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Integrated analysis of multi-omics data for the discovery of biomarkers and therapeutic targets for juvenile idiopathic arthritis

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ABSTRACT

Background: Juvenile idiopathic arthritis (JIA) is a prevalent chronic rheumatic disease affecting children. Current medications merely alleviate symptoms rather than curing the disease. Hence, the identification and development of novel drug targets and biomarkers for JIA are imperative for enhancing treatment efficacy.
Methods: We employed two-sample Mendelian randomization (MR) analysis to investigate the causal effects of plasma proteins on JIA. Additionally, colocalization, bulk RNA-seq, and single-cell RNA-seq analyses were conducted to further investigate and validate the potential of candidate proteins as drug targets.
Results: Through MR analysis, we successfully identified five plasma proteins that are causally linked to JIA. Genetically inferred lower levels of AIF1, TNF, and TNFSF11 were associated with an elevated risk of JIA, while higher levels of AGER and GP1BA proteins were positively correlated with JIA risk. Colocalization analysis further supported our findings on GP1BA (OR = 9.26, 95 % CI: 2.30–37.20) and TNFSF11 (OR = 0.18, 95 % CI: 0.07–0.45). Based on this evidence, we classified these five proteins into two tiers. Finally, we conducted a systematic evaluation of the druggability and current drug development progress for these identified candidate proteins.
Conclusions: This study employed MR analysis to reveal causal relationships between plasma proteins and JIA, identifying five potential candidate proteins as promising drug targets for JIA, particularly focusing on GP1BA and TNFSF11.

1. Introduction

Juvenile idiopathic arthritis (JIA) is a common chronic rheumatic disease in childhood. Clinical symptoms of JIA include joint pain, stiffness, and inflammation [1]. The diagnostic criteria for JIA require onset before the age of 16 years and arthritis lasting more than 6 weeks [2,3]. It affects approximately 16–150 children per 100,000 individuals [3]. While remission can be achieved in some JIA patients, a significant proportion, ranging from 30 % to 60 %, continue to experience active disease into adulthood [4,5]. Furthermore, although remission can often be achieved with specific medications, there is currently no cure for JIA [6]. As a result, patients with JIA often require lifelong medication, which can be a significant burden. Thus, it is imperative to identify and develop new drug targets and biomarkers for JIA to enhance therapeutic outcomes.

Blood plasma proteins play a vital role in various biological processes such as signaling, transportation, and inflammation [7]. Proteomics research improves our understanding of molecular mechanisms and identifies potential therapeutic targets. For example, certain drug classes used in JIA target specific proteins. Etanercept binds specifically to TNF-α and lymphotoxin and infliximab binds specifically to TNF-α [8]. Recently, multiple genome-wide association studies (GWASs) have discovered protein quantitative trait loci (pQTLs) for numerous plasma proteins [9–12]. The majority of circulating proteins are linked to cis-pQTLs, which are located within 1 Mbp of the gene encoding the protein. These cis-pQTLs explain a substantial proportion of the

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protein's variance [12]. These pQTLs offer the opportunity to employ Mendelian randomization (MR) as potent instrumental variables for causal inference.

MR is a powerful method that utilizes genetic variants extracted from GWAS summary statistics as instrumental variables to assess the causal impact of exposure (plasma proteins) on outcome (JIA) [13,14]. Two-sample MR can calculate the causal impact of exposure on outcome by using genetic variants that are only associated with the exposure and solely affect the outcome through the exposure. Compared to observational studies, MR can avoid confounding factors from the environment and reverse causality, as the genetic variants used in MR are less susceptible to environmental changes [15]. Furthermore, the efficiency and cost-effectiveness of MR make it suitable for large-scale causal relationship screening.

This study aimed to investigate potential therapeutic targets for JIA by employing MR to identify potential causal plasma proteins using GWAS data. Initially, we utilized MR to identify plasma proteins potentially causally linked to JIA as the primary findings. Subsequently, external datasets and colocalization analysis were employed to validate these primary findings. To gain deeper insights into the functions and interactions of candidate plasma proteins, Protein-Protein Interaction (PPI) network analysis, bulk RNA-seq analysis, and single cell RNA-seq analysis were conducted. Additionally, the druggability of these candidate proteins was assessed using the DrugBank database.

2. Materials and methods

2.1. Proteomic data source

The proteomic data are derived from the UK Biobank Pharma Proteomics Project (UKB-PPP) [12]. Researchers conducted proteomic profiling on blood plasma samples collected from 54,219 UKB participants using the antibody-based Olink Explore 3072 proximity extension assay (PEA), measuring 2941 protein analytes and capturing 2923 unique proteins. For each plasma protein, cis-pQTLs (within a ±1 MB window of the gene encoding the target protein) from the corresponding GWAS were utilized as genetic instruments. The selection of pQTL instruments involved the following steps: (i) exhibited genome-wide significant association ($P < 5 \times 10^{-8}$), (ii) demonstrated independent association (LD clumping $r^2 < 0.001$), and (iii) assessed the strength of genetic instruments using the F-statistic, with a threshold of greater than 10 (calculated using the formula: $R^2 = 2 \times EAF \times (1\text{-}EAF) \times beta^2$; $F = R^2 \times (N-2)/(1-R^2)$).

2.2. Outcome data sources

The primary analysis of the outcome GWAS data was sourced from the research conducted by Anne Hinks et al., which involved 15,872 individuals of European ancestry (2816 cases and 13,056 controls) [16]. The GWAS summary data for another JIA study were sourced from the study conducted by Elena Lopez-Isac et al., which comprised 12,501 individuals of European ancestry (3305 cases and 9196 controls) [17]. The summary-level data of JIA download links were displayed in Table S1.

2.3. Mendelian randomization analysis

MR serves as a robust tool for evaluating the causal effects of exposure factors on outcomes [18]. In this study, we utilized the "TwoSampleMR" package (https://github.com/MRCIEU/TwoSampleMR) to conduct MR analysis and explore the association between plasma proteins and JIA. The wald ratio was employed when only one pQTL was available for a specific protein, while the inverse variance weighted (IVW) method was applied when two or more genetic instruments were available. To correct for multiple testing, we adopted the false discovery rate (FDR). Proteins with an FDR <0.05 were considered to have a causal effect on JIA. Furthermore, only proteins demonstrating significance in both the discovery and replication cohorts were deemed to be associated with JIA.

2.4. Colocalization analysis

The colocalization analysis, a statistical approach grounded in GWAS data, utilizes summary statistics from single variants to determine if two genetically correlated traits with independent associations share a common genetic locus. This process aids in confirming the causal relationship between genetic variation and the outcome, rather than being a result of LD or other confounding factors. To validate the MR results and investigate the genetic links between plasma proteins and JIA, we employed the coloc R package to conduct Bayesian colocalization analysis using GWAS summary statistics of plasma proteins and JIA [19]. The colocalization analysis comprised five hypotheses: (i) H0, no causal variant existed for either protein or JIA in the genomic locus; (ii) H1, there was a single causal variant for the protein only; (iii) H2, there was a single causal variant for JIA only; (iv) H3, two distinct causal variants existed for both protein and JIA; (iv) H4, a shared causal variant was present for both protein and JIA. We computed the posterior probabilities (PPs) for every hypothesis and ascertained the evidence of colocalization for a protein by establishing a protein-based PP threshold of 0.6 [20].

2.5. Protein-protein interaction network

GeneMANIA (https://genemania.org/) is a sophisticated tool that amalgamates diverse genomic and proteomic data to formulate hypotheses regarding gene function, including protein-protein interactions, co-expression relationships, genetic interactions, pathways, and protein domain similarities [21]. In this research, we utilized GeneMANIA to conduct a protein-protein interaction (PPI) network analysis to explore potential interactions among candidate proteins.

2.6. Bulk RNA-seq analysis

Transcriptome expression profiles of whole blood were sourced from the Gene Expression Omnibus (GEO) database (accession number: GSE112057), which includes 12 healthy control samples and 115 samples from individuals with JIA [22]. RNA extracted from whole blood underwent RNA sequencing (RNA-Seq) for profiles of gene expression. The sequences were aligned to the human reference genome (hg19), and the aligned reads were quantified as read counts per gene using SAMtools and HTSeq with default parameters. The raw read counts were then normalized to transcripts per million (TPM) for further analysis.

2.7. Single cell RNA-seq analysis

Single-cell RNA-seq data of human whole blood samples from individuals with JIA and normal samples were accessed through the GEO database with the accession number GSE205095, [23]. The dataset consisted of 6 JIA samples and 2 healthy control samples. Quality control, filtering, and clustering analyses were conducted using the R package Seurat [24]. Cells with fewer than 200 genes were excluded to remove low-quality cells. Probable doublets were filtered out by excluding cells with more than 2000 genes or 10,000 UMIs. Additionally, cells with a mitochondrial fraction exceeding 5 % were excluded. In total, 20,858 cells successfully passed the filtering process.

2.8. Druggable proteins identification

The DrugBank database is a comprehensive resource offering detailed insights into drugs, encompassing their mechanisms of action, pharmacology, interactions, and therapeutic applications [25]. We utilized the DrugBank database to evaluate the druggability of candidate

proteins. This database offers extensive documentation on these proteins, including information about associated drugs and their developmental pathways. This approach enables the identification of opportunities for drug repurposing, wherein medications originally intended for other medical conditions show promise for treating JIA.

3. Results

3.1. MR analysis revealed 5 plasma proteins causally associated with JIA

The study design flowchart is detailed in Fig. 1. Through MR analysis, we successfully identified 11 plasma proteins with a causal relationship with JIA after adjusting for False Discovery Rate (FDR <0.05) in the Discovery outcome dataset (Fig. 2A, Table S2 and Table S3). Among these proteins, five were validated in the replication dataset ($P < 0.05$) (Fig. 2B, C, 2D, and Table S4). Notably, MR analysis suggested that genetically inferred lower levels of AIF1, TNF, and TNFSF11 were associated with an elevated risk of JIA. In contrast, higher levels of AGER and GP1BA proteins were positively correlated with JIA risk.

3.2. Colocalization analysis identified candidate therapeutic targets for JIA

We performed colocalization analysis on the 5 proteins identified in the MR results to ascertain the potential shared causal genetic variations associated with JIA and pQTLs. The analysis confirmed the validation of 2 proteins (PP.H4 > 0.6) (Table S5). Among the 5 candidate proteins, TNFSF11 (PP.H4 $= 0.89$) and GP1BA (PP.H4 $= 0.68$) emerged as the most prominent candidates for JIA risk (Figure S1 and Figure S2). Combining the above evidence, we classified these 5 proteins into two tiers. Two proteins (GP1BA and TNFSF11) passed all tests and were classified into tier 1 (Table 1). The remaining three proteins (AGER, AIF1, and TNF) were assigned to tier 2.

3.3. Network functional analysis of candidate proteins

To further explore the interactions among candidate proteins and their involvement in functional pathways, we performed the PPI network analysis (Fig. 3). This analysis disclosed that the majority of the connections were due to physical interactions, co-expression relationships, and genetic interactions. The functional profiling of the network highlighted the roles of AGER and AIF1 in mononuclear cell migration, and TNF and TNFSF11 in the modulation of inflammatory responses and mononuclear cell migration. Additionally, GP1BA was identified as a node in multiple functional pathways, including blood coagulation and the protein activation cascade.

3.4. Bulk RNA analysis of candidate proteins

To further investigate the expression of candidate proteins at the transcriptome level, we conducted Bulk RNA analysis using data from the GEO database. The results revealed a significant upregulation of GP1BA in JIA compared to healthy individuals, whereas TNF exhibited a significant downregulation in JIA (Fig. 4A and B). However, the expression levels of AGER, AIF1, and TNFSF11 did not show significant differences between the JIA and control groups (Fig. S3).

3.5. Cell-specific expression of five candidate proteins in JIA

To investigate whether the coding genes of the 5 circulating proteins exhibit cell type-specific expression, we conducted single-cell-type expression analysis using single-cell RNA-seq data from GEO. The scRNA-seq data comprised 8 samples, consisting of human peripheral blood mononuclear cells from 2 healthy controls and 6 JIA patients. After meticulous preprocessing of the data, incorporating stringent quality control metrics, we applied the UMAP technique based on the top 10 principal components to visualize the high-dimensional scRNA-seq data. Subsequently, we precisely classified the cells into 10

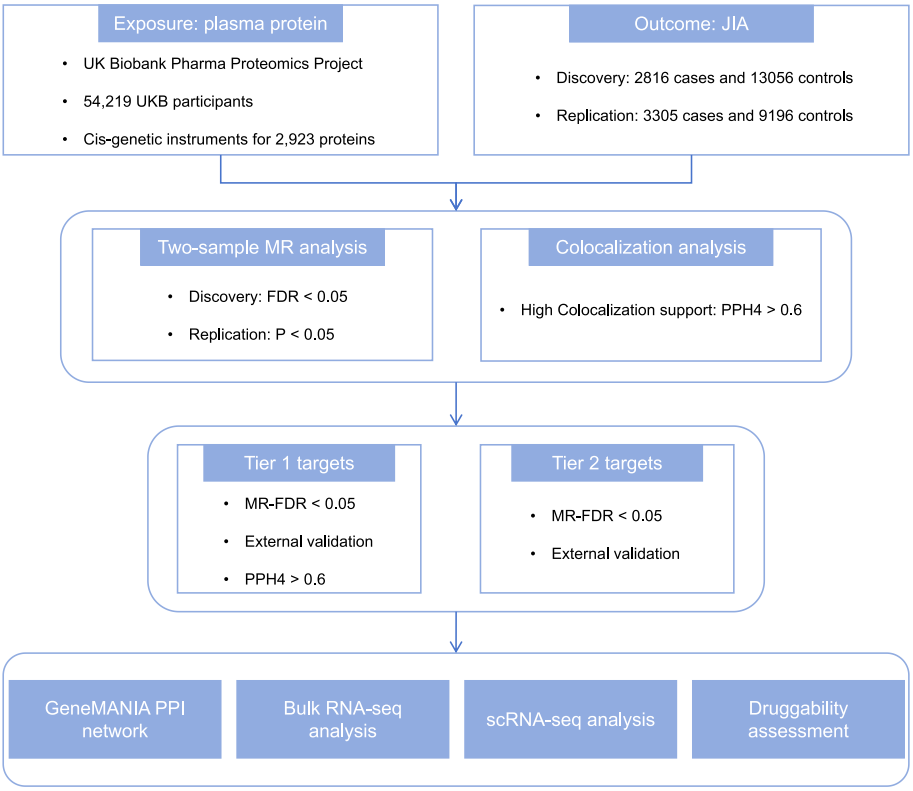

Fig. 1. Study overview.

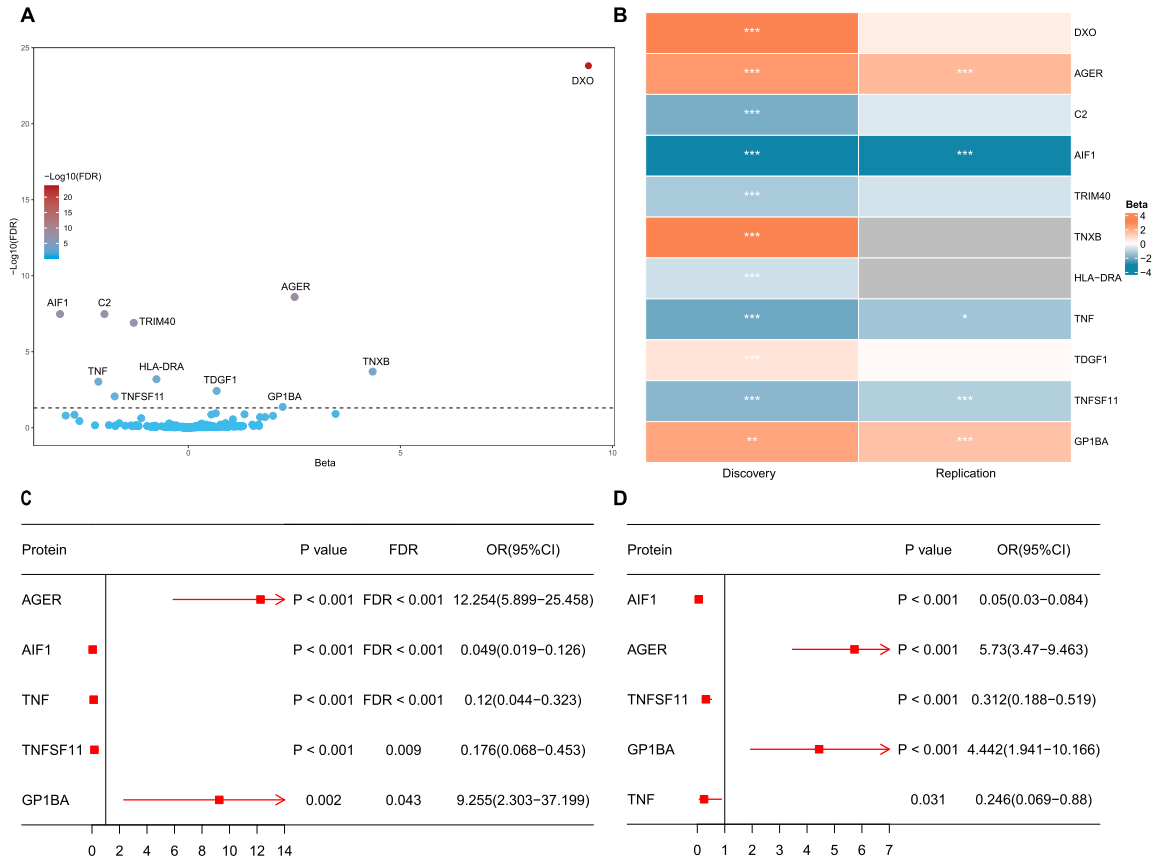

Fig. 2. **A** The volcano plot illustrates the impact of 11 candidate plasma proteins associated with JIA. **B** The heatmap illustrates the estimated effects of the 11 candidate proteins identified in MR analysis. **C** displays the results from the discovery dataset. **D** displays the results from the replication dataset.

Table 1
Evidence supporting potential proteins significantly associated with JIA.

Tissue	Protein	Protein full name	Uniprot ID	method	nsnps	pvalue	OR (95%CI)	FDR	Replication MR analysis	Colocalization	Category
Blood	AGER	Advanced glycosylation end product-specific receptor	Q15109	Wald ratio	1	1.85E-11	12.254 (5.899–25.458)	2.53E-09	Yes	No	tier 2
Blood	AIF1	Allograft inflammatory factor 1	P55008	Wald ratio	1	4.88E-10	0.049 (0.019–0.126)	3.33E-08	Yes	No	tier 2
Blood	TNFSF11	Tumor necrosis factor ligand superfamily member 11	O14788	Inverse variance weighted	2	2.76E-05	0.12 (0.044–0.323)	9.41E-04	Yes	Yes	tier 1
Blood	GP1BA	Platelet glycoprotein Ib alpha chain	P07359	Wald ratio	1	3.18E-04	0.176 (0.068–0.453)	8.68E-03	Yes	Yes	tier 1
Blood	TNF	Tumor necrosis factor	P01375	Wald ratio	1	1.72E-03	9.255 (2.303–37.199)	4.26E-02	Yes	No	tier 2

subclusters and identified their respective cell types using the SingleR R package (Fig. 4C and Fig. S4) [26]. The predominant cell types identified included B cells, monocytes, T cells, and NK cells (Fig. 4D). Interestingly, AIF1 exhibited specific expression in T cells and monocytes, as depicted in Fig. 5. In contrast, GP1BA, AGER, TNF, and TNFSF11 demonstrated negligible expression in any specific cell type, as illustrated in Fig. 5, 5 and 6.

3.6. Assessment of potential therapeutic targets druggability

We conducted a systematic evaluation of the druggability and drug development progress for the five identified candidate proteins using the DrugBank database (Fig. S6). Notably, therapeutics targeting TNFSF11, such as recombinant osteoprotegerin and thiocolchicoside, have been approved for treating bone-related conditions. Lenalidomide is also used in the management of multiple myeloma. For AGER, oxytocin-based drugs are utilized for various purposes, including postpartum hemorrhage management. GP1BA-targeted medications, including dexibuprofen for pain and inflammation and ibuprofen for joint symptoms, are commonly prescribed. The drug development landscape for TNF includes diverse therapeutics, such as certolizumab pegol for autoimmune and autoinflammatory disorders and dilmapimod for arthritis treatment. Etanercept is applied in the treatment of severe rheumatoid arthritis and moderate to severe plaque psoriasis. However, no drugs targeting AIF1 are currently available.

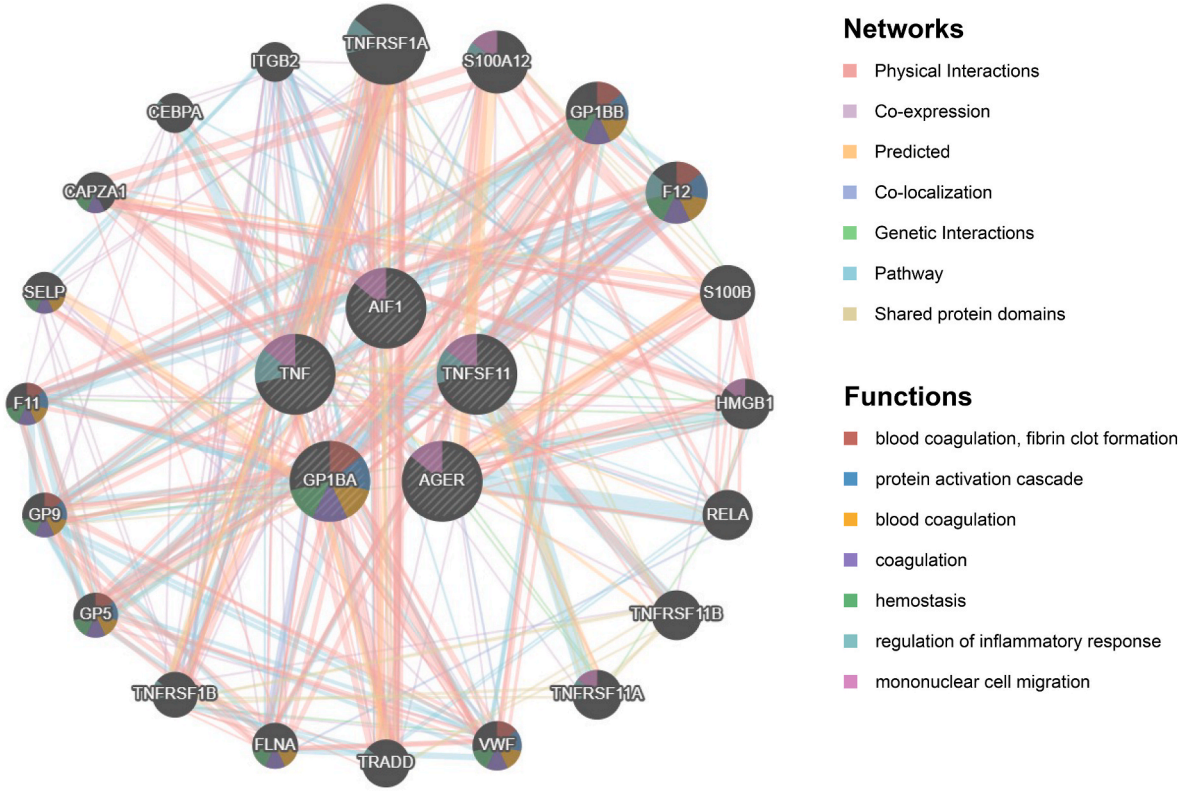

Fig. 3. The PPI network was constructed using GeneMANIA.

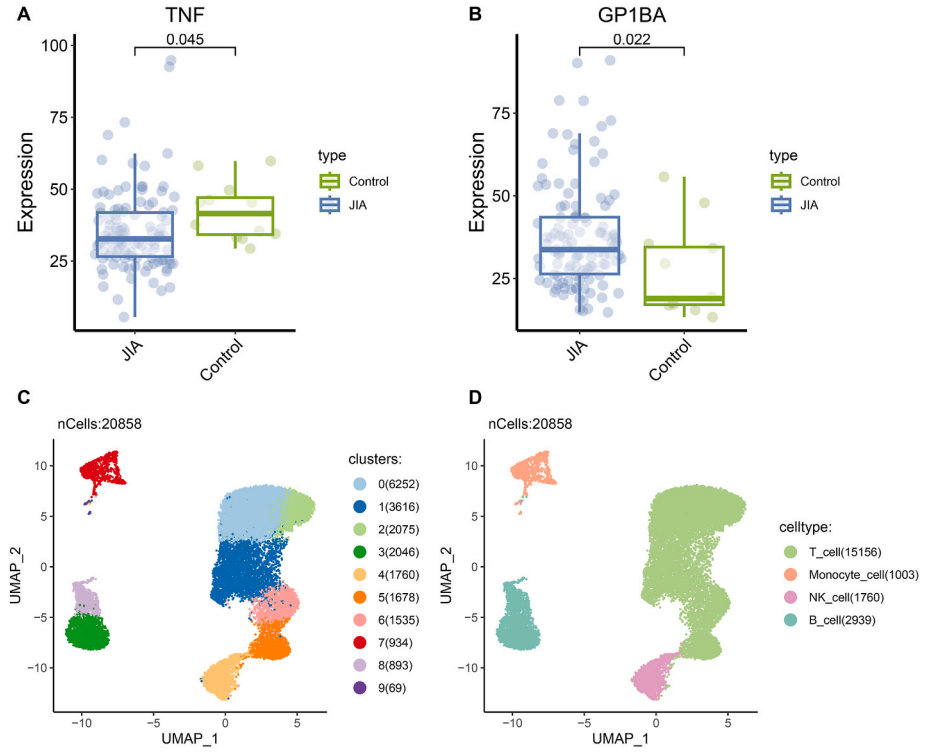

Fig. 4. **A** The box plot illustrates the expression levels of TNF gene in both normal and JIA samples in bulk RNA-seq analysis. **B** The box plot illustrates the expression levels of GP1BA gene in both normal and JIA samples in bulk RNA-seq analysis. **C** 2D visualization of single-cell types by UMAP, with a total of 10 cell clusters. **D** UMAP plots revealing 4 cell types of all groups.

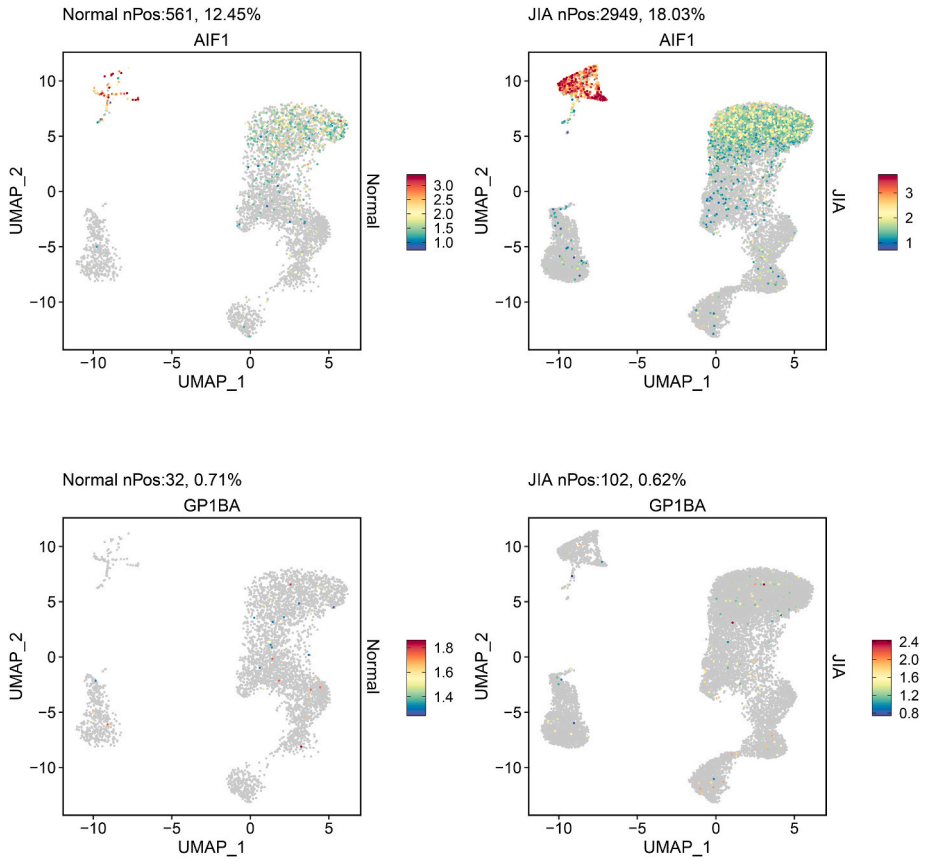

Fig. 5. The UMAP plot illustrates the expression patterns of the AIF1 and GP1BA proteins across various cell types in both normal and JIA samples.

4. Discussion

In this study, we thoroughly investigated the causal relationships between 2923 plasma proteins and the risk of JIA. Utilizing both discovery and replication phases of proteome-wide MR analysis, we found five proteins with significant associations. Elevated levels of AGER and GP1BA, as well as reduced levels of AIF1, TNF, and TNFSF11, correlated with an increased risk of JIA. To substantiate these findings, colocalization analysis was conducted, confirming the reliability of these associations. Our findings classify the five proteins with causal links to JIA into two tiers: GP1BA and TNFSF11 in Tier 1, presenting the most robust evidence, while AGER, AIF1, and TNF are placed in Tier 2, indicating strong evidence. Moreover, we conducted a PPI network for the JIA-associated proteins to elucidate their roles in biological pathways. Additionally, we performed bulk RNA-seq analysis and single-cell RNA-seq analysis to explore the cell types exhibiting specific expression of the five proteins. Finally, we utilized the DrugBank database to assess their potential as therapeutic targets for treating JIA.

JIA is a heterogeneous disease, with current therapeutic efforts primarily focused on symptom relief. However, targeted treatments based on etiology and pathological mechanisms hold promise for improving outcomes in JIA patients. Despite the identification of numerous novel targets for JIA in recent years, leveraging these discoveries to develop new drugs or repurpose existing ones for JIA treatment remains challenging. Our study utilized GWAS and pQTL data to identify a range of potential drug targets for JIA. Furthermore, we assessed their druggability as potential therapeutic targets using the DrugBank database, which is crucial for successful market approval.

Glycoprotein Ib platelet subunit alpha (GP1BA) is a platelet surface membrane glycoprotein consisting of a heterodimer, comprised of an alpha chain and a beta chain connected by disulfide bonds [27]. Studies have demonstrated that during the initial adhesion of platelets, a crucial platelet membrane glycoprotein, known as GP1BA, efficiently binds with von Willebrand factor (VWF) and fibrinogen to create the glycoprotein Ib/IX/V complex, contributing to platelet aggregation [28,29]. Currently, there are numerous drugs developed targeting GP1BA. Among them, Ibuprofen, a nonsteroidal anti-inflammatory drug (NSAID) and non-selective cyclooxygenase (COX) inhibitor, is utilized to alleviate mild to moderate pain, fever, and inflammation. Additionally, a study by Shan Luo et al. suggests that platelets may play a causal role in the pathogenesis of oligoarticular and RF-negative polyarticular JIA, highlighting GP1BA as a potential therapeutic target for these JIA subtypes [30]. Therefore, GP1BA represents a promising therapeutic target for the treatment of JIA.

Tumor necrosis factor ligand superfamily member 11 (TNFSF11), belonging to the tumor necrosis factor (TNF) cytokine family, acts as a ligand for osteoprotegerin, playing a pivotal role in osteoclast differentiation and activation [31,32]. Additionally, TNFSF11 functions as a survival factor for dendritic cells and contributes to the regulation of T cell-dependent immune responses. The cytokine receptor activator of nuclear factor kappa B ligand (RANKL), encoded by the TNFSF11 gene, plays a crucial role in osteoclastogenesis, and bone loss is a prominent feature in early JIA, often associated with severe polyarticular disease [33,34]. Various forms of bone loss may manifest, such as focal articular erosions, periarticular osteopenia, and generalized osteoporosis. Throughout adulthood, the skeletal system undergoes continuous renewal via bone remodeling, where old or damaged bone is removed by osteoclasts through mechanisms largely unknown. Osteoblasts regulate bone remodeling by producing the osteoclast differentiation factor RANKL, encoded by the TNFSF11 gene. Currently, drugs targeting TNFSF11 include denosumab, lenalidomide, and thiocolchicoside, with denosumab specifically functioning as a RANKL inhibitor used to manage osteoporosis in high-risk patients. But there are no approved medications targeting TNFSF11 for treating JIA. Additional mechanistic

studies are needed to clarify TNFSF11's role in JIA bone metabolism, potentially establishing it as a promising therapeutic target for future treatment.

Aside from the two proteins (GP1BA and TNFSF11) validated through external validation and colocalization analysis, the remaining three proteins (AGER, AIF1, and TNF) continue to exhibit strong potential. TNF, also referred to as TNFα, earned its name due to its capacity to induce hemorrhagic necrosis of tumors in vivo [35]. It acts as a proinflammatory cytokine, exerting diverse inflammatory effects locally and systemically [36]. The primary source of TNF is activated mononuclear phagocytes, which are stimulated by lipopolysaccharide or endotoxin. Currently, TNF inhibitors used to treat JIA include etanercept, infliximab, and adalimumab. Etanercept was the first TNF inhibitor approved by the US FDA (in May 1999) for children with moderate to severe polyarticular JIA [37]. While not formally approved by the FDA for JIA, infliximab is commonly used and shows a positive response [38]. Adalimumab obtained FDA approval in February 2008 for treating moderately to severely active polyarticular-course JIA in children aged $\geq$4 years [39]. However, most TNF inhibitors share similar side effects [40,41]. Common adverse events include local injection site reactions, gastrointestinal discomfort, headaches, and rashes. Additionally, responses to TNF inhibitors may vary depending on the category of JIA. Patients with systemic juvenile idiopathic arthritis may respond poorly to TNF inhibitor therapy [42,43]. Therefore, further exploration of new drug targets and the development of novel medications are necessary. One gene linked to various disorders, including autoimmune conditions, is AGER, responsible for encoding multiple isoforms of a cell surface receptor known as the receptor for advanced glycation end-products (RAGE) [44]. RAGE, the protein produced by this gene, possesses immunoglobulin-like domains, rendering it a multiligand receptor with the ability to bind numerous molecules, primarily advanced glycation end-products (AGEs) - proteins and lipids that accumulate due to factors like hyperglycemia, oxidative stress, aging, and inflammation [45]. Moreover, a study indicates that the AGER rs1035798 AA genotype is associated with an increased risk of JIA in Belarusian children [46]. Allograft inflammatory factor 1 (AIF1) is a 17 kDa cytosolic protein that binds calcium and actin [47]. Its expression is induced by cytokines such as IFN-γ. Numerous studies have associated AIF1 with immune-inflammatory disorders, including kidney disease, rheumatoid arthritis, cancer, and cardiovascular diseases [48–51]. AIF1 plays a crucial role in immune responses and inflammatory processes. Some research suggests its involvement in the pathogenesis of rheumatoid arthritis, potentially as an inflammation factor that activates macrophages and whose expression can be upregulated by other pro-inflammatory factors [52,53]. Currently, research on the association between AIF1 and JIA is limited. Future studies are needed to comprehensively investigate its specific mechanisms in inflammatory responses, particularly in the context of JIA.

There are several limitations in this study. Firstly, the GWAS data used in our study exclusively derive from European populations, potentially limiting the generalizability of our findings to other ethnic groups. Secondly, further cell and animal experiments are needed to elucidate the biological mechanisms behind the in vitro and in vivo experimental results, providing a better understanding of the therapeutic efficacy of the targeted proteins studied. Thirdly, the data utilized in bulk RNA-seq analysis and single cell RNA-seq analysis originate from peripheral blood samples, necessitating future research to validate experimental results using JIA synovial fluid samples. Lastly, this study did not further investigate the subtypes of JIA, indicating the necessity for more comprehensive research in the future.

In summary, this study utilized proteome-wide MR to identify five plasma proteins associated with JIA, enhancing our understanding of its pathogenesis. These findings offer guidance and new directions for targeted therapeutic strategies. Further experimental research is warranted to assess the efficacy and feasibility of these candidate therapeutic targets.

5. Conclusions

Employing proteome-wide MR, this study evaluated the genetically inferred causal associations between a multitude of proteins and JIA, culminating in the identification of five potential druggable targets. Our research highlights the value of amalgamating extensive GWAS data with transcriptomic and proteomic information to accurately determine feasible drug targets.

CRediT authorship contribution statement

Yi-Xin Cai: Writing – original draft, Investigation, Formal analysis. **Xiao-Li Chen:** Writing – original draft, Investigation, Formal analysis. **Dai-Shan Zheng:** Investigation. **Yue-Zhong Huang:** Formal analysis. **Zhan-Pei Bai:** Formal analysis. **Xiu-Feng Huang:** Writing – review & editing, Funding acquisition.

Ethics approval and consent to participate

This study exclusively used publicly available data which was approved by the ethical reviews of the studies cited in the Methods section.

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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.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtauto.2024.100256.

Data availability

All data used in this study are publicly available data at the summary level, with citations to the relevant studies.

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