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ORIGINAL RESEARCH

Evidence from a Comprehensive Bioinformatics Analysis Point to Possible Therapeutic Targets for Vitiligo

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Background: Vitiligo is an autoimmune, hypopigmented dermatological disease for which the pathogenesis remains unclear. With limited therapeutic options, it has a significant treatment burden and adverse psychological effects for patients.

Methods: The core method employed was Mendelian randomization (MR), which was used to assess the impact of over 2,000 plasma proteins on generalized vitiligo. To further enhance the reliability of the MR conclusions, we conducted a series of comprehensive analyses, including reverse MR, the Steiger test, colocalization analysis, and phenotype scanning. Subsequently, we employed Phenome-wide MR (PheW-MR) analysis to exclude targets associated with adverse effects and evaluated the druggability of these targets through drug gene list mapping and molecular docking. Additionally, we employed protein-protein interaction (PPI) analysis to elucidate the interactions between the target proteins and existing vitiligo treatments. Finally, pathway enrichment analysis and transcriptome-proteome correlation analysis provided further biological insight into the target proteins.

Results: Following a series of comprehensive analyses, we identified three potential drug targets for the treatment of vitiligo: GZMB, FCRL3, and ULK3, each of which is associated with an increased risk of the disease. Validation across different cohorts confirmed the significance of GZMB and FCRL3. Colocalization analysis indicated that these targets share common variants with vitiligo, while PheW-MR analysis suggested that targeting these proteins would not result in significant side effects. Furthermore, molecular docking demonstrated stable binding between the target proteins and predicted drugs. The PPI network revealed that GZMB, FCRL3, and ULK3 interact with the target proteins of existing vitiligo treatments.

Conclusion: Our study has identified three promising drug target proteins for the treatment of vitiligo, which merit prioritization in drug development efforts. These targets warrant further investigation to elucidate their underlying mechanisms.

Keywords: Mendelian randomization, vitiligo, drug target, protein quantitative trait loci, Bayesian colocalization, molecular docking

Introductions

Localized or widespread depigmentation of the skin and mucosa is the predominant symptom of vitiligo, a dermatological disorder linked to oxidative stress, cytokine involvement, melanocyte self-destruction, autoimmune, and genetic predispositions.[1] It is reported that vitiligo affects 0.5% to 2% of people worldwide. Roughly half of the cases present before the age of 20, and between 70 and 80% of cases before the age of 30.[2,3] Vitiligo has garnered increasing attention both academically and societally due to its significant impact on quality of life and its associated economic burden.[4,5] The etiology of vitiligo is complex. Based on research, the main mechanism of action is the attack of melanocytes by autoreactive cytotoxic CD8+ T lymphocytes, which facilitates the local generation of IFN-γ, hence promoting the course of the disease. A positive feedback loop is then created when nearby keratinocytes release chemokines that are triggered by IFN-γ, which attract more T cells to the skin and intensify the inflammatory and immunological responses.[6] Current

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treatments for vitiligo are suboptimal, as they are not universally effective across all patients and fail to achieve repigmentation in all anatomical regions.[7] Moreover, within the first year after stopping therapy, 40% of patients relapse.[8] Therefore, despite the development of safer therapeutic options, investigating the underlying mechanisms and potential drug targets of vitiligo remains of significant value.

Proteins are fundamental to all life processes, playing pivotal roles in both health and disease.[9] They execute specific tissue functions and mediate cellular communication,[10] therefore, when dysregulation occurs, it can trigger the onset and progression of diseases, making proteins critical drug targets.[11] Advances in large-scale proteomics have enabled genome-wide association studies (GWAS) of circulating protein levels, which can identify protein quantitative trait loci (pQTLs) of significant importance.[12]

Mendelian randomization (MR) is a genetic instrumental variable study that typically estimates the causal influence of an exposure on an outcome by using single nucleotide polymorphisms (SNPs) from GWAS as genetic instruments.[13] Unlike observational studies, MR effectively addresses confounding factors and reverse causation, as SNPs follow the principles of random assortment during genetic inheritance and precede the phenotype.[14] By lowering the possibility that linkage disequilibrium (LD) influenced the MR results, Bayesian colocalization has been utilized to support inference.-[15,16] To link genetic variation to disease mechanisms through protein, MR studies combined with colocalization using pQTLs as instrumental variables (IVs) might be valuable.[17]

Several studies have examined the association between circulating proteins and vitiligo; however, most relied on low-throughput protein measurements and limited sample sizes, where confounding biases and reverse causation were inescapable.[18,19] Consequently, we undertook an investigation to elucidate the causal relationship between a broad array of circulating proteins and vitiligo using a MR-based analytical framework, with the goal of identifying potential therapeutic targets for the treatment and prevention of vitiligo.

Methods

This study was approved by the independent ethics committee of the Second Affiliated Hospital of Wenzhou Medical University and Yuying Children's Hospital.

Plasma Protein pQTL Datasets Used in This Analysis

We selected circulating plasma proteins as the exposure, with pQTL data for these proteins obtained from nine large genome-wide association studies,[12,20–27] primarily involving European populations (Table S1). The inclusion criteria for pQTLs were as follows: First, pQTLs were required to have genome-wide significant associations, as defined by the p-values in the original GWAS for the respective plasma proteins; second, they had to be located outside the major histocompatibility complex (MHC) region (Chr6, 26–34 Mb); third, pQTLs were independent from one another (LD clumping $r2 < 0.01$, window size = 5000 kb); fourth, they were cis-acting pQTLs, which are pQTLs that fall within a 500 kb window of the protein-coding region that corresponds to them; and fifth, pQTLs were excluded if they exhibited potential pleiotropy (defined as pQTLs associated with five or more proteins simultaneously).

GWAS Summary Statistics of Generalized Vitiligo

Employing the results of the biggest case inclusion in a vitiligo GWAS research to date—4,680 patients and 39,586 controls—we used data from the genetic analysis carried out by Jin et al[28] on generalized vitiligo (GV) in a European population. Given that GV is associated with more severe disease and suboptimal treatment outcomes, there is a heightened urgency to develop effective therapeutic interventions for this phenotype, which we believe holds significant clinical value.

MR Analysis

In this study, we employed circulating plasma proteins as the exposure and GV as the outcome, conducting MR analysis using the "TwoSampleMR" package in R (https://github.com/MRCIEU/TwoSampleMR). The MR effect estimates were calculated using the Wald ratio for cases with a single pQTL serving as the instrumental variable for a particular plasma protein, or by applying the inverse-variance weighted (IVW) method under a random-effects model when multiple

genetic instruments were available. Given the large-scale nature of our analysis, which encompassed potential causal associations between thousands of circulating proteins and GV, the significance threshold was set at 0.05 divided by the total number of proteins tested in order to account for the possibility of false-positive results from multiple testing using the Bonferroni correction. Plasma proteins that met this stringent criterion were designated as MR-identified proteins and subsequently underwent rigorous quality screening and druggability assessments.

Reverse MR Analysis and Steiger Test

In order to avoid the association between MR-identified proteins and GV losing its significance as a drug target due to the existence of reverse causality, we selected IVs from the GWAS of GV according to the criterion of $p<5x10^{-8}$ and the same LD conditions as that of pQTL and carried out reverse MR with MR-identified proteins as the endpoints. In addition, we performed the Steiger test using the "TwoSampleMR" package to further clarify the direction of causality, and if the direction of causal effect was judged to be Ture and the p-value was <0.05, then it could be assumed that there was no reverse causality.[29]

Bayesian Colocalization Analysis

In order to reduce the risk of false-positive results owing to LD, we evaluated MR-identified proteins utilizing Bayesian colocalization analysis. The posterior probability that a certain genomic locus contains a single mutation affecting plasma protein levels and GV is estimated by this approach. We conducted these colocalization analyses using the "coloc" R package (http://cran.r-project.org/web/packages/coloc).[30] The package provides posterior probabilities for five hypotheses, with posterior probability hypothesis 4 (PPH4) indicating that both traits are associated with the same genetic variant. A PPH4 value greater than 0.8 suggests that the two traits share a common genetic variant.[31] MR-identified proteins that met this threshold were considered as candidate proteins.

Phenotype Scanning and Phenome-Wide MR Analysis (PheW-MR)

We applied PhenoScanner (www.phenoscanner.medschl.cam.ac.uk) to recognize and eliminate pleiotropic pQTLs that might be correlated with GV for the purpose of making sure that IVs influenced the outcomes solely through exposure and not via probable confounders. Specifically, we excluded pQTLs linked to any known risk factor for GV, such as metabolic profiles, proteins, or clinical features, applying a correlation threshold of $P<5x10^{-8}$. Additionally, recognizing the importance of minimizing side effects for any potential drug target, we assessed the candidate proteins for GV treatment by conducting PheW-MR analysis against 525 diseases characterized by ICD-10 diagnostic codes from the UK Biobank (Table S2). To control for multiple testing, we applied a significance threshold of $P<9.52x10^{-5}$ (0.05/525), thereby reducing the likelihood of false-positive findings.

Mapping of the Candidate Proteins with Existing Therapeutic Targets

To evaluate the potential of candidate proteins as therapeutic targets, we examined their overlap with the list of druggable genes compiled by Finan et al.[32] In their study, Finan et al systematically classified 4,479 druggable genes into three tiers. Tier 1, consisting of 1,427 genes, includes efficacy targets of approved small-molecule drugs, biotherapeutic agents, and clinical-phase drug candidates. Tier 2 comprises 682 genes that either encode targets with known bioactive, drug-like small-molecule binding partners or share significant sequence identity (≥50%) with approved drug targets. Tier 3, the largest group with 2,370 genes, encompasses those encoding secreted or extracellular proteins, proteins with more distant similarity to approved drug targets, and members of key druggable gene families not already included in the first two tiers. Our analysis specifically focused on the stratification of candidate proteins and their potential to be targeted by small-molecule drugs or biologics.

Molecular Docking

In order to gain further insight into the druggability of the candidate proteins, molecular docking was conducted at the atomic level in the present study. Adopting this method allowed for the evaluation of the binding affinity and pattern of interaction between targeted proteins and the predicted drugs. In this study, the Drug Signature Database (DSigDB, http://dsigdb.tanlab.org/DSigDBv1.0/) will be employed for the purpose of drug prediction.[33] Subsequently, we used the software AutodockVina

1.2.2 (http://autodock.scripps.edu/) to perform molecular docking of candidate proteins and drugs predicted based on DSigDB. The PubChem Compound Database[34] (https://pubchem.ncbi.nlm.nih.gov/) provided the drug structural data, and Table S3 shows the matching IDs. Protein structure data were obtained from the PDB (Protein Data Bank, http://www.rcsb.org/), and Table S3 displays the associated PDB IDs. The FCRL3 protein, on the other hand, has Alphafold-based structural data from EMBL-EBI (https://www.ebi.ac.uk/). The final structures were obtained for 3 proteins and 10 drugs. We calculated the binding energy between them, and in general a binding energy less than 0 corresponds to a meaningful docking result, while smaller binding energies represent a more stable bond between the two.

Protein-Protein Interaction (PPI) Network and Gene Ontology Enrichment Analysis

We conducted a PPI network analysis to elucidate the relationships among candidate target proteins and their associated proteins, providing insights into their cellular roles. For this analysis, we employed GeneMANIA (https://genemania.org/).[35] Furthermore, we relied on the Drugbank database (https://www.drugbank.ca) to find the corresponding therapeutic targets (Table S4) for the 16 drugs that were obtained from a recent review[7] in order to investigate the interactions between candidate proteins and marketed drug targets for the therapy of vitiligo. Their interactions were constructed using the Search Tool for the Retrieval of Interacting Genes (STRING, V11.5, https://string-db.org/), and the minimum requirement for an interaction score was 0.4.[36]

Additionally, to investigate possible enriched pathways linked to proteins, Gene Ontology (GO) pathway studies were carried out using the "ClusterProfiler" R package.[37] Three terms are included in GO: cellular component (CC), molecular function (MF), and biological process (BP). Furthermore, the enrichment of nominally significant plasma proteins ($p<0.05$) in BP in MR analysis was the main focus of our investigation.

Correlation Analysis between Transcriptomic and Proteomic Associations

Existing expression quantitative trait loci (eQTLs) corresponding to pQTLs can contribute to the biological interpretability of the former by demonstrating that the effect of genes on protein expression is mediated by modulating the transcription of the corresponding mRNAs.[30,38] We utilized data provided by the Genotype-Tissue Expression (GTEx) project (https://www.gtexportal.org), selecting eQTLs data for sun-exposed and non-sun-exposed skin tissues. Employing a threshold of $p < 5x10^{-8}$, we screened IVs (Tables S5 and S6) and conducted two-sample MR analysis with GV as the outcome. Subsequently, we employed MR effect estimates of genes nominally significant ($p < 0.05$) in both pQTL-based MR and eQTL-based MR analyses. The transcriptome and proteome corresponding to these genes were correlated using Pearson correlation analysis; the degree of link between the two was measured by the correlation coefficient, R, with $p < 0.05$ being deemed statistically significant. Figure 1 summarizes the entire workflow of our study design.

Results

Identifying IVs for Plasma Proteins

A total of 3811 cis-pQTL were selected from the nine plasma protein datasets, involving 2958 proteins (1558 unique proteins). The selection was made according to the screening criteria, which are detailed in Table S7. Subsequently, the aforementioned instrumental variables were merged with GV's GWAS. The final cis-pQTL included in the MR analysis totaled 3556, involving 2826 proteins (1491 unique proteins), as detailed in Table S8.

Screening the MR-Identified Proteins for GV

After Bonferroni correction ($p < 1.77\times10^{-5}$), the two-sample MR analysis found 14 proteins (9 unique proteins) with a significant causal relationship with GV, which we termed MR-identified proteins. Specifically, elevated levels of Agouti-signaling protein (ASP), Receptor tyrosine-protein kinase erbB-3 (ERBB3), and Ribonuclease T2 (RNASET2) were associated with a reduced risk of GV. Conversely, increased levels of Granzyme B (GZMB), Interferon regulatory factor 3 (IRF3), Fc receptor-like protein 3 (FcRL3), UNC-51 like kinase 3 (ULK3), Grancalcin (GCA), and Aldehyde dehydrogenase-mitochondrial (ALDH2) were linked to an elevated risk of GV (Figure 2 and Table 1).

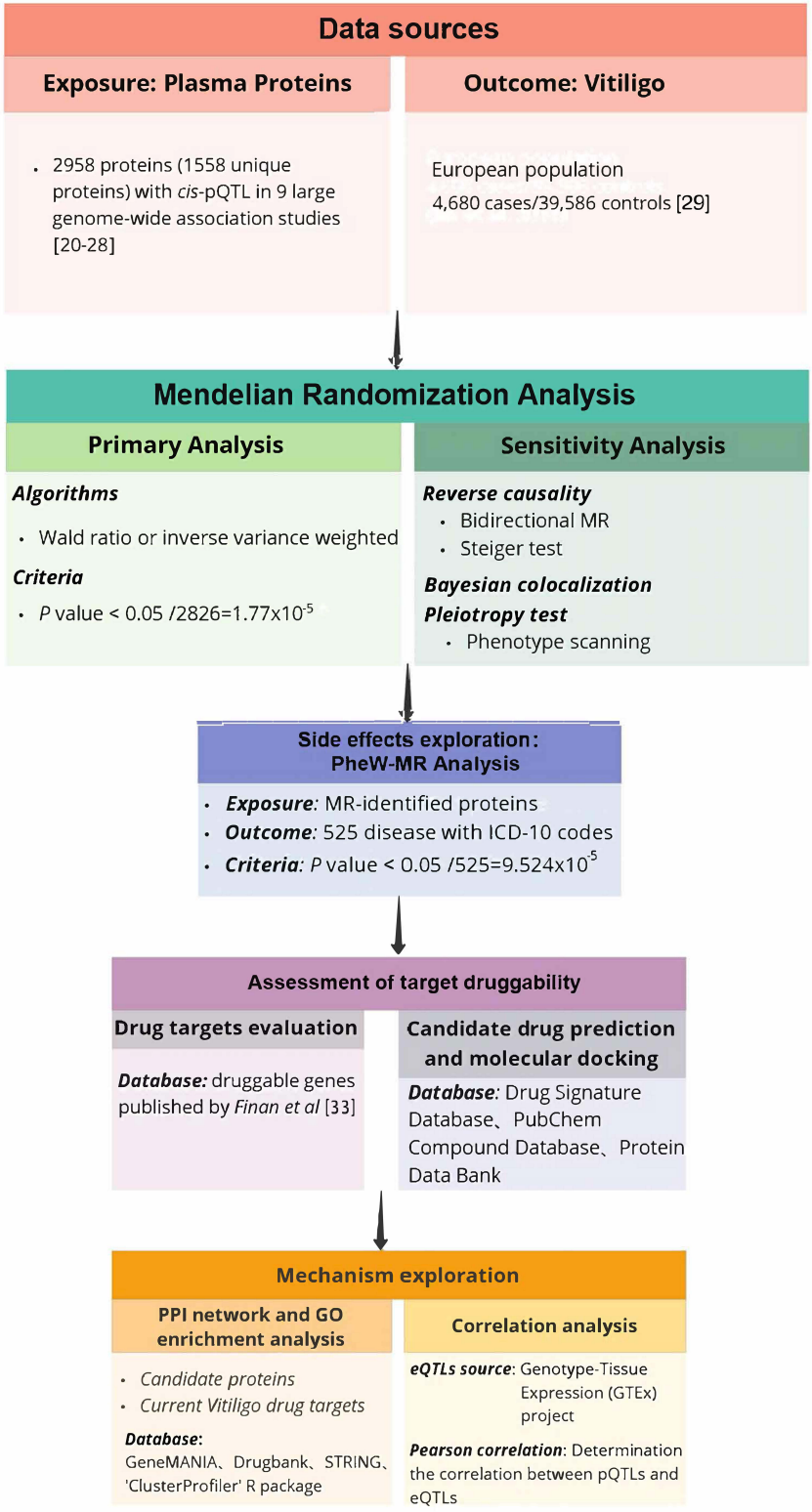

Figure 1 Study design of the MR study to reveal potential drug targets for GV.

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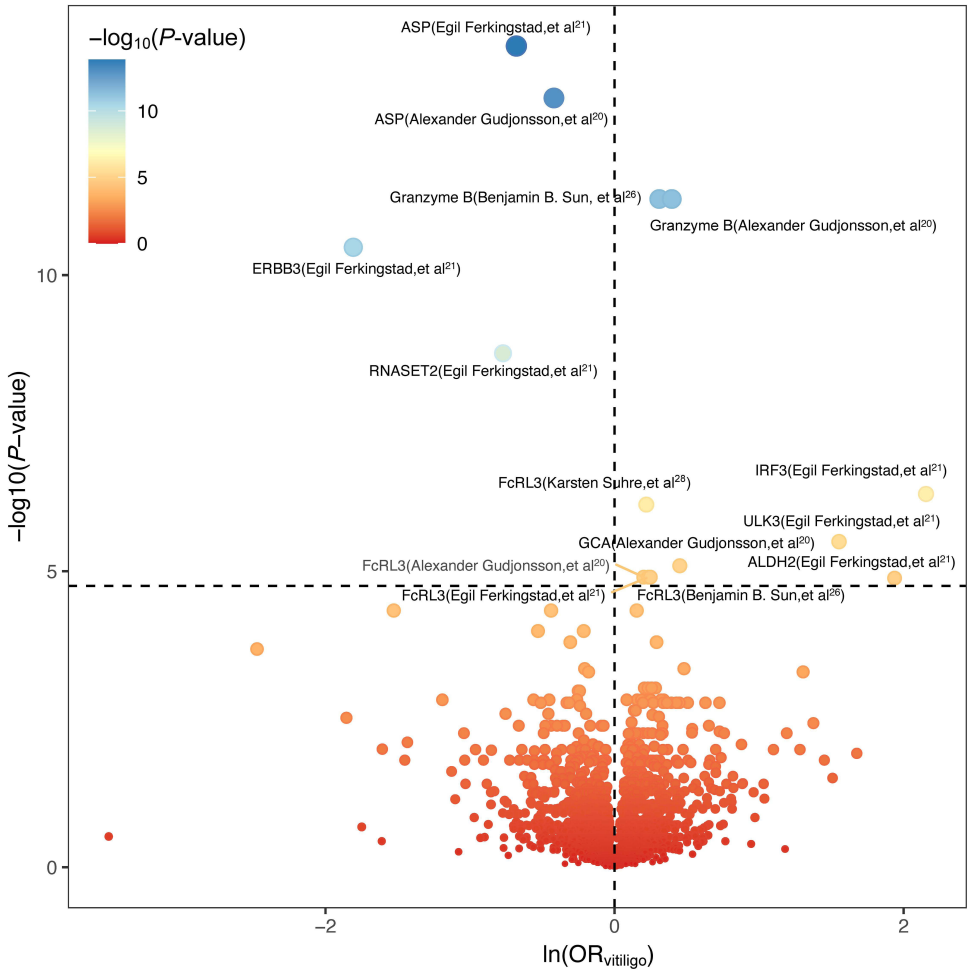

Figure 2 Volcano plots of the MR results for 2826 plasma proteins on the risk of GV.

Reverse MR Analysis and Steiger Test

We extracted 41 SNPs (Table S9) as IVs from the GWAS pooled data of GV, put 14 MR-identified proteins as endpoints, and performed reverse MR analysis, which showed that there was no causal effect of GV on plasma concentrations of MR-identified proteins (Table S10 and Figure S1). Furthermore, results from the Steiger test confirmed that the causal direction of all 14 significant associations identified in the MR analysis was correctly oriented from protein levels to GV risk (Table S11).

Colocalization Analysis

We employed Bayesian colocalization analysis to determine whether shared SNPs underlie both the risk of GV and the expression of plasma proteins, whereas MR-identified proteins that also passed colocalization analysis were more likely to be drug targets.[31] Our analysis showed that of the 14 MR-identified associations, 8 associations (4 unique proteins, namely ASP, ULK3, FCRL3, and GZMB) showed strong evidence of colocalization with GV. In contrast, the PPH4 values for ERBB3, IRF3, GCA, and ALDH2 were below 0.8, indicating that these associations might be influenced by LD or pleiotropy rather than true causal relationships. It is important to acknowledge that the fundamental assumption of colocalization is violated when a causative locus contains multiple SNPs simultaneously, which represents an inherent limitation of the analysis. Consequently, we were unable to further evaluate RNASET2 as a potential therapeutic target for GV (Table 1 and Figure S2).

Table 1 Summary of Primary Analysis, Reverse Causality Detection, Bayesian Colocalization Analysis, Phenotype Scanning, and PheW-MR on the MR-Identified Proteins

Gene	Protein	Protein full name	UniProt	Data source	MR Estimate			Steiger test(pval)	Bidirectional MR		Bayesian Colocalization PPH4	Phenotype Scanning	PheW-mr	Evidence of Potential Drug Target
					Method	OR(95% CI)	pval		OR(95% CI)	pval				
ASIP	ASP	Agouti-signaling protein	P42127	Egil Ferkingstad, et al[12]	Wald ratio	0.507 (0.426,0.603)	1.35E-14	2.73E-70	0.962 (0.881,1.05)	0.382	9.99E-01	Pass	NO	NO
				Alexander Gudjonsson, et al[20]	Wald ratio	0.657 (0.589,0.734)	1.02E-13	1.10E-155	0.907 (0.782,1.053)	0.201	9.88E-01	Pass	NO	
GZMB	Granzyme B	Granzyme B	P10144	Benjamin B. Sun, et al[25]	Wald ratio	1.362 (1.248,1.487)	5.18E-12	3.14E-102	0.926 (0.749,1.144)	0.477	9.95E-01	Pass	YES	YES
				Alexander Gudjonsson, et al[20]	Wald ratio	1.486 (1.328,1.663)	5.18E-12	7.05E-99	0.999 (0.888,1.123)	0.986	9.94E-01	Pass	YES	
ERBB3	ERBB3	Receptor tyrosine-protein kinase erbB-3	P21860	Egil Ferkingstad, et al[12]	Wald ratio	0.164 (0.096,0.28)	3.39E-11	5.16E-08	0.997 (0.978,1.017)	0.789	9.63E-08	Pass	YES	NO
RNASET2	RNASET2	Ribonuclease T2	O00584	Egil Ferkingstad, et al[12]	IVW	0.462 (0.359,0.595)	2.09E-09	9.87E-78	0.974 (0.923,1.029)	0.345	NA*	Pass	YES	NO
IRF3	IRF3	Interferon regulatory factor 3	Q14653	Egil Ferkingstad, et al[12]	Wald ratio	8.625 (3.723,19.978)	4.97E-07	1.80E-03	1.013 (0.997,1.029)	0.119	5.05E-03	Pass	YES	NO
ULK3	ULK3	UNC-51 like kinase 3	Q6PHR2	Egil Ferkingstad, et al[12]	Wald ratio	4.725 (2.459,9.082)	3.18E-06	2.40E-05	1.005 (0.992,1.019)	0.448	9.52E-01	Pass	YES	YES
GCA	GCA	Grancalcin	P28676	Alexander Gudjonsson, et al[20]	Wald ratio	1.57 (1.288,1.914)	8.09E-06	1.29E-17	1.015 (0.988,1.044)	0.272	5.38E-13	Pass	YES	NO
FCRL3	FcRL3	Fc receptor-like protein 3	Q96P31	Karsten Suhre, et al[27]	Wald ratio	1.246 (1.142,1.359)	7.52E-07	7.27E-53	0.962 (0.823,1.124)	0.625	Lack of data	Pass	YES	YES
				Alexander Gudjonsson, et al[20]	Wald ratio	1.224 (1.118,1.34)	1.26E-05	5.05E-166	1.009 (0.983,1.035)	0.514	9.54E-01	Pass	YES	
				Egil Ferkingstad, et al[12]	Wald ratio	1.264 (1.138,1.404)	1.26E-05	7.36E-85	1.008 (0.997,1.019)	0.167	9.55E-01	Pass	YES	
				Benjamin B. Sun, et al[25]	Wald ratio	1.28 (1.146,1.431)	1.26E-05	2.21E-96	1.004 (0.971,1.037)	0.827	9.69E-01	Pass	YES	
ALDH2	ALDH2	Aldehyde dehydrogenase, mitochondrial	P05091	Egil Ferkingstad, et al[12]	Wald ratio	6.94 (2.904,16.583)	1.31E-05	1.36E-04	1.011 (0.991,1.031)	0.292	5.41E-05	Pass	YES	NO

Notes: *Because the number of SNPS in the original analysis was 2, the colocalization analysis could not be performed.

Phenotype Scanning and PheW-MR

After phenotype scanning, ASP (rs62209647, rs62211989) was found to be associated with traits such as self-reported basal cell carcinoma and basal metabolic rate; ULK3 (rs936228) was found to be associated with traits such as diastolic blood pressure, systolic blood pressure, and self-reported hypertension; FCRL3 (rs2210913, rs7522061, rs7528684) was found to be associated with traits such as rheumatoid arthritis, Grave's disease, diabetes mellitus type 1; Fc receptor-like protein 1 (FCRL1); CD5 antigen-like (CD5L); It should be noted in particular that we found a direct association between GZMB (rs8192917) and GV, but this does not seem to violate the exclusivity assumption of Mendelian randomization, since in the case of our selected instrumental variable (rs8192917), its correlation with GZMB (p = 7.2×10-124 from Benjamin B. Sun et al;[25] p = 4.61×10-131 from Alexander Gudjonsson et al[20]) far exceeds its correlation with GV (p = 9.00×10-9 from Jin 2016)[28] (Table S12). To further evaluate the probable adverse effects of the four candidate proteins on additional phenotypes, we performed PheW-MR analysis using 525 diseases from the UK Biobank as endpoints. The results indicated that targeting ASP may lead to several adverse outcomes, including atrophic skin disorders, malignant melanoma, and other skin-related malignancies. These findings suggest significant risks associated with ASP-targeted therapies, leading us to conclude that ASP is not a suitable candidate for drug development (Table S13).

Assessment of Target Druggability

The three candidate proteins obtained from MR analysis combined with multiple sensitivity analyses (Table 1 shows our screening process and results well), namely ULK3, FCRL3, and GZMB, are candidate targets that we believe can be used for the treatment of vitiligo (Figure 3). We further evaluated them to determine whether they could be used for actual clinical treatment. First, we contrasted the candidate proteins with the druggable genes reported by Finan et al.[32] All three proteins were classified as druggable targets, with one each falling into tiers 1, 2, and 3. Notably, each protein could be targeted by at least one small molecule drug or biologic (Table S14).

We also performed drug prediction using the DSigDB database, and we listed the top 10 most predictive drugs based on corrected p-values (Table S15), which showed that amiodarone (amiodarone CTD 00005381) interacts with both FCRL3 and ULK3. Subsequently, based on the structure data we obtained, molecular docking was performed between these 10 predicted drugs and their interacting candidate proteins and their binding energies were calculated, and a total of 9 proteins were effectively docked with the drugs (Table S3 and Figure S3), which further confirmed the druggability of the candidate proteins at the microscopic level. The lowest binding energy (−6.57 kcal/mol) was shown between ULK3 and XMD13-2 LINCS, indicating that ULK3 has an excellent small molecule binding capacity.

PPI Network and GO Enrichment Analysis

As shown in Figure 4, the PPI network constructed by GeneMANIA, contains 3 candidate proteins as well as 20 potential interacting proteins, which constitute 179 interaction links between them. Functional analysis of the PPI network revealed that the candidate proteins are primarily involved in the positive regulation of mitochondrial membrane permeability during apoptosis, protein insertion into membranes, and mitochondrial outer membrane permeabilization, among other processes. Then, after a comprehensive analysis of the PPI network involving established drug targets for GV, we identified significant interactions between three candidate proteins and the 14 known therapeutic targets. Notably, GZMB exhibited associations with 11 known vitiligo targets, with the most robust interactions observed between GZMB

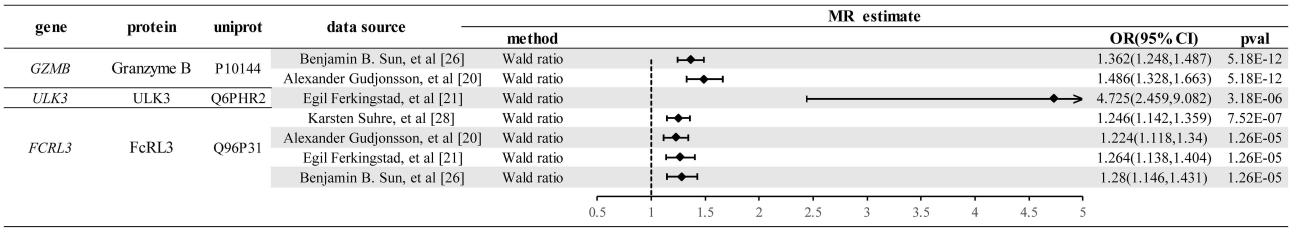

Figure 3 Forest plot for the MR result between candidate proteins and GV.

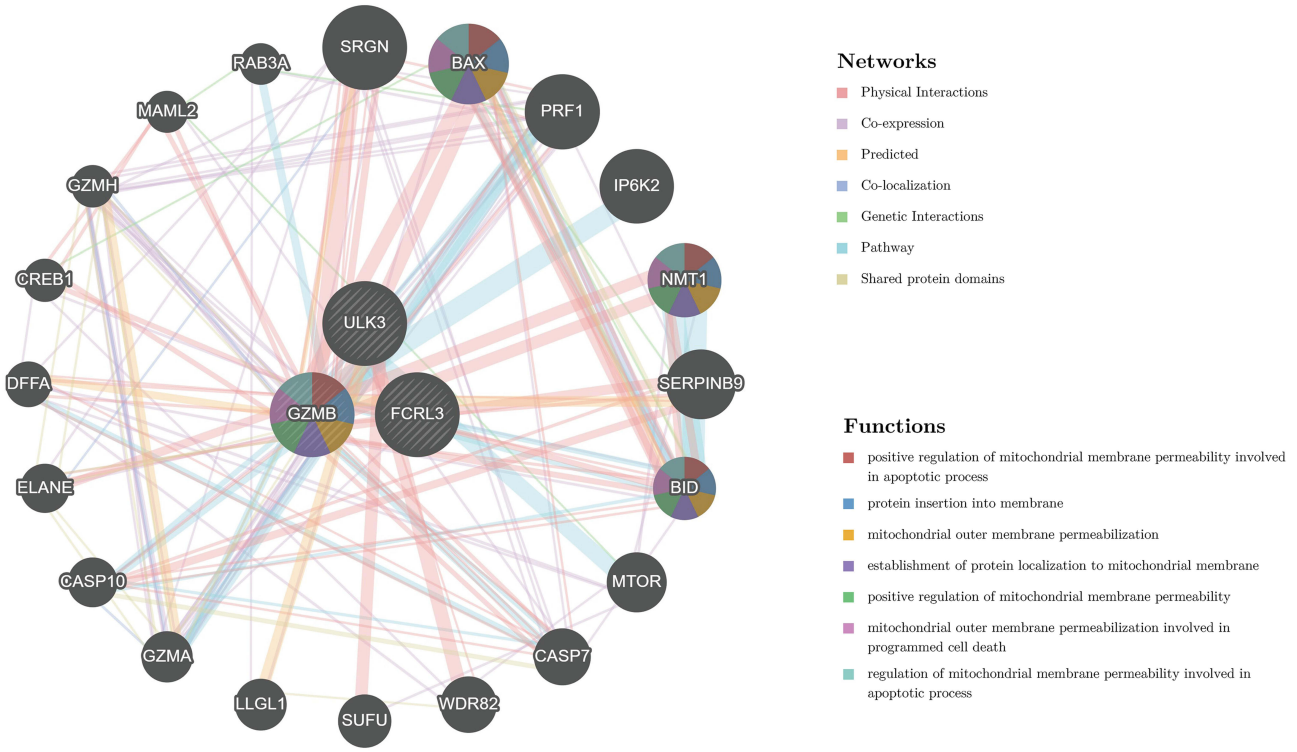

Figure 4 Protein-protein interaction network between three candidate proteins and 20 potential interacting proteins.

and Janus Kinases 1, 2, and 3 (JAK1, JAK2, JAK3), tumor necrosis factor (TNF), and Low-affinity immunoglobulin gamma Fc region receptor 1A (FCGR1A), as these were experimentally confirmed. Additionally, FCRL3 showed known associations with B-lymphocyte antigen CD20 (MS4A1) and FCGR3A, which are targeted by Rituximab and Etanercept, respectively. ULK3 was associated with mammalian target of rapamycin (MTOR), targeted by pimecrolimus (Figure 5). On one hand, investigating the efficacy of these known clinical drugs for vitiligo within the framework of protein interactions is meaningful. On the other hand, it indicates the promising drug exploration value of candidate proteins.

In GO pathway enrichment analyses, we observed that pathways for a number of GV-related biological processes were enriched by nominally significant plasma proteins including immune system process, adaptive immune response, regulation of immune system process, regulation of lymphocyte activation, regulation of T cell activation, response to stress, and so forth (Table S16).

Correlation Analysis

In order to investigate the correlation between the transcriptome and the proteome, a correlation analysis was performed on the MR effect estimates of the transcriptome and the proteome (see Tables S17 and S18 for the results of the MR analysis of eQTL-GV). The results demonstrated a robust and strong positive correlation between the two. For sun-exposed skin tissues, the Pearson correlation was R = 0.74, with a p-value of 4.5×10^{-9} and a total of 46 genes (Figure S4; Table S19). In non-sun-exposed skin tissues, the Pearson correlation was R = 0.62, with a p-value of 2.4×10^{-4} and a total of 30 genes (Figure S5; Table S20). The results indicate a high degree of consistency between the MR effects of the transcriptome and plasma proteins.

Discussion

Our study presents a comprehensive analysis integrating multiple GWAS datasets on plasma proteins and GV. Using MR as the central analytical approach, we employed a range of complementary methods, including reverse MR, the Steiger test, colocalization analysis, phenotype scanning, PheW-MR, drug gene list mapping, molecular docking, PPI, pathway

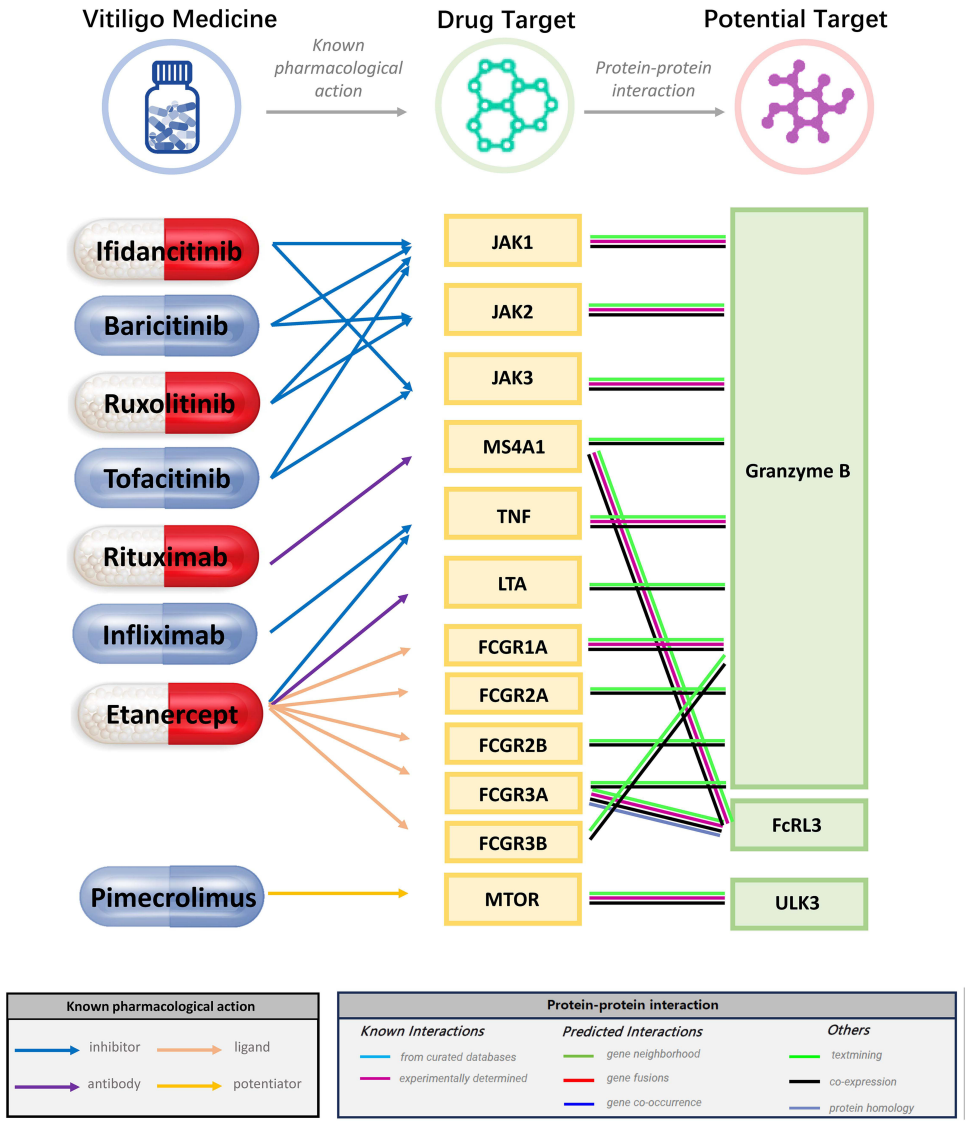

Figure 5 Interaction between current vitiligo medication targets and three candidate proteins.
Abbreviations: JAK1, Janus Kinase 1; JAK2, Janus Kinase 2; JAK3, Janus Kinase 3; MS4A1, B-lymphocyte antigen CD20; TNF, tumor necrosis factor; LTA, Lymphotoxin-alpha; FCGR1A, Low-affinity immunoglobulin gamma Fc region receptor 1A; FCGR2A, Low-affinity immunoglobulin gamma Fc region receptor 2A; FCGR2B, Low-affinity immunoglobulin gamma Fc region receptor 2B; FCGR3A, Low-affinity immunoglobulin gamma Fc region receptor 3A; FCGR3B, Low-affinity immunoglobulin gamma Fc region receptor 3B; MTOR, mammalian target of rapamycin; FcRL3, Fc receptor-like protein 3; ULK3, UNC-51 like kinase 3.

enrichment analysis, as well as eQTL and pQTL correlation analysis. The primary objective was to identify potential therapeutic targets for the treatment of GV. Ultimately, our findings highlight three plasma candidate proteins—GZMB, FCRL3, and ULK3—that demonstrate substantial evidence supporting their potential as therapeutic targets.

Plasma proteins are a popular area for drug therapeutic target development nowadays,[11] and MR has emerged as one of the key instruments for drug targets prediction.[39,40] To enhance the clinical applicability of the GWAS findings, we conducted a series of sensitivity and druggability analyses. First, we specifically selected cis-pQTLs as IVs, given their proximity to the genes encoding the proteins of interest and their direct, independent influence on protein expression.[41] More than one GWAS dataset of plasma proteins were included in this study, but some similar results were obtained, eg, GZMB yielded positive results in 2 cohorts and FCRL3 yielded consistent conclusions in 4 cohorts, implying that they can act as a validation of each other to make the conclusions more reliable. Both reverse MR analysis and the Steiger test confirmed the unidirectional causal relationship of the MR-identified proteins on vitiligo, addressing limitations inherent in traditional observational studies. In addition, Bayesian colocalization further identified four candidate proteins (ASP, GZMB, FCRL3, and ULK3) sharing the

same genetic variants as GV with high probability, eliminating the bias that might be introduced by LD. Furthermore, phenotype scanning revealed that these candidate proteins were linked to various other traits, though these associations alone could not fully explain their relationship with vitiligo. For instance, the SNP associated with FCRL3 was also linked to rheumatoid arthritis, Grave's disease, and type 1 diabetes mellitus, all of which are reported complications of vitiligo, suggesting these conditions may share common autoimmune pathways.[42–44] To ensure that treatment of the drug target does not result in undesired side effects, ASP, which has the potential to cause skin tumorigenesis, was excluded from our candidate pool despite the significant association between ASP and a reduced risk of GV development. Finally, drug prediction, molecular docking, and interactions with known vitiligo drug targets demonstrate the favorable druggability of the candidate protein; the PPI network shows that the candidate protein and its potential associated proteins are relevant to alterations in mitochondrial membrane permeability during apoptosis or programmed cell death, which is in line with previous studies reporting that mitochondrial dysfunction is involved in the pathogenesis of vitiligo;[45] The results of the enrichment analysis demonstrated that nominally significant plasma proteins were enriched in "immune system process", "regulation of T cell activation" and other biological processes that are closely related to the pathogenesis of vitiligo. The high correlation between the transcriptome and proteome of the nominally significant genes increased the biological interpretability of pQTL-based MR. Collectively, these analyses offer robust support for the validity of our conclusions.

GZMB encodes Granzyme B, a serine protease secreted by natural killer (NK) cells and cytotoxic T-lymphocytes (CTLs), which plays a pivotal role in the induction of apoptosis by CTLs through both caspase-dependent and caspase-independent pathways.[46,47] Beyond its role in apoptosis, Granzyme B has been implicated in promoting inflammation by upregulating pro-inflammatory cytokines such as IL-1α and IL-18 and contributing to extracellular matrix protein degradation.[46,48,49] Previous observational studies have demonstrated that Granzyme B can be employed as a potential biomarker for the severity and clinical prognosis of vitiligo, with elevated levels observed in skin and plasma samples from vitiligo patients. However, the causal relationship remains undetermined.[50] Our MR analysis provides evidence for causality from a novel perspective. Furthermore, our conclusions are consistent with previous genetic studies. For instance, Jeong et al's[51] study on gene polymorphisms found a significant association between the occurrence of non-segmental vitiligo (NSV) and a haplotype (T-A-G-T-T) composed of five SNPs (rs2236337, rs2236338, rs11539752, rs10909625, and rs8192917) located within a LD block encoding Granzyme B (OR = 1.19, p = 0.002). Similarly, Xu et al[52] identified that the C allele of rs8192917 increased the risk of vitiligo by approximately 40% in the Chinese Han population (OR = 1.39, $P = 1.92\times10^{-8}$), and suggested that LD alone cannot explain this effect in the Han population. Investigating its mechanisms, some studies suggest that Skin-homing CTLs are one of the main causes of melanocyte depletion in patients with vitiligo, and the high expression of Granzyme B in plasma Skin-homing CTLs of active vitiligo patients may represent cell cytotoxicity-induced melanocyte damage and apoptosis.[53] In addition, the recurrence of vitiligo has been linked to tissue-resident memory T (Trm) cells, which express CD103, CD69, CD49a, and melanocyte-specific T cell receptors (TCRs). Upon IL-15 stimulation, Trm cells secrete interferon-γ, perforin, and Granzyme B, potentially exacerbating melanocyte destruction.[54,55] In fact, Granzyme B has minimal physiological function in normal skin tissues, suggesting that targeting this molecule could be therapeutically beneficial without causing harm to the skin.[46,56] Our PPI analysis also revealed interactions between Granzyme B and various JAK inhibitors. Recently developed biologics targeting JAK, such as Ruxolitinib, Tofacitinib, and Baricitinib, have been reported clinically for the treatment of vitiligo.[7] Therefore, Granzyme B holds promise as a new drug target for vitiligo treatment and merits further investigation.

The human FCRL3 protein belongs to the immunoglobulin receptor superfamily, which comprises six transmembrane proteins and two cytoplasmic proteins. B cells, NK cells, regulatory T cells (Treg), and CD8+ T cells are the main sorts of cells that express it. At the moment, it is believed that FCRL3 regulates B cell activity in two separate manners.[57–60] FCRL3 has been reported to be associated with various autoimmune diseases, including rheumatoid arthritis,[61] auto-immune thyroid diseases,[62] and systemic lupus erythematosus.[63] The novel conclusion of this study is that FCRL3 is a risk factor for the onset and progression of vitiligo. It has been postulated that an imbalance in the Th17/Treg ratio may be involved in the aetiology of vitiligo. In peripheral blood mononuclear cells (PBMC) from patients with vitiligo, elevated levels of the Th17-specific transcription factor RORγt have been observed, while the levels of the Treg-specific transcription factors FOXP3, HELIOS and EOS were significantly reduced. Additionally, increased levels of IL-17 were

also noted.[64] It has been demonstrated that FCRL3 stimulates Tregs to express RORγt and mediates their transformation into a Th17-like phenotype thereby inhibiting the normal function of Tregs as evidenced by the secretion of pro-inflammatory cytokines associated with Th17, including IL-17, IL-26 and IFN-γ.[57] It is therefore proposed that FCRL3 may be involved in the development of vitiligo by inhibiting the function of Treg cells and promoting the expression of Th17-related pro-inflammatory cytokines. Moreover, transcriptome sequencing of skin tissues by Yang et al revealed that high expression of the FCRL3 gene was linked to a shorter duration of vitiligo and a higher response rate to phototherapy in combination with topical drug therapy.[65] This indicates that treatments targeting FCRL3 may be capable of achieving enhanced efficacy by regulating the Th17/Treg balance, a hypothesis that warrants further investigation. ULK3 (UNC-51 like kinase 3) is a member of a small kinase subfamily.[66] Our study has identified ULK3 as a potential risk factor for vitiligo; however, there is currently no published literature reporting a relationship between ULK3 and vitiligo. According to the previous studies, ULK3 is one of the fundamental building blocks of the autophagy.[67,68] An autophagosome-dependent lysosomal process known as autophagy is a conserved catabolic pathway that targets cytoplasmic components for breakdown and recycling. This mechanism guarantees the preservation of cellular homeostasis under physiological settings.[68] In a related study, Patricia González-Rodríguez et al[67] demonstrated that ULK3 plays a crucial role in regulating autophagy. Specifically, ULK3 activates GLI1 (GLI family zinc finger 1), which subsequently upregulates the expression of the DNMT3A (DNA methyltransferase 3 alpha) gene. This results in the downregulation of MAP1LC3 (microtubule-associated protein 1 light chain 3) transcription, a gene that has been demonstrated to be positively associated with autophagic activity. Consequently, the net effect of ULK3 is a reduction in autophagy. Emanuela Bastonini et al[69] found that autophagy is a protective role for vitiligo. Thus the pathophysiology of vitiligo may be attributed to autophagy malfunction, whereby the melanocytes' redox equilibrium may be upset.[70] Therefore, we speculated that ULK3 might influence the pathogenesis of vitiligo by affecting the activity of autophagy, but it needs to be confirmed by further studies.

It is inevitable that this study will have some shortcomings. Firstly, the abundance of plasma proteins differs from that of skin tissue proteins, and we were unable to explore the more compelling MR relationship between skin tissue proteins and vitiligo. Secondly, alterations in plasma protein levels resulting from pQTL modifications do not necessarily indicate that these changes will lead to alterations in protein function or impact the progression of the disease. Thirdly, the plasma proteins analysed were derived from nine distinct studies. While they can serve as mutual corroboration, the inconsistency of measurement methods across studies may introduce some bias into the results. Fourthly, our investigation of adverse effects was confined to 525 common disease characteristics, which may have resulted in the omission of other unfavourable effects. Furthermore, due to methodological constraints, there is a possibility that some potential targets may have been overlooked, such as RNASET2, which has been identified by previous studies as a promising candidate for the treatment of vitiligo.[71,72] Furthermore, the application of stringent screening conditions, such as genome-wide significant association and cis-acting pQTL, resulted in the utilization of a limited number of SNPs as IVs. This would render some classical MR methodologies inapplicable. Lastly, the drug targets derived in this study are applicable only to European populations and are not sufficiently generalizable to other races. Similarly, this study focused on GV, and thus the conclusions do not apply to other types of vitiligo, such as segmental vitiligo, so there is no guarantee that these candidate proteins can be used in the same way for the development of drugs for patients with other types of vitiligo, given the differences in the pathogenesis of these different types of vitiligo.

Conclusions

This study identified three promising candidate drug targets for the treatment of generalized vitiligo: GZMB, FCRL3, and ULK3. Targeting these plasma proteins may increase the likelihood of success in clinical trials and help streamline the prioritization of drug development, potentially reducing associated costs. To fully comprehend the molecular processes that underlie these proteins' involvement in the pathophysiology of vitiligo, more investigation is necessary.

Data Sharing Statement

All original data used in this study were obtained from publicly available databases. Detailed sources are provided in the relevant sections of the manuscript, references, or supplementary materials.

Ethics Approval

This study is a secondary analysis based on publicly available databases, approved by the Research Ethics Committee of the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University.

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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.

Disclosure

All authors declare that no commercial or financial relationships that could be construed as potential conflicts of interest were present during the conduct of this study.

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