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ORIGINAL RESEARCH

Identification of Novel Protein Biomarkers and Drug Targets for Acne Vulgaris by Integrating Human Plasma Proteome with Genome-Wide Association Data

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Background: Despite the availability of numerous therapies, the treatment of acne vulgaris remains challenging. Novel drug targets for acne vulgaris are still needed.

Methods: We conducted a Mendelian randomization analysis to explore possible drug targets for acne vulgaris. We utilized summary statistics obtained from the dataset of acne vulgaris, including 399,413 individuals of European ancestry. We gathered genetic instruments for 566 plasma proteins from genome-wide association studies. In order to strengthen the findings from Mendelian randomization, various methods were employed, including bidirectional Mendelian randomization analysis, Bayesian co-localization, phenotype scanning, and single-cell analysis. These methods facilitated the identification of reverse causality, the search for reported variant-trait associations, and the determination of the cell types that is the primary source of protein. Furthermore, using the plasma proteins in the deCODE cohort, we conducted a replication of the Mendelian randomization analysis as an external validation.

Results: At the significance level of Bonferroni ($P < 8.83\times10^{-5}$), a protein-acne pair was discovered through Mendelian randomization analysis. In plasma, increasing TIMP4 (OR = 1.15; 95% CI, 1.09–1.21; $P = 1.01\times10^{-7}$) increased the risk of acne vulgaris. The absence of reverse causality was observed in the TIMP4 protein. According to Bayesian co-localization analysis, TIMP4 shared the same variant with acne vulgaris (PPH4 = 0.93). TIMP4 was replicated in deCODE cohort (OR = 1.17; 95% CI, 1.10–1.24; $P = 1.48\times10^{-7}$). Single-cell analysis revealed that TIMP4 was predominantly detected in myeloid cells in blood, and was detected in almost all cell types in skin tissue.

Conclusion: The integrative analysis revealed that the level of plasma TIMP4 has a direct influence on the risk of developing acne vulgaris. This implies that TIMP4 protein could serve as a potential target for the development of drugs aimed at treating acne vulgaris.

Keywords: plasma proteome, acne vulgaris, drug target, biomarker, Mendelian randomization, TIMP4

Introduction

Acne vulgaris is characterized by the appearance of comedones, papules, and pustules. The global prevalence of acne vulgaris is estimated to be 9.38% across all age groups, with over 85% of adolescents affected.[1] It affects the pilosebaceous unit and is characterized by both non-inflammatory and inflammatory lesions, as well as the subsequent pigmentation and scarring that persist throughout a lifetime. The etiology of acne vulgaris is complex and not fully understood, but increasing evidence points to the importance of inflammation and sebum secretion in its development.[2] Acne can be promoted by many potential risk factors, including high glycemic index foods, psychological stress,

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comedogenic make-up, and any factor that increases androgen production.[3] Common acne treatments involve the use of synthetic chemical agents such as antibiotics, isotretinoin, and retinoids.[4,5] For example, isotretinoin is effective for moderate to severe acne, but it has been associated with side effects that may result in teratogenicity, dyslipidemia, and liver enzyme abnormalities.[6,7] Therefore, it is essential to identify new treatment targets for the development of novel anti-acne agents in order to reduce the risk of these side effects while maintaining the same level of effectiveness.

Human proteins are essential for a wide range of biological functions and are also the main targets for drug development.[8] In recent years, the method of Mendelian randomization (MR) analysis has gained a significant amount of attention and has become a popular technique for drug repurposing and drug target development.[9,10] MR typically relies on the utilization of single nucleotide polymorphisms (SNPs), which are genetic markers that are obtained from large-scale genome-wide association studies (GWAS). SNPs serve as instrumental variables in MR studies. The purpose of utilizing these instruments is to assess the causal impact of an exposure on an outcome. These randomly assigned SNPs are not influenced by environmental factors, which renders them as suitable tools for inferring causality. Compared with observational studies, other confounding factors have less influence on MR.[11,12] MR-based strategies have identified potential therapeutic candidates for various disorders, including multiple sclerosis and alzheimer's disease,[13,14] due to recent progress in high-throughput proteomic methods in plasma. Nevertheless, few MR studies have actually established the casual linkage between circulating proteins and acne vulgaris through MR.

In this investigation, our objective was to identify plasma proteins that could serve as potential targeting of treatment for acne vulgaris. The investigation design is summarized in Figure 1. First, MR was employed to uncover plausible plasma protein candidates associated with acne vulgaris by employing an extensive GWAS dataset of acne vulgaris, encompassing 399,413 individuals,[15] and plasma protein quantitative trait loci (pQTL) data provided by Lin's investigation.[13] Second, we corroborated the initial findings through various methods including Bayesian co-localization analysis, reverse MR, and phenotype scanning. Third, we conducted an external validation by utilizing the plasma pQTL data from the deCODE cohort[16] to identify potential therapeutic drug targets. Finally, single-cell analysis was performed to determination of the cell types that is the primary source of protein.

Materials and Methods

Plasma Protein Quantitative Trait Loci

For the primary analysis, the plasma pQTL data were obtained from the investigation conducted by Lin,[13] which incorporated five previously published Genome-Wide Association Studies (GWAS) conducted in European populations.[17–21] Firstly, it is essential to identify instrumental variables (IVs) that exhibit a strong and independent association with the exposure under investigation. These IVs will serve as the genetic instruments to be utilized in the subsequent analysis. Secondly, it is crucial to ensure that these genetic instruments satisfy the independence assumption, which implies that they should not be correlated with any factors that might confound the association with exposure and outcome. This ensures that the genetic instruments solely capture the variation in the exposure of interest. Lastly, the third assumption in Mendelian randomization (MR) analysis, known as the exclusion restriction assumption, necessitates a null association between the genetic instrument and the outcome variable, unless this association can be attributed to the genetic instrument's impact on the exposure variable being investigated. This assumption is crucial in order to establish a causal relationship between the exposure and the outcome variables under investigation.[22]

To fulfill the requirement of the relevance assumption in MR, only pQTLs that met specific criteria were selected for analysis. These criteria included: (i) a significant genome-wide association with the protein of interest, indicated by a P-value less than 5×10^{-8} and an F statistic greater than 10; (ii) situated away from the major histocompatibility complex (MHC) areas, specifically on chromosome 6 between 26 and 34 Mb; (iii) exhibiting independent association, as determined by a linkage disequilibrium (LD) clumping r^2 value less than 0.001 within a 10MB window; (iv) not demonstrating a genome-wide significant association with the outcome of interest, as indicated by a P-value greater than 5×10^{-8}; and (v) being a cis-acting pQTL, meaning that it regulates the expression of a nearby gene.[23,24] By applying these stringent screening criteria, a total of 570 cis-acting SNPs associated with 566 proteins were included in the analysis, as detailed in Supplementary Table S1.

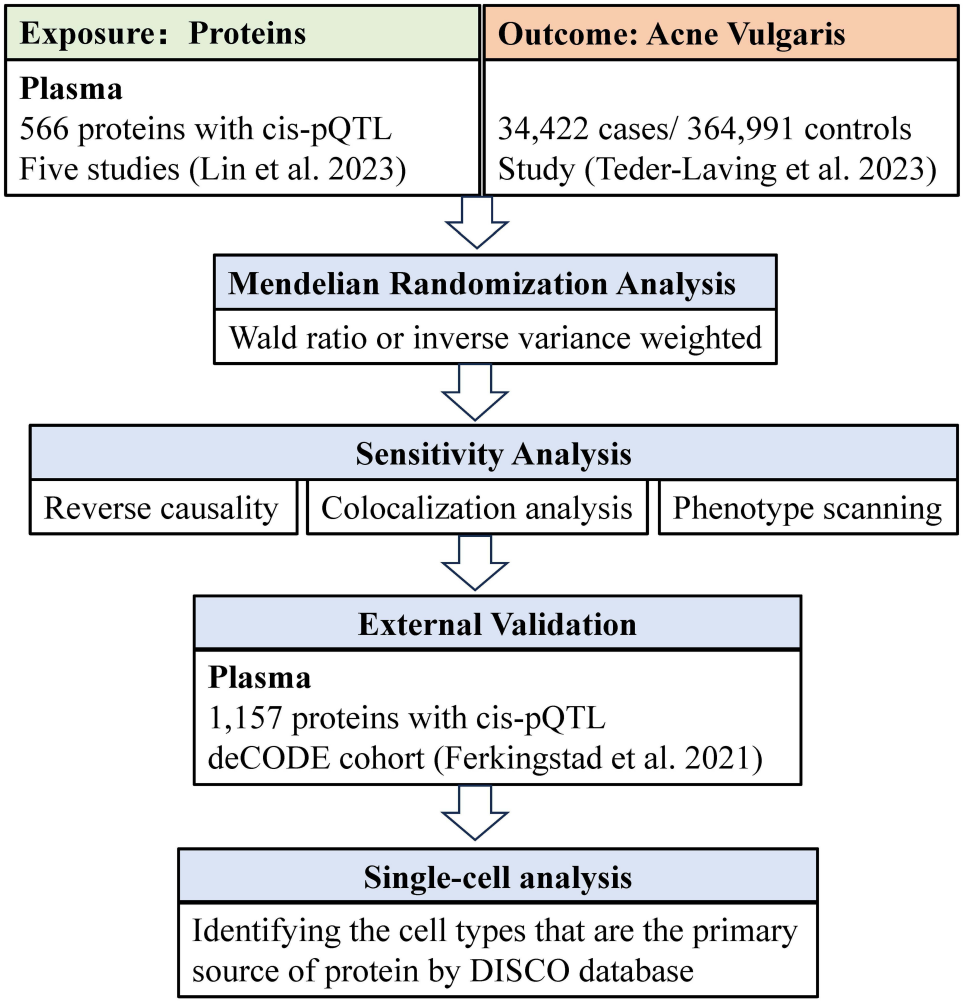

Figure 1 Study design to identify plasma proteins associated with acne vulgaris.

To further validate the findings, an external dataset consisting of plasma pQTL data from the deCODE cohort, as reported by Ferkingstad et al, was utilized.[16] This dataset encompassed measurements of 4907 plasma proteins in a large cohort of 35,559 participants. By employing the same screening criteria as the primary analysis, a total of 1374 cis-acting SNPs associated with 1157 proteins were included for external validation, as outlined in Supplementary Table S2. This external validation dataset provides an additional layer of confidence in the observed associations between the identified pQTLs and their respective proteins.

GWAS Summary Statistics of Acne Vulgaris

Summary statistics were extracted from the GWAS dataset pertaining to the dermatological condition known as acne vulgaris, encompassing a substantial cohort of 399,413 individuals, with a specific breakdown of 34,422 individuals classified as cases and 364,991 individuals classified as controls, all of whom belonged to the European ancestral group.[15]

Mendelian Randomization Analysis

In this particular investigation, we opted to utilize the plasma proteins as the exposure variable, while acne vulgaris was considered the outcome variable. To carry out this analysis, we employed the "TwoSampleMR" package,[25] which can be found at the following GitHub repository: https://github.com/MRCIEU/TwoSampleMR. It is worth mentioning that in cases where only one pQTL (protein quantitative trait locus) was available for a specific protein, we relied on the Wald

ratio. However, in situations where two or more instruments were at our disposal, we employed inverse variance weighted MR (MR-IVW) and subsequently conducted heterogeneity analysis to account for potential differences among instruments.[26]

For the primary analysis, we employed Bonferroni correction to account for multiple testing, ensuring that the statistical significance threshold was appropriately adjusted. Specifically, we set a threshold P-value of $P < 0.05/566 = 8.83 \times 10^{-5}$, which allowed us to prioritize the results in a statistically rigorous manner. Moreover, we conducted MR for external validation purposes, employing a distinct P-value threshold of $P < 0.05/1157 = 4.32 \times 10^{-5}$. This stringent threshold was selected to ensure that the external validation results were robust and reliable, thus further bolstering the credibility of our findings.

Bidirectional MR Analysis

To investigate the possibility of a reverse causal relationship between acne vulgaris and plasma proteins, we conducted a bidirectional MR analysis.[27] In order to properly orient the causal relationship, we followed the same rigorous inclusion criteria for identifying genetic instruments associated with acne vulgaris, resulting in the selection of 15 appropriate instruments from the previous GWAS on this dermatological condition. These instruments were then utilized in the bidirectional MR analysis, as outlined in Supplementary Table S3. Comprehensive summary statistics for proteins were obtained from previous investigation.[18] By employing the MR-IVW method, we were able to assess the potential reverse causality between acne vulgaris and plasma proteins. Reverse causality would be confirmed if the bidirectional MR analysis yielded statistically significant results, as determined by a P-value less than 0.05.

Colocalization Analysis

Colocalization analysis was conducted in order to enhance the reliability of the causal inference, thereby further solidifying the findings of the study. To estimate the posterior probability that the genetic associations with both the protein and the phenotype shared the same causal variant, a stringent Bayesian model was employed. This model was implemented using the "coloc" package, which is a widely recognized and respected tool in the field (https://github.com/chr1swallace/coloc).[28] The default arguments provided by the package were utilized to ensure consistency and comparability across analyses. As part of this investigation, the researchers specifically examined the posterior probability of hypothesis 4 (PPH4), which posited that both the protein and acne vulgaris were linked to the same region through shared genetic variants. To evaluate this hypothesis, the coloc.abf algorithm was employed. It is noteworthy that a PPH4 value exceeding 80% was considered to provide substantial support for the assertion that plasma protein and acne vulgaris were indeed causally related through the same variant.[29,30]

Phenotype Scanning

We utilized the tool known as "phenoscanner", which can be accessed at the web address https://github.com/phenoscanner/phenoscanner, in order to elucidate any potential pleiotropy exhibited by the SNPs that were employed in our primary analysis.[31,32] In essence, phenoscanner diligently combed through previously conducted GWAS to ascertain any associations between the identified pQTLs and other phenotypic traits. For an SNP to be classified as pleiotropic, it had to satisfy a set of stringent criteria: firstly, the association between the SNP and the various traits had to be deemed statistically significant at the genome-wide level, denoted by a P-value of less than 5×10^{-8}; secondly, the GWAS in which the association was observed had to have been conducted within a population of European ancestry.

Single-Cell Analysis

A single-cell analysis utilizing the Deeply Integrated human Single-Cell Omics (DISCO) database (https://www.immunesinglecell.org/) was performed, which contains whole blood and skin single-cell RNA sequencing (scRNA-seq) data, in order to investigate the expression patterns of genes encoding plasma proteins.[33]

Results

Primary Result of MR

Using either the Wald ratio or IVW method, it was found that only one protein, namely tissue inhibitors of metalloproteinase 4 (TIMP4), exhibited a significant association with the risk of developing acne vulgaris after applying the Bonferroni correction ($P < 8.83\times10^{-5}$) (Supplementary Table S4 and Figure 2). In particular, an increase in TIMP4 levels (OR = 1.15; 95% CI, 1.09–1.21; $P = 1.01\times10^{-7}$) was found to elevate the risk of developing acne vulgaris (Figure 3). It is noteworthy that this association remained robust even after applying a stringent correction for multiple testing, further strengthening the credibility of the observed relationship between TIMP4 and acne vulgaris risk. This finding suggests that the expression of TIMP4 might play a crucial role in the pathogenesis of acne vulgaris.

Sensitivity Analysis

In order to strengthen the findings from Mendelian randomization, various methods were employed, including bidirectional Mendelian randomization analysis, bayesian co-localization, and phenotype scanning. First and foremost, it is imperative to note that the bidirectional MR analysis did not uncover any conclusive evidence indicating a causal effect between acne vulgaris and the level of the identified protein TIMP4. Second, Bayesian co-localization analysis has provided compelling evidence suggesting that TIMP4, with a coloc.abf-PPH4 value of 0.93, shares the same genetic

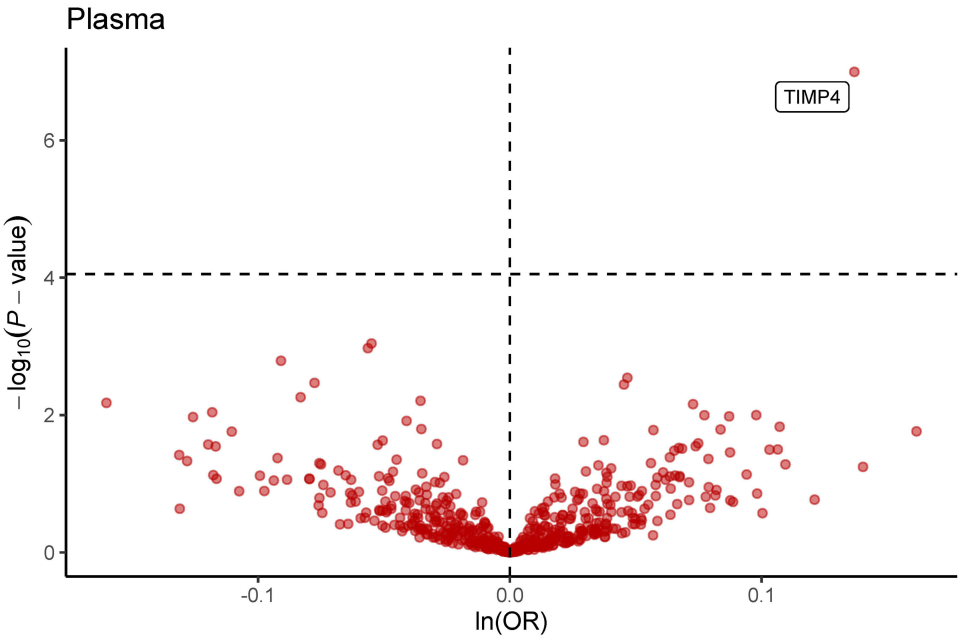

Figure 2 Volcano plots of the MR results for 566 plasma proteins on the risk of acne vulgaris. Dashed horizontal black line corresponded to $P = 8.83\times10^{-5}$ (0.05/566). ln: natural logarithm.

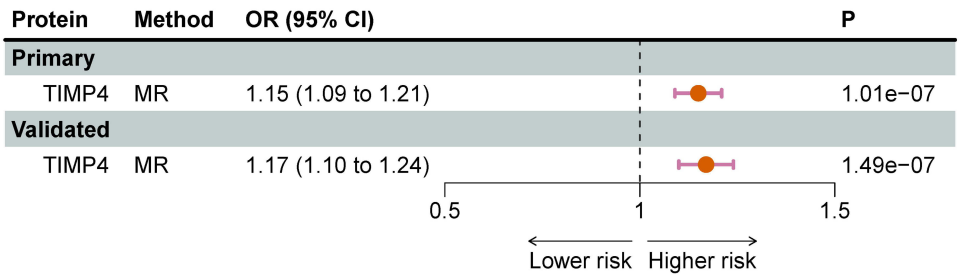

Figure 3 The causal relationship between potential causal protein and acne vulgaris by MR analysis.

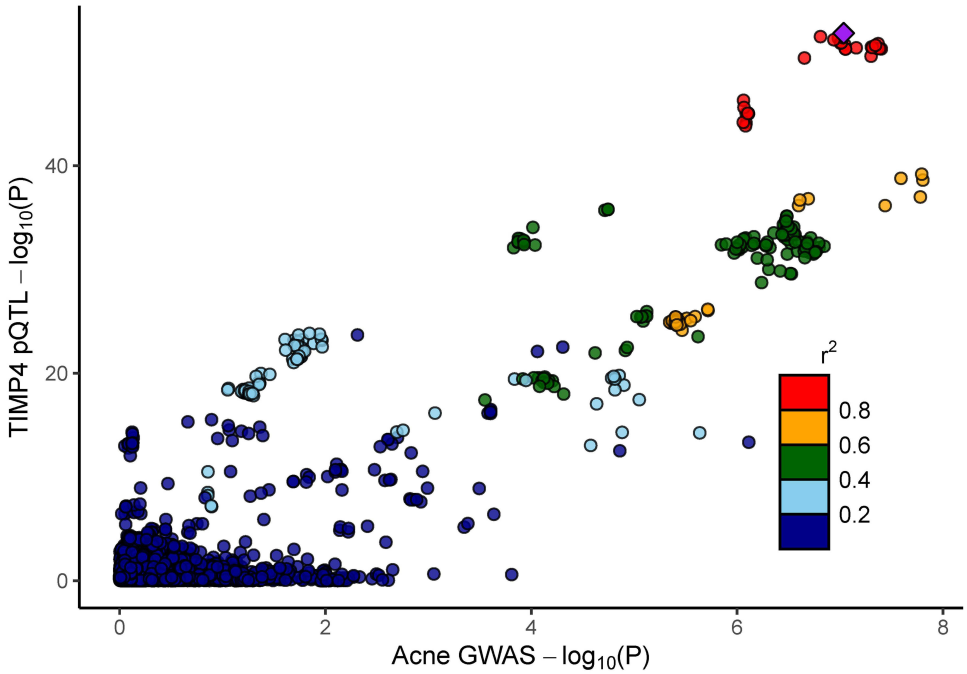

Figure 4 Bayesian colocalization analysis of potential causal protein and acne vulgaris. Diamond purple point represented the SNP that used for the primary MR analysis.

variant with acne vulgaris (Figure 4). It is possible that this shared genetic variant may serve as a potential link between the pathogenesis of acne vulgaris and the regulation of TIMP4. Furthermore, it is of utmost importance to consider the implications of phenotype scanning in relation to TIMP4. Through meticulous examination, it has been determined that TIMP4, specifically the variant rs454615, exhibits a significant association with plateletcrit (Table 1). Plateletcrit is not a reported risk factor for acne vulgaris, indicating that the instrumental variables are not pleiotropic.

External Validation of TIMP4 for Acne Vulgaris

Using MR in the deCODE cohort, we were able to replicate the primary findings and establish a correlation between the protein TIMP4 and acne vulgaris at a statistically significant level according to the Bonferroni correction ($P < 4.32\times10^{-5}$) as depicted in Figure 3. This corroborates the initial findings and strengthens the evidence for the involvement of TIMP4 in the development of acne vulgaris. Specifically, the study found that an increase in TIMP4 levels was associated with an elevated risk of acne vulgaris, as indicated by an odds ratio of 1.17 (with a 95% CI ranging from 1.10 to 1.24) and a P-value of 1.48×10^{-7}. In contrast, it is worth noting that no significant association was observed between other proteins and acne vulgaris in the deCODE cohort, as shown in Supplementary Table S5.

Single-Cell Analysis of TIMP4

To determine which cell type was the primary source of TIMP4 in blood and skin tissue, we performed single-cell analyses using the DISCO database. As illustrated in Figure 5A, TIMP4 was predominantly detected in myeloid cells, including monocytes and megakaryocytes, in blood samples. In contrast, TIMP4 was detected in almost all immune cells,

Table 1 Summary of Reverse Causality Testing, Bayesian Co-Localization Analysis and Phenotypic Scanning of Potentially Disease-Causing Protein

Protein	SNP	Bidirectional MR (P value)	Co-Localization PPH4 (coloc.abf)	Phenotype Scanning
TIMP4	rs454615	0.57	0.93	Plateletcrit

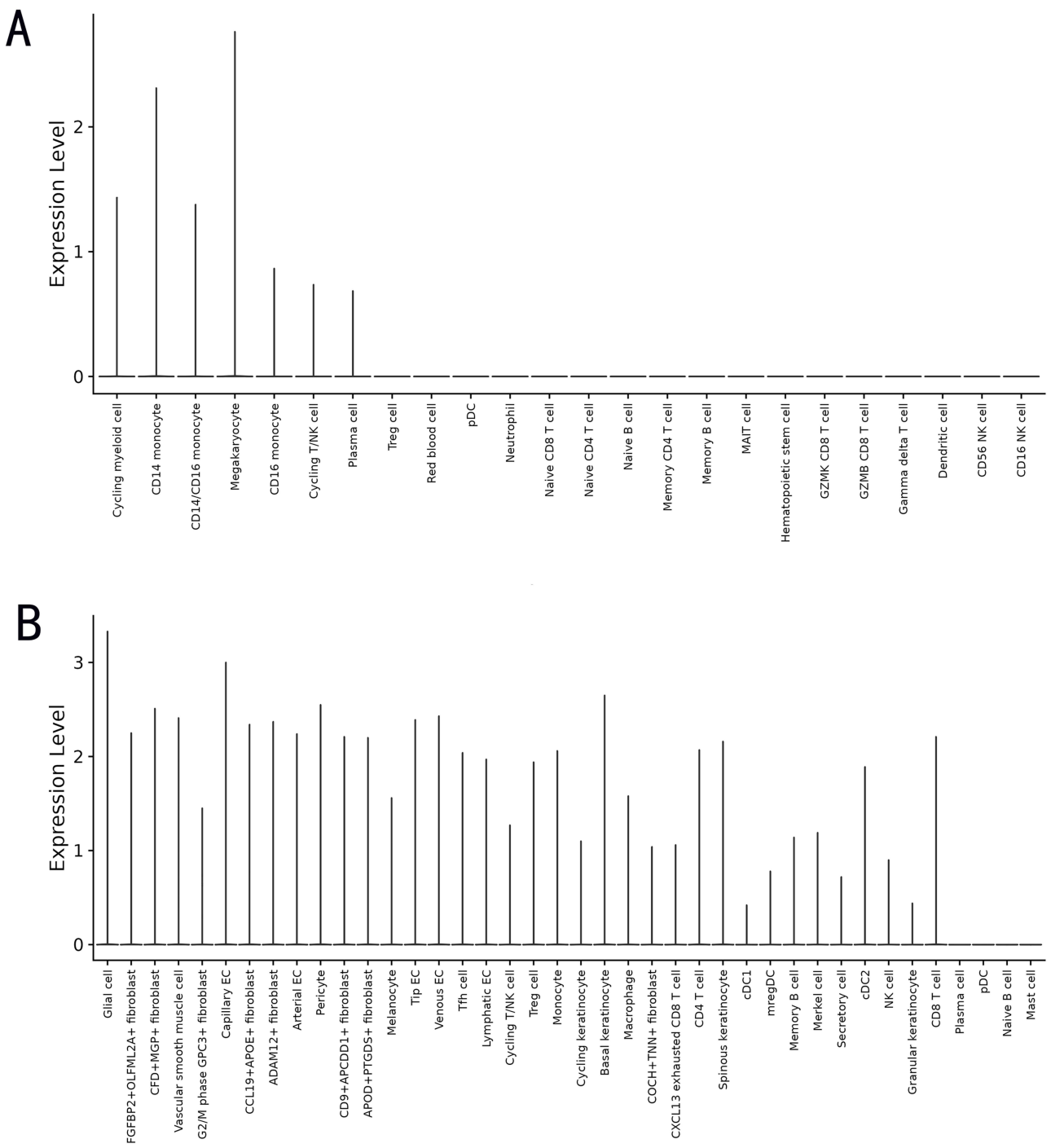

Figure 5 Single-cell analysis using the DISCO database revealed the expression patterns of TIMP4 in different cell types. (**A**) The violin plots of TIMP4 expression in blood. (**B**) The violin plots of TIMP4 expression in skin.

fibroblasts, keratinocytes, melanocytes, and endothelial cells in skin samples (Figure 5B). It is worth noting that in the cell types where TIMP4 gene expression could be detected, only some of the cells expressed the gene.

Discussion

In this study, we aim to present a comprehensive analysis concerning the causal connections that exist between plasma proteins and the risk of developing acne vulgaris. To accomplish this, we employed a discovery proteome-wide MR,

which ultimately led to the identification of a noteworthy protein known as TIMP4. It is worth noting that genetically determined higher levels of TIMP4 were found to be significantly associated with an increased susceptibility to acne vulgaris. However, it is crucial to acknowledge that the concept of "causality" as identified by MR may potentially involve reverse causality.[8] Consequently, in order to address this potential issue, we proceeded to conduct a bidirectional MR analysis. Upon further analysis, it was found that the proteins identified in the primary MR analysis did not show any indications of reverse causality. Furthermore, in an effort to eliminate any potential bias introduced by linkage disequilibrium, we also employed Bayesian co-localization. Through this approach, we were able to determine that TIMP4 is likely to share the same variant with acne vulgaris, thereby highlighting the causal effects of the TIMP4 protein in the development of this skin condition. It is worth mentioning that TIMP4 has also been discovered to exhibit connections with other characteristics during phenotype scanning. However, it is important to note that none of these connections were able to comprehensively elucidate the link between the identified protein and the development of acne vulgaris. Using a similar methodology in the deCODE cohort, we undertook additional verification of the causal effects between TIMP4 and acne vulgaris. This investigation further reinforces the dependability and validity of the potential drug targets that have been identified in the present study. To identify the cell types that are the primary source of TIMP4 protein, single cell analysis revealed that TIMP4 was predominantly detected in myeloid cells in the blood and in almost all cell types in skin tissue. In aggregate, our research has successfully pinpointed a protein, namely TIMP4, which exhibits the most persuasive and compelling evidence. Notably, this protein represents a plasma protein marker that is intimately associated with the pathogenesis of acne vulgaris.

Recent studies have provided evidence to support the potential involvement of inflammatory mediators in the progression of acne lesions, including the presence of metalloproteinases (MMPs).[34,35] MMPs are a type of extracellular proteinase that rely on zinc for their enzymatic activity. The activities of these MMPs are carefully regulated by tissue inhibitors of metalloproteinases (TIMPs).[36,37] Until now, there has been a lack of research exploring the relationship between TIMP4 and acne vulgaris. Our current investigation aims to bridge this knowledge gap by establishing a direct association between TIMP4 and this dermatological condition. It is important to note that TIMP4 is a member of the TIMP gene family, which is widely recognized for its ability to modulate the functions of MMPs. The delicate balance between MMPs and TIMPs is crucial for the proper remodeling of the extracellular matrix (ECM). In instances where this equilibrium is disrupted, inflammatory diseases, such as acne vulgaris, can manifest.[38] TIMPs are multifaceted proteins that exhibit their biological effects through various pathways, including both MMP-dependent and MMP-independent mechanisms. Five transcription factors (p50, p52, p65, c-REL, and ReIB) involved in immunological and inflammatory responses constitute the NF-κB family.[39,40] Interestingly, a recent investigation has proposed that TIMP4 may serve as a negative regulator of adipogenesis by modulating the NF-κB cascade in adipocytes.[41] In addition, the findings discovered in experiments conducted on mice provide evidence indicating that the absence of TIMP4, a specific protein, can potentially hinder the process of lipid absorption, thereby impair the development of obesity induced by a diet high in fat content.[42] Furthermore, it is worth noting that acne, a common skin condition, is not solely associated with inflammation, but also with the excessive production of lipids in the skin. With this in mind, we can postulate that TIMP4 may potentially play a significant role in both of these interconnected pathways. It is important to mention that TIMP4 has also been linked to the occurrence of other diseases, such as ulcerative colitis and cardiovascular disease, which further emphasizes its relevance in the field of medical research. An imbalance between TIMP1 and TIMP4 serum levels is present in patients of ulcerative colitis. Patients of ulcerative colitis had significantly lower serum TIMP4 levels when compared to healthy controls.[43,44] As a matter of fact, TIMP4 is considered one of the intrinsic key regulators of MMP9, another protein that is known to be activated in atrial fibrosis, a condition characterized by abnormal tissue growth in the heart's atria.[45] Interestingly enough, studies have shown that MMP9 has the ability to activate latent TGF-β1, transforming it into its active form, and also to induce the production of TGF-β1, a protein that plays a crucial role in the communication between neighboring fibroblasts and the stimulation of collagen synthesis, as well as the initiation of cardiac fibrosis.[46] Considering all the evidence presented, it can be argued that TIMP4 holds great potential as a therapeutic target for the treatment of acne vulgaris.

Our study carries a number of limitations which must be acknowledged. Firstly, it must be acknowledged that our analysis was conducted solely on populations of European ancestry, thus impeding the generalizability of our results to

other ancestral groups. Consequently, further studies focusing on non-European ancestry are imperative in order to effectively translate these findings into meaningful clinical applications. Secondly, it is important to note that all prioritized proteins in our analysis were found to have only a single cis-acting SNP and were lacking any trans-pQTLs. This unfortunate limitation restricts the range of analyses that can be applied, including alternative MR algorithms, heterogeneity tests, and pleiotropy tests. Nevertheless, it is worth highlighting that we did conduct a thorough investigation into the SNPs associated with our top findings. Remarkably, the majority of these SNPs exhibited strong instrument strength, as indicated by their F statistics surpassing the threshold of 10. Third, regrettably, we were unable to gauge the quantities of these relevant proteins in other tissues, which could have potentially enriched our understanding of the perplexing nature of acne vulgaris, particularly in relation to the skin tissue. Lastly, our exploration did not encompass an exploration of the intricate pharmaceutical mechanisms targeting the identified protein. Therefore, it is imperative to undertake further in-depth studies and meticulously designed clinical trials to ascertain the viability and credibility of our research findings, thereby ensuring their practical applicability and potential therapeutic benefits in the context of acne vulgaris.

Conclusion

Our investigation successfully identified a circulating protein, known as TIMP4, which has been found to be causally linked to the risk of acne vulgaris. This breakthrough discovery not only sheds new light on the underlying causes of this common skin condition, but also emphasizes the potential of TIMP4 as a promising target for future drug development in the treatment of acne vulgaris. However, it is essential to conduct further rigorous experimental and clinical studies to fully assess the practicality and effectiveness of these potential candidates and validate the current findings.

Data Sharing Statement

The original contributions presented in the study are included in the article/Additional file, further inquiries can be directed to the corresponding author.

Ethics Statement

China's scientific and technological authorities released the "Measures for Ethical Review of Life Science and Medical Research Involving Human Beings" in 2023. According to clause 32 of the Measures for Ethical Review, ethical review can be exempted if research is conducted using legally obtained public data.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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