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Original Research

Exploring novel drug targets for erectile dysfunction through plasma proteome with genome

Zeming Qiu, MM[1,2,†], Long Cheng, MD[1,2,†], Qinyuan Wang, MM[2,3], Zhilong Dong, PhD, MD[1,2,*]

[1]Department of Urology, The Second Hospital & Clinical Medical School, Lanzhou University, Lanzhou 730030, Gansu, China
[2]The Second Hospital & Clinical Medical School, Lanzhou University, Lanzhou 730030, Gansu, China
[3]Department of Plastic Surgery, The Second Hospital & Clinical Medical School, Lanzhou University, Lanzhou 730030, Gansu, China
*Corresponding author: Department of Urology, The Second Hospital & Clinical Medical School, Lanzhou University, Lanzhou 730030, Gansu, China.
Email: dongzhl@lzu.edu.cn

Abstract

Background: Currently, the treatment and prevention of erectile dysfunction (ED) remain highly challenging.

Aim: This study conducted a systematic druggable genome-wide Mendelian randomization (MR) analysis to identify potential therapeutic targets for ED.

Methods: A proteome-wide MR approach was employed to investigate the causal effects of plasma proteins on ED. Subsequently, summary data–based MR (SMR) analysis was performed to identify potential drug targets for ED. Enrichment analysis and protein–protein interaction (PPI) networks revealed the functional characteristics and biological relevance of these potential therapeutic targets. Drug prediction and molecular docking studies were conducted to validate the pharmacological activity of these identified targets. Finally, a systematic MR analysis was conducted to assess upstream intervention factors, such as lifestyles and diseases, associated with these targets, providing insights for the prevention and treatment of ED.

Outcomes: This study identified several potential therapeutic targets for ED.

Results: Proteome-wide MR analysis revealed that 126 genetically predicted plasma proteins were causally associated with ED. SMR analysis indicated that TMEM9 was associated with an increased risk of ED, while MDH1, NQO1, QDPR, ARL4D, TAGLN2, and PPP1R14A were associated with a decreased risk of ED. These potential targets were primarily enriched in metabolic and redox-related biological processes. Molecular docking indicated that the predicted drugs had favorable binding affinities with the proteins, further confirming the pharmacological value of these targets. Finally, 6 plasma proteins (MDH1, NQO1, QDPR, ARL4D, TAGLN2, and TMEM9) could be modulated by lifestyle- and disease-related factors.

Clinical Implications: This study provides new insights into the etiology and potential drug targets of ED and contributes to the development of more effective treatments for ED and reducing the cost of drug development.

Strengths and Limitations: This is a systematic and extensive study exploring the causal relationship between plasma proteins and ED, which helps to provide a comprehensive perspective to understand the role of potential targets in ED. However, we did not conduct this study in different types of ED or different stages of ED progression.

Conclusion: In summary, this study identified 7 plasma proteins causally associated with ED and provided new insights into the etiology and potential drug targets for ED.

Keywords: drug targets; plasma proteins; erectile dysfunction; Mendelian randomization.

Introduction

Erectile dysfunction (ED) is defined as the persistent inability to achieve and/or maintain a strong-enough erection for satisfactory sexual activity.[1] Epidemiological studies indicate that approximately 150 million men worldwide suffer from varying degrees of ED, with the number of affected individuals expected to increase to 300 million by 2025.[2] The rising incidence of ED diminishes the quality of life for patients and their partners and imposes a significant medical and economic burden on both individuals and society. Unfortunately, due to the complex etiology and pathogenesis of ED, the development of related therapeutic drugs has been relatively slow. Consequently, the treatment and prevention of ED remain highly challenging. Although several drugs (such as phosphodiesterase type 5 inhibitors) have been developed and offer significant benefits to ED patients, there are still many challenges, including side effects and suboptimal response rates.[3,4] Therefore, it is of great value to discover more promising drug targets and develop more effective drugs accordingly.

Proteins can be regarded as the ultimate participants in all biological processes related to disease and health.[5] They often possess specific binding sites or regions that can be targeted by small molecules or biologics, making them crucial drug targets.[6] Given the accessibility of blood compared to other

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tissues, plasma proteome represents an attractive resource for identifying disease biomarkers in large-scale cohorts.[7] Recently, genome-wide association studies (GWAS) of plasma proteins have identified thousands of protein quantitative trait loci (pQTLs).[8] These studies can assess the causal effects of plasma proteins on outcomes and screen for biomarkers that could serve as drug targets.

Mendelian randomization (MR) employs single-nucleotide polymorphisms (SNPs) from GWAS as instrumental variables (IVs) to estimate the causal relationship of exposures on outcomes. Compared to observational studies, MR helps to mitigate the impact of confounding factors, thereby enhancing the reliability and accuracy of causal inference.[9] A large-scale proteome-wide MR using pQTLs as IVs has been conducted to determine the causal relationship between plasma proteins and outcomes.[10] When combined with proteomics, MR provides new insights into the etiology and treatment of diseases. This study aims to answer the following questions: What are the potential ED drug targets identified through plasma proteomics? Do these targets have biological significance and druggability?

Methods

Study design

We first conducted a 2-sample MR analysis using 4907 plasma proteins from the deCODE genetics dataset and ED GWAS dataset from the FinnGen consortium to identify plasma proteins causally associated with ED. Subsequently, summary data–based Mendelian randomization (SMR) analysis was performed to further filter proteins that could serve as drug targets. Enrichment analysis and protein–protein interaction (PPI) networks revealed the functional characteristics and biological relevance of these potential therapeutic targets. Furthermore, drug prediction and molecular docking studies were employed to validate the pharmacological activity of the identified targets. Finally, we performed a systematic MR analysis of 35 lifestyle- and disease-related factors and plasma proteins to identify upstream interventions for these targets, providing new insights into the prevention and treatment of ED. The overview of this study is presented in Figure 1.

Data source

We obtained the summary statistics of genetic associations for plasma proteins from a large-scale proteomics study (deCODE genetics), which performed whole-genome sequencing on 35 559 individuals of Icelandic ancestry, generating their genotype and phenotype data.[10] SOMAscan was employed to provide comprehensive pQTLs for 4907 plasma proteins. The summary statistics for ED were sourced from the latest GWAS summary data (2024) from FinnGen R11 (https://r11.finngen.fi/), including 2548 cases and 196 451 controls.[11] Summary-level data for lifestyle and disease-related factors were obtained from representative large-scale GWAS cohorts; detailed information regarding the relevant GWAS is provided in Supplementary Table 1. All participants were of European descent and provided informed consent. Given that our study is based on publicly available summary data, no additional ethical review was required.

Proteome-wide MR analysis

We performed a 2-sample MR analysis with plasma proteins as exposures and ED as the outcome. The criteria for selecting pQTLs as IVs were as follows: First, SNPs within ±1 Mb of the gene region (cis-pQTLs) were included. Second, SNPs significantly associated with plasma proteins were required to have a genome-wide significance threshold of $P < 5 \times 10^{-8}$. The independence of SNPs was confirmed by testing linkage disequilibrium (LD) ($r^2 < 0.001$, kb = 10 000). In addition, we removed SNPs associated with potential risk factors related to outcome based on results searching in PhenoScanner. Finally, the *F*-statistic was used to assess the statistical strength of the IVs, retaining only strong instruments ($F > 10$).[12] For specific proteins with only 1 SNP, we applied the Wald ratio method. For proteins with 2 or more available SNPs, the inverse variance weighting (IVW) method was employed, with 3 other methods (MR–Egger, weighted median, and weighted mode) used for complementary analyses. The heterogeneity of SNPs in IVW estimates was assessed using Cochran's *Q* test ($P < .05$ indicates heterogeneity). The potential pleiotropy of these SNPs was assessed by MR–Egger regression intercepts.[13]

Summary data–based Mendelian randomization analysis

The SMR method was used to evaluate the association between ED and the expression levels of plasma protein-coding genes. Compared to traditional MR analysis, SMR can be used to explore the relationship between gene expression levels and outcomes. Top-associated cis-pQTLs within a ± 1000 kb window centered on the corresponding gene were identified,[14] with a significance threshold set at 5×10^{-8}. HEIDI testing was employed to distinguish between causal relationship and pleiotropy from linkage. A *P*-value for HEIDI (P-HEIDI) > .01 indicates no heterogeneity.[15] A *P*smr-value <0.05 was considered statistically significant. SMR and HEIDI tests were performed using the SMR software tool (SMR v1.3.1).

Enrichment analysis

To better understand the biological functions and metabolic pathways of potential target genes, we conducted Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. Gene Ontology analysis investigates the commonalities of genes across biological processes (BPs), molecular functions (MFs), and cellular components (CCs). It determines the enrichment of genes in each GO annotation by comparing the differences between the analyzed genes and the reference genome. Kyoto Encyclopedia of Genes and Genomes analysis provides information on metabolic pathways.

Protein–protein interaction network construction

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://string-db.org/) integrates predictive information from experimental data, text mining, and homologous data. We used the STRING database to explore potential interactions among the 7 drug target proteins and related upstream and downstream candidate proteins, constructing a PPI network. A confidence score of 0.4 was set as the minimum required interaction score.[16]

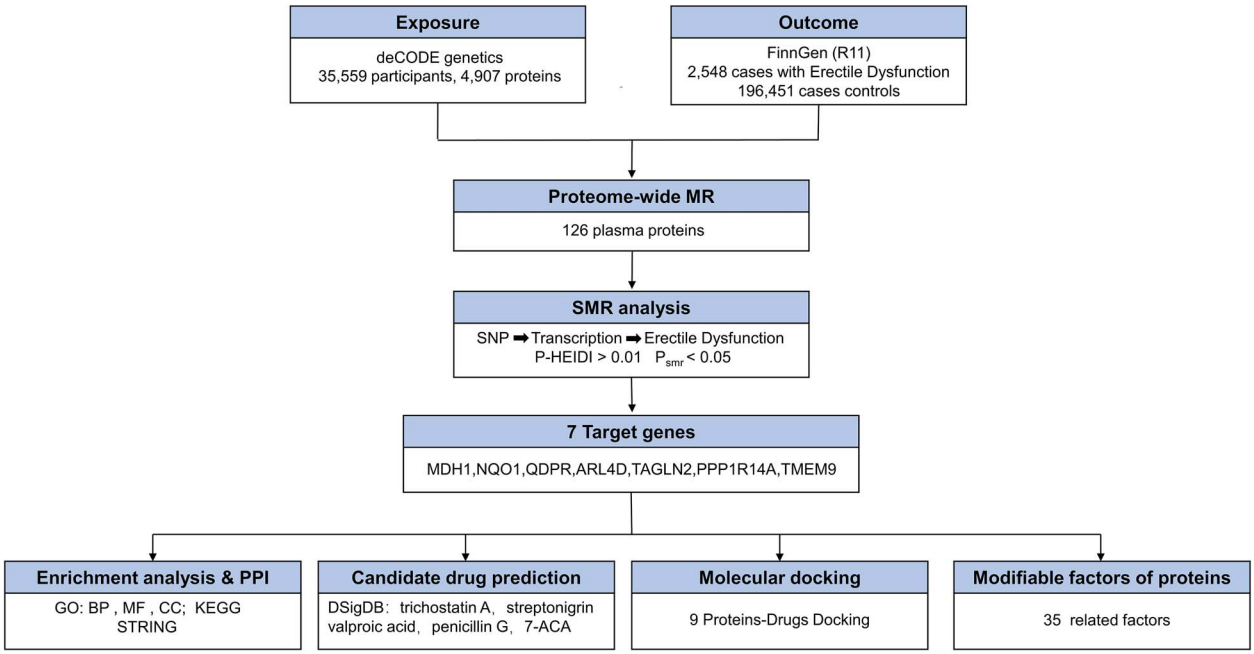

Figure 1. Overview of the study design.

Candidate drug prediction

We utilized the Drug Signature Database (DSigDB, http://dsigdb.tanlab.org/DSigDBv1.0/) for this study.[17] DSigDB contains 22 527 gene sets and 17 389 different compounds, covering 19 531 genes, making it a vast database capable of linking drugs and other compounds to their target genes. The DSigDB gene sets were derived and compiled from quantitative inhibition data of drugs/compounds sourced from various databases and publications (eg, PubChem and ChEMBL). These genes represent the direct targets of drugs/compounds, identified through automated computational methods and manual curation. The DSigDB gene set seamlessly integrates with GSEA software to link gene expression with drugs/compounds, ranking them by *P*-value for use in drug discovery and translational research. Specifically, we uploaded the genes of the target proteins identified through SMR analysis to DSigDB. This allowed us to predict candidate drugs that can bind to the target genes and assess their druggability, aiming to achieve targeted gene therapy.

Molecular docking

To further explore the impact of candidate drugs on drug target proteins and their druggability, we conducted molecular docking at the atomic level to evaluate the binding affinity and interaction patterns between candidate drugs and their targets. Molecular docking simulations can analyze ligand–receptor binding affinity and interaction patterns. By identifying ligands with high binding affinity and favorable interaction patterns, we can prioritize relevant drug targets and optimize the design of potential candidate drugs.

Drug structure data were sourced from the PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/),[18] and protein structure data were downloaded from the Protein Data Bank (http://www.rcsb.org/). We utilized the computerized protein–ligand docking software CB-Dock2 (http://autodock.scripps.edu/) to conduct molecular docking of the top 5 important drugs and the proteins encoded by their corresponding target genes.[19] CB-Dock2 employed its curvature-based cavity detection method (CurPocket) to predict the binding sites of target proteins and used AutoDock Vina to predict the binding positions of query ligands. CB-Dock2 selected several top-ranked cavities for further analysis based on cavity size (cavity ranking), calculated docking centers and adjusted the docking box size accordingly. Docking results were visualized in the model. Binding energies below -5 kcal/mol were considered successful for ligand–receptor interactions.

Modulation of ED-related plasma proteins by lifestyle- and disease-related factors

A total of 35 lifestyle- and disease-related factors were used to assess their association with ED-related plasma proteins. The MR analysis methods were consistent with those described in the proteome-wide MR analysis.

All statistical analyses were conducted using R software version 4.1.0, with the TwoSampleMR package version 0.6.8.

Results

Proteome-wide MR analysis

After rigorous screening, a total of 1677 plasma proteins were included in the MR analysis. MR analysis based on the IVW or Wald ratio method revealed that 126 plasma proteins were associated with ED. Among them, 60 plasma proteins were positively correlated with ED, while 66 proteins were negatively correlated (Figure 2A). Detailed information on the IVs is provided in Supplementary Table 2. The results of the MR analysis are presented in Supplementary Table 3. Furthermore, several heterogeneity was detected among the IVs (Supplementary Table 4), and no horizontal pleiotropy was observed (Supplementary Table 5).

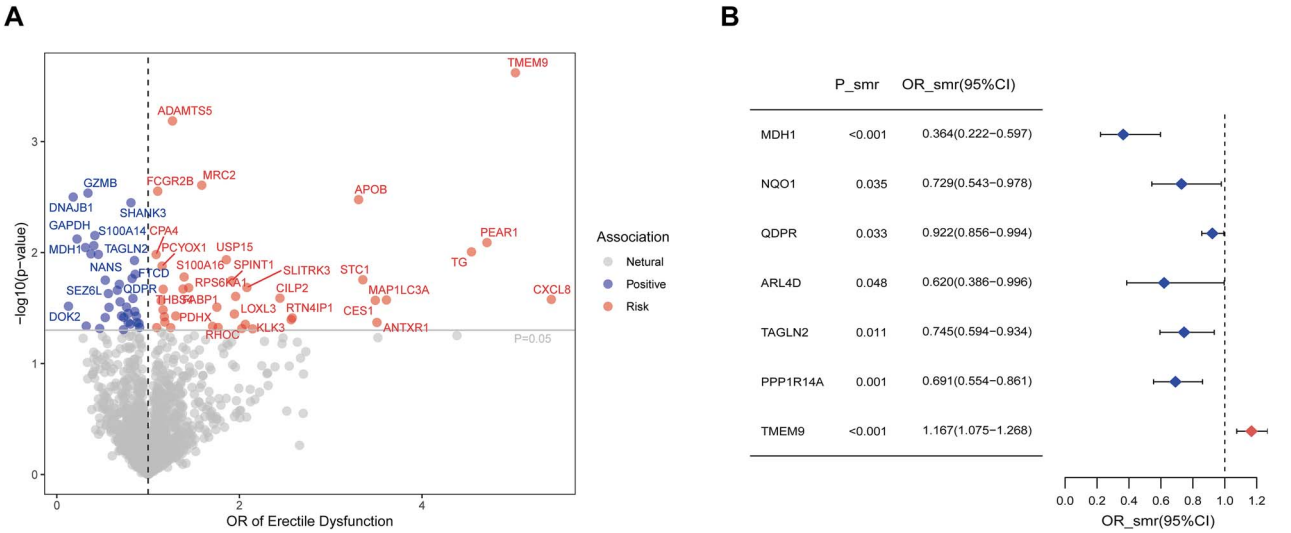

Figure 2. (A) A volcano plot displaying the MR results of plasma proteins on erectile dysfunction. (B) A forest plot displaying the SMR results for 7 drug targets.

SMR analysis

Subsequently, we performed SMR analysis on the 126 identified proteins to further refine potential targets. The results showed that 7 target genes had a significant causal relationship with ED (*P*smr <.05), indicating their potential as drug target candidates. Among these, TMEM9 was identified as a pathogenic gene for ED, which may exacerbate the progression of ED. Additionally, we identified 6 protective genes that might reduce the risk of ED (MDH1, NQO1, QDPR, ARL4D, TAGLN2, and PPP1R14A) (Figure 2B). All 7 target genes successfully passed the HEIDI test. Detailed results of the SMR analysis are provided in Supplementary Table 6.

Enrichment analysis and PPI network

According to GO enrichment analysis, biological processes such as regulation of biological quality, response to chemical substances and stimuli, response to metal ions, and small molecule metabolic processes are significantly enriched in BP. In the CC category, drug target genes are primarily enriched in cytoplasm-associated components, while in MF, they are mainly involved in oxidoreductase activity and metal ion binding. KEGG enrichment analysis indicates that drug target genes are primarily involved in pathways related to amino sugar and nucleotide sugar metabolism, mineral absorption, folate biosynthesis, and the biosynthesis of ubiquinone and other terpenoid-quinones (Figure 3B).

A PPI network diagram for the proteins encoded by these differential genes was constructed using the STRING website (Figure 3A). Due to the absence of MDH1 in the STRING database, it was temporarily excluded from the PPI network. Network nodes represent proteins, with line colors indicating evidence of interactions between different proteins. The PPI network illustrates the interactions of the 6 drug targets and their associated proteins. Key molecules like NQO1 act as hub proteins with high centrality, suggesting their strong connections with other proteins.

Candidate drug prediction

In this study, we used DSigDB to predict potential intervention drugs and listed the top 5 candidate drugs based on their *P*-values (Table 1). The results indicate that trichostatin A is the most significant drug associated with NQO1, MDH1, and TAGLN2. Additionally, both trichostatin A and valproic acid (VPA) were found to be associated with most of the drug target genes.

Molecular docking

Molecular docking was performed in this study to assess the affinity of candidate drugs for their targets and evaluate their druggability. We utilized CB-Dock2 to obtain binding sites and interaction patterns between the top 5 candidate drugs and their respective target proteins. The binding affinities of each interaction were generated, resulting in 9 successful docking results between proteins and drugs (Table 1 and Figure 4). Among these, TAGLN2 showed the lowest binding energy with trichostatin A (-91 kcal/mol), indicating a highly stable interaction.

Effects of 35 lifestyle- and disease-related factors on ED-associated proteins

Among the 35 common lifestyle- and disease-related factors analyzed, smoking initiation correlated positively with NQO1 and TMEM9. Cigarettes per day was positively associated with ARL4D. Age first had sexual intercourse showed a negative association with NQO1. Lifetime number of sexual partners was positively associated with MDH1. Both body mass index and weight were positively associated with MDH1, NQO1, QDPR, ARL4D, TAGLN2, and TMEM9. Hip circumference was positively correlated with ARL4D. Non-alcoholic fatty liver disease was positively correlated with NQO1 and QDPR. Asthma and insomnia both exhibited negative correlations with NQO1. Metformin use exhibited a negative correlation with MDH1 (Figure 5). Detailed results are presented in Supplementary Table 7.

Discussion

Penile erection is mediated through the vascular dilation of the corpus cavernosum, primarily regulated by the nervous and circulatory systems.[20] The circulatory system is the primary

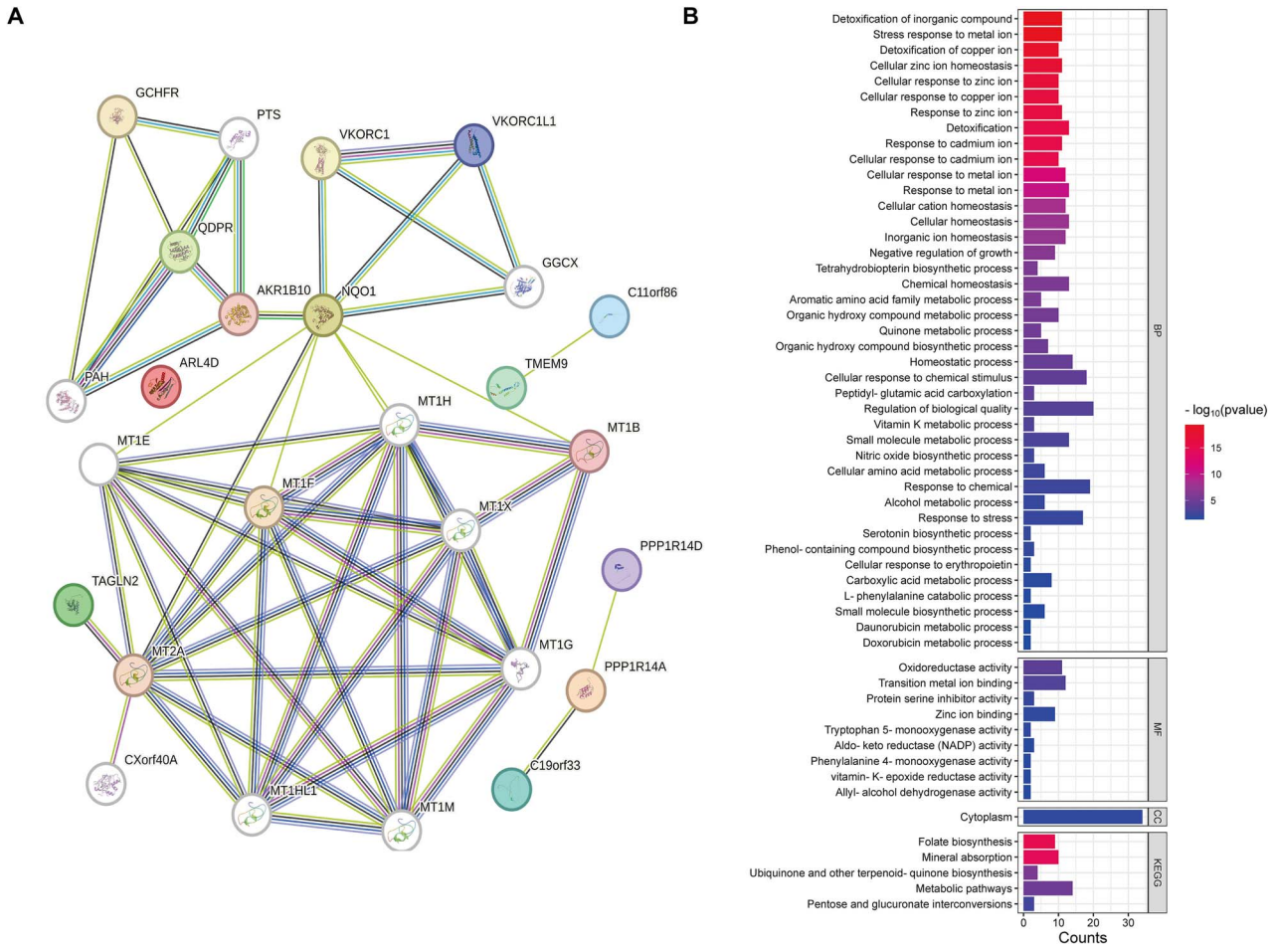

Figure 3. (A) Protein–protein interaction (PPI) network. (B) The results of the gene-enrichment analysis.

Table 1. Molecular docking results of available proteins and candidate drugs.

Drug	PubChem ID	*P*-value	Protein	PDB ID	Binding energy(kcal/mol)
Trichostatin A	444732	.0017	MDH1	7rm9	-7.5
			NQO1	8ok0	-8.5
			TAGLN2	1wym	-91
Streptonigrin	5298	.0049	NQO1	8ok0	-10
Valproic acid	3121	.0050	MDH1	7rm9	-5.4
			NQO1	8ok0	-5.7
			TAGLN2	1wym	-58
Penicillin G	5904	.0058	NQO1	8ok0	-8.8
7-ACA	441328	.0063	NQO1	8ok0	-7.1

object that comes into contact with and potentially affects the penis, and blood samples are relatively easy to obtain and less invasive to the patient. Therefore, studying the association between plasma proteins and ED, and identifying targets in the blood, has significant and practical clinical implications for predicting and treating ED. Our study identified 7 genes (MDH1, NQO1, QDPR, ARL4D, TAGLN2, PPP1R14A, and TMEM9) as potential drug target genes through SMR analysis. Moreover, to explore the biological significance and interaction mechanisms of these drug targets, we conducted an enrichment analysis and built a PPI network. Subsequently, we conducted drug prediction and molecular docking to further validate the pharmacological and therapeutic value of these important target genes. Finally, a systematic MR analysis of 35 lifestyle and disease-related factors and targets was performed to determine which related factors may serve as upstream intervention factors for the targets, thereby providing new insights for the prevention and treatment of ED.

In this study, 6 plasma proteins (MDH1, NQO1, QDPR, ARL4D, TAGLN2, and PPP1R14A) predicted by genetics were found to be negatively associated with ED. Malate dehydrogenase 1 (MDH1) is an NAD(H)-dependent enzyme that may regulate metabolism between the cytosol and mitochondria.[21] MDH1 deficiency can lead to severe neurodevelopmental disorders, and the resultant cavernous nerve damage is a common cause of neurogenic erectile dysfunction.[22] During sexual stimulation, erection begins with nerve conduction, with nitric oxide (NO) and acetylcholine released

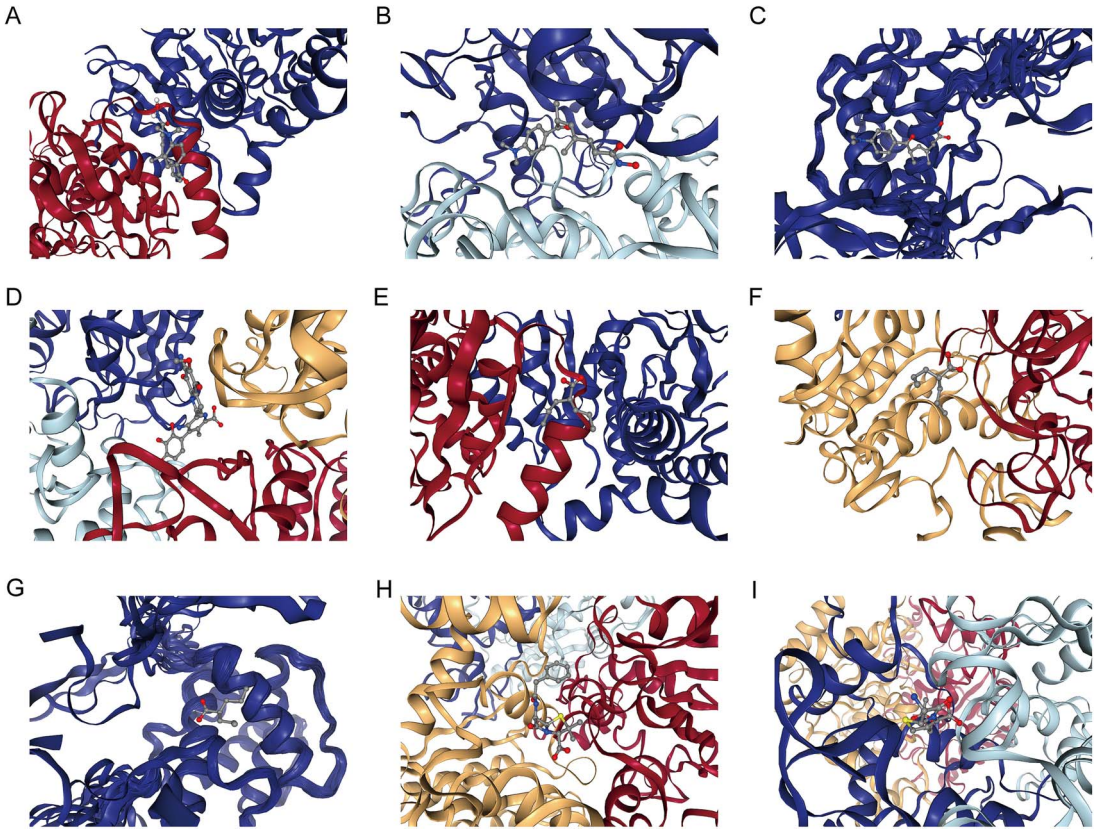

Figure 4. Molecular docking results of available proteins and drugs. (A) MDH1 docking trichostatin A. (B) NQO1 docking trichostatin A. (C) TAGLN2 docking trichostatin A. (D) NQO1 docking streptonigrin. (E) MDH1 docking valproic acid. (F) NQP1 docking valproic acid. (G) TAGLN2 docking valproic acid. (H) NQO1 docking penicillin G. (I) NQO1 docking 7-ACA.

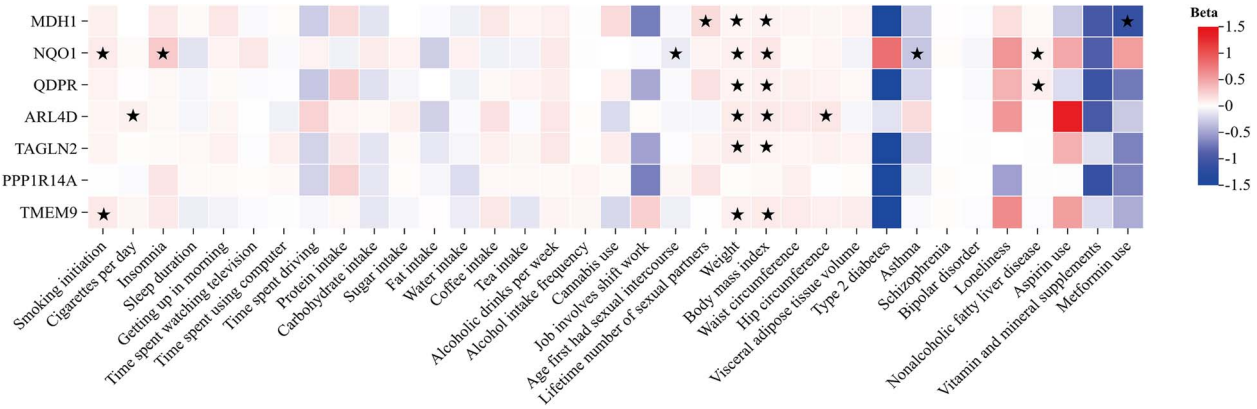

Figure 5. Heatmap displaying results from Mendelian randomization between 35 lifestyle- and disease-related factors and 7 proteins associated with erectile dysfunction.

from the parasympathetic nerves, mediating vascular dilation through the relaxation of vascular smooth muscle cells in the corpora cavernosa, leading to increased blood flow, filling the corpora cavernosa, and supporting penile erection. Additionally, MDH1 plays a crucial role in cellular aging, characterized by reduced MDH1 activity and a subsequent decrease in the NAD/NADH ratio in fibroblasts of elderly individuals. Aging-related declines in androgen levels inhibit NO production, leading to endothelial dysfunction and smooth muscle cell apoptosis, which can progress to age-related ED.[23] The prevalence and severity of ED increase with age.[24] These studies highlight the significant potential of MDH1 as a therapeutic target for ED. NADH Quinone Dehydrogenase 1 (NQO1) is an enzyme involved in cellular detoxification and defense against oxidative stress (OS). It plays a crucial role in the regulation of neuronal function and synaptic plasticity in the central nervous system (CNS), cellular adaptation to OS, neuroinflammatory and degenerative processes, and tumourigenesis.[25] Impaired NQO1 activity in the CNS not only leads to abnormal release and clearance of crucial neurotransmitters (such as NO) but also results in increased OS.[26] OS can damage endothelial function and NO signaling, which are crucial for erectile function. Reactive oxygen species can degrade NO, reducing its availability, impairing vasodilation, increasing vascular tone, and causing inflammation. This results in reduced blood flow to the tissues and an inability to achieve or maintain an erection, which further leads to endothelial dysfunction and eventually

ED.[27] Therefore, NQO1-mediated oxidative activation may influence the physiological processes of erection. Additionally, abnormal NQO1 enzyme activity is associated with the pathophysiological mechanisms of various neurological diseases, including Parkinson's disease, Alzheimer's disease, epilepsy, multiple sclerosis, cerebrovascular disease, traumatic brain injury, and malignant brain tumors.[28] These conditions are also significant factors in neurogenic and vasculogenic ED.[24] In summary, NQO1 may play a critical role in the treatment of ED. Quinoid dihydropteridine reductase (QDPR) is an enzyme that regulates tetrahydrobiopterin (BH4), which is a cofactor for enzymes involved in neurotransmitter synthesis and blood pressure regulation. Reduced QDPR activity can lead to the accumulation of dihydrobiopterin and depletion of BH4, resulting in impaired neurotransmitter synthesis, oxidative stress, and an increased risk of Parkinson's disease.[29] Enrichment analysis of drug target genes involved in the biological processes of redox enzymes also supports this conclusion. However, there is currently a lack of substantial direct evidence linking QDPR to ED, and further research is needed to explore the potential of QDPR as a therapeutic target for ED. ADP-ribosylation factor-like 4D (ARL4D), an Arf-like small GTPase, is involved in regulating cell morphology, cell migration, and actin cytoskeleton remodeling.[30] Activated ARL4D can recruit cytohesin-2/ARNO to the plasma membrane to facilitate Arf6 activation, leading to the regulation of actin dynamics and affecting cellular processes such as cell migration and neurite growth,[31,32] which may impact penile erection function. Transgelin-2 (TAGLN2) is a member of the calmodulin family and regulates the actin cytoskeleton of various immune cells, thereby modulating the activation, differentiation, and phagocytosis of lymphocytes and macrophages. It stabilizes the cortical interaction with F-actin at the immunological synapse and activates leukocyte function-associated antigen-1 following T-cell receptor stimulation, regulating T-lymphocyte activation, cytokine production, and the interaction between T lymphocytes and antigen-presenting cells.[33] Studies have shown that TAGLN2 knockout mice are more susceptible to bacterial infections and have a higher mortality rate during bacteremia. These results support the role of TAGLN2 in macrophage phagocytosis and bacterial clearance.[34] Reduced TAGLN2 activity, which can lead to bacterial infections and even immune dysregulation, may have significant impacts on penile erection. Furthermore, TAGLN2 acts as a receptor for extracellular metallothionein-2, which relaxes the myosin cytoskeleton of smooth muscle cells.[35] Therefore, TAGLN2 represents a promising therapeutic target for diseases such as ED and asthma. Protein Phosphatase 1 Regulatory Inhibitor Subunit 14A (PPP1R14A) is a widely expressed serine–threonine phosphatase and an inhibitor of protein phosphatase 1.[36] Previous studies have demonstrated that PPP1R14A plays a critical role in the occurrence and progression of tumors, including gliomas and schwannomas.[37,38] PPP1R14A may serve as a significant diagnostic marker and a potential therapeutic target for gliomas and schwannomas. Additionally, existing research suggests that gliomas and schwannomas could be risk factors for ED.[24]

In this study, genetically predicted TMEM9 was positively associated with the risk of ED. Transmembrane Protein 9 (TMEM9) is a type I transmembrane protein primarily located in lysosomes, late endosomes, and multivesicular bodies.[39] It is closely associated with inflammation and can regulate TNF-α-enhanced cytokine secretion through the classical Wnt/β-catenin pathway. Overexpression of TMEM9 may increase the levels of IL-6 and IL-1β in LX-2 cells.[40] Erectile dysfunction is associated with elevated levels of several pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-8.[41] Damaged endothelial cells stimulate an inflammatory response within the vascular walls by increasing the production of inflammatory cytokines and cell adhesion molecules. This leads to the formation of atherosclerotic plaques and a reduction in NO release in the inflammatory state, ultimately impairing erectile function.[42,43] Furthermore, existing studies have confirmed that history of prostatitis is an independent risk factor for ED.[44] Chronic low-grade inflammation is crucial to the pathogenesis of ED and the intermediate stages of possible endothelial dysfunction.[45] Thus, the upregulation of TMEM9 may be 1 of the mechanisms through which local inflammation from prostatitis exacerbates ED.

In this study, we utilized DSigDB to predict potential therapeutic drugs for ED. Some of our predictions have already been confirmed by related research. Valproic acid, an FDA-approved drug, has been widely used for nearly 40 years to treat epilepsy and other neuropsychiatric disorders.[46,47] Beyond its anticonvulsant properties, a published animal study on ED treatment has shown that early intervention with VPA may serve as a potential treatment strategy for ED by maintaining penile morphology and erectile function in rat models subjected to prostatectomy-induced ED. Valproic acid can be used to treat men undergoing prostatectomy to prevent the occurrence of ED, without concerns about its impact on prostate cancer recurrence.[48] Additionally, VPA has been found to reduce the protein expression of fibroblast growth factor. One study indicated that VPA improves erectile function and reduces fibrosis in diabetic rat models.[49] In summary, VPA is a safe and widely used drug that may offer a new avenue for ED treatment. Further clinical studies are needed to assess the long-term effects of VPA on erectile function. Moreover, trichostatin A, streptonigrin, penicillin G, and 7-aminocephalosporanic acid (7-ACA) play roles in immune system modulation, exhibiting anti-inflammatory, antimicrobial, and neuroprotective properties.[50-53] However, we have not found any clinical trials for these drugs in the treatment of ED. Hence, the potential connection between trichostatin A, streptonigrin, penicillin G, and 7-ACA with ED deserves continued attention and exploration.

Our study has several strengths. Firstly, this is a systematic and extensive study exploring the causal relationship between plasma proteins and ED, which helps to provide a comprehensive perspective to understand the etiologic role of circulating proteins in ED. Moreover, the SMR analysis helps minimize false positives, ensuring the robustness of the results. Secondly, we conducted drug prediction based on these potential drug targets, revealing their pharmacological potential, and assessed the affinity of candidate drugs with their targets through molecular docking techniques. Finally, we performed a comprehensive assessment of potential upstream intervention factors related to ED-associated target genes. However, this study also has certain limitations. Firstly, all GWAS participants were of European descent, which not only minimizes regional variation but also restricts the generalizability of the findings. Secondly, the accuracy of molecular docking analysis largely depends on the quality of protein structures and ligands. Although this method identifies potential drug targets, it does not guarantee their efficacy in clinical settings. Thirdly, ED can be classified into psychogenic, organic, or

mixed psychogenic and organic types. The expression levels or predicted values of the target genes we identified may fluctuate in different types of ED or at different stages of ED progression. This limitation hinders the ability to provide personalized treatment strategies for the unique variations observed in ED. Therefore, future studies would be best conducted in specific types or stages of ED.

Conclusion

In summary, this study identified 7 plasma proteins causally associated with ED and provided new insights into the etiology and potential drug targets for ED. Additionally, through drug prediction and molecular docking, we validated the therapeutic value of these targets, which may contribute to the development of more effective ED treatments and reduce drug development costs.

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Author contributions

Conceptualization, Z.D. and Z.Q.; methodology, Z.Q. and L.C.; data curation, Q.W.; writing—original draft preparation, Z.Q.; writing—review and editing, Z.D. Zeming Qiu and Long Cheng contributed to the work equally and should be regarded as co-first authors.

Supplementary material

Supplementary material is available at *Sexual Medicine* online.

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Conflicts of interest

None declared.

Data availability

All the summary-level GWAS data in this study are publicly available for download by qualified researchers. All data generated in this study can be obtained from the Supplementary Information.

Ethical approval and consent to participate

All the summary-level GWAS and pQTLs data used in the analyses are publicly available, and therefore, ethical approval was not imperative for this study. Ethical approval for the GWAS can be found in the corresponding GWAS publications cited in the manuscript.

Consent for publication

All authors have read and agreed to the published version of the manuscript.

References

1. Najari BB, Kashanian JA. Erectile dysfunction. *JAMA*. 2016;**316**(17):1838. https://doi.org/10.1001/jama.2016.12284.
2. Ayta IA, JB MK, Krane RJ. The likely worldwide increase in erectile dysfunction between 1995 and 2025 and some possible policy consequences. *BJU Int*. 1999;**84**(1):50-56. https://doi.org/10.1046/j.1464-410x.1999.00142.x.
3. De Nunzio C, Nacchia A, Grimaldi MC, *et al.* Major adverse cardiovascular events related to phosphodiesterase 5 inhibitors: analysis of real-life data from Eudra-Vigilance database. *Minerva Urol Nephrol*. 2024;**76**(2):203-209. https://doi.org/10.23736/S2724-6051.23.05611-2.
4. Ohl DA, Carlsson M, Stecher VJ, Rippon GA. Efficacy and safety of sildenafil in men with sexual dysfunction and spinal cord injury. *Sex Med Rev*. 2017;**5**(4):521-528. https://doi.org/10.1016/j.sxmr.2017.01.007.
5. Emilsson V, Gudmundsdottir V, Gudjonsson A, *et al.* Coding and regulatory variants are associated with serum protein levels and disease. *Nat Commun*. 2022;**13**(1):481. https://doi.org/10.1038/s41467-022-28081-6.
6. Sun BB, Maranville JC, Peters JE, *et al.* Genomic atlas of the human plasma proteome. *Nature*. 2018;**558**(7708):73-79. https://doi.org/10.1038/s41586-018-0175-2.
7. Gudjonsson A, Gudmundsdottir V, Axelsson GT, *et al.* A genome-wide association study of serum proteins reveals shared loci with common diseases. *Nat Commun*. 2022;**13**(1):480. https://doi.org/10.1038/s41467-021-27850-z.
8. Emilsson V, Ilkov M, Lamb JR, *et al.* Co-regulatory networks of human serum proteins link genetics to disease. *Science*. 2018;**361**(6404):769-773. https://doi.org/10.1126/science.aaq1327.
9. Burgess S, Timpson NJ, Ebrahim S, Davey SG. Mendelian randomization: where are we now and where are we going? *Int J Epidemiol*. 2015;**44**(2):379-388. https://doi.org/10.1093/ije/dyv108.
10. Ferkingstad E, Sulem P, Atlason BA, *et al.* Large-scale integration of the plasma proteome with genetics and disease. *Nat Genet*. 2021;**53**(12):1712-1721. https://doi.org/10.1038/s41588-021-00978-w.
11. Kurki MI, Karjalainen J, Palta P, *et al.* FinnGen provides genetic insights from a well-phenotyped isolated population. *Nature*. 2023;**613**(7944):508-518. https://doi.org/10.1038/s41586-022-05473-8.
12. Burgess S, Thompson SG, CRP CHD Genetics Collaboration. Avoiding bias from weak instruments in Mendelian randomization studies. *Int J Epidemiol*. 2011;**40**(3):755-764. https://doi.org/10.1093/ije/dyr036.
13. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through egger regression. *Int J Epidemiol*. 2015;**44**(2):512-525. https://doi.org/10.1093/ije/dyv080.
14. Zhu Z, Zhang F, Hu H, *et al.* Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. *Nat Genet*. 2016;**48**(5):481-487. https://doi.org/10.1038/ng.3538.
15. Wu Y, Zeng J, Zhang F, *et al.* Integrative analysis of omics summary data reveals putative mechanisms underlying complex traits. *Nat Commun*. 2018;**9**(1):918. https://doi.org/10.1038/s41467-018-03371-0.
16. Szklarczyk D, Kirsch R, Koutrouli M, *et al.* The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res*. 2022;**51**(D1):D638-D646. https://doi.org/10.1093/nar/gkac1000.
17. Yoo M, Shin J, Kim J, *et al.* DSigDB: drug signatures database for gene set analysis. *Bioinformatics*. 2015;**31**(18):3069-3071. https://doi.org/10.1093/bioinformatics/btv313.
18. Kim S, Chen J, Cheng T, *et al.* PubChem 2023 update. *Nucleic Acids Res*. 2023;**51**(D1):D1373-D1380. https://doi.org/10.1093/nar/gkac956.

19. Liu Y, Yang X, Gan J, Chen S, Xiao ZX, Cao Y. CB-Dock2: improved protein–ligand blind docking by integrating cavity detection, docking and homologous template fitting. *Nucleic Acids Res*. 2022;**50**(W1):W159-W164. https://doi.org/10.1093/nar/gkac394.
20. Guimaraes EL, Dias DO, Hau WF, *et al*. Corpora cavernosa fibroblasts mediate penile erection. *Science*. 2024;**383**(6683):eade8064. https://doi.org/10.1126/science.ade8064.
21. Oh SJ, Gu DR, Jin SH, Park KH, Lee SH. Cytosolic malate dehydrogenase regulates RANKL-mediated osteoclastogenesis via AMPK/c-Fos/NFATc1 signaling. *Biochem Biophys Res Commun*. 2016;**475**(1):125-132. https://doi.org/10.1016/j.bbrc.2016.05.055.
22. Broeks MH, Shamseldin HE, Alhashem A, *et al*. MDH1 deficiency is a metabolic disorder of the malate-aspartate shuttle associated with early onset severe encephalopathy. *Hum Genet*. 2019;**138**(11-12):1247-1257. https://doi.org/10.1007/s00439-019-02063-z.
23. Yu W, Wang J, Dai YT, *et al*. Modulation of SIRT1 expression improves erectile function in aged rats. *Asian J Androl*. 2022;**24**(6):666-670. https://doi.org/10.4103/aja202199.
24. Shamloul R, Ghanem H. Erectile dysfunction. *Lancet*. 2013;**381**(9861):153-165. https://doi.org/10.1016/S0140-6736(12)60520-0.
25. Siegel D, Ross D. Immunodetection of NAD(P)H:quinone oxidoreductase 1 (NQO1) in human tissues. *Free Radic Biol Med*. 2000;**29**(3-4):246-253. https://doi.org/10.1016/s0891-5849(00)00310-5.
26. Ross D, Siegel D. The diverse functionality of NQO1 and its roles in redox control. *Redox Biol*. 2021;**41**(2021):101950. https://doi.org/10.1016/j.redox.2021.101950.
27. Roychoudhury S, Chakraborty S, Choudhury AP, *et al*. Environmental factors-induced oxidative stress: hormonal and molecular pathway disruptions in hypogonadism and erectile dysfunction. *Antioxidants*. 2021;**10**(6):837. https://doi.org/10.3390/antiox10060837.
28. Yuhan L, Khaleghi Ghadiri M, Gorji A. Impact of NQO1 dysregulation in CNS disorders. *J Transl Med*. 2024;**22**(1):4. https://doi.org/10.1186/s12967-023-04802-3.
29. Girish A, Sutar S, Murthy TPK, *et al*. Comprehensive bioinformatics analysis of structural and functional consequences of deleterious missense mutations in the human QDPR gene. *J Biomol Struct Dyn*. 2024;**42**(11):5485-5501. https://doi.org/10.1080/07391102.2023.2226740.
30. Lin SJ, Huang CF, Wu TS, Li CC, Lee FJS. Arl4D-EB1 interaction promotes centrosomal recruitment of EB1 and microtubule growth. Chang F, ed. *Mol Biol Cell*. 2020;**31**(21):2348-2362. https://doi.org/10.1091/mbc.E18-10-0611.
31. Li CC, Chiang TC, Wu TS, Pacheco-Rodriguez G, Moss J, Lee FJS. ARL4D recruits cytohesin-2/ARNO to modulate actin remodeling. Barr F, ed. *Mol Biol Cell*. 2007;**18**(11):4420-4437. https://doi.org/10.1091/mbc.e07-02-0149.
32. Yamauchi J, Miyamoto Y, Torii T, *et al*. Valproic acid-inducible Arl4D and cytohesin-2/ARNO, acting through the downstream Arf6, regulate neurite outgrowth in N1E-115 cells. *Exp Cell Res*. 2009;**315**(12):2043-2052. https://doi.org/10.1016/j.yexcr.2009.03.012.
33. Na BR, Kim HR, Piragyte I, *et al*. TAGLN2 regulates T cell activation by stabilizing the actin cytoskeleton at the immunological synapse. *J Cell Biol*. 2015;**209**(1):143-162. https://doi.org/10.1083/jcb.201407130.
34. Kim HR, Lee HS, Lee KS, *et al*. An essential role for TAGLN2 in phagocytosis of lipopolysaccharide-activated macrophages. *Sci Rep*. 2017;**7**(1):8731. https://doi.org/10.1038/s41598-017-09144-x.
35. Yin LM, Xu YD, Peng LL, *et al*. Transgelin-2 as a therapeutic target for asthmatic pulmonary resistance. *Sci Transl Med*. 2018;**10**(427):eaam8604. https://doi.org/10.1126/scitranslmed.aam8604.
36. Wang Z, Huang R, Wang H, *et al*. Prognostic and immunological role of PPP1R14A as a pan-cancer analysis candidate. *Front Genet*. 2022;**13**(2022):842975. https://doi.org/10.3389/fgene.2022.842975.
37. Hagel C, Dornblut C, Schulz A, *et al*. The putative oncogene CPI-17 is up-regulated in schwannoma. *Neuropathol Appl Neurobiol*. 2016;**42**(7):664-668. https://doi.org/10.1111/nan.12330.
38. Xu J, Zhang Y, Shi Y, *et al*. CPI-17 overexpression and its correlation with the NF2 mutation Spectrum in sporadic vestibular Schwannomas. *Otol Neurotol*. 2020;**41**(1):e94-e102. https://doi.org/10.1097/MAO.0000000000002430.
39. Baek S, Chang JW, Yoo SM, *et al*. TMEM9 activates Rab9-dependent alternative autophagy through interaction with Beclin1. *Cell Mol Life Sci*. 2024;**81**(1):322. https://doi.org/10.1007/s00018-024-05366-1.
40. Wei W, Jiang F, Liu XC, Su Q. TMEM9 mediates IL-6 and IL-1β secretion and is modulated by the Wnt pathway. *Int Immunopharmacol*. 2018;**63**(2018):253-260. https://doi.org/10.1016/j.intimp.2018.07.036.
41. Vlachopoulos C, Aznaouridis K, Ioakeimidis N, *et al*. Unfavourable endothelial and inflammatory state in erectile dysfunction patients with or without coronary artery disease. *Eur Heart J*. 2006;**27**(22):2640-2648. https://doi.org/10.1093/eurheartj/ehl341.
42. Matos G, Hirotsu C, Alvarenga TA, *et al*. The association between TNF-α and erectile dysfunction complaints. *Andrology*. 2013;**1**(6):872-878. https://doi.org/10.1111/j.2047-2927.2013.00136.x.
43. Luboshitzky R, Aviv A, Hefetz A, *et al*. Decreased pituitary-gonadal secretion in men with obstructive sleep apnea. *J Clin Endocrinol Metab*. 2002;**87**(7):3394-3398. https://doi.org/10.1210/jcem.87.7.8663.
44. Ma C, Cai Z, Xiong J, Li H. History of prostatitis is an independent risk factor for erectile dysfunction: a cross-sectional study. Khurshid M, ed. *Biomed Res Int*. 2020;**2020**(1):8964673. https://doi.org/10.1155/2020/8964673.
45. Kaya-Sezginer E, Gur S. The inflammation network in the pathogenesis of erectile dysfunction: attractive potential therapeutic targets. *Curr Pharm Des*. 2020;**26**(32):3955-3972. https://doi.org/10.2174/1381612826666200424161018.
46. Bowden CL. Valproate. *Bipolar Disord*. 2003;**5**(3):189-202. https://doi.org/10.1034/j.1399-5618.2003.00031.x.
47. Peterson GM, Naunton M. Valproate: a simple chemical with so much to offer. *J Clin Pharm Ther*. 2005;**30**(5):417-421. https://doi.org/10.1111/j.1365-2710.2005.00671.x.
48. Hannan JL, Kutlu O, Stopak BL, *et al*. Valproic acid prevents penile fibrosis and erectile dysfunction in cavernous nerve-injured rats. *J Sex Med*. 2014;**11**(6):1442-1451. https://doi.org/10.1111/jsm.12522.
49. Kutlu O, Karaguzel E, Gurgen SG, *et al*. Antifibrogenic role of valproic acid in streptozotocin induced diabetic rat penis. *Andrologia*. 2016;**48**(4):453-463. https://doi.org/10.1111/and.12465.
50. Shen MJ, Sun LC, Liu XY, *et al*. Trichostatin a improves the inflammatory response and liver injury in septic mice through the FoxO3a/autophagy signaling pathway. *World J Emerg Med*. 2022;**13**(3):182-188. https://doi.org/10.5847/wjem.j.1920-8642.2022.056.
51. Nabihah, Nasir N, Sekar M, Ravi S, *et al*. Chemistry, biosynthesis and pharmacology of streptonigrin: an old molecule with future prospects for new drug design, development and therapy. *Drug Des Dev Ther*. 2023;**17**(2023):1065-1078. https://doi.org/10.2147/DDDT.S388490.
52. Zhang M, Powell CA, Guo Y, Benyon L, Duan Y. Characterization of the microbial community structure in Candidatus Liberibacter asiaticus-infected citrus plants treated with antibiotics in the field. *BMC Microbiol*. 2013;**13**(1):112. https://doi.org/10.1186/1471-2180-13-112.
53. Lin X, Kück U. Cephalosporins as key lead generation beta-lactam antibiotics. *Appl Microbiol Biotechnol*. 2022;**106**(24):8007-8020. https://doi.org/10.1007/s00253-022-12272-8.

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