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Original Article

A Mendelian randomisation approach to explore genetic factors associated with erectile dysfunction based on pooled genomic data

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Abstract: Background: Genetic factors are thought to play a major role in erectile dysfunction (ED), but the search for specific ED-related genes remains a mysterious area characterised by limited and inconclusive research. Methods: Whole blood expression quantitative trait loci (eQTLs) and the GWAS data related to the genetics of ED are derived from a Finnish database, Finngen, which contains a dataset of 1154 cases and 94024 controls, culminating in a total of 95178 individuals under scrutiny. Based on these pooled data, a Mendelian randomisation (MR) analysis of ED was performed. Subsequent analyses of PPI and single cell type expression help identify potential pathogenic genes, revealing the function of genes and their association with phenotypes. Results: After SMR analysis, 110 ED-associated genes were screened, of which MDM4 Degree had the highest value with an OR of 1.8453076, was displaced on chromosome 1, and had a risk of promoting ED. Single-cell sequencing analysis results demonstrate the expression of the MDM4 gene in six cell types, further confirming the role of the MDM4 gene in ED. Conclusions: Our study showed that among the 110 genes associated with ED, MDM4 was highly associated with an increased risk of ED. These findings strongly support personalised treatment strategies decision-making for ED patients.

Keywords: Erectile dysfunction, Mendelian randomization, MDM4 gene

Introduction

Erectile Dysfunction (ED) is a common and distressing condition affecting the male population worldwide, characterised by the consistent inability to achieve or maintain an erection sufficient for satisfactory sexual performance [1, 2]. Its effects extend beyond the physical to psychological well-being, self-esteem and intimate relationships [3]. ED affects individuals and places a significant burden on healthcare systems and society as a whole [4]. The aetiology of ED is multifactorial and involves a complex interplay of physiological, psychological, and environmental factors [5]. While factors such as age, co-morbidities (e.g., diabetes, cardiovascular diseases), lifestyle choices (e.g., smoking, obesity), and psychological stressors are known to contribute to ED, emerging evidence suggests a substantial genetic component underlying its pathogenesis [6-8].

Genetic predisposition has been increasingly implicated in the susceptibility to ED, with familial aggregation studies demonstrating an increased risk in individuals with a family history of the condition [7]. Genome-wide association studies (GWAS) and candidate gene approaches have identified several genetic loci associated with ED [9], shedding light on potential biological mechanisms underlying erectile function. However, translation of these findings into clinical practice and therapeutic interventions remains limited, mainly due to the complexity of genetic interactions and the challenges associated with establishing causal relationships. Mendelian randomisation (MR) is emerging as a robust epidemiological tool in this context [10], providing a method to examine the causal relationship between potentially modifiable risk factors and ED using genetic variants as instrumental variables [11]. The key advantage of MR is its ability to minimise confounding

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and reverse causation, which are common problems in observational studies [12]. By using genetic variants that are randomly assigned at conception, MR provides a natural experiment that can mimic the randomness of randomised controlled trials, providing evidence that is less likely to be biased by confounding factors.

This paper explores the feasibility and advantages of using MR methodology to elucidate the genetic influences on ED. By using MR, we aim to unravel the causal effects of genetic variants associated with ED and identify potential targets for therapeutic intervention. Research indicates that there are 110 genes associated with erectile dysfunction (ED), of which MDM4 plays a critical role in influencing ED. Through a comprehensive analysis of genetic factors using MR, we aim to advance personalised medicine approaches to the management and treatment of ED, ultimately improving outcomes and quality of life for those affected.

Materials and methods

eQTL data

Expression Quantitative Trait Loci (eQTL) are genetic variants or single nucleotide polymorphisms (SNPs) that influence gene expression levels. These genetic loci are associated with the expression levels of specific genes in individual genomes. Studying eQTL provides insight into the genetic factors that regulate gene expression, revealing gene functionality and its association with phenotypes. A key resource is the Genotype-Tissue Expression (GTEx) project, which collects tissue samples from a variety of healthy individuals, including organs such as heart, liver, kidney, lung and brain [13]. With contributions from thousands of donors, GTEx provides extensive eQTL data, elucidating the relationship between genotypes and gene expression levels. Our analysis focuses on GTEx V8 whole blood eQTL summary statistics ($P < 1 \times 10^{-5}$) for SMR analysis.

Single-cell data

Single cell data were obtained from the public database GEO dataset GSE206528. This dataset includes three standard tissue samples obtained from the tumour margin of cancer resections, with all patients reporting good erectile function and morning erections. The five ED CC tissues were obtained from biopsies of implanted penile prostheses. All ED patients were diagnosed with organic ED by nocturnal tumescence and intracavernosal injection testing rather than psychogenic ED [14].

SMR analysis

In this analysis, we used summary data-based Mendelian randomisation (SMR) to investigate whether single nucleotide polymorphisms (SNPs) influencing phenotype are mediated by gene expression. We used summary data from genome-wide association studies (GWAS) and expression quantitative trait loci (eQTL) studies to explore the association between gene expression and erectile dysfunction (ED). We also performed a Heterogeneity in Dependent Instruments (HEIDI) test to assess whether the observed associations were influenced by linkage disequilibrium. A P_HEIDI value of less than 0.05 indicates that the observed associations may be due to two independent genetic variants in linkage disequilibrium. A P_SMR less than 0.05 is considered statistically significant. The analysis was performed using version 1.3.1 of the SMR software tool [15].

Functional enrichment analysis

The Gene Ontology (GO) system provides structured, computable information about the functionality of genes and gene products. Functional enrichment analysis was performed using the R package clusterProfiler, and the enrichment analysis results were visualised. A significance threshold of $P < 0.05$ was used.

Pathway enrichment analysis

The Kyoto Encyclopedia of Genes and Genomes (KEGG) database systematically analyses gene function and links genomic and functional information. It includes metabolic pathways, hierarchical classifications, gene databases and genome databases. The pathway database within KEGG is the most widely used public database for metabolic pathways. Functional enrichment analysis was performed using the R package clusterProfiler, and the enrichment analysis results were visualised. A significance threshold of $P < 0.05$ was used.

PPI analysis

Following the SMR analysis, a protein-protein interaction (PPI) analysis was performed for

each of the three diseases. The basic principle of PPI is that physical or chemical interactions occur between different proteins, potentially leading to structural changes that may affect their function. PPI analysis was performed using the online STRING database (string-db.org), with a low confidence score threshold of 0.15 to account for the limited number of target genes and to avoid neglecting important genes. Topological analysis methods, in particular the degree algorithm, were used to validate hub genes. The CytoHubba plugin integrated into Cytoscape 3.9.1 (University of California, San Diego) was used. The topological structure of the PPI network was analysed to identify critical nodes.

Genotype analysis and screening

The analysis and selection of genetic loci are based on two methods combined with SMR analysis: (1) Selection of critical loci in the disease: In SMR analysis, loci are selected that meet the criteria of FDR < 0.01 and HEIDI_P > 0.01. These loci serve as markers that influence the disease at the genetic level. (2) Single-cell sequencing analysis of the expression of genes at relevant loci in different cells: By analysing the changes in the number of cells in the ED group by cell proportion analysis and then examining their expression patterns by single-cell sequencing, this approach provides insights into drug design [16].

Statistical analysis

Statistical analysis was performed using SMR to investigate whether the effects of SNPs on phenotypes are mediated by gene expression. We analysed summary data from GWAS and eQTL studies to investigate the association between gene expression and erectile dysfunction (ED). We also used the Heterogeneity in Dependent Instruments (HEIDI) test to assess whether linkage effects confounded the observed associations. A *p*-value < 0.01 and HEIDI > 0.01 were used as criteria for identifying disease-related genes. Single-cell analysis used Principal Component Analysis (PCA) to reduce the dimensionality of the raw data set and Uniform Manifold Approximation and Projection (UMAP) to map high-dimensional data into a low-dimensional space for visualisation and analysis. All statistical procedures were performed using R software (version 4.3.1).

Result

Screening of genes associated with ED

In order to screen out genes associated with ED, we performed summary data-based MR analysis (SMR analysis) for ED. By summarizing the results of genome-wide association studies (GWAS), we applied a specific MR analysis technique, the Summary data-based Mendelian Randomization (SMR) analysis. A Manhattan plot of genes analyzed for association with erectile dysfunction (ED) was obtained after SMR analysis (**Figure 1**), where the x-axis represents chromosome numbering, the y-axis shows an indication of the significance of the association of each gene with ED, i.e., the negative logarithmic (base 10) value of the *p*-value (-log10(p)), and the red dots represent the disease-associated targets. In determining statistical significance, we set a significance threshold, wherein a *P*-value less than 0.01 was deemed significant, suggesting that genes with *P*-values below this threshold have a significant association with the diseases. Additionally, to ensure consistency and reduce the likelihood of spurious results, we assessed the heterogeneity of the genes. A heterogeneity *P*-value greater than 0.01 indicates no significant inconsistency among the analyzed genes. Finally, 110 erectile dysfunction-related genes were obtained.

Enrichment analysis of genes associated with ED

To further investigate how the genes associated with erectile dysfunction (ED) exert their influence, we performed a Gene Ontology (GO) enrichment analysis on the 110 ED-related genes. We generated pie charts and bubble plots by examining the GO enrichment analysis results, as shown in **Figure 2A** and **2B**. In **Figure 2A**, different colours indicate different Gene Ontology categories: biological processes (green), cellular components (yellow) and molecular functions (purple). The size and intensity of the colour in each sector indicates the degree of gene enrichment and statistical significance within that functional category. **Figure 2B** shows a bubble plot of these enrichment results, with the GeneRatio represented on the x-axis and different GO terms on the y-axis. The size of the bubbles corresponds to the number of enriched genes. At the same time, the colour

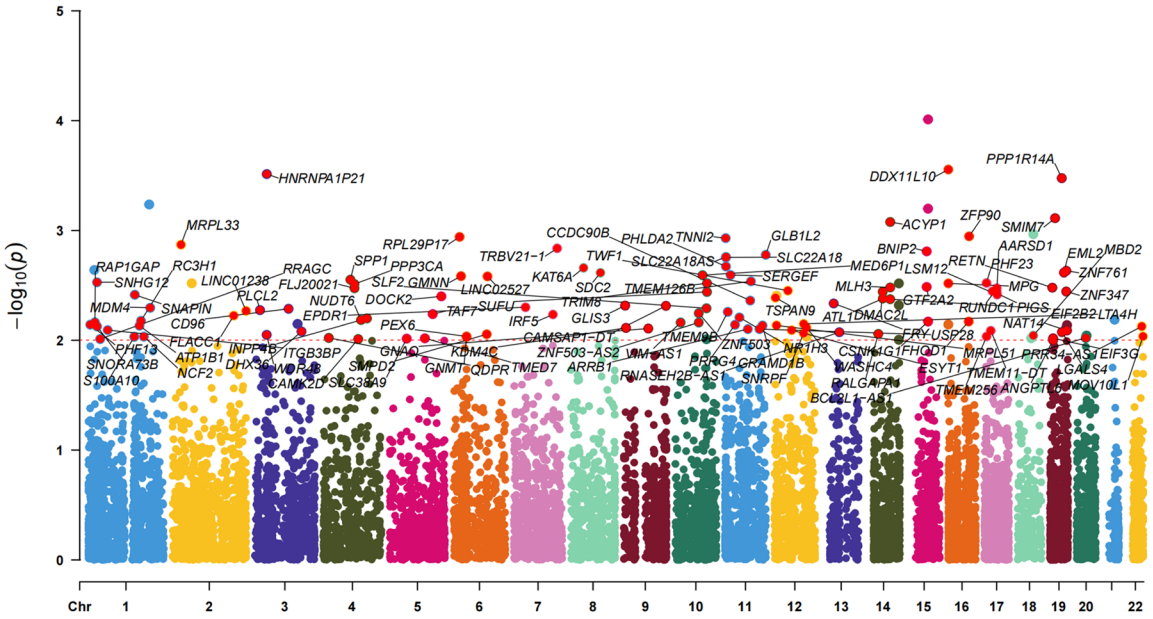

Figure 1. Screening of genes associated with ED. Manhattan plot of the SMR analysis of the effect of genes of interest on the outcome of erectile dysfunction in whole blood, where the horizontal coordinate is the chromosome number, the vertical coordinate is the significance of the gene pair *p* value taken as log10 transformed to represent the genes, and the red dots represent the disease-associated targets.

depth indicates the *p*-value, i.e. the level of significance, with darker colours indicating greater significance due to lower *p*-values.

Notably, several GO terms in **Figure 2B** are marked by larger, darker bubbles, indicating robust enrichment of genes and a statistically significant association with ED. Specifically, we observed significant enrichment in areas related to cell growth, developmental processes, metabolic pathways and signalling mechanisms. Terms such as 'positive regulation of cardiomyocyte hypertrophy', 'positive regulation of muscle hypertrophy' and 'positive regulation of RNA polymerase II-mediated mRNA transcription' suggest the involvement of cardiovascular functions and muscle activities in ED pathology. In the cellular component category, there is a marked enrichment associated with structures such as 'RNA polymerase II transcriptional regulatory complex', 'myofibrils' and 'gene-specific intranuclear bodies', suggesting an important role for ED-associated genes in cellular structure and subcellular localisation.

For molecular functions, we see enrichments related to "GTPase regulatory activity", "nucleotide triphosphatase regulatory activity", "hormone binding", etc. Among them, the enrichment analysis in the biological process (BP) part of **Figure 2B** shows that ED-related genes are most enriched in two molecular functions: regulation of GTPase activity and regulation of nucleotide triphosphatase activity (the enormous bubbles) and all of them have the highest gene ratios (around 0.1) and the most significant correlations (red bubbles). This suggested possible interactions and biochemical processes at the molecular level in which the proteins encoded by these genes might be involved. We then used KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis to investigate the enrichment of genes associated with erectile dysfunction (ED) in specific pathways and biological processes. This approach explored how essential genes might influence ED by affecting these pathways or biological processes. This approach explored how essential genes might influence ED by affecting these pathways or biological processes. Bubble plots of FEGG enrichment analysis are shown in **Figure 2C**, and ED-related genes were mainly enriched in signalling pathways and biological processes, such as Dopaminergic Synapse, Hedgehog signalling pathway, Gonadotropin-releasing hormone (GnRH) secretion, and Long-Term Potentiation signalling pathways and bio-

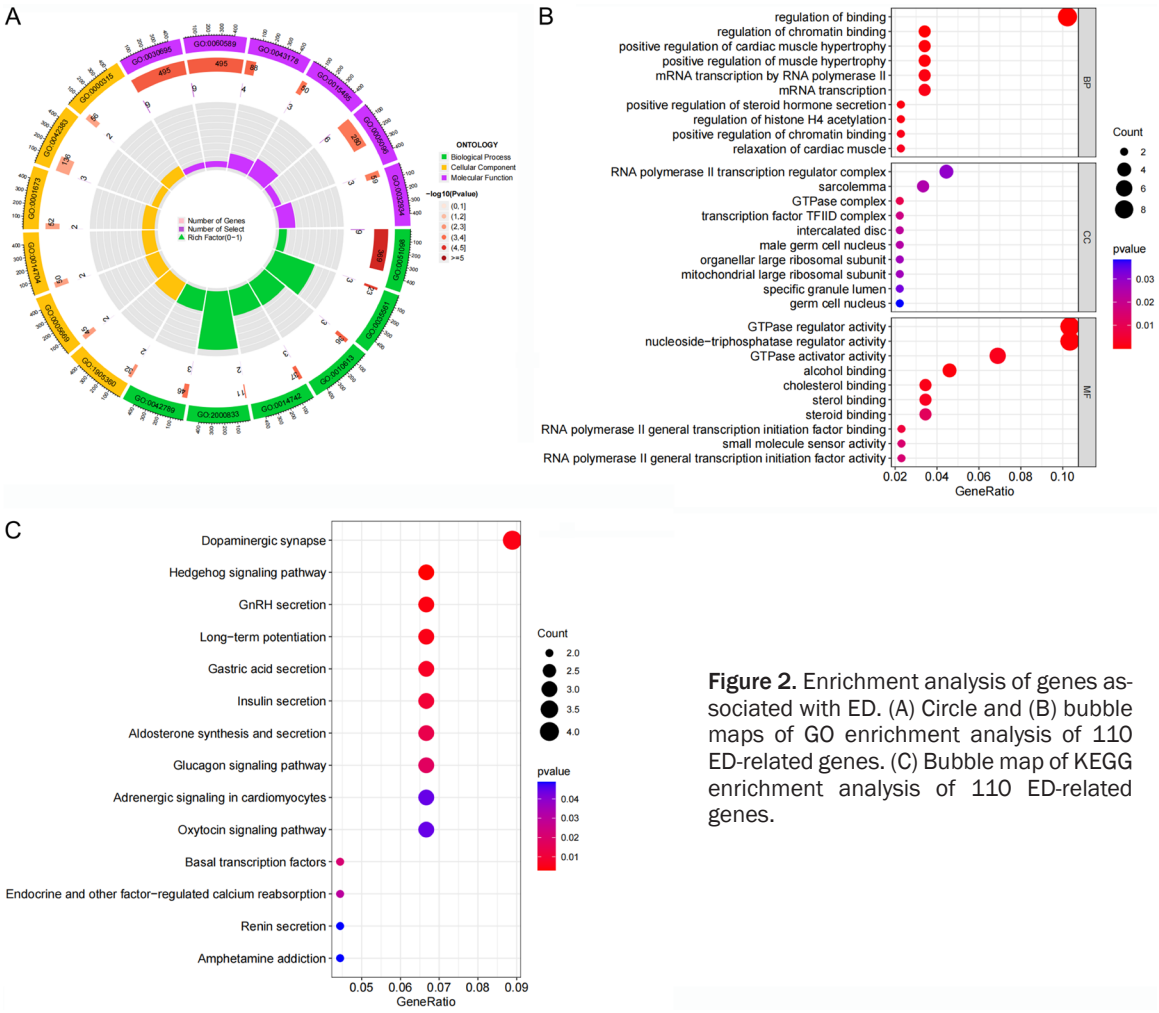

Figure 2. Enrichment analysis of genes associated with ED. (A) Circle and (B) bubble maps of GO enrichment analysis of 110 ED-related genes. (C) Bubble map of KEGG enrichment analysis of 110 ED-related genes.

logical processes. Dopaminergic synapse had the highest gene enrichment, significant gene ratio, and robust correlation significance. Genetic enrichment of dopaminergic synapses suggests that dopamine may play an essential role in the pathogenesis of ED, which may involve modulation of dopamine levels or functional abnormalities of dopamine receptors.

PPI in combination with SMR results to further search for disease-associated genes

We used the String website to construct a protein-protein interaction (PPI) network for the genes identified in the SMR analysis (excluding genes that cannot be encoded into proteins) to further search for critical genes associated with ED. This interaction network was visualised using Cytoscape software. As can be seen in **Figure 3A**, proteins such as SPP1, MDM4, and IRF5 have larger nodes and darker colours in the network, with multiple interacting proteins; they also have a high density of network connections, indicating that there are a large number of interactions with other proteins, suggesting that they may be essential players in the biological process and perhaps the critical regulators in the development of ED. We then selected 14 genes associated with ED with large degree values for forest mapping and analysis. The results are shown in **Figure 3B**, where the OR value indicates the ratio of the odds of ED occurring when a gene is present to the odds of ED occurring when the gene is absent. OR values greater than 1 indicate an increased risk of ED occurring when the gene is present, and OR values less than 1 indicate a decreased risk of ED occurring when the gene is present. p_SMR values reflect the statistical significance of gene expression and ED associations. *P* values less than 0.05 are usually considered statistically significant. As shown in

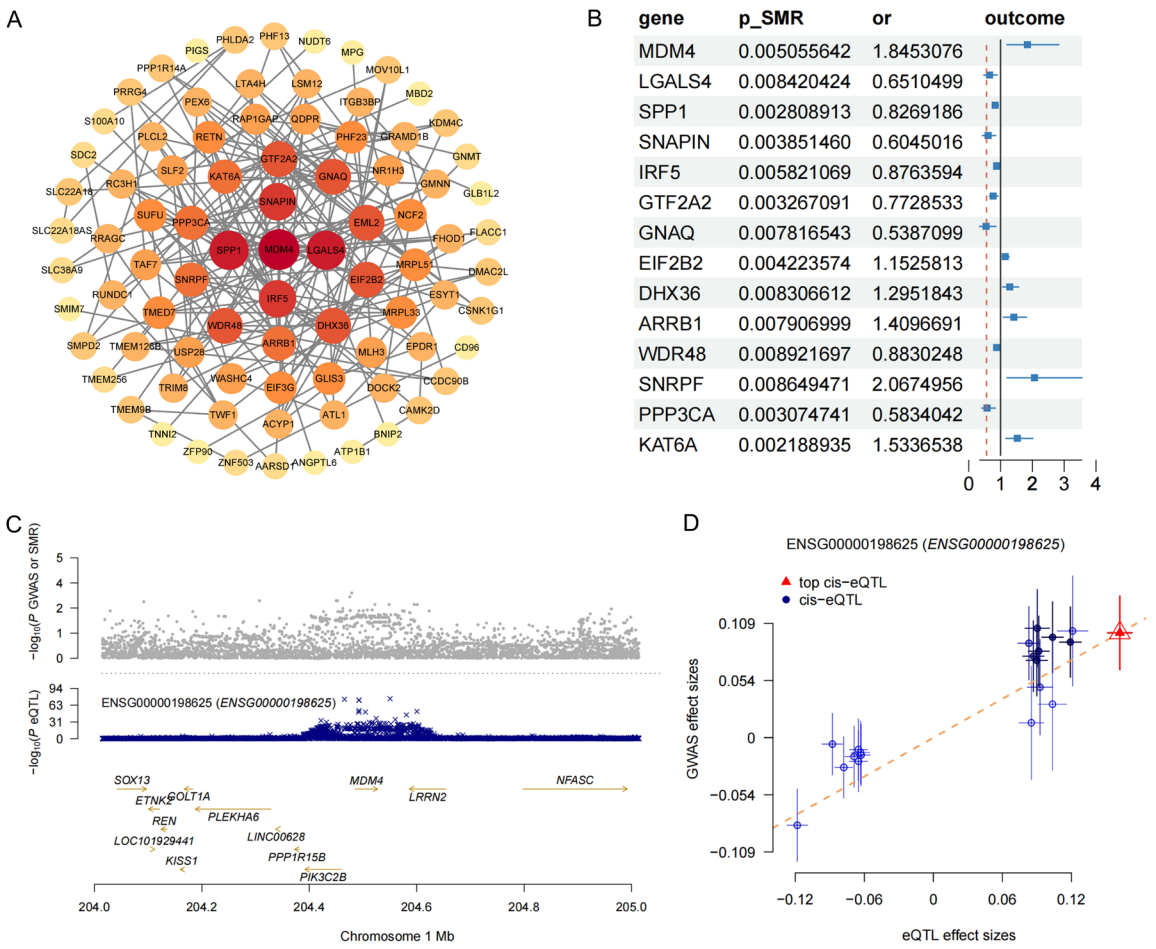

Figure 3. PPI combined with SMR results to further search for disease-associated genes. A. PPI network map of ED-associated genes. B. Forest plot obtained by selecting high degree genes from the PPI network map for analysis. C. View of localised chromosomal regions including the MDM4 gene as part of the GWAS and SMR analyses. The upper part is a scatterplot of SNPs showing how strongly each SNP is associated with ED. The lower part is the -log10 *p*-value from the eQTL analysis, showing the strength of the association between each SNP and the expression level of the MDM4 gene. D. The distribution of GWAS and eQTL effect loci of trip10 gene, the dotted line represents the linear trend, and the red triangle represents the maximum effect loci of eQTL.

Figure 3B, six genes increase the risk of developing ED and eight genes decrease the risk of developing ED, as indicated by the ORs, because this paper aims to screen for genes that promote the development of ED. Therefore, we focused on the six genes with OR values greater than 1 (MDM4, EIF2BR, DHX36, ARPB1, SNRPF, KAT6A). The p-SMR values of the above six genes are then compared. Among the remaining three genes, MDM4 has the highest degree value and its effect on the disease is also high (OR.1.8453076), suggesting that MDM4 is most associated with an increased risk of ED in this group of genes.

The gene expression association analysis shows that the dot plot at the top of **Figure 3B** indicates the association between individual SNPs and MDM4 gene expression. Higher dots indicate stronger associations, so it can be seen that certain SNPs in the plot have higher associations with MDM4 gene expression levels. Expression quantitative locus (eQTL) analysis shows that the scatter plot at the bottom of **Figure 3B** shows the effect of specific SNPs as eQTL on MDM4 gene expression. The vertical position of the dots represents significance, with higher values indicating greater significance. The gene localisation in **Figure 3B** shows that MDM4 is located within the 204.4-204.5 Mb interval of chromosome 1. Finally, the eQTL effect size and GWAS effect size of each cis-eQTL and the top cis-eQTL in MDM4 were analysed to investigate their effects on

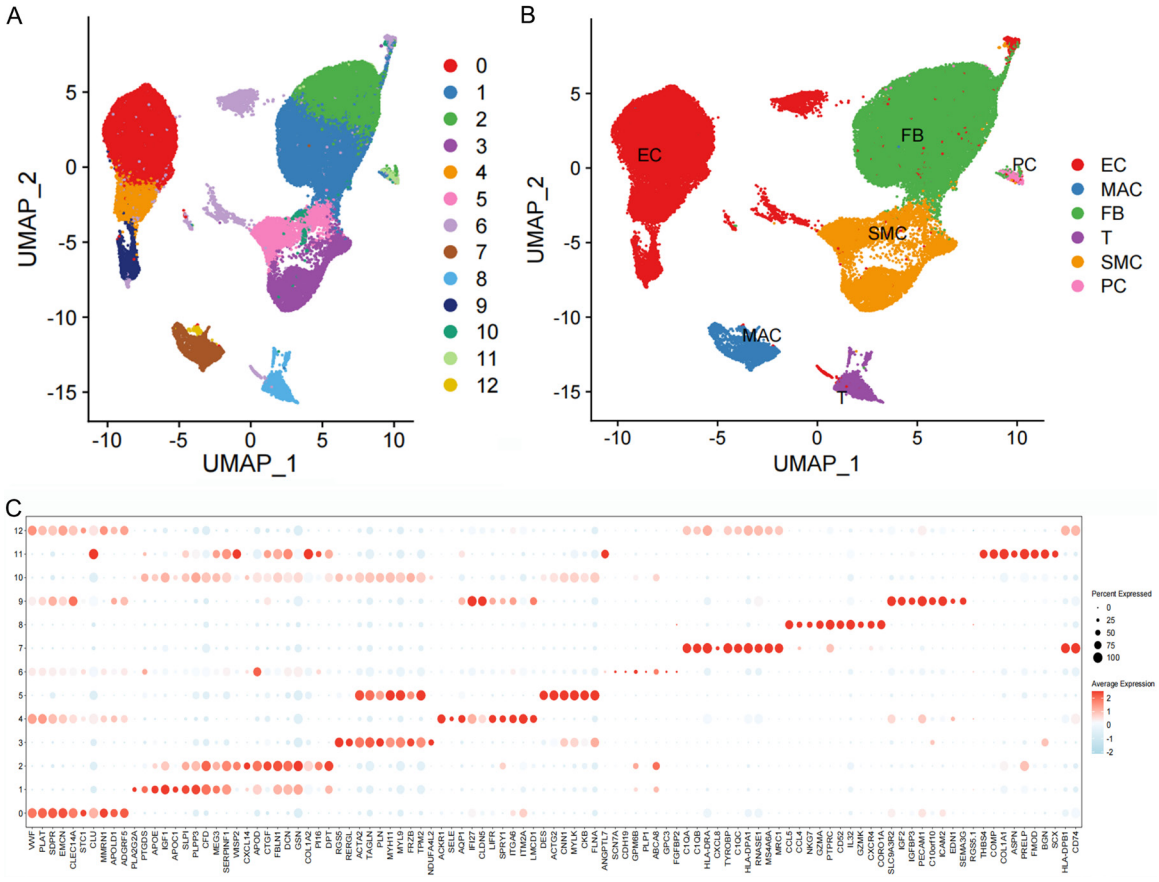

Figure 4. Single-cell sequencing analysis. A. UMAP plot of multidimensional clustering results for cells in single-cell sequencing data. B. Annotated UMAP plots for 13 clusters. C. Dot plots of single-cell sequencing data showing expression levels and expression percentages of selected genes in different cell clusters. The size of the dots in the graph indicates the percentage of expression of the gene in the corresponding clusters, i.e., what percentage of cells express the gene; the colour of the dots indicates the average expression level, with red representing high expression, blue representing low expression, and darker colours indicating higher expression levels.

MDM4 gene expression and ED shape (GWAS effect size). As shown in **Figure 3C**, the eQTL effect size and GWAS effect size of the top cis-eQTL were much more significant than 0, suggesting that this variant increased the risk of ED by increasing MDM4 expression. In addition, the top cis-eQTL lies exactly on the dashed line with a slope of 1 in the figure, demonstrating that its effect on MDM4 gene expression (eQTL effect size) and its effect on ED traits (GWAS effect size) are consistent (**Figure 3D**).

Single-cell sequencing analysis

We performed a comprehensive analysis of gene expression in cells associated with ED using single-cell sequencing technology. As shown in **Figure 4A**, cells from the whole sample were effectively classified into 13 distinct clusters, which were uniquely mapped in two-dimensional space using the Uniform Manifold Approximation and Projection (UMAP) algorithm. We annotated these clusters by analysing the expression of marker genes across these 13 clusters, as shown in **Figure 4B** and **4C**. We successfully identified six major cell types: endothelial cells (EC), macrophages (MAC), fibroblasts (FB), T cells (T), smooth muscle cells (SMC), and prostate cells (PC).

Cell ratio analysis and expression of disease-associated genes

We then analysed the spatial distribution of six major cell types (endothelial cells, macrophages, fibroblasts, T-cells, smooth muscle cells, and prostate cells) in the erectile dysfunction (ED) group and the healthy (normal) control

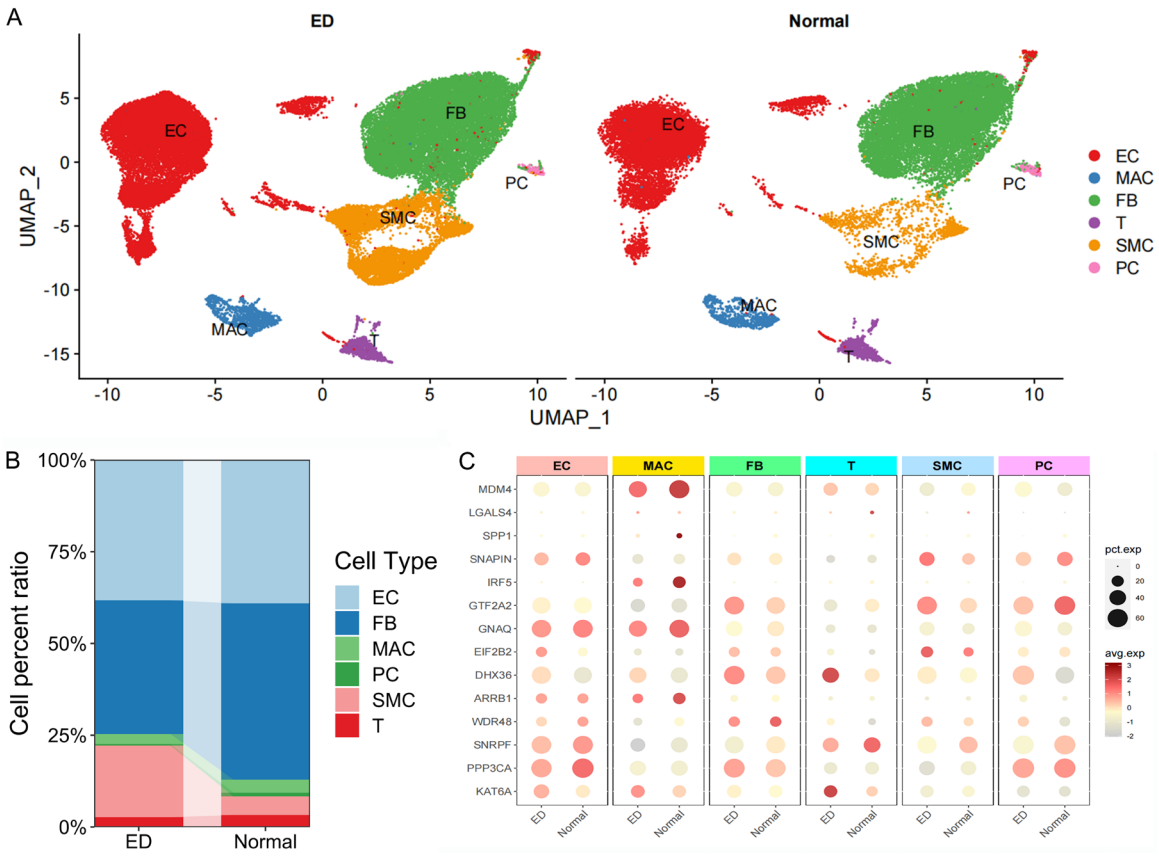

Figure 5. Cell ratio analysis and expression of disease-associated genes. A. UMAP plot of the differences in cell distribution between the erectile dysfunction (ED) group and the standard (normal) group. B. Histogram of the proportions of the six cell types. C. A dot plot of the expression of selected genes in six different cell types in the erectile dysfunction (ED) group versus the normal healthy group. Each circle represents the expression of the gene in a specific cell type, where the size of the circle indicates the percentage of gene expression in the corresponding cell type (pct. exp) and the colour shade indicates the average expression (avg. exp). The colour from red to grey indicates high to low expression.

group. Using the UMAP method (**Figure 5A**), In the ED group, the distribution area of endothelial cells (EC) is significantly more significant than that of the normal group, suggesting an increase in the number of phenotypic changes of these cells under the conditions of erectile dysfunction. Smooth muscle cells (SMC) also show a more dispersed distribution in the ED group, which may be related to the pathophysiological processes of erectile dysfunction, such as alterations in vascular smooth muscle function. Meanwhile, the distribution of macrophages (MAC) is similar between the two groups, although slightly more compact in the normal group. Furthermore, fibroblasts (FB) are widely distributed in the normal group. At the same time, they appear more concentrated in the ED group, which may reflect changes in the role of fibroblasts in the local environment in erectile dysfunction. T cells (T) and prostate cells (PC) are present in both groups, but their distribution is more dispersed in the ED group, which may be related to changes in immune responses or local microenvironmental changes.

Comparing the cell content between the ED group and the standard group (**Figure 5B**), it can be seen that in the ED group the proportion of smooth muscle cells (SMC) is relatively high at approximately 20%. In contrast, in the standard group the proportion of SMC is only about 7%. Conversely, the proportion of fibroblasts (FB) shows the opposite trend, with the proportion of FB cells in the ED group being significantly lower than in the standard group. In addition, the percentage of prostate cells (PC) in the ED group is almost zero compared to the normal group. The role of MDM4 in ED was further

analysed by analysing the expression of the above ED-related genes in six cells. As shown in **Figure 5C**, the expression of MDM4 genes in macrophages and smooth muscle cells was more significant in the ED group, especially in macrophages, which showed a higher average expression. The expression in the other four cells was similar to that in the normal group.

Discussion

Erectile dysfunction (ED) is a type of male sexual dysfunction that affects the quality of a man's sex life and overall physical and mental health. Although the causes of ED are many, including psychological, endocrine, vascular, and neurological factors, increasing research suggests that genetic factors also play a significant role in the development of ED [1-4]. Recent genetic studies, particularly genome-wide association studies (GWAS), have identified several genetic variants associated with an increased risk of ED. However, the genetic mechanisms underlying ED are not fully understood [7, 9]. To further explore the genetic basis of ED, we used the method of Mendelian randomisation (MR). MR is a method that uses genetic variants as instrumental variables to estimate the causal effect of an exposure (such as gene expression levels) on a specific outcome (such as ED). The advantage of this method lies in its ability to reduce confounding bias and reverse causation issues common in traditional observational studies. By integrating GWAS data and gene expression databases, MR analysis proved to be instrumental in revealing genes potentially associated with ED. During the gene screening process, a total of 110 genes emerged as potential candidates associated with ED. The identification of these genes provides deeper insights into the pathogenesis of ED and lays the foundation for future functional studies and the development of potential therapeutic targets. Notably, among these findings, striking associations have emerged between changes in gene expression and the propensity for ED, supporting the hypothesis that these genes play a pivotal role in the trajectory of ED development. Such findings provide invaluable clues to unravelling the complex genetic basis of ED and pave the way for targeted interventions to mitigate its effects.

GO enrichment analysis showed that these genes were associated with specific biological processes, cellular components, and molecular functions. First, the biological processes "positive regulation of cardiomyocyte hypertrophy", "positive regulation of muscle hypertrophy", and "positive regulation of RNA polymerase II-mediated mRNA transcription" were associated. These processes involve changes in the function of heart and muscle tissue and the regulation of gene expression, which could directly affect blood flow and vascular health, thereby affecting erectile function. For example, blood flow velocity and vessel diameter have been shown to be valid parameters for assessing arterial erectile dysfunction [1]. Regulation of muscle hypertrophy could affect penile tissue dynamics, particularly trabecular smooth muscle mass, which is critical for the veno-occlusive mechanism necessary to maintain an erection [17]. An imbalance in the regulatory mechanisms that control muscle growth could affect the structural and functional integrity of penile tissue, thereby affecting erectile function [18]. The analysis also identified a significant enrichment in the cellular component category related to the "RNA polymerase II transcriptional regulatory complex". This finding implies that the genes associated with ED may play a critical role in cell transcriptional regulation processes, affecting how genes are expressed in response to physiological demands. Such regulation is essential for maintaining cellular function and could influence the development of ED by affecting cellular health and responses.

In terms of molecular function, "GTPase regulatory activity" and "nucleotide triphosphatase regulatory activity" are significantly enriched. These molecular functions are involved in energy transfer and molecular signalling and are critical for cellular signalling pathways. GTPases are essential molecular switches that control various cellular processes by switching between active (GTP-bound) and inactive (GDP-bound) states. In the context of erectile dysfunction (ED), GTPase modulators may affect signalling pathways that control vascular tone and smooth muscle contraction, which are critical for erectile function. Abnormal GTPase signalling may disrupt normal vasodilation and lead to ED. In addition, nucleoside triphosphatases (GTPases), such as ATPases and GTPases, play a crucial role in cells, regulating energy conversion and signalling, which is essential for erectile function (ED) [19]. These enzymes affect blood flow by regulating the contraction

and relaxation of vascular smooth muscle. ATPases control smooth muscle contraction by managing calcium ion pumping [20, 21], and GTPases play a role in cytoskeletal reorganisation, supporting penile vascular dilation and occlusion mechanisms [22, 23]. In addition, ATPases are involved in energy metabolism, providing the necessary energy for the erectile process [24]. Thus, any dysfunction in GTPases may affect vascular and smooth muscle activity, thereby affecting erectile function. Understanding how NTPase regulators control these processes may provide new approaches to the treatment of ED.

In the KEGG enrichment analysis of genes related to Erectile Dysfunction (ED), we observed significant enrichment in several signalling pathways and biological processes, including the dopaminergic synapse pathway. This finding suggests that the dopaminergic synaptic pathway may be involved in the development of ED and may influence ED through effects on neural transmission and vascular regulatory mechanisms. Dopamine is a key neurotransmitter involved in the regulation of mood, reward and motivational behaviour, and also affects sexual function [25]. Activation of the dopaminergic synaptic pathway can increase the sensation of sexual arousal by regulating neural signals between the hypothalamus and the gonads, affecting the release of sex hormones [26]. This mechanism may be important in regulating penile blood flow and erectile status. Dopamine, via its receptors (such as D1 and D2 receptors), modulates vascular dilation and contraction in the smooth muscle of the penile corpus cavernosum [27, 28], thereby affecting the physiological process of erection. According to one study, dopamine receptor agonists may promote relaxation of penile smooth muscle and increase arterial blood flow, thereby facilitating erection [29]. In addition, the role of dopamine in regulating sex hormones and mood may also indirectly affect function and erection [27]; for example, dopamine reuptake inhibitors found in antidepressants have been shown to improve sexual dysfunction caused by depression [30, 31]. These studies suggest that regulation of the dopaminergic synaptic pathway may be an important biological mechanism affecting ED. Understanding the specific actions of this pathway may help in the development of new therapeutic strategies for ED.

The PPI network analysis revealed several important nodal proteins, including SPP1, MDM4, and IRF5. These appeared as larger nodes and darker colours in the network, suggesting that these proteins are highly interactive with many other proteins. The high connectivity density of these hubs suggests that they may play important regulatory roles in biological processes and may be critical factors in the development of ED. The results of the forest plot analysis of ED-related genes with high degree values then showed that genes such as MDM4, EIF2B2 and KAT6A played a significant role in promoting the ED process. In particular, MDM4 not only had the highest degree value in the network, but was also most strongly associated with an increased risk of ED, suggesting that MDM4 may play a central role in the development of ED. Expression quantitative loci (eQTL) analysis also revealed that specific SNPs in the MDM4 gene significantly affected its gene expression, further confirming the critical role of MDM4 in regulating ED-related biological processes. The effect size of the top cis eQTL and GWAS effect size analyses indicated that this variant may increase the risk of ED by increasing MDM4 expression.

The MDM4 gene, also known as MDMX or HDMX, is located at the 1q32 locus on the human genome [32]. It encodes a protein that can form a heterodimer with MDM2, an E3 ubiquitin ligase known to bind to the tumour suppressor protein p53 and promote its proteasomal degradation [33]. This interaction between MDM4 and MDM2 is critical for the regulation of the p53 pathway [34], which plays an important role in cell cycle regulation and apoptosis [35-37]. Regarding the impact of MDM4 on erectile dysfunction (ED), while specific studies directly linking MDM4 gene function to ED have not been extensively documented, the involvement of MDM4 in cell cycle control and apoptosis may suggest potential mechanisms by which it may influence ED [38]. For example, inhibition of the MDM4/p53 and Bax signalling pathways has been shown to reduce cell growth and induce apoptosis in colon cancer cells [39]. In addition, Wang et al. showed that HT22 apoptosis and oxidative stress could be slowed down by inhibiting MDM4 expression [40]. Regulation of the cell cycle and apoptotic processes in penile tissue could affect cellular health and integrity, which are essential for normal erectile function [41, 42]. For instance,

proper vascular and smooth muscle function in the penis, which is crucial for achieving and maintaining an erection, is highly dependent on the balance between cell proliferation and cell death. Single-cell sequencing analysis and MDM4 gene expression in six cell types further confirmed the role played by the MDM4 gene in ED. The MDM4 gene may play an essential role in the development of ED by regulating the activity or apoptosis of cellular macrophages and smooth muscle cells through the P53 pathway. Further research into how MDM4, in particular its regulatory effects on the p53 pathway, might influence the pathophysiology of ED may provide new insights into the molecular mechanisms underlying this condition. Such studies could potentially identify novel therapeutic targets within the p53 regulatory network that could be exploited in the treatment of ED.

Conclusion

In summary, the pathogenesis of erectile dysfunction (ED) is complex, involving psychological, endocrine, vascular, and neurological factors, with increasing evidence suggesting an important role for genetic factors. Using Mendelian randomisation (MR) analysis, we have successfully identified 110 genes associated with ED. The identification of these genes not only deepens our understanding of the pathological basis of ED but also lays the groundwork for future functional studies and the development of potential therapeutic targets. In addition, through GO and KEGG enrichment analysis, we have delved deeper into the functional roles of these genes, unravelling their implications in various biological processes, cellular components and molecular functions. Of particular importance is the elucidation of the central role of the dopaminergic synaptic pathway in modulating hormone release and erectile function, highlighting it as a potential new target for the treatment of ED. The MDM4 gene, a focus of our study, suggested possible mechanisms by which it might indirectly affect ED through its role in cell cycle control and apoptosis. These findings offer new perspectives for the treatment of ED and highlight the importance of further research into specific genetic factors in the development of ED and the development of therapeutic strategies targeting these factors.

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Disclosure of conflict of interest

None.

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References

[1] Huang WL, Tung SY, Tseng CS, Wang TD, Lee WJ, Chen JH, Su YR, Chang HC and Chang YK. The flow index provides a comprehensive assessment of erectile dysfunction by combining blood flow velocity and vascular diameter. Sci Rep 2022; 12: 16099.

[2] Bertolotto M, Campo I, Sachs C, Ciabattoni R, Bucci S, Cova MA and Van Nieuwenhove S. Sonography of the penis/erectile dysfunction. Abdom Radiol (NY) 2020; 45: 1973-1989.

[3] Mobley DF, Khera M and Baum N. Recent advances in the treatment of erectile dysfunction. Postgrad Med J 2017; 93: 679-685.

[4] Kessler A, Sollie S, Challacombe B, Briggs K and Van Hemelrijck M. The global prevalence of erectile dysfunction: a review. BJU Int 2019; 124: 587-599.

[5] Barassi A, Corsi Romanelli MM, Pezzilli R, Dozio E, Damele CAL, Vaccalluzzo L, Di Dario M, Goi G, Papini N, Massaccesi L, Colpi GM and Melzi d'Eril GV. Levels of uric acid in erectile dysfunction of different aetiology. Aging Male 2018; 21: 200-205.

[6] Jorgenson E, Matharu N, Palmer MR, Yin J, Shan J, Hoffmann TJ, Thai KK, Zhou X, Hotaling JM, Jarvik GP, Ahituv N, Wessells H and Van Den Eeden SK. Genetic variation in the SIM1 locus is associated with erectile dysfunction. Proc Natl Acad Sci U S A 2018; 115: 11018-11023.

[7] Kałka D, Gebala J, Biernikiewicz M, Mrozek-Szetela A, Rożek-Piechura K, Sobieszczańska M, Szuster E, Majchrowska M, Miętka A and Rusiecka A. Erectile dysfunction in men burdened with the familial occurrence of coronary artery disease. J Clin Med 2021; 10: 4046.

[8] Rastrelli G, Yannas D, Mucci B, Corona G and Maggi M. Family history for cardio-metabolic diseases: a predictor of major adverse cardio-

vascular events in men with erectile dysfunction. J Sex Med 2020; 17: 2370-2381.
[9] Kazemi E, Zargooshi J, Kaboudi M, Heidari P, Kahrizi D, Mahaki B, Mohammadian Y, Khazaei H and Ahmed K. A genome-wide association study to identify candidate genes for erectile dysfunction. Brief Bioinform 2021; 22: bbaa338.
[10] Birney E. Mendelian randomization. Cold Spring Harb Perspect Med 2022; 12: a041302.
[11] Sanderson E, Glymour MM, Holmes MV, Kang H, Morrison J, Munafò MR, Palmer T, Schooling CM, Wallace C, Zhao Q and Smith GD. Mendelian randomization. Nat Rev Methods Primers 2022; 2: 6.
[12] Holmes MV, Ala-Korpela M and Smith GD. Mendelian randomization in cardiometabolic disease: challenges in evaluating causality. Nat Rev Cardiol 2017; 14: 577-590.
[13] Tao Y, Zhao R, Yang B, Han J and Li Y. Dissecting the shared genetic landscape of anxiety, depression, and schizophrenia. J Transl Med 2024; 22: 373.
[14] Zhao L, Han S, Su H, Li J, Zhi E, Li P, Yao C, Tian R, Chen H, Chen H, Luo J, Shi C, Ji Z, Hu J, Wu G, Zhou W, Tang Y, Chen Y, Lin G, Lue TF, Wu D and Li Z. Single-cell transcriptome atlas of the human corpus cavernosum. Nat Commun 2022; 13: 4302.
[15] Zhu Z, Zhang F, Hu H, Bakshi A, Robinson MR, Powell JE, Montgomery GW, Goddard ME, Wray NR, Visscher PM and Yang J. Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. Nat Genet 2016; 48: 481-7.
[16] Peng Y, Dong Y, Sun Q, Zhang Y, Zhou X, Li X, Ma Y, Liu X, Li R, Guo F and Guo L. Integrative analysis of single-cell and bulk RNA-sequencing data revealed T cell marker genes based molecular sub-types and a prognostic signature in lung adenocarcinoma. Sci Rep 2024; 14: 964.
[17] Reece JC, Dangerfield DC and Coombs CJ. End-to-side somatic-to-autonomic nerve grafting to restore erectile function and improve quality of life after radical prostatectomy. Eur Urol 2019; 76: 189-196.
[18] Andersson KE. Erectile physiological and pathophysiological pathways involved in erectile dysfunction. J Urol 2003; 170: S6-13; discussion S13-4.
[19] Kozlova MI, Shalaeva DN, Dibrova DV and Mulkidjanian AY. Common patterns of hydrolysis initiation in P-Loop fold nucleoside triphosphatases. Biomolecules 2022; 12: 1345.
[20] Xu H and Van Remmen H. The SarcoEndoplasmic reticulum calcium ATPase (SERCA) pump: a potential target for intervention in aging and skeletal muscle pathologies. Skelet Muscle 2021; 11: 25.
[21] Elnatan D and Agard DA. Calcium binding to a remote site can replace magnesium as cofactor for mitochondrial Hsp90 (TRAP1) ATPase activity. J Biol Chem 2018; 293: 13717-13724.
[22] Durand-Onaylı V, Haslauer T, Härzschel A and Hartmann TN. Rac GTPases in hematological malignancies. Int J Mol Sci 2018; 19: 4041.
[23] Cuartas-López AM, Hernández-Cuellar CE and Gallego-Gómez JC. Disentangling the role of PI3K/Akt, rho GTPase and the actin cytoskeleton on dengue virus infection. Virus Res 2018; 256: 153-165.
[24] Li Q, Zhang S, Yang G, Wang X, Liu F, Li Y, Chen Y, Zhou T, Xie D, Liu Y and Zhang L. Energy metabolism: a critical target of cardiovascular injury. Biomed Pharmacother 2023; 165: 115271.
[25] Melis MR, Sanna F and Argiolas A. Dopamine, erectile function and male sexual behavior from the past to the present: a review. Brain Sci 2022; 12: 826.
[26] Heaton JP. Central neuropharmacological agents and mechanisms in erectile dysfunction: the role of dopamine. Neurosci Biobehav Rev 2000; 24: 561-9.
[27] Giuliano F and Allard J. Dopamine and male sexual function. Eur Urol 2001; 40: 601-8.
[28] Hyun JS, Bivalacqua TJ, Baig MR, Yang DY, Leungwattanakij S, Abdel-Mageed A, Kim KD and Hellstrom WJ. Localization of peripheral dopamine D1 and D2 receptors in rat corpus cavernosum. BJU Int 2002; 90: 105-12.
[29] Gratzke C, Angulo J, Chitaley K, Dai YT, Kim NN, Paick JS, Simonsen U, Uckert S, Wespes E, Andersson KE, Lue TF and Stief CG. Anatomy, physiology, and pathophysiology of erectile dysfunction. J Sex Med 2010; 7: 445-75.
[30] Meston CM and McCall KM. Dopamine and norepinephrine responses to film-induced sexual arousal in sexually functional and sexually dysfunctional women. J Sex Marital Ther 2005; 31: 303-17.
[31] Simonsen U, García-Sacristán A and Prieto D. Penile arteries and erection. J Vasc Res 2002; 39: 283-303.
[32] Bardot B and Toledo F. Targeting MDM4 splicing in cancers. Genes (Basel) 2017; 8: 82.
[33] Yang J, Jin A, Han J, Chen X, Zheng J and Zhang Y. MDMX recruits UbcH5c to facilitate MDM2 E3 ligase activity and subsequent P53 degradation in vivo. Cancer Res 2021; 81: 898-909.
[34] Zhang Q, He X, Chen L, Zhang C, Gao X, Yang Z and Liu G. Synergistic regulation of P53 by Mdm2 and Mdm4 is critical in cardiac endocardial cushion morphogenesis during heart development. J Pathol 2012; 228: 416-28.
[35] Han X, Wei W and Sun Y. PROTAC degraders with ligands recruiting MDM2 E3 ubiquitin ligase: an updated perspective. Acta Mater Med 2022; 1: 244-259.

[36] Mitobe Y, Suzuki S, Nakagawa-Saito Y, Togashi K, Sugai A, Sonoda Y, Kitanaka C and Okada M. Antagonizing MDM2 overexpression induced by MDM4 inhibitor CEP-1347 effectively reactivates wild-type P53 in malignant brain tumor cells. Cancers (Basel) 2023; 15: 4326.
[37] Timmerman DM, Remmers TL, Hillenius S and Looijenga LHJ. Mechanisms of TP53 pathway inactivation in embryonic and somatic cells-relevance for understanding (germ cell) tumorigenesis. Int J Mol Sci 2021; 22: 5377.
[38] Espadinha M, Lopes EA, Marques V, Amaral JD, Dos Santos DJVA, Mori M, Daniele S, Piccarducci R, Zappelli E, Martini C, Rodrigues CMP and Santos MMM. Discovery of MDM2-P53 and MDM4-P53 protein-protein interactions small molecule dual inhibitors. Eur J Med Chem 2022; 241: 114637.
[39] Chen W, Cai G, Liao Z, Lin K, Li G and Li Y. miRNA-766 induces apoptosis of human colon cancer cells through the P53/bax signaling pathway by MDM4. Exp Ther Med 2019; 17: 4100-4108.
[40] Wang W, Hu Y and Zhang Y. FTX attenuates cerebral ischemia-reperfusion injury by inhibiting apoptosis and oxidative stress via miR-186-5p/MDM4 pathway. Neurotox Res 2022; 40: 542-552.
[41] Hagiuda J, Ueno M, Ashimine S, Kuroda I, Ishizawa K and Deguchi N. Langerhans cell histiocytosis on the penis: a case report. BMC Urol 2006; 6: 28.
[42] Zhao W, Sun J, Yao LY, Hang D, Li YQ, Chen CP, Zhou YW, Chen X, Tao T, Wei LS, Zheng YY, Ge X, Li CJ, Xin ZC, Pan Y, Wang XZ, He WQ, Zhang XN, Yao B and Zhu MS. MYPT1 reduction is a pathogenic factor of erectile dysfunction. Commun Biol 2022; 5: 744.