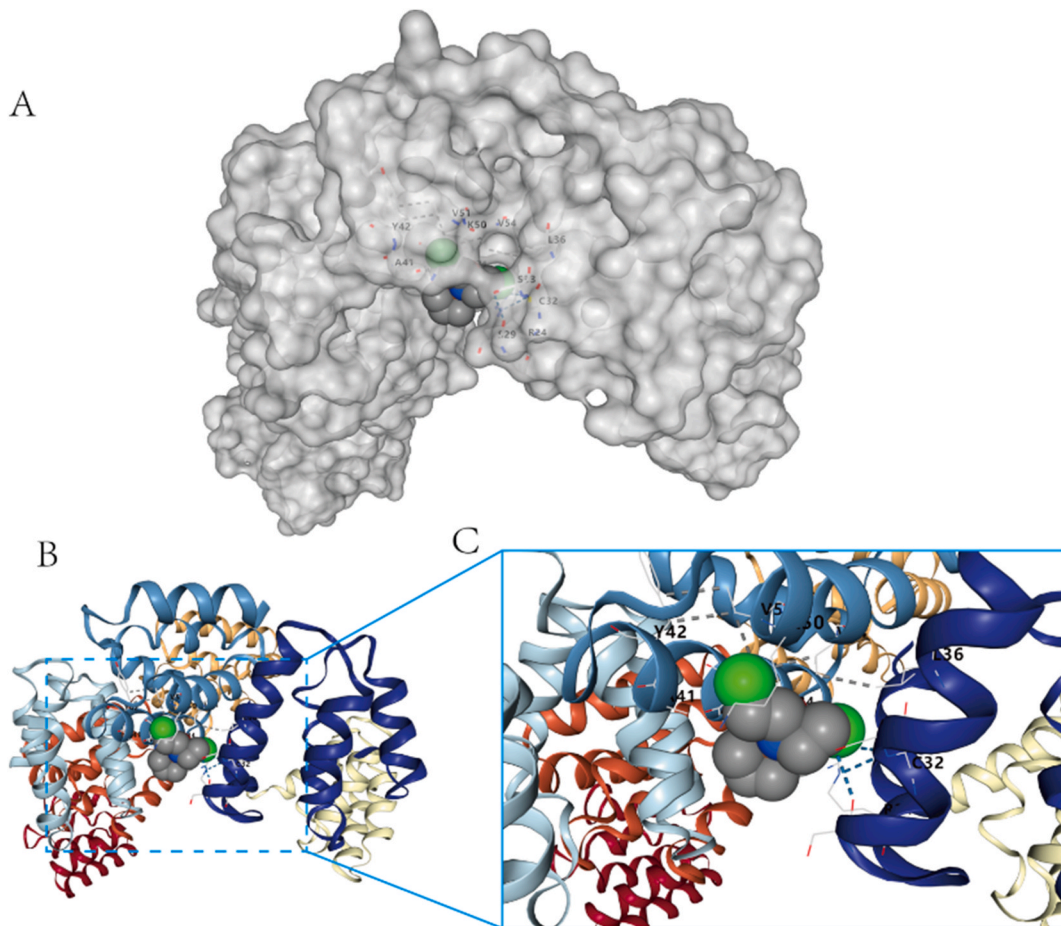**Fig. 5.** Results of targeted drug prediction.

Fig. 6. Illustrates the binding mode of RTEL1 with Cyclophosphamide through molecular docking. (A) The three-dimensional structure of the binding pocket of RTEL1 with Cyclophosphamide. (B) The cartoon representation of the optimal molecular conformation overlaid on the structure shown in Panel A. (C) The enlarged view of the pocket's three-dimensional structure as highlighted in Panel B.

interplay between risk genes CARD9, RTEL1, STMN3, ARFRP1, and their adjacent genes, as illustrated in Fig. 7. Network analysis identified 20 genes frequently altered in close association with the target genes, unveiling a web of interactions encompassing direct protein interactions, shared protein domains, gene co-expression, co-localization, and their correlations in biological functions. Furthermore, the network was linked to numerous pivotal biological pathways, encompassing aspects from the disassembly of cellular protein complexes, disaggregation of proteins, dynamics of microtubules, to the regulation of nucleotide-binding domains. Notably, the network also encompassed the modulation of the I-kappaB kinase/NF-kappaB signaling pathway and the organization and regulation of the microtubule cytoskeleton, core biological processes. These profound network analysis insights afford us a valuable perspective to more comprehensively understand the roles of these key genes in cellular functional regulation and the progression of IBD.

4. Discussion

This study comprehensively analyzed the blood eQTL data and GWAS summary data of IBD (including UC and CD) using three key methodologies: TWAS, SMR and Bayesian colocalisation analysis. Our objective was to pinpoint potential causative genes and identify drug candidates for IBD. In total, we identified 21 causative genes for IBD, along with 18 for UC and 15 for CD. By intersecting these gene sets, we derived four genes, CARD9, RTEL1, STMN3 and ARFRP1. Among these, CARD9, RTEL1 and STMN3 exhibited a positive correlation with IBD (including UC and CD), whereas ARFRP1 demonstrated the ability to inhibit the pathogenesis of IBD (including UC and CD). Additionally, we identified cyclophosphamide as a potential therapeutic agent for IBD (including UC and CD).

The CARD9 gene plays an essential role in the immune system, particularly in antifungal immunity and C-type lectin signal transduction. It is closely linked to the development of various inflammatory diseases, including rheumatoid arthritis, IgA nephropathy, ankylosing spondylitis, and inflammatory bowel disease (IBD) [25]. In the context of IBD research, an abundance of genome-wide association studies (GWAS) and case studies have illustrated CARD9's involvement in the disease's pathophysiological processes [26–29] [26–29] [26–29]. Specific CARD9 gene variants, such as rs10870077 and rs10781499, have been identified as
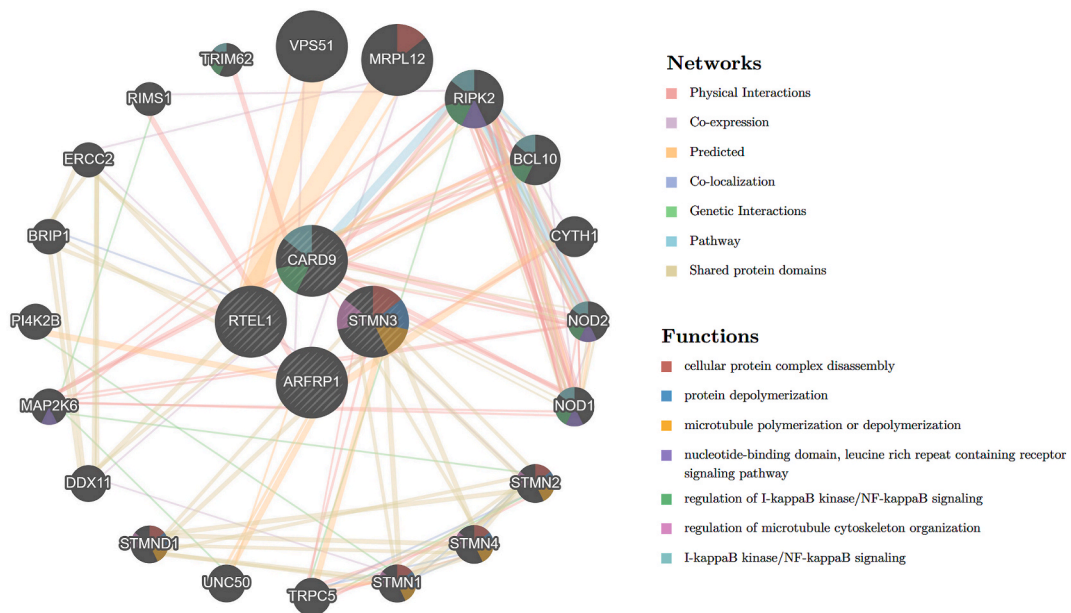
Fig. 7. Gene-gene interaction networks.

genetic risk factors for IBD, showing a strong association with ulcerative colitis (UC) and a moderate association with Crohn's disease (CD) [30,31]. Additionally, CARD9 influences the immune response by promoting the production of pro-inflammatory cytokines like TNFα and IL-6, exacerbating IBD [32]. It also plays a role in the regulation of the gut microbiome, further impacting the development of IBD [33]. Given the strong association between CARD9 and IBD and the extensive research already conducted, identifying CARD9 as a risk gene for IBD through TWAS and Mendelian randomisation analysis may not provide novel insights.

Genome-wide association studies (GWAS) have uncovered several genetic loci associated with susceptibility to inflammatory bowel disease (IBD), with newly identified susceptibility regions in pediatric patients at 20q13 and 21q22. These discoveries provide fresh insights into the molecular mechanisms underlying IBD and pave the way for novel therapeutic strategies. The complex telomeric region of 20q13 encompasses multiple genes, including STMN3, RTEL1, and ARFRP1, implicated in a range of biological processes such as cell cycle regulation, DNA repair, protein transport, and immune responses. Furthermore, a study by Kugathasan et al., in 2008, through GWAS analysis on a large cohort, first identified the association between the 20q13 locus and susceptibility to pediatric IBD, underscoring the significance of these genes in the pathogenesis of IBD [34].

RTEL1, a helicase associated with replication, plays a crucial role in telomere and genome-wide replication [35]. Mutations in RTEL1 have been linked to diarrhea and immune deficiencies, particularly in infancy [36,37]. Studies on patients with congenital dyskeratosis in Ashkenazi Jewish populations have revealed that mutations in RTEL1 lead to infantile IBD and severe immunodeficiencies [38]. Specifically, an early-onset ulcerative colitis and significant alterations in the immune system were observed in an Ashkenazi Jewish infant with a missense mutation in the RTEL1 gene (C.3791G > A), highlighting the need for heightened vigilance regarding the association between RTEL1 mutations and IBD in this population [38]. Disruptions in RTEL1's interaction with PCNA can lead to accelerated cellular aging [39], and although this mutation is found in the ExAC database, it is not present in the 1000 Genomes Project [35]. Patients with dyskeratosis congenita carrying this mutation exhibited telomere shortening [40]. The p.P1034H variant in RTEL1 might be disease-associated, but its pathogenic effect remains unclear. Knockdown of RTEL1 has been shown to alleviate chronic obstructive pulmonary disease by regulating macrophage polarization and inflammation [41]. Additionally, RTEL1 mutations are associated with various diseases, including Hoyeraal-Hreidarsson syndrome, pulmonary fibrosis, and myelodysplastic syndromes [42–45] [42–45] [42–45]. Telomere dysfunction is related to several chronic inflammatory diseases, including IBD [46–48] [46–48] [46–48].

ARFRP1, a small GTPase closely related to the ARF family, plays a pivotal role in the trans-Golgi network (TGN) and is essential for the regulation of membrane traffic. It activates its GTP-bound state, recruiting ARL1 and its effectors to the trans-Golgi, influencing the localization of specific Golgi proteins. While the role of ARFRP1 in intracellular protein transport and Golgi dynamics is known, its direct link to IBD remains unclear. Inflammation involves complex biological responses with numerous cellular signaling pathways, and ARFRP1 could influence inflammatory responses by regulating the transport of intracellular inflammatory mediators or receptors. The Golgi function supported by ARFRP1 is crucial for protein post-modification and sorting, potentially involving immune responses. Although there's no direct link between ARFRP1 and inflammation, its role in the TGN could affect the sorting and secretion of cytokines, key mediators of inflammation. Dysregulation of vesicular transport could trigger abnormal immune responses, leading to inflammatory diseases [49]. As a member of the ARF family involved in vesicular transport and membrane fusion, ARFRP1 affects key sites in the TGN for intracellular protein sorting and modification [50]. It also plays a role in maintaining organelle structure and lipid metabolism, crucial for cellular homeostasis and responses to external stimuli, such as pathogen-triggered immune responses [51,52].

Inhibition of ARFRP1 reduces triglyceride release in Caco-2 cells, increases the abundance of lipolysis-related proteins, and decreases the use of fatty acids for triglyceride synthesis and their release via chylomicrons [53].

STMN3, part of the stathmin protein family, plays a key role in regulating microtubule dynamics. It disrupts the orderly assembly of microtubules by forming complexes with tubulin, affecting the cell cycle and movement. Although the direct role of STMN3 in inflammation is not well-defined, its involvement in key processes of immune cell function suggests it may indirectly impact inflammatory responses [53]. STMN3's effect on microtubule stability is crucial for immune cell migration to inflammation sites, and changes in its expression or function could alter immune cell behavior during inflammation. Stathmin-3 can be phosphorylated by JNK, a kinase involved in stress and inflammatory responses, and this post-translational modification may regulate responses to inflammatory stimuli [54]. Interactions between STMN3 and other proteins and signaling pathways could influence inflammatory diseases, such as airway inflammation and remodeling in asthma [55]. Furthermore, STMN3 is regulated by ID1, a transcription factor induced by nicotine or EGF stimulation in non-small cell lung cancer, promoting cell proliferation and invasion. Given the role of inflammation in cancer progression, ID1 regulation of STMN3 might link inflammatory signals to cancer cell behavior.

Cyclophosphamide, an oxazaphosphorus-substituted nitrogen mustard alkylating agent with immunosuppression properties accompanied by large drug toxicity [56], was first reported by Arnold and Bourseaux in 1958. Currently, cyclophosphamide is primarily used in the treatment of malignant tumours and certain autoimmune diseases with severe extrinsic manifestations like lupus, many types of vasculitis and systemic sclerosis [57]. In this study, drug predictions revealed cyclophosphamide as a potential therapeutic agent for IBD treatment. Moreover, several prospective uncontrolled pilot studies have found that intravenous shock cyclophosphamide is effective in treating patients with severe IBD who do not respond to steroid therapy and have a high safety profile [58, 59]. Similarly, another retrospective multi-centre case study reported an effectiveness rate of 68% for cyclophosphamide pulse therapy in patients with CD who failed to respond to treatment with biologics or steroids [60].

Our study has certain limitations. First, the limited sample size of the IBD (including UC and CD) GWAS dataset and blood eQTL data cannot guarantee the robustness of the study results. Thus, large-scale IBD (including UC and CD) GWAS and eQTL datasets should be used in the future, along with relevant analyses such as TWAS, SMR, and colocalisation to provide reliable and significant results. Second, the populations involved in this study are all European, limiting the generalisation of the results in terms of the global population. Hence, more diverse population data is required to verify our results. Third, We only analyzed eQTL data for blood and did not perform analysis of data from other sites, especially eQTL data for intestinal sites and IBD (including UC and CD).

Our study has unveiled the causal associations between CARD9, RTEL1, STMN3, ARFRP1, and inflammatory bowel disease, offering novel insights into the disease's mechanisms. Future research should delve deeper into the functionality and mechanisms of action of these genes, as well as the efficacy of Cyclophosphamide as a potential treatment option, thereby paving new avenues for IBD therapy.

5. Conclusion

In summary, this study, through the integration of blood eQTL data with GWAS meta-data for IBD, including UC and CD, and employing a variety of research analytical methods such as TWAS, SMR, and Bayesian colocalisation analysis, has not only reconfirmed known genes associated with IBD, such as CARD9, but also uncovered new potential pathogenic genes like RTEL1, STMN3, and ARFRP1. Notably, while CARD9 has been widely associated with IBD in prior research, our study further supports its significance in the disease mechanism and underscores the importance of discovering and elucidating new functions for known genes. Additionally, our analysis suggests cyclophosphamide as a potential therapeutic for IBD, offering fresh avenues for drug development in IBD.

Particularly, beyond further substantiation of CARD9, this study's emphasis on unveiling the potential roles of the other three genes, especially ARFRP1, suggests its possible inhibitory role in the pathogenesis of IBD. These insights not only augment our understanding of the complex pathological mechanisms of IBD but also pave new paths for future targeted therapies. In essence, our research highlights the importance of delving into known associated genes while also exploring and validating new pathogenic genes and therapeutic targets in IBD research. These achievements provide novel perspectives and directions for the study of the pathogenesis and clinical treatment of IBD, including UC and CD.

Data availability statement

IBD data can be obtained from https://gwas.mrcieu.ac.uk/datasets/, whereas blood eQTL data can be obtained from http://gusevlab.org/projects/fusion/. SMR import data format was obtained from https://yanglab.westlake.edu.cn/software/smr/#eQTLsummarydata.

Ethics statement

Not applicable.

Funding statement

CRediT authorship contribution statement

Qinghua Luo: Writing – original draft, Methodology, Formal analysis, Data curation. **Jiawen Wang:** Writing – original draft, Visualization, Funding acquisition. **Wei Ge:** Writing – original draft, Visualization, Data curation. **Zihao Li:** Writing – original draft, Visualization. **Yuanting Mao:** Writing – original draft, Visualization. **Chen Wang:** Writing – review & editing, Visualization, Data curation. **Leichang Zhang:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28944.

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