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Uncovering drug targets for cluster headache through proteome-wide Mendelian randomization analysis

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Abstract

Background Cluster headache (CH) is a highly disabling primary headache disorder with a complex underlying mechanism. However, there are currently no effective targeted therapeutic drugs available. Existing medications often have limited efficacy and numerous side effects, which frequently fail to meet clinical needs. This study aims to identify potential new therapeutic targets for CH through proteome-wide mendelian randomization (PWMR).

Methods We used PWMR to estimate the causal effects of plasma proteins on CH. This analysis integrated plasma protein quantitative trait loci (pQTL) data with genome-wide association study (GWAS) results of CH phenotypes. In addition, we conducted various sensitivity analyses, enrichment analyses, phenome-wide MR assessments, protein–protein interaction network construction, and mediation MR analyses to further validate the drug potential of the identified protein targets.

Results We identified 11 protein targets for CH ($p < 2.41 \times 10^{-5}$), with high-priority candidates exhibiting minimal side effects. Phenome-wide MR revealed novel targets—PXDNL, CCN4, PKD1, LGALS9, and MRC1—that show no significant disease-related adverse effects and interact with established preventive CH drug targets. Notably, PXDNL interacts with both acute and preventive CH drug targets. Furthermore, the causal effect of plasma proteins on CH is partially mediated by cortical surface area, with mediation proportions ranging from 3.2% to 10.0%.

Conclusions We identified a set of potential protein targets for CH, characterized by rare side effects and a strong association with the biological mechanisms underlying the disorder. These findings offer valuable insights for the development of targeted drug therapies in the treatment of CH.

Keywords Cluster headache, Drug targets, Mendelian randomization, Proteomics

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Introduction

Cluster headache (CH), a debilitating primary headache disorder, is marked by severe unilateral pain predominantly around the orbital region, often accompanied by ipsilateral cranial autonomic symptoms, restlessness, or both [1, 2]. Widely regarded as one of the most agonizing pain conditions—exceeding the pain of childbirth, bone fractures, or gunshot wounds [3]—CH poses significant challenges to effective management. Current treatments frequently fail to provide sufficient relief while presenting notable side effects. For instance, rebound headache is a recognized adverse event associated with the acute therapeutic use of oxygen [4]. Similarly, the prophylactic drug verapamil, while effective in 50–80% of patients, often necessitates very high doses, which frequently limit its use due to significant side effects [5]. This underscores the urgent need to identify novel drug targets for CH and to develop treatments that combine efficacy with minimal side effects.

Proteins play a central role in biological processes and represent key targets for drug development [6]. While observational studies have suggested associations between plasma proteins and CH [7, 8], their findings are often limited by confounding factors and reverse causality, making it unclear whether these proteins are causal drivers or consequences of the disease. Mendelian randomization (MR) offers a robust method to these challenges in drug target discovery and repurposing [9]. By using genetic variations as instrumental variables, MR estimates the causal relationship between an exposure and an outcome, effectively minimizing biases from confounding and reverse causality. This approach mirrors the rigor of randomized controlled trials and enhances the likelihood of success in drug development [10]. Recent advancements in high-throughput proteomics applied to plasma have further enabled MR-based strategies to identify promising therapeutic targets across a range of diseases [11–13].

In this study, we employed proteome-wide mendelian randomization (PWMR) to investigate the causal effects of plasma proteins on CH, leveraging the largest available datasets of pQTL and GWAS data for CH. To prioritize potential drug targets, we integrated phenome-wide MR with protein–protein interaction (PPI) analyses. Additionally, functional enrichment and two-step MR approaches were utilized to uncover biological pathways underlying the identified causal associations.

Methods

An overview of the study design is presented in Fig. 1. The investigation proceeded in four key stages: 1) Causal Relationship Identification: MR analysis was performed to assess causal relationships between exposures (plasma protein cis-pQTLs) and outcomes (CH). Enrichment analysis of identified drug target proteins was conducted to explore potential biological mechanisms. 2) Phenome-Wide MR Analysis: We evaluated potential side effects associated with significant proteins to ensure their safety profiles. 3) PPI: The relationship between proteins without significant side effects and established acute or preventive drug targets was examined through PPI analysis. 4) Mediation Analysis: To elucidate intermediate mechanisms, we identified cortical structure that mediate the effects of recognized drug target proteins on CH outcomes.

Ethical compliance was ensured, with informed consent obtained from all participants in the original GWAS. Ethical review and approval details for these datasets are available in the original studies.

Data sources for plasma proteins

Plasma protein were sourced from the comprehensive dataset conducted by the UK Biobank Pharma Proteomics Project (UKB-PPP) [14]. This initiative utilized the Olink platform to perform proteomic profiling on blood plasma samples from 54,219 participants, encompassing a total of 2,923 proteins.

Data sources for CH

Summary statistics for the MR analysis were sourced from the most extensive GWAS dataset on CH to date [15], comprising 25,772 individuals of European ancestry, including 4,043 cases and 21,729 controls. This pioneering dataset represents the largest meta-analysis of CH aimed at uncovering genetic risk variants and advancing our understanding of the disorder's biological underpinnings.

Mendelian randomization analysis

We conducted a two-sample MR analysis to investigate the causal effects of plasma proteins on CH, utilizing the TwoSampleMR package (https://github.com/MRCIEU/TwoSampleMR). For each protein, single-nucleotide polymorphisms (SNPs) within 1 Mb of the encoding gene, associated with plasma protein levels at genome-wide significance ($p < 5 \times 10^{-8}$), were identified. Linkage disequilibrium (LD) pruning was performed ($R^2 < 0.001$, window size = 10,000 kb) using European samples from the 1000 Genomes Project, and SNPs with minor allele frequency (MAF) < 0.01 were excluded. To avoid weak instrument bias, only SNPs with an F statistic > 20 were included as instrumental variables (IVs) [16, 17]. Causal effects were estimated using the inverse variance-weighted (IVW) method with a random-effects model for proteins with more than three IVs [18, 19], and a fixed-effect IVW method for proteins with two or three

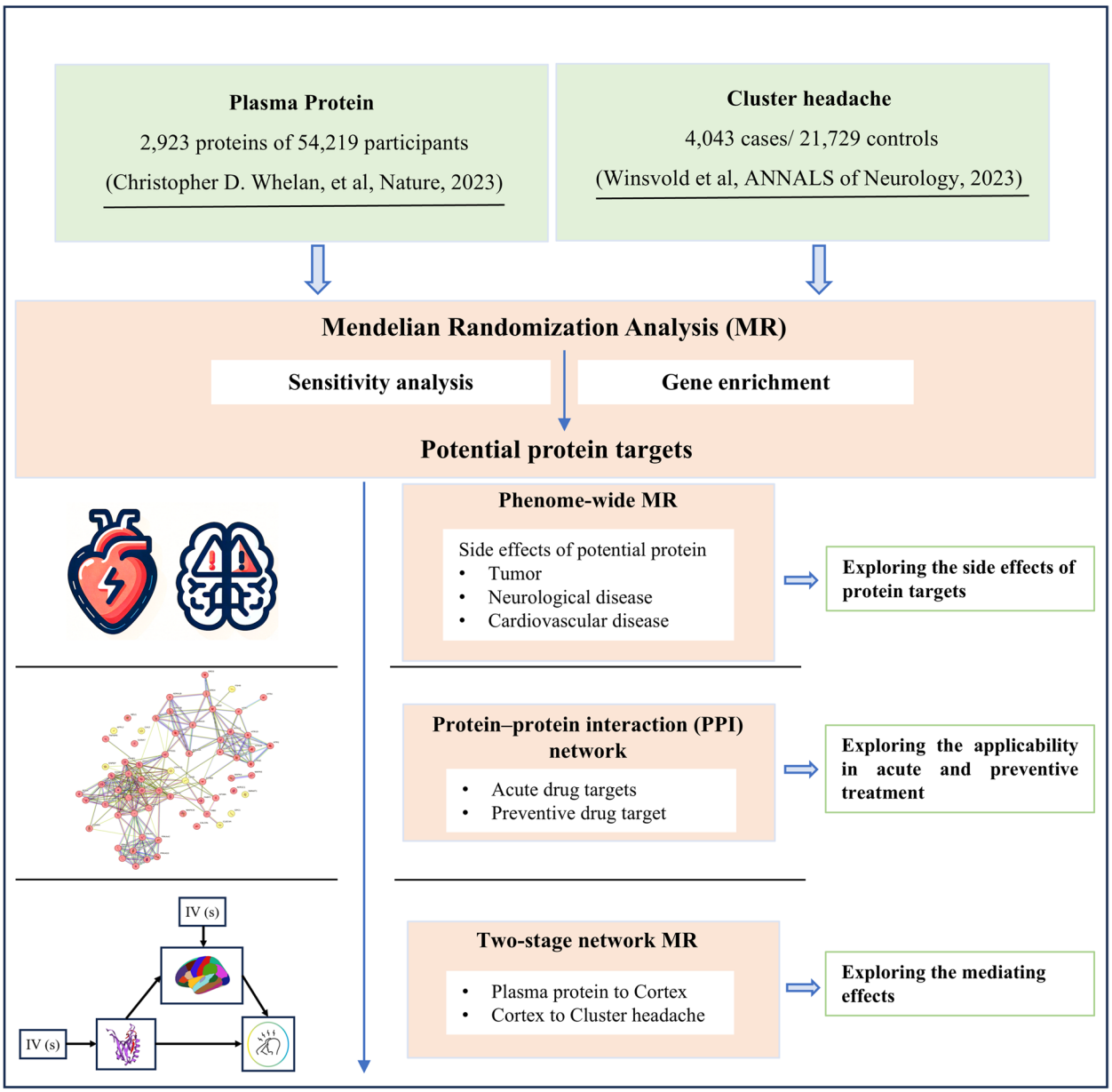

Fig. 1 Study design for identification of plasma proteins causally associated with CH

IVs [20]. For proteins with a single IV, the Wald ratio was applied. Odds ratios (OR) for CH risk were reported per standard deviation (SD) increase in plasma protein levels. Bonferroni correction was applied to account for multiple testing ($p < 2.41 \times 10^{-5}$, 0.05/2073; where 2073 is the number of proteins with at least one IV). To refine genetic instruments, heterogeneity was assessed, and outliers were adjusted or excluded using the ivw_radial and egger_radial functions from the RadialMR v0.4R package (https://github.com/WSpiller/RadialMR) [21], applying modified Q and Q' tests at a nominal significance level of 0.05. Ambiguous SNPs with non-concordant alleles (e.g., A/G vs. A/C) and palindromic SNPs with ambiguous strands (A/T or G/C) were corrected or excluded during harmonization to ensure alignment of exposure and outcome effects to the same allele. The study's robustness was ensured through comprehensive sensitivity analyses. Horizontal pleiotropy was assessed using MR-Egger regression and the MR-PRESSO Global test [22, 23], while Cochran's Q statistic evaluated heterogeneity across SNPs [24]. Reverse MR analyses were conducted to examine potential reverse causation between CH

liability and protein levels [25], further strengthening the validity of our findings.

Phenome-wide MR analysis

To ensure the safety and efficacy of potential drug targets, it is crucial that the identified proteins do not exhibit side effects that could lead to other major diseases, such as cancer, benign tumors, neurological disorders, or cardiovascular diseases. To evaluate the potential side effects of identified protein targets, we conducted phenome-wide MR analyses using disease outcomes from the FinnGen cohort (R8, total sample size = 342,499). FinnGen, a large-scale genomics initiative, integrates genetic data from over 500,000 Finnish biobank samples with health records to elucidate disease mechanisms and predispositions (https://finngen.gitbook.io/documentation) [26]. For this analysis, we focused on 1,421 non-headache disease traits with more than 500 cases to ensure sufficient statistical power. Multiple testing was addressed using Bonferroni correction.

To prioritize PWMR significant proteins as drug targets, we classified them based on the presence or absence of side effects associated with major diseases. Specifically: 1) Primary drug targets were defined as proteins entirely free of any side effects. 2) Secondary drug targets included proteins with potential side effects, provided these side effects were not linked to the aforementioned major diseases (cancer, benign tumors, neurological disorders, or cardiovascular diseases). Proteins associated with side effects related to these major diseases were excluded from further PPI analyses. This approach enhances the translational relevance of our findings by focusing on proteins with the highest potential as safe and effective therapeutic targets.

Gene enrichment

We employed GeneMANIA (http://www.genemania.org) to predict the functions and networks of cis-genes associated with proteins linked to CH. Detailed information on the datasets integrated into GeneMANIA can be found in the referenced literature [27]. Pathways demonstrating enriched functions were considered statistically significant if they exhibited a false discovery rate (FDR) below 0.05, ensuring the robust identification of biologically relevant interactions.

Protein–protein interaction network

We conducted a PPI network analysis to explore the potential mechanisms of both primary and secondary proteins, in conjunction with established CH drug targets. CH therapies are classified into acute and preventive categories. To investigate the interactions between the identified proteins and current therapeutic targets, we constructed PPI networks for CH. A recent guideline identified 6 acute drugs and 9 preventive drugs for CH [28], with corresponding protein targets retrieved from DrugBank (https://www.drugbank.ca) [29]. This guideline provides evidence-based recommendations for the treatment of CH, derived from a systematic review of the literature and the consensus of the European Society of Neurology Task Force expert panel. All PPI analyses were performed using the STRING database (version 12.0, https://string-db.org/), with a minimum interaction score of 0.4 to ensure the precision and relevance of the results [30].

Mediation analysis

Building on evidence that differential protein expression influences cortical structure [31, 32] and that cortical changes are implicated in the pathogenesis of CH [33, 34], we utilized cortical structure GWAS to explore the mediating role of cortical features in the relationship between protein targets with PPI interaction effects and CH. This analysis incorporated a meta-analysis of brain MRI data from 51,665 individuals across 60 cohorts, assessing both the surface area and average thickness of the entire cortex as well as 34 cortical regions with established functional specializations, as detailed in prior research [35]. We began by investigating the causal effects of cortical surface area and thickness on CH. Next, we evaluated the causal relationships between identified protein targets and cortical structures influencing CH, assessing whether cortical changes mediate the effect of proteins on CH. Finally, we quantified the indirect effect of protein targets on CH through cortical structures. The "product of coefficients" method was used to calculate indirect effects [36], with the "delta" method employed to estimate standard errors [37]. To ensure robust results, FDR correction was applied at each step. Mediation effects were defined as the ratio of the indirect effect to the total effect of plasma proteins on CH.

Results

Causal role of plasma proteins in CH

MR analysis identified 11 plasma proteins with significant causal links to CH ($p < 2.41 \times 10^{-5}$) (Fig. 2). Odds ratios ranged from 0.55 (95% confidence interval [CI], 0.40–0.70) for Macrophage Mannose Receptor 1 (MRC1) to 1.54 (95% CI, 1.28–1.80) for Polycystin-1 (PKD1) (Fig. 2 and Table S1). Of these, eight proteins acted as protective factors, while three emerged as risk factors.

Pathway enrichment and phenome-wide MR findings

The identified CH-related proteins formed interconnected networks involving co-expression, shared protein domains, and genetic interactions. Gene enrichment

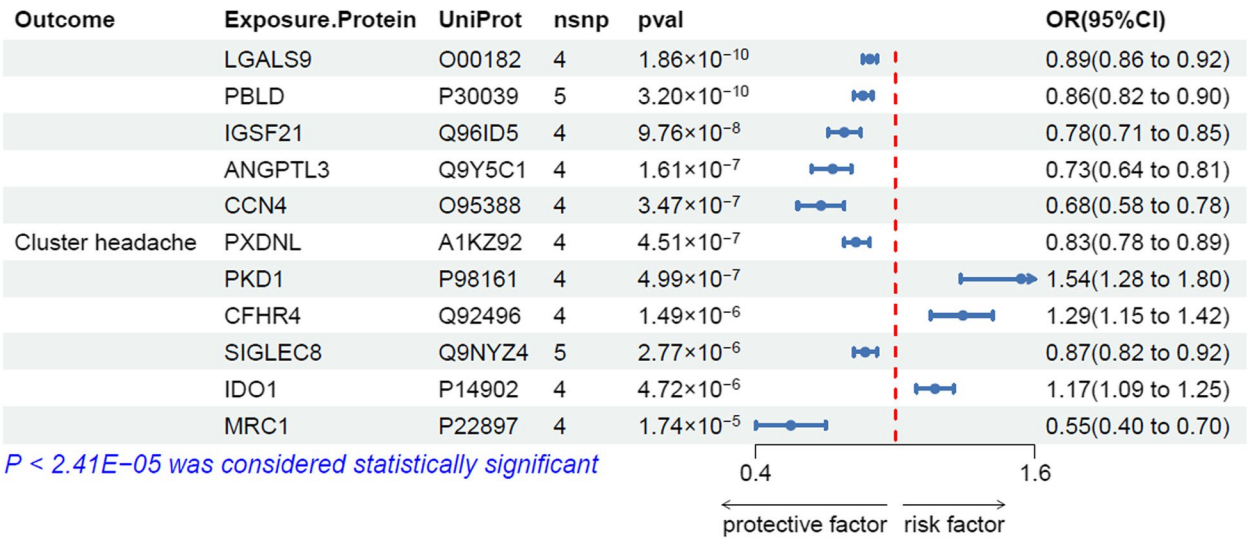

Fig. 2 MR results for plasma proteins and the risk of CH. The forest plot of the MR results for 2073 plasma proteins on the risk of CH. Each box represents the effect (i.e., OR change) per 1 SD change in the respective plasma proteins on CH and the error bars represent 95% CI. Arrows indicate that 95% CI exceeds the x axis. Bonfferoni corrected *P* = 2.41E-05 (0.05/2073). Abbreviations: OR, odds ratio; CI, confidence interval

analysis of cis-genes revealed pathways such as complement activation, regulation of humoral immune response, killing by host of symbiont cells, cytolysis, killing of cells in other organism involved in symbiotic interaction, killing of cells of other organism, alpha-amino acid catabolic process, and humoral immune response (Fig. 3A and Table S2).

Phenome-wide MR analysis further classified the proteins into primary and secondary drug targets. Key primary targets included MRC1, PKD1, CCN4, and PXDNL, meeting stringent Bonferroni correction thresholds ($p < 3.20 \times 10^{-6}$, 0.05/1421 diseases/11 total proteins). Secondary targets encompassed CFHR4 and LGALS9. These findings laid the groundwork for subsequent PPI analyses (Fig. 3B and Table S3).

Relevance to current CH medications

Comprehensive data on CH medications and their targets are presented in Table S4. Five identified proteins—PXDNL, CCN4, PKD1, LGALS9, and MRC1—interacted with established drug targets. These interactions align with preventive therapies such as verapamil, topiramate, melatonin, valproic acid, and lithium. Remarkably, PXDNL also showed associations with acute treatment targets, including oxygen and lidocaine (Fig. 4, Figure S1 and S2).

Mediation effect of proteins on CH outcomes via cortical structures

Our analysis revealed significant associations between cortical structure and CH, specifically involving the supramarginal surface areas, isthmus cingulate thickness and posterior cingulate surface areas (FDR $p = 5.39 \times 10^{-5}$, 0.002, and 0.049, respectively). Correlation analyses further identified CFHR4 as linked to the posterior cingulate surface area (FDR $p = 0.0086$) and PXDNL as associated with the supramarginal surface area (FDR $p = 0.0083$). Mediation analysis demonstrated that the influence of CFHR4 on CH was partially mediated by the posterior cingulate surface area, accounting for 3.2% of the effect (FDR $p = 0.016$). As CFHR4 has not been linked to any known drug targets, it was excluded from our analysis. PXDNL's impact on CH was mediated through the supramarginal surface area, with a mediation effect of 10.0% (FDR $p = 0.004$, Fig. 5, Table 1 and Table S5 and Table S6).

Sensitivity analysis

The robustness of the primary MR analyses was supported by multiple sensitivity tests. No evidence of heterogeneity was observed for any protein associations, as indicated by Cochran Q statistics ($p > 0.05$; Table S7). Instrumental variables demonstrated no horizontal pleiotropy, as confirmed by MR-Egger intercept values ($p > 0.05$) and the MR-PRESSO Global test ($p > 0.05$; Table S7). Additionally, reverse causation effects were ruled out, as shown in Table S8.

Discussion

This study employed two-sample MR to explore the causal effects of plasma proteins on CH, identifying 11 proteins with significant causal associations. These proteins are primarily enriched in complement activation

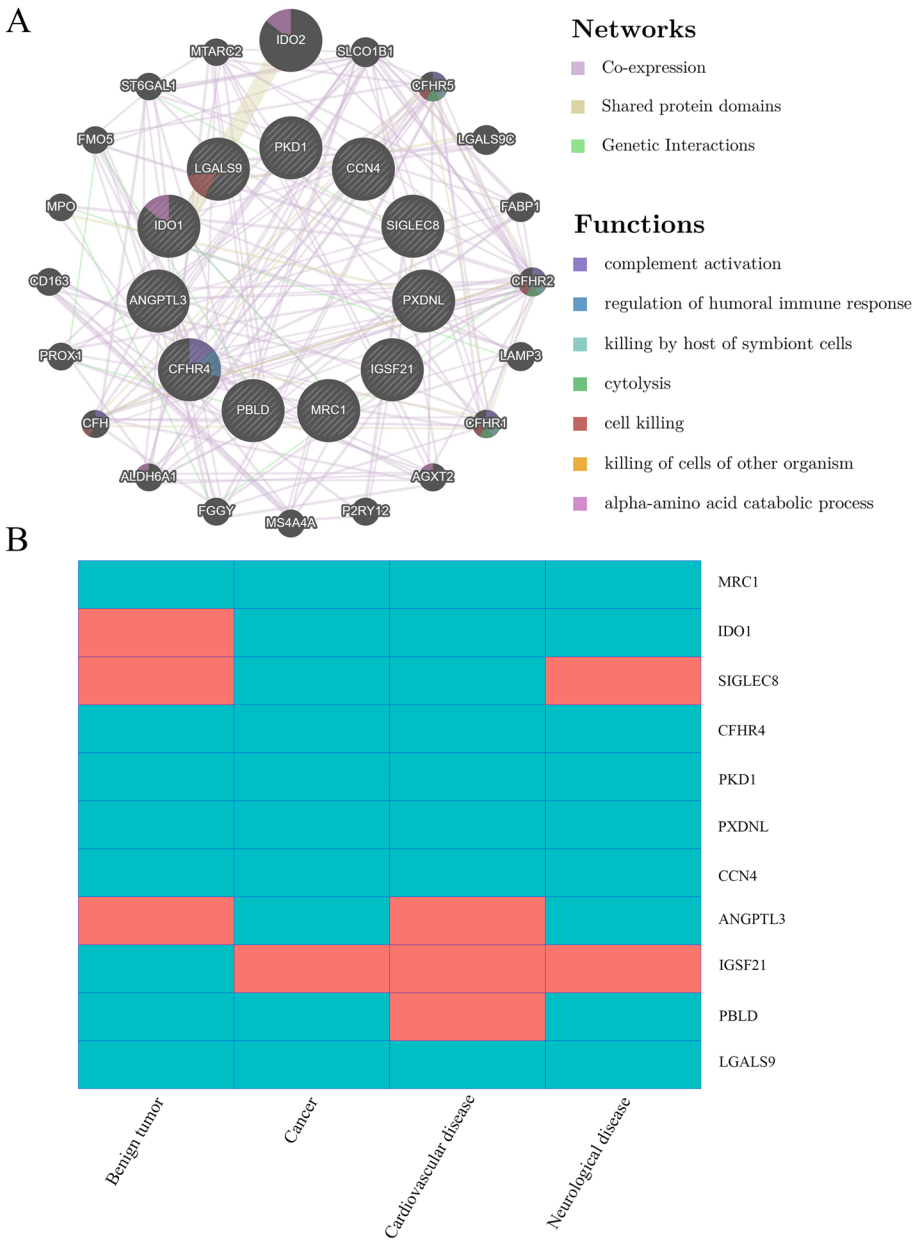

Fig. 3 Results of enrichment analysis and phenome-wide MR analysis. **A** Networks and functions of identified proteins associated with CH. The colors of the line represent different networks. The network and function prediction was based on an online tool: GeneMANIA (http://www.genemania.org). **B** Heatmap of phenome-wide MR results. Heat map of phenome-wide MR Results between CH related proteins and the Finngen disease database. Red squares indicate that the protein is associated with the disease. Bonferroni correction $p < 3.20 \times 10^{-6}$ (0.05/1421 diseases/11 total proteins)

and immune response regulation. Among these, primary drug targets include PXDNL, MRC1, PKD1, and CCN4, while LGALS9 was categorized as a secondary drug target. Furthermore, specific cortical structures were found to partially mediate the influence of these protein targets on CH, highlighting their potential role in the pathophysiology of the disorder.

PXDNL and peroxidasin (PXDN) are members of the peroxidase-cyclooxygenase superfamily [38]. Our findings suggest that elevated expression of PXDNL may mitigate the risk of CH. The PXDNL gene encodes a peroxidase protein secreted into the extracellular matrix, where it plays a crucial role in heme oxygenase-1 (HO-1)-dependent cell adhesion and enhances cellular

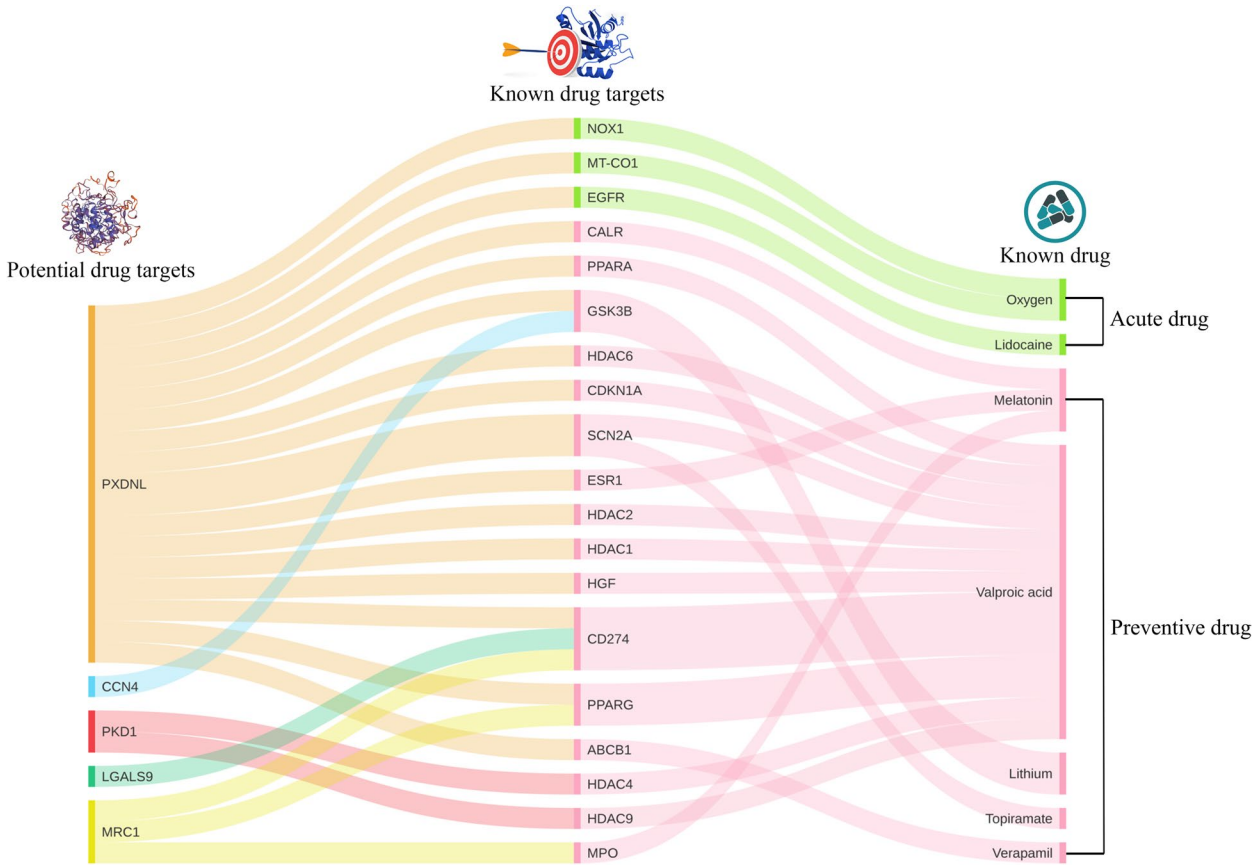

Fig. 4 Interaction between commonly used acute and preventive CH medications targets and identified potential drug targets. The CH-associated proteins, including PXDNL, CCN4, PKD1, LGALS9, and MRC1 were linked to preventive CH drugs. PXDNL is also associated with acute CH drugs

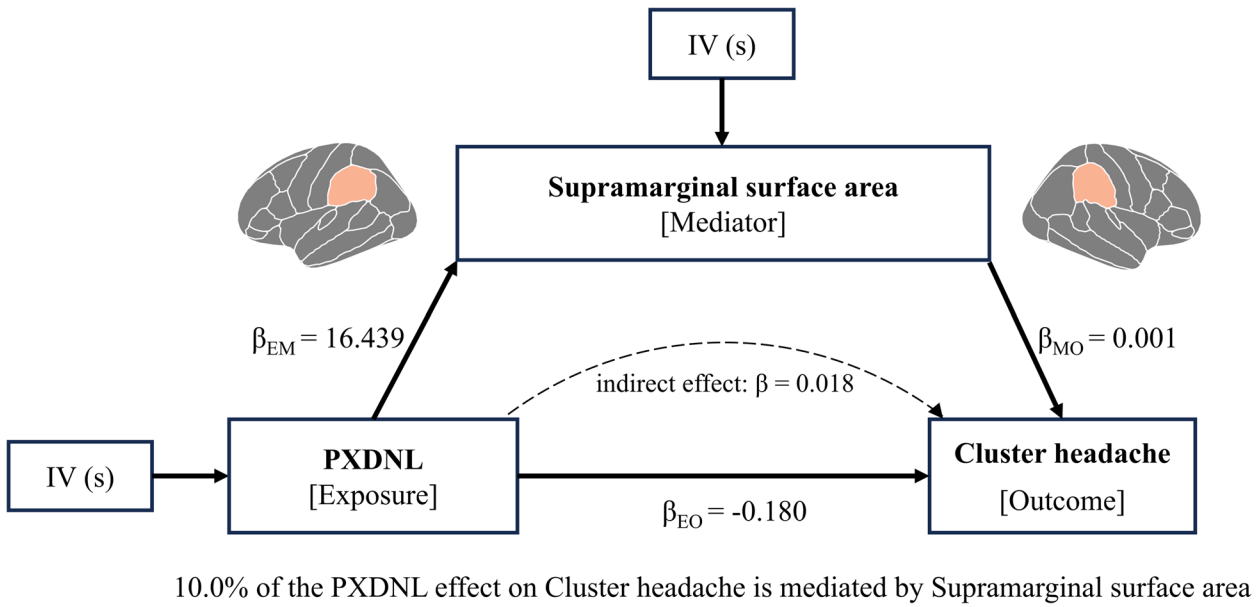

Fig. 5 Mediation analyses to quantify the effects of proteins with PPI on CH via cortical structure. PXDNL effect on CH mediated by supramarginal surface area. β_{EM} effects of exposure on mediator, β_{MO} effects of mediator on outcome, β_{EO} effects of exposure on outcome

Table 1 The mediation effect of protein on cluster headache via affecting cortical thickness and surface area

Cluster headache										
Protein	**Cortical Thickness and Surface Area**	**β1**	**se1**	**β2**	**se2**	**mediation_effect**	**se**	**total_effect**	***p*_value**	**Proportion**
PXDNL	Supramarginal surface area	16.439	4.695	0.001	2.25×10^{-4}	0.018	0.006	−0.180	0.004	10.0%

β1 and se1 denotes the MR effect of plasma proteins on Cortical Thickness and Surface Area. β2 and se2 represents the MR effect of these Cortical Thickness and Surface Area on Cluster headache. se: standard errors of indirect effects

resilience to metabolic and oxidative stress [39]. These stressors, particularly excitotoxic oxidative stress and increased neuronal excitability, are thought to contribute to the onset of primary headache attacks [40, 41]. Therefore, we hypothesize that the elevated expression of PXDNL may reduce the risk of CH attacks by decreasing factors such as metabolism and oxidative stress. Notably, the supramarginal surface area of the cortex plays a masking mediating role between the two. Increased functional connectivity between the supramarginal cortical region and the right hypothalamus was observed during spontaneous CH attacks, compared to the same subjects during interictal periods [42]. PXDNL is closely related to the existing acute and preventive drug targets for CH, and the specific mechanisms involved warrant further investigation.

Four proteins—CCN4, MRC1, LGALS9, and PKD1—showed no potential side effects in the Phenome-wide MR assessment, and all of them showed protein interactions with targets of current preventive CH drugs. CCN4, also known as WISP1, is a matricellular protein and a member of the cellular communication network family. It plays a role in various pathological conditions, including osteoarthritis, cancer, and tissue fibrosis [43]. Our findings suggest that elevated CCN4 expression is associated with a reduced risk of CH. Overexpression of CCN4 can downregulate matrix metalloproteinase-1 (MMP-1) [44], a protein elevated in CH that is associated with neuroinflammation and may contribute to the pathogenesis of the condition [45]. Therefore, increased CCN4 expression may regulate neuroinflammation by reducing MMP-1 levels, thereby lowering the risk of CH. Mannose receptor (MRC1/CD206), a key C-type lectin receptor and essential member of the innate pattern recognition receptor family, is predominantly expressed on macrophages [46]. Our results suggest that an increase in MRC1 expression may reduce the risk of CH. Studies have shown that the expression of MRC1 can be elevated by FOXP3 signaling, playing a role in promoting clearance and inflammation resolution following stroke [47]. Other research supports the critical role of MRC1 in preventing allergen-induced pulmonary inflammation [48]. Furthermore, it is well established that inflammatory responses are actively involved in the pathogenesis of CH, with some evidence indicating that neuroinflammation may contribute to the pathophysiology of CH [49]. Therefore, it is reasonable to hypothesize that MRC1 may influence CH by modulating neuroinflammatory and immune mechanisms. LGALS9 (Galectin-9) has garnered significant attention due to its various biological functions and potent immune-regulatory effects. Research suggests that the LGALS9 signaling pathway regulates autoimmune responses through its interaction with T-cell immunoglobulin mucin-3 (TIM-3) or other yet-to-be-identified receptors, while also altering macrophage anti-inflammatory activities, reducing effector T cell numbers, and increasing regulatory T cells [50]. Additionally, studies have shown that LGALS9 plays a role in promoting functional recovery of neuroinflammation, a key pathological event following ischemic stroke [51]. Therefore, it is plausible that LGALS9 may reduce the risk of CH through its modulation of immune responses and inflammation. LGALS9 is also recognized in cancer research for its potential as a therapeutic target due to its ability to induce apoptosis [52]. However, there is no related research linking apoptosis to CH.

PKD1 is an ion-channel regulator and a component of a heteromeric calcium-permeable ion channel, formed in conjunction with PKD2, which is activated through the interaction between PKD1 and a Wnt family member, such as WNT3A and WNT9B [53]. PKD1 may also interact with polycystin-1-interacting protein, which plays a role in detecting, isolating, and exocytosing aged mitochondria [54]. Given the paroxysmal character of CH, ion channel genes emerge as potential candidates involved in the pathogenesis of CH [8, 55]. Additionally, preventive treatments for CH, such as topiramate and valproic acid, likely exert their effects by modulating voltage-gated sodium and calcium ion channels [8]. Thus, modulation of PKD1 may provide a feasible and promising strategy for the treatment of CH by controlling ion channel activity.

We acknowledge several limitations in our study. Firstly, the study population was restricted to individuals of European ancestry, limiting the generalizability of our findings to other ethnic or racial groups. Secondly, our analysis focused solely on cis-pQTLs, excluding trans-pQTLs. While this approach minimizes the risk of violating MR assumptions, it also overlooks more intricate

regulatory mechanisms that could influence protein expression. Third, due to gaps in the GWAS data for these subgroups, we were unable to perform an analysis linking these proteins to episodic or chronic CH. Furthermore, the drug targets we have identified remain hypothetical, as they are based solely on a analysis of GWAS data. Future validation through fundamental experimental studies and prospective randomized controlled trials is required.

In conclusion, our analysis highlights a set of circulating proteins—CCN4, MRC1, LGALS9, and PKD1—as promising targets for the prevention of CH. PXDNL, in particular, shows potential as a dual-purpose target for both acute and preventive CH treatments. The involvement of these candidate proteins in CH pathophysiology warrants further exploration to fully understand their therapeutic potential.

Abbreviations

CH	Cluster headache
MR	Mendelian randomization
pQTL	Protein quantitative trait loci
GWAS	Genome-wide association studies
PPI	Protein–protein interaction
UKB-PPP	UK Biobank Pharma Proteomics Project
SNPs	Single-nucleotide polymorphisms
IVs	Instrumental variables
IVW	Inverse variance-weighted
OR	Odds ratios
SD	Standard deviation
FDR	False discovery rate

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s10194-025-01999-0.

Supplementary Material 1: Figure S1. Networks of CH-associated proteins and targets of commonly used acute CH drugs (A, B). Red solid circles represented current acute CH drugs targets. Yellow solid circles represented identified plasma proteins.

Supplementary Material 2: Figure S2. Networks of CH-associated proteins and targets of commonly used preventive CH drugs (A, B). Red solid circles represented current preventive CH drugs targets. Yellow solid circles represented identified plasma proteins.

Supplementary Material 3.

Supplementary Material 4.

Supplementary Material 5.

Supplementary Material 6.

Supplementary Material 7.

Supplementary Material 8.

Supplementary Material 9.

Supplementary Material 10.

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Authors' contributions
All authors contributed to the study conception and design. ZHX and ZG wrote the original draft. ZHX, ZG, LZ, DQ, YLM, XSL, PZ, MTZ, GYL and TSG analyzed the data. ZHX prepared Figures and tables. YGW, XYY, ZG, LZ, DQ, YLM, XSL, PZ, MTZ, GYL and TSG reviewed and edited the final draft. All authors contributed to the article and approved the submitted version.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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