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Comparison of critical biomarkers in 2 erectile dysfunction models based on GEO and NOS-cGMP-PDE5 pathway

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Abstract

Background: Erectile dysfunction is a disease commonly caused by diabetes mellitus (DMED) and cavernous nerve injury (CNIED). Bioinformatics analyses including differentially expressed genes (DEGs), enriched functions and pathways (EFPs), and protein-protein interaction (PPI) networks were carried out in DMED and CNIED rats in this study. The critical biomarkers that may intervene in nitric oxide synthase (NOS, predominantly nNOS, ancillary eNOS, and iNOS)-cyclic guanosine monophosphate (cGMP)-phosphodiesterase 5 enzyme (PDE5) pathway, an important mechanism in erectile dysfunction treatment, were then explored for potential clinical applications.

Methods: GSE2457 and GSE31247 were downloaded. Their DEGs with a |logFC (fold change)| > 0 were screened out. Database for Annotation, Visualization and Integrated Discovery (DAVID) online database was used to analyze the EFPs in Gene Ontology enrichment and Kyoto Encyclopedia of Genes and Genomes networks based on down-regulated and up-regulated DEGs respectively. PPI analysis of 2 datasets was performed in Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) and Cytoscape. Interactions with an average score greater than 0.9 were chosen as the cutoff for statistical significance.

Results: From a total of 1710 DEGs in GSE2457, 772 were down-regulated and 938 were up-regulated, in contrast to the 836 DEGs in GSE31247, from which 508 were down-regulated and 328 were up-regulated. The 25 common EFPs such as aging and response to hormone were identified in both models. PPI results showed that the first 10 hub genes in DMED were all different from those in CNIED.

Conclusions: The intervention of iNOS with the hub gene complement component 3 in DMED and the aging process in both DMED and CNIED deserves attention.

Abbreviations: BP = biological processes, C3 = complement component 3, CC = cell component, CNIED = cavernous nerve injury induced erectile dysfunction, DEGs = differentially expressed genes, DMED = diabetes mellitus-related erectile dysfunction, DN = diabetic nephropathy, ED = erectile dysfunction, EFPs = enriched functions and pathways, GEO = Gene Expression Omnibus, GO = Gene ontology, GRP = gastrin-releasing peptide, IGF-1 = insulin-like growth factor-1, KEGG = Kyoto Encyclopedia of Genes and Genomes, MF = molecular function, NO = nitric oxide, NOS-cGMP-PDE5 pathway = nitric oxide synthase-cyclic guanosine monophosphate-phosphodiesterase 5 enzyme pathway, PPI = protein-protein interaction, Ubb = ubiquitin B.

Keywords: bioinformatics analysis, cavernous nerve injury-induced erectile dysfunction, diabetes mellitus-induced erectile dysfunction, NOS-cGMP-PDE5 pathway, rat model

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The above information was supplied regarding data availability: Raw data is available at NCBI GEO: GSE2457 and GSE31247.

The datasets generated during and/or analyzed during the current study are publicly available.

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1. Introduction

Erectile dysfunction (ED) is the persistent inability to attain and maintain an erection sufficient for a satisfactory sexual performance. Epidemiologic studies of ED suggest that approximately 5% to 20% of men have moderate to severe ED.[1] The etiology of ED is multifactorial. Compared with the general population, the prevalence of ED is higher and occurs earlier in diabetic patients.[2] With the development of prostatectomy and the increase of patients with pelvic fracture and urethral injury, cavernous nerve injury induced erectile dysfunction (CNIED) is gaining popularity.[3]

Because of many limitations of direct study in humans, it is important to establish ED animal models. The most frequently reported ED models can be classified into traumatic ED such as CNIED, and metabolic ED such as diabetes mellitus-related erectile dysfunction (DMED).[4] At present, the most classical modeling method is the rat model of DMED, induced by streptozotocin.[5] The commonly used method to create CNIED model is to squeeze, freeze, or cut off unilateral or bilateral cavernous nerves.[6] Vascular and psychological ED animal models were also established according to different etiology.[7,8] Many bioinformatics technologies have been used to compare the above ED models from the point of genetics,[9–11] such as differentially expressed genes (DEGs) analysis between CNIED and DMED rat models.[11] By utilizing Gene Expression Omnibus (GEO), we further compared the enriched functions and pathways with Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and the protein-protein interaction (PPI) networks based on down-regulated and up-regulated DEGs respectively in DMED and CNIED.

Till now, bioinformatic strategies play important roles in the diagnosis and therapeutics of many diseases. Familial hypercholesterolemia is one of the known genetic causes of premature cardiovascular disease. Seven core genes were identified to represent potential molecular biomarkers for the diagnosis of atherosclerosis and might serve as the developing therapeutics against familial hypercholesterolemia.[12] Long non-coding RNAs, acting as competing endogenous RNAs, play important roles in the regulation of the expressions of genes involved in cancer. A recent research showed that ADAMTS9-AS1, a competing endogenous RNA, could accelerate biomarker discovery and therapeutic strategies development on prostate cancer.[13] Furthermore, the research on some essential signals which critical genes are involved in will help with the understanding of the molecular mechanisms and the discovery of potential targets. To give 2 examples, the first one is about the genes enriched in the insulin signaling pathway and the potential targets for diabetes and obesity treatment[14]; and the second one is about immune-related DEGs-based immune signature in the recognition of disease progression and the prognosis of lung squamous cell carcinoma patients.[15]

In ED treatment, nitric oxide synthase (NOS)-cyclic guanosine monophosphate (cGMP)-phosphodiesterase 5 enzyme (PDE5) pathway is an important pathophysiological basis in different types of ED.[16] The normal erectile function involves the synthesis of nitric oxide (NO) from the activation of 3 subtypes of NOS (predominantly nNOS, ancillary eNOS, and iNOS), and the subsequent accumulation of cGMP, whereas cGMP breakdown is controlled by PDE5, which terminates erection. It has been shown that in CNIED rat model, local up-regulation of insulin-like growth factor-1 (IGF-1) promoted an up-regulation of nNOS.[9] Serum IGF-1 level appears to be a specific predictor of ED in male population.[17] An obvious decrease in cavernous IGF-1 levels might play an important role in spontaneously hypertensive rats with ED.[18] The above research also suggested that up-regulated IGF-1 induced nNOS may help with preserving ED after pelvic surgery.[9] However, little is known about the relationship of critical biomarkers in different models with the other 2 subtypes of NOS, especially the activities of iNOS. The construction of critical biomarkers-based NOS-cGMP-PDE5 signature may help us understand the underlying genetic influence and serve as a platform for developing therapeutics against both DMED and CNIED.

2. Materials and methods

2.1. Microarray data

GEO (http://www.ncbi.nlm.nih.gov/geo/),[19] a public functional genomics data repository, provides chips, microarrays, and high throughput gene expression data. Two gene expression datasets, namely GSE2457 entitled "Transcription profiling of rat penis samples from animals with diabetes-induced erectile dysfunction" and GSE31247 entitled "Gene expression profile on the penile tissue of erectile dysfunction (ED) in cavernous nerve injury (CNI) rat model", were downloaded from GEO (Affymetrix GPL571 platform, Affymetrix Human Genome U133A 2.0 Array).[9,11] The probes were transformed into corresponding gene symbols on the basis of the provided annotation information on the platform. The ED samples compared with the non-ED samples in GSE2457 and GSE31247 were standardized separately. The model descriptions of GSE2457 and GSE31247 were compared as shown in Figure 1.

2.2. Identification of DEGs

The DEGs in the ED and non-ED specimen were picked out from GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r), which is a platform for examining DEGs across experimental conditions by comparing multiple datasets in GEO series. The genes with multiple probes were averaged, and the probes that lacked gene symbols were removed. The genes with a $|\log FC \text{ (fold change)}| > 0$ and $P < .05$ were screened out and $P < .05$ represented statistical significance.

2.3. GO and KEGG enrichment analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID: http://david.ncifcrf.gov) (version 6.8)[20] offers an analytical platform with a comprehensive source of annotated information of proteins and genes which can be extracted and analyzed. Besides, GO, a significant bioinformatics tool enables us to annotate genes according to biological processes (BP).[21] KEGG is a knowledge-based platform for systematic analysis of gene functions, linking genomic information with higher order functional information.[22,23] Database for Annotation, Visualization and Integrated Discovery (DAVID) online database was used in the study of the functions of DEGs. $P < .05$ was taken to represent statistical significance. ImageGP (http://www.ehbio.com/ImageGP/) was used to draw enrichment plots for GO and KEGG.

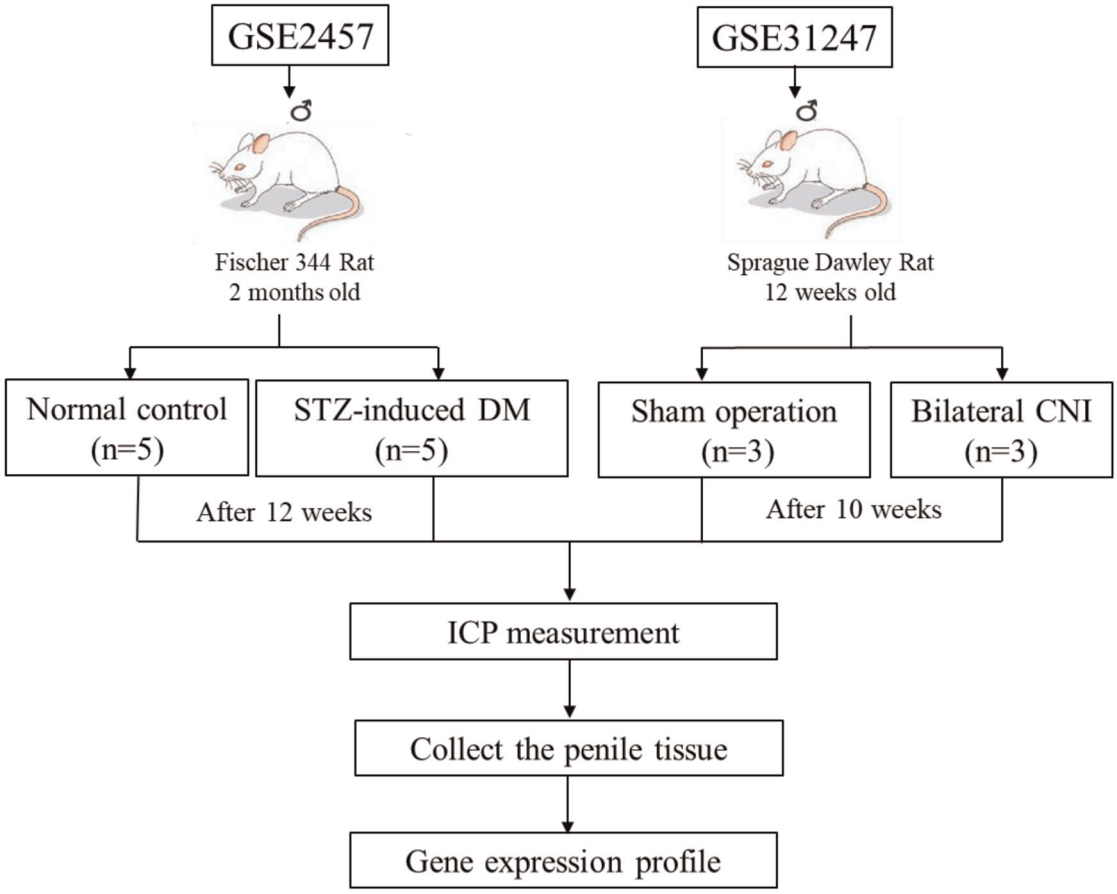

Figure 1. Comparison of erectile dysfunction rat model in GSE2457 and GSE31247. CNI = cavernous nerve injury, DM = diabetes mellitus, ICP = intracavernosal pressure, STZ = streptozotocin.

2.4. PPI networks construction and module analysis

In this study, the Search Tool for the Retrieval of Interacting Genes/Proteins database (STRING: https://string-db.org/, version 10.0)[24] was used to predict PPI networks, determine PPI and investigate the molecular basis of diseases. The interactions with an average score greater than 0.9 were chosen as the cutoff for statistical significance. Cytoscape (https://cytoscape.org/, version 3.4.0) is a bioinformatic software often utilized to visualize molecular interaction networks. This software contains an APP known as the plug-in Molecular Complex Detection (MCODE) (version 1.4.2), which is used to cluster specific networks according to the topology to revel the regions that are densely connected. Cytoscape was used to draw the PPI networks. The selection criteria were: k-score = 2, max depth = 100, node score cutoff = 0.2, degree cutoff = 2, and Molecular Complex Detection (MCODE) scores > 5.

3. Results

3.1. Identification of DEGs in GSE2457 and GSE31247

Figure 2A and B shows that DEGs in GSE2457 and GSE31247 were identified after standardization of the microarray data. The dataset consisted of 772 down-regulated and 938 up-regulated DEGs between DMED rat model and normal rat (control), as well as 508 down-regulated and 328 up-regulated DEGs identified between CNIED rat model and sham operation control.

Figure 3A shows that a total of 76 DEGs existed commonly in DMED and CNIED including 21 down-regulated, 22 up-regulated (Fig. 3B), and 33 opposite expression genes (Fig. 3C). According to their *P* value and logFC, gastrin in common down-regulated DEGs, IGF binding protein 3 in up-regulated DEGs, and androgen regulated protein in opposite-regulated DEGs caught our attention. The identified 22 up- and 21 down-regulated DEGs in DMED and CNIED along with their *P* values and logFC values are shown in Table S1, Supplemental Digital Content, http://links.lww.com/MD/G437 and Table S2, Supplemental Digital Content, http://links.lww.com/MD/G438, respectively.

3.2. KEGG and GO enrichment analyses of DEGs

Functional and pathway enrichment analyses were conducted on down-regulated and up-regulated DEGs respectively for further analyses of biological classification. The cytological component (CC), molecular function (MF), BP, and KEGG comparisons of down-regulated and up-regulated DEGs in GSE2457 and GSE31247 are shown separately in Figure 4.

Tables 1 to 4 show the first 20 down- and up-regulated items according to their *P* value in CC, MF, BP, and KEGG in GSE2457 and GSE31247, respectively. Table 1 shows that down-regulated

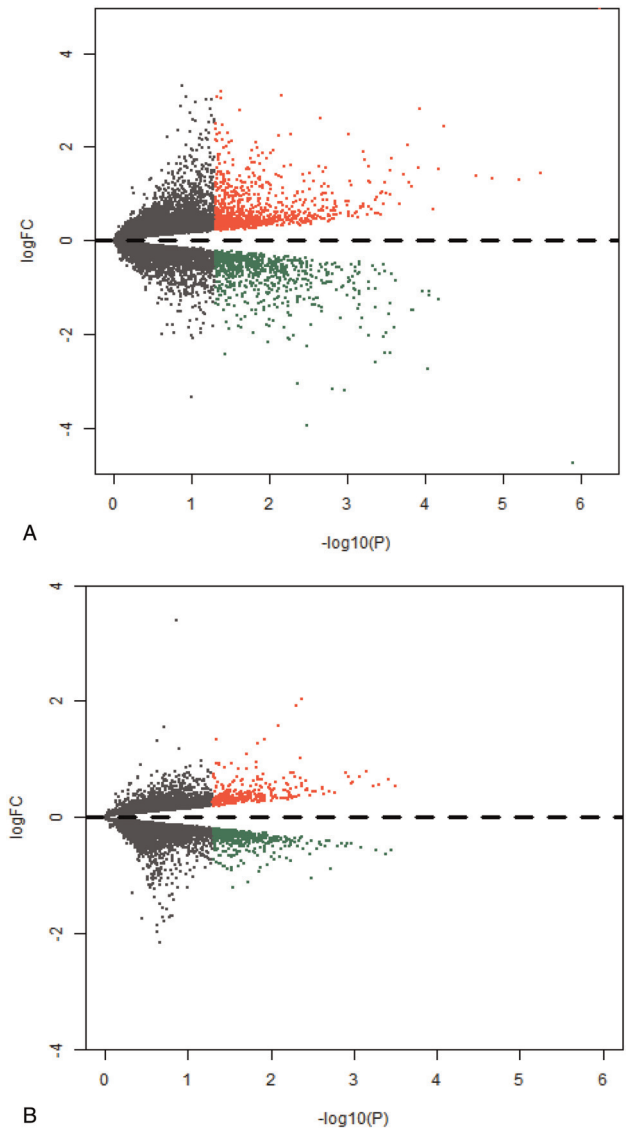

Figure 2. (A) All DEGs in diabetes-induced erectile dysfunction (GSE2457) and (B) all DEGs in cavernous nerve injury-induced erectile dysfunction (GSE31247). DEGs were selected with a |logFC (fold change)| > 0 and $P < .05$ among the mRNA expression profiling set GSE2457 and GSE31247. DEGs = differentially expressed genes.

DEGs in DMED mainly had enrichment in oxidative phosphorylation, osteoblast differentiation, Alzheimer disease, and metabolic pathways. Table 2 shows that up-regulated DEGs in DMED mainly had enrichment in the regulation of transcription from DNA and RNA and Adenosine 5'-monophosphate-activated protein kinase signaling pathway.

The results of GSE31247 demonstrated the down-regulated detection of chemical stimulus involved in the sensory perception of smell and G-protein coupled receptor signaling pathway, as well as up-regulated cGMP biosynthetic process and positive regulation of NOS activity in BP. KEGG pathway analyses showed down-regulated olfactory transduction and up-regulated staphylococcus aureus infection (Tables 3 and 4).

The results also displayed the 6 common items in CC: extracellular exosome, extracellular space, extracellular matrix, proteinaceous extracellular matrix, neuron projection, and sarcolemma; the 2 common items in MF: calcium ion binding and heparin binding; the 4 common items in BP: collagen fibril organization, aging, cell adhesion, and wound healing; and the common item in KEGG: focal adhesion.

Also according to their *P* value, Tables 2 and 4 show the 4 common items in CC including extracellular exosome, extracellular space, neuron projection, and cell surface, alongside the 3 common items in BP including aging, wound healing, and cell adhesion.

On the other hand, response to hormone in BP was down-regulated in GSE2457, but up-regulated in GSE31247. Phagosome in KEGG was up-regulated in GSE2457, but down-regulated in GSE31247.

3.3. PPI network construction and module analysis

The constructed PPI networks of DEGs including the top 50 hub genes in GSE2457 are shown in Figure 5 and DEGs in GSE31247 are shown in Figure 6. The top 10 hub genes in DMED and CNIED are listed in Table S3, Supplemental Digital Content, http://links.lww.com/MD/G439 and Table S4, Supplemental Digital Content, http://links.lww.com/MD/G440, respectively. Ubiquitin B (Ubb) was the first hub gene in CNIED, and ring-box 1, S-phase kinase-associated protein 1, and complement component 3 (C3) were the top 3 in DMED.

4. Discussion

Twenty years ago, when Viagra, the brand name of sildenafil, received FDA approval, a true revolution started for the treatment of ED and in sexual medicine.[25] During the past decades, much progress has been made for a deeper understanding of the regulatory factors that mediate normal erectile function. The discovery of the NOS-cGMP-PDE5 pathway has been shown to be critical in the normal physiological functions and the pathophysiology of ED.[26,27]

At present, bioinformatics analysis and microarray technology has been widely used on ED study.[9–11,28–30] Studies investigating the genetics of ED are mostly derived from animal models and candidate gene approaches.[28] Rodents are the most widely used animals to establish ED model.[4] Both DMED and CNIED rat models are commonly used.[5,6,29,30] Using CNIED rat model, a study showed that sildenafil, as a PDE5 inhibitor (PDE5i), can attenuate gene expression on inflammatory and oxidative stress related-pathways.[29] Some genome-wide and computational studies provided the groundwork for understanding complex mechanisms and molecular signature changes in different ED,[11] but little is known from the point of NOS-cGMP-PDE5 pathway, especially the subtypes of NOS.

Our research demonstrated that gastrin showed the lowest *P* value in both DMED and CNIED among common down-regulated DEGs. Gastrin-releasing peptide (GRP) is a mammalian neuropeptide that acts through the G protein-coupled receptor. It was suggested that the sexually dimorphic GRP/G protein-coupled receptor system in the lumbosacral spinal cord played a critical role in the regulation of male sexual function.[31] Double immunofluorescence of GRP and nNOS showed that GRP-positive fibers in the sacral autonomic nucleus were more prominent in male than in female monkeys.[32] Another important down-regulated DEG, imbalanced matrix metalloproteinases, associated with ED via vascular alterations.[33]

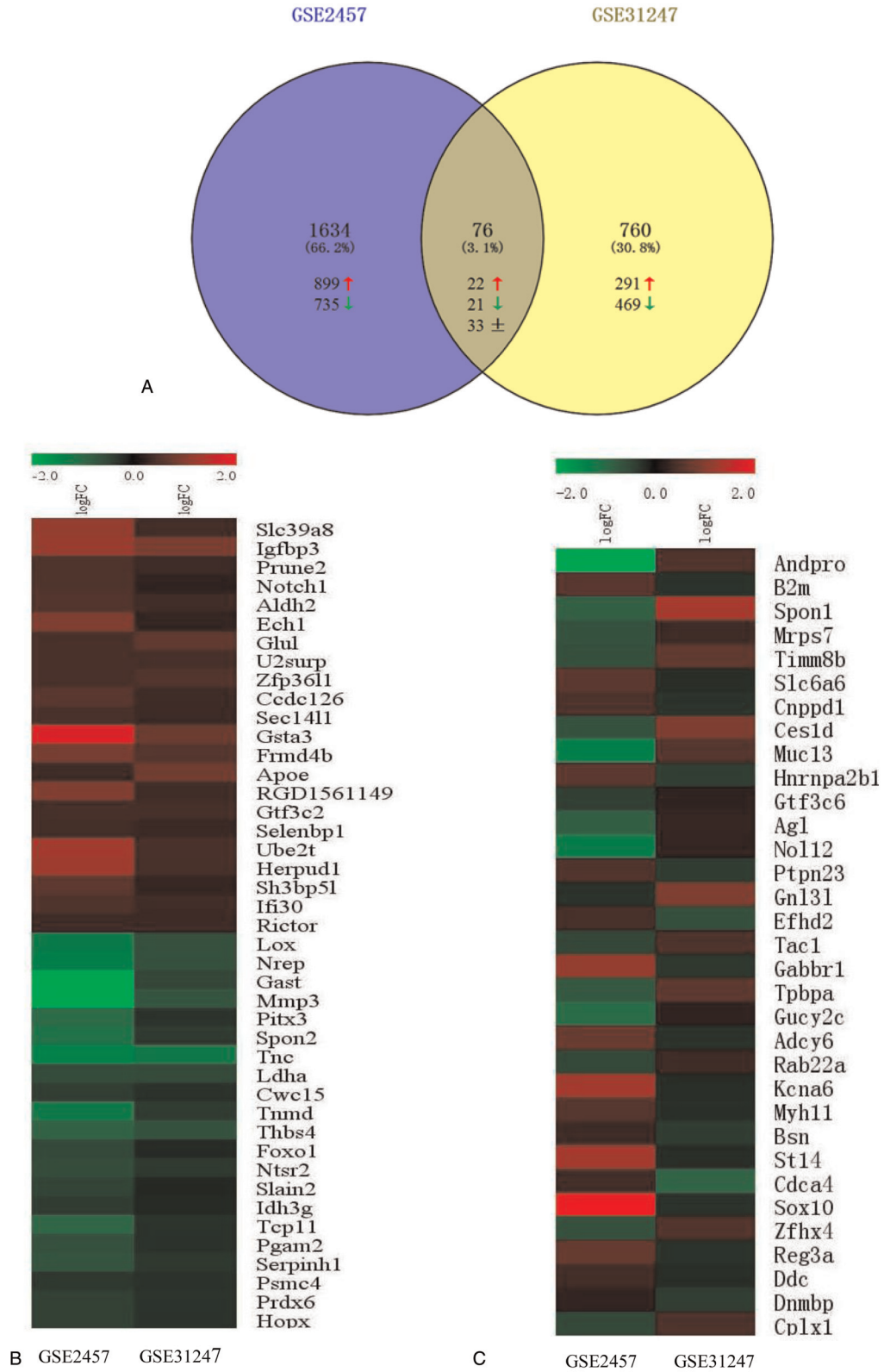

Figure 3. Co-existing DEGs in diabetes-induced and cavernous nerve injury-induced ED. (A) DEGs were selected with a |logFC (fold change)| > 0 and $P < .05$ among the mRNA expression profiling set GSE2457 and GSE31247. (B) Up-regulated DEGs and down-regulated DEGs in both GSE2457 and GSE31247. (C) Opposite gene regulation in GSE2457 and GSE31247. DEGs = differentially expressed genes, ED = erectile dysfunction.

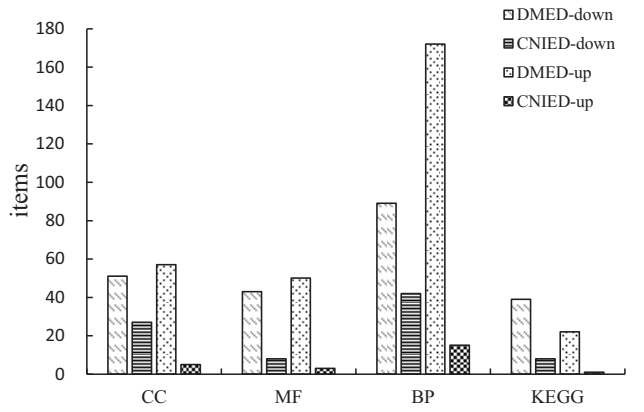

Figure 4. Comparison of down-regulated and up-regulated GO & KEGG pathway enrichment analysis of DMED and CNIED. DEGs were selected with a |logFC (fold change)| > 0 and $P < .05$ among the mRNA expression profiling set GSE2457 and GSE31247. BP = biological processes, CC = cytological component, CNIED = cavernous nerve injury induced erectile dysfunction, DEGs = differentially expressed genes, DMED = diabetes mellitus-related erectile dysfunction, GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, MF = molecular function.

Relaxin signals through a nNOS-cGMP-dependent pathway to up-regulate matrix metalloproteinases had the additional involvement of iNOS.[34]

Up-regulated insulin-like growth factor binding protein 3 showed a high logFC in both DMED and CNIED. It was reported that the expression levels of insulin-like growth factor binding protein 3 of mRNA and protein were greatly increased while the activity of NOS and concentration of cGMP decreased, alongside a significant reduction in the intracavernous pressure in aging rats compared with young control rats.[35] A further elucidation of the particular subtype of NOS should be conducted.

Some reports have shown that testosterone deficiency decreased NO production by altering the expression and activity of eNOS and nNOS.[36] Testosterone also regulates the expression of PDE5. In aged rats, exercise training had beneficial effects on erectile function through increased testosterone production and activated eNOS.[37] A systematic review showed that testosterone supplements enhanced response to PDE5i in men with ED.[38] However, some inconsistencies on testosterone supplement and ED treatment have also been noted,[36] and our present study interestingly showed this inconsistency in 2 ED models: androgen regulated protein showed the lowest *P* value in down-regulated DEGs in DMED but up-regulated DEGs in CNIED. Our functional enrichment analysis further confirmed

Table 1

GO and KEGG pathway enhancement analysis of down-regulated DEGs in DMED (GSE2457).

Term	Description	Count in gene set	Gene ratio	*P* value
GOTERM_CC_DIRECT*				
GO:0005739	Mitochondrion	130	17.038	1.65E-17
GO:0031012	**Extracellular matrix**	41	5.374	1.11E-14
GO:0005747	Mitochondrial respiratory chain compl	20	2.621	7.03E-14
GO:0005578	**Proteinaceous extracellular matrix**	36	4.718	2.37E-11
GO:0070062	**Extracellular exosome**	157	20.577	2.06E-09
GO:0005743	Mitochondrial inner membrane	37	4.849	2.28E-09
GO:0005615	**Extracellular space**	93	12.189	3.68E-09
GO:0005581	Collagen trimer	16	2.097	3.77E-09
GO:0000502	Proteasome complex	13	1.704	1.53E-06
GO:0005604	Basement membrane	15	1.966	8.91E-06
GO:0005737	Cytoplasm	245	32.110	2.66E-05
GO:0016529	Sarcoplasmic reticulum	10	1.310	2.99E-05
GO:0005753	Mitochondrial proton-transporting ATP	7	0.917	1.20E-04
GO:0043025	Neuronal cell body	38	4.980	3.11E-04
GO:0009986	Cell surface	41	5.374	4.72E-04
GO:0005839	Proteasome core complex	6	0.786	8.93E-04
GO:0005829	Cytosol	84	11.009	1.17E-03
GO:0042383	Sarcolemma	13	1.704	1.56E-03
GO:0005614	Interstitial matrix	5	0.655	3.13E-03
GO:0005761	Mitochondrial ribosome	5	0.655	3.91E-03
GOTERM_MF_DIRECT†				
GO:0005201	Extracellular matrix structural constituent	16	2.097	4.53E-11
GO:0005509	**Calcium ion binding**	52	6.815	2.14E-06
GO:0005515	Protein binding	92	12.058	1.18E-05
GO:0008137	NADH dehydrogenase (ubiquinone) activity	8	1.048	4.09E-04
GO:0005518	Collagen binding	10	1.311	4.11E-04
GO:0048407	Platelet-derived growth factor binding	5	0.655	4.85E-04
GO:0001968	Fibronectin binding	7	0.917	6.72E-04
GO:0008201	**Heparin binding**	15	1.966	9.86E-04
GO:0004298	Threonine-type endopeptidase activity	6	0.786	1.57E-03
GO:0008233	Peptidase activity	13	1.704	2.19E-03
GO:0044325	Ion channel binding	13	1.704	2.51E-03
GO:0042802	Identical protein binding	39	5.111	3.10E-03

(*continued*)

Table 1

(continued).

Term	Description	Count in gene set	Gene ratio	*P* value
GO:0042803	Protein homodimerization activity	46	6.029	3.47E-03
GO:0043394	Proteoglycan binding	4	0.524	3.52E-03
GO:0003899	DNA-directed RNA polymerase activity	6	0.786	5.08E-03
GO:0004129	Cytochrome-c oxidase activity	6	0.786	5.84E-03
GO:0050998	Nitric-oxide synthase binding	5	0.655	7.80E-03
GO:0005178	Integrin binding	10	1.311	1.13E-02
GO:0016887	ATPase activity	14	1.835	1.45E-02
GO:0008083	Growth factor activity	12	1.573	1.58E-02
GOTERM_BP_DIRECT‡				
GO:0030199	**Collagen fibril organization**	14	1.835	1.28E-09
GO:0001503	Ossification	15	1.966	1.75E-05
GO:0001649	Osteoblast differentiation	16	2.097	4.05E-05
GO:0071300	Cellular response to retinoic acid	11	1.442	3.90E-04
GO:0071230	Cellular response to amino acid stimulus	11	1.442	4.86E-04
GO:0001501	Skeletal system development	13	1.704	4.89E-04
GO:0009612	Response to mechanical stimulus	13	1.704	5.34E-04
GO:0009725	***Response to hormone***‖	12	1.573	1.23E-03
GO:0006979	Response to oxidative stress	15	1.966	1.24E-03
GO:0046034	ATP metabolic process	8	1.048	1.55E-03
GO:0002931	Response to ischemia	8	1.048	1.76E-03
GO:0007568	**Aging**	24	3.145	1.95E-03
GO:0060325	Face morphogenesis	7	0.917	2.39E-03
GO:1902600	Hydrogen ion transmembrane transport	9	1.180	2.66E-03
GO:0007029	Endoplasmic reticulum organization	6	0.786	3.56E-03
GO:0010388	Cullin deneddylation	4	0.524	3.76E-03
GO:0060316	Positive regulation of ryanodine-sensitive	4	0.524	3.76E-03
GO:0051384	Response to glucocorticoid	13	1.704	4.44E-03
GO:0010033	Response to organic substance	14	1.835	4.94E-03
GO:0060351	Cartilage development involved in endochon	4	0.524	5.22E-03
KEGG_PATHWAY§				
rno00190	Oxidative phosphorylation	34	4.456	2.83E-16
rno05010	Alzheimer disease	34	4.456	4.30E-13
rno04932	Non-alcoholic fatty liver di	30	3.932	1.71E-11
rno05012	Parkinson disease	29	3.801	1.90E-11
rno05016	Huntington disease	33	4.325	4.88E-11
rno04974	Protein digestion and absorption	20	2.621	2.82E-09
rno03050	Proteasome	13	1.704	3.15E-07
rno01100	Metabolic pathways	88	11.533	1.09E-06
rno04260	Cardiac muscle contraction	15	1.966	3.29E-06
rno04261	Adrenergic signaling in card	19	2.490	1.52E-05

BP = biological processes, CC = cytological component, CNIED = cavernous nerve injury induced erectile dysfunction, DEGs = differentially expressed genes, DMED = diabetes mellitus-related erectile dysfunction, GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, MF = molecular function.

* The first 20 of 51 are displayed according to their *P* value. The 6 common down-regulated items with CNIED in CC include extracellular exosome, extracellular space, extracellular matrix, proteinaceous extracellular matrix, neuron projection, and sarcolemma.

† The first 20 of 43 are displayed according to their *P* value. The 2 common down-regulated items with CNIED in MF include calcium ion binding and heparin binding.

‡ The first 20 of 89 are displayed according to their *P* value. The 4 common down-regulated items with CNIED in BP include collagen fibril organization, aging, cell adhesion, and wound healing.

§ The first 20 of 39 are displayed according to their *P* value. The 1 common down-regulated item with CNIED in KEGG is focal adhesion.

‖ The up-regulated item in CNIED (refer to Table 4).

that response to hormone was down-regulated in DMED, but up-regulated in CNIED. In this case, testosterone treatment should be considered according to different etiology, and the activities of eNOS, nNOS, and PDE5 need to be compared in different models.

The present study showed that changed extracellular exosomes existed in CC of both CNIED and DMED models. Exosomes are cell-derived vesicles with a diameter 30 to 120 nm. Improvement of therapeutic potential and delivery efficiency of exosomes is important for their therapeutic application.[39] The research showed that nNOS expression in the penile dorsal nerves of CNIED model[40] and in the cavernous tissues of DMED[41] was obviously lower than in their control group. Exosomes derived from adipose-derived mesenchymal stem cells enhanced nNOS-positive nerve regeneration and nNOS expression. Adipose-derived mesenchymal stem cells might be a common potential agent for CNIED and DMED treatment,[40,41] and nNOS might be their common target.

The relationship between the down-regulated collagen fibril organization and DMED model has been well studied.[42–44] Isoforms of collagen that are precursors to fibril-forming collagen type 1 were reported to be down-regulated with diabetes.[42] Icarisid II, a PDE5i from *Epimedium wanshanense*, increased cellular cGMP by enhancing NOS in the corpus cavernosum

Table 2

GO and KEGG pathway enhancement analysis of up-regulated DEGs in DMED.

Term	Description	Count in gene set	Gene ratio	*P* value
GOTERM_CC_DIRECT*				
GO:0005654	Nucleoplasm	143	15.426	1.95E-12
GO:0005913	Cell-cell adherens junction	42	4.531	2.23E-12
GO:0005634	Nucleus	303	32.686	6.50E-11
GO:0005737	Cytoplasm	315	33.981	1.88E-09
GO:0070062	**Extracellular exosome**	181	19.525	5.25E-09
GO:0016020	Membrane	143	15.426	6.37E-06
GO:0030054	Cell junction	40	4.315	1.02E-05
GO:0000790	Nuclear chromatin	26	2.805	1.50E-05
GO:0043231	Intracellular membrane-bounded organelle	57	6.149	1.54E-05
GO:0043005	**Neuron projection**	38	4.099	2.13E-05
GO:0005925	Focal adhesion	38	4.099	2.54E-05
GO:0016328	Lateral plasma membrane	11	1.187	1.33E-04
GO:0005730	Nucleolus	60	6.472	1.69E-04
GO:0045202	Synapse	29	3.128	1.72E-04
GO:0030424	Axon	33	3.560	2.31E-04
GO:0043025	Neuronal cell body	44	4.746	2.38E-04
GO:0005911	Cell-cell junction	21	2.265	3.72E-04
GO:0044297	Cell body	14	1.510	4.21E-04
GO:0042612	MHC class I protein complex	7	0.755	4.39E-04
GO:0005856	Cytoskeleton	24	2.589	6.14E-04
GOTERM_MF_DIRECT†				
GO:0005515	Protein binding	144	15.534	1.01E-14
GO:0098641	Cadherin binding involved in cell-cell adhesion	33	3.560	1.28E-08
GO:0003682	Chromatin binding	46	4.962	1.60E-06
GO:0042802	Identical protein binding	58	6.257	3.94E-06
GO:0043295	Glutathione binding	7	0.755	8.46E-05
GO:0003677	DNA binding	79	8.522	8.63E-05
GO:0000978	RNA polymerase II core promoter proximal region sequence-specific DNA binding	34	3.668	9.92E-05
GO:0044822	Poly(A) RNA binding	82	8.846	1.04E-04
GO:0005102	Receptor binding	34	3.668	2.20E-04
GO:0032403	Protein complex binding	33	3.560	6.81E-04
GO:0031491	Nucleosome binding	6	0.647	8.81E-04
GO:0001102	RNA polymerase II activating transcription factor binding	8	0.863	1.26E-03
GO:0005524	ATP binding	91	9.817	1.50E-03
GO:0003700	Transcription factor activity, sequence-specific DNA binding	54	5.825	2.10E-03
GO:0019903	Protein phosphatase binding	12	1.294	3.01E-03
GO:0019901	Protein kinase binding	35	3.776	3.05E-03
GO:0019899	Enzyme binding	32	3.452	3.12E-03
GO:0003779	Actin binding	23	2.481	3.56E-03
GO:0043565	Sequence-specific DNA binding	42	4.531	4.44E-03
GO:0001077	Transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding	23	2.481	4.51E-03
GOTERM_BP_DIRECT‡				
GO:0045893	Positive regulation of transcription, DNA-templated	56	6.041	3.29E-07
GO:0000122	Negative regulation of transcription from RNA polymerase II promoter	67	7.228	4.98E-07
GO:0098609	Cell-cell adhesion	29	3.128	6.63E-07
GO:0006351	Transcription, DNA-templated	68	7.335	1.66E-06
GO:0045944	Positive regulation of transcription from RNA polymerase II promoter	81	8.738	4.33E-06
GO:0045892	Negative regulation of transcription, DNA-templated	49	5.286	1.11E-05
GO:0032355	Response to estradiol	25	2.697	2.50E-05
GO:0006397	mRNA processing	19	2.050	2.93E-05
GO:2000811	Negative regulation of anoikis	7	0.755	3.62E-05
GO:0042493	Response to drug	47	5.070	4.04E-05
GO:0014070	Response to organic cyclic compound	29	3.128	8.34E-05
GO:0007155	**Cell adhesion**	28	3.020	1.02E-04
GO:0006635	Fatty acid beta-oxidation	10	1.079	2.41E-04
GO:0046320	Regulation of fatty acid oxidation	4	0.431	3.96E-04
GO:0001889	Liver development	18	1.942	4.55E-04
GO:0038083	Peptidyl-tyrosine autophosphorylation	9	0.971	4.56E-04
GO:0030324	Lung development	17	1.834	4.57E-04
GO:0043065	Positive regulation of apoptotic process	31	3.344	6.33E-04

(*continued*)

Table 2

(continued).

Term	Description	Count in gene set	Gene ratio	*P* value
GO:0001701	In utero embryonic development	27	2.913	7.32E-04
GO:0032092	Positive regulation of protein binding	11	1.187	1.06E-03
KEGG_PATHWAY[§]				
rno04520	Adherens junction	16	1.726	4.61E-06
rno05168	Herpes simplex infection	25	2.697	4.16E-04
rno04612	Antigen processing and presentation	15	1.618	5.24E-04
rno00071	Fatty acid degradation	10	1.079	6.62E-04
rno00480	Glutathione metabolism	10	1.079	3.09E-03
rno04068	FoxO signaling pathway	16	1.726	5.23E-03
rno04940	Type I diabetes mellitus	11	1.187	6.31E-03
rno01212	Fatty acid metabolism	9	0.971	6.91E-03
rno03320	PPAR signaling pathway	11	1.187	6.92E-03
rno04152	AMPK signaling pathway	15	1.618	7.37E-03

AMPK = Adenosine 5'-monophosphate-activated protein kinase, BP = biological processes, CC = cell component, CNIED = cavernous nerve injury induced erectile dysfunction, DEGs = differentially expressed genes, DMED = diabetes mellitus-related erectile dysfunction, GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, MF = molecular function.

[*] The first 20 of 57 are displayed according to their *P* value. The 4 common up-regulated items with CNIED in CC include extracellular exosome, extracellular space, neuron projection, and cell surface.

[†] The first 20 of 50 are displayed according to their *P* value.

[‡] The first 20 of 172 are displayed according to their *P* value. The 3 common up-regulated items in BP with CNIED include aging, wound healing, and cell adhesion.

[§] The first 20 of 22 are displayed according to their *P* value.

Table 3

GO and KEGG pathway enhancement analysis of down-regulated DEGs in CNIED (GSE31247).

Term	Description	Count in gene set	Gene ratio	*P* value
GOTERM_CC_DIRECT[*]				
GO:0005615	**Extracellular space**	53	11.134	1.11E-04
GO:0005886	Plasma membrane	124	26.050	1.98E-04
GO:0070062	**Extracellular exosome**	87	18.277	5.95E-04
GO:0016020	Membrane	73	15.336	1.50E-03
GO:0005578	**Proteinaceous extracellular matrix**	15	3.151	2.42E-03
GO:0005887	Integral component of plasma membrane	36	7.563	4.12E-03
GO:0016021	Integral component of membrane	158	33.193	5.72E-03
GO:0042383	**Sarcolemma**	9	1.891	6.20E-03
GO:0016023	Cytoplasmic, membrane-bounded vesicle	8	1.681	7.74E-03
GO:0005925	Focal adhesion	18	3.782	1.13E-02
GO:0071598	Neuronal ribonucleoprotein granule	3	0.630	1.37E-02
GO:0005623	Cell	8	1.681	2.08E-02
GO:0043005	**Neuron projection**	17	3.571	2.15E-02
GO:0043195	Terminal bouton	8	1.681	2.17E-02
GO:0005791	Rough endoplasmic reticulum	6	1.261	2.18E-02
GO:0042995	Cell projection	7	1.471	2.41E-02
GO:0030659	Cytoplasmic vesicle membrane	6	1.261	2.71E-02
GO:0005769	Early endosome	11	2.311	2.72E-02
GO:0043234	Protein complex	23	4.832	2.81E-02
GO:0005794	Golgi apparatus	29	6.092	2.91E-02
GOTERM_MF_DIRECT[†]				
GO:0004930	G-protein coupled receptor activity	57	11.975	3.43E-03
GO:0004984	Olfactory receptor activity	51	10.714	3.68E-03
GO:0005525	GTP binding	18	3.782	1.06E-02
GO:0032403	Protein complex binding	17	3.571	1.71E-02
GO:0008201	**Heparin binding**	9	1.891	2.48E-02
GO:0005200	Structural constituent of cytoskeleton	6	1.261	2.94E-02
GO:0005509	**Calcium ion binding**	26	5.462	3.03E-02
GO:0043621	Protein self-association	5	1.050	4.79E-02
GOTERM_BP_DIRECT[‡]				
GO:0007155	**Cell adhesion**	16	3.361	1.49E-03
GO:0001525	Angiogenesis	13	2.731	1.51E-03
GO:0050911	Detection of chemical stimulus involved in sensory perception of smell	51	10.714	2.61E-03
GO:0042060	**Wound healing**	10	2.101	3.13E-03
GO:0007186	G-protein coupled receptor signaling pathway	68	14.286	3.30E-03

(*continued*)

Table 3

(continued).

Term	Description	Count in gene set	Gene ratio	*P* value
GO:0045779	Negative regulation of bone resorption	4	0.840	5.90E-03
GO:0043029	T cell homeostasis	5	1.050	6.64E-03
GO:0048514	Blood vessel morphogenesis	5	1.050	8.25E-03
GO:0007602	Phototransduction	4	0.840	8.30E-03
GO:0007204	Positive regulation of cytosolic calcium ion concentration	10	2.101	1.22E-02
GO:0002076	Osteoblast development	4	0.840	1.28E-02
GO:0030036	Actin cytoskeleton organization	9	1.891	1.29E-02
GO:0030216	Keratinocyte differentiation	6	1.261	1.38E-02
GO:0030199	**Collagen fibril organization**	5	1.050	1.46E-02
GO:0045909	Positive regulation of vasodilation	5	1.050	1.46E-02
GO:0051592	Response to calcium ion	7	1.471	1.56E-02
GO:0033189	Response to vitamin A	5	1.050	1.58E-02
GO:0045786	Negative regulation of cell cycle	5	1.050	1.58E-02
GO:0010595	Positive regulation of endothelial cell migration	5	1.050	1.58E-02
GO:0006629	Lipid metabolic process	7	1.471	1.92E-02
KEGG_PATHWAY				
rno04740	Olfactory transduction	48	10.084	3.56E-03
rno04145	***Phagosome***[||]	13	2.731	4.50E-03
rno05416	Viral myocarditis	8	1.681	8.87E-03
rno00590	Arachidonic acid metabolism	7	1.471	1.87E-02
rno05219	Bladder cancer	5	1.050	1.97E-02
rno04530	Tight junction	7	1.471	3.26E-02
rno04972	Pancreatic secretion	7	1.471	3.90E-02
rno04510	**Focal adhesion**[§]	11	2.311	4.75E-02

BP = biological processes, CC = cell component, CNIED = cavernous nerve injury induced erectile dysfunction, DEGs = differentially expressed genes, DMED = diabetes mellitus-related erectile dysfunction, GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, MF = molecular function.

[*] The first 20 items of 27 are displayed according to their *P* value. The 6 common down-regulated items with DMED in CC include extracellular exosome, extracellular space, extracellular matrix, proteinaceous extracellular matrix, neuron projection, and sarcolemma.

[†] The 2 common down-regulated items with DMED in MF include calcium ion binding and heparin binding.

[‡] The first 20 items of 42 are displayed according to their *P* value. The 4 common down-regulated items with DMED in BP include cell adhesion, collagen fibril organization, wound healing, and aging.

[§] The 1 common item with DMED in KEGG is focal adhesion.

[||] The up-regulated item in DMED.

tissue of diabetic ED rats, and also increased smooth muscle cell/collagen fibril proportions.[43,44] Little is known about the relationship between collagen fibril, CNIED and the role of NOS/PDE5 till now.

"Aging" appeared in down-regulated BP in both DMED and CNIED, but it showed the highest gene ration in DMED. Also in DMED, down-regulated metabolic pathways (the highest gene ration), Alzheimer disease (the second gene ration), oxidative phosphorylation (the second gene ration) in KEGG, and down-regulated response to hormone, osteoblast differentiation and response to oxidative stress in BP caught our attention. Many aging-related disorders have been reported such as ED, androgen deficiency, and decreased bone density.[45–47] Current studies suggested strong correlations between low testosterone, metabolic syndrome and aging.[47] Oxidative stress, balance between superoxide and NO, and deficiency of testosterone led to metabolic impairment and accelerated aging.[48] The above aging-related problems are also the damaging factors of sexual function.

Among the 3 subtypes of NOS, the effect of iNOS was specific in aging-related ED, osteoblast differentiation in diabetes, and even Alzheimer disease.[25,45,49,50] The pharmacologically up-regulation of iNOS in smooth muscle cells of corpus cavernosum of penis led to the reversal of aging-related changes in the corpora with correction of the venous leakage.[45] In osteoblast-like cells, iNOS and eNOS stimulated by metformin inhibited the GSK3β/Wnt/β-catenin pathway, and promoted osteogenic differentiation of osteoblasts.[49] The role of iNOS will be explored in our future research.

Compared to DMED model, CNIED seemed to have its special functional enrichment on down-regulated olfactory receptor activity in MF, down-regulated detection of chemical stimulus involved in sensory perception of smell in BP, and down-regulated olfactory transduction in KEGG. It was reported that olfactory sensitivity was related to erectile function in adult males.[51] In smoking men the reduction of olfactory acuity could adversely affect sexuality.[52] On the other hand, penile erection occurs in response to visual, olfactory and tactile stimuli initiated within the brain and/or on the periphery.[53] It was reported that cGMP had essential and distinctive functions in olfactory sensation and adaptation.[54] Based on cGMP signal, the relationship between olfactory receptor activity in penile tissue and olfactory sensation within the brain based on the function of NOS/PDE5 should be deeply investigated.

PPI results showed the specific hub gene in different ED models. C3 was only shown in DMED, but not in CNIED. It has been reported that islet C3 expression was up-regulated in human type 2 diabetes and rodent models of diabetes.[56] The correlation analysis and subgroup analysis of 7 complement cascade-related hub genes and the clinical characteristics of diabetic nephropathy (DN) showed that C3 may participate in the development of DN, one of the main complications of diabetes.[57] Some research suggested that identification of C3 might be a therapeutic target for DN.[58] We will pay attention to the possibility of C3 as a

Table 4

GO and KEGG pathway enhancement analysis of up-regulated DEGs in CNIED (GSE31247).

Term	Description	Count in gene set	Gene ratio	*P* value
GOTERM_CC_DIRECT*				
GO:0042613	MHC class II protein complex	3	1.034	1.45E-02
GO:0005887	Integral component of plasma membrane	22	7.586	1.50E-02
GO:0005576	Extracellular region	18	6.207	1.57E-02
GO:0005763	Mitochondrial small ribosomal subunit	3	1.034	3.43E-02
GO:0009986	**Cell surface**	15	5.172	3.57E-02
GOTERM_MF_DIRECT				
GO:0016595	Glutamate binding	3	1.034	1.54E-02
GO:0070402	NADPH binding	3	1.034	1.99E-02
GO:0004351	Glutamate decarboxylase activity	2	0.690	4.07E-02
GOTERM_BP_DIRECT†				
GO:0019886	Antigen processing and presentation of exogenous peptide antigen via MHC class II	5	1.724	3.23E-05
GO:0050671	Positive regulation of lymphocyte proliferation	3	1.034	6.46E-03
GO:0048660	Regulation of smooth muscle cell proliferation	3	1.034	8.00E-03
GO:0009725	***Response to hormone***‡	6	2.069	1.22E-02
GO:0070534	protein K63-linked ubiquitination	4	1.379	1.55E-02
GO:0050679	Positive regulation of epithelial cell proliferation	5	1.724	2.22E-02
GO:0019233	Sensory perception of pain	5	1.724	2.51E-02
GO:0006182	cGMP biosynthetic process	3	1.034	2.53E-02
GO:0051000	Positive regulation of nitric-oxide synthase activity	3	1.034	2.53E-02
GO:0070848	Response to growth factor	3	1.034	3.38E-02
GO:0030521	Androgen receptor signaling pathway	3	1.034	3.68E-02
GO:0009449	Gamma-aminobutyric acid biosynthetic process	2	0.690	4.10E-02
GO:0035461	Vitamin transmembrane transport	2	0.690	4.10E-02
GO:0070979	Protein K11-linked ubiquitination	3	1.034	5.00E-02
GO:0002052	Positive regulation of neuroblast proliferation	3	1.034	5.00E-02
KEGG_PATHWAY				
rno05150	Staphylococcus aureus infection	4	1.379	4.00E-02

BP = biological processes, CC = cytological component, cGMP = cyclic guanosine monophosphate, CNIED = cavernous nerve injury induced erectile dysfunction, DEGs = differentially expressed genes, DMED = diabetes mellitus-related erectile dysfunction, GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, MF = molecular function.

* The 1 common item in CC is cell surface.

† The 3 common up-regulated items in BP with CNIED include aging, wound healing, and cell adhesion.

‡ The down-regulated item in DMED (refer to Table 1).

therapeutic target for DMED. Myeloid-derived suppressor cells played a role in resistance to diabetes in the absence of C3. Myeloid-derived suppressor cells number was significantly increased, accompanied by highly expressed arginase1 and iNOS in streptozotocin-treated C3–/– mice.[55] Further relationship between C3, iNOS and DMED should be explored.

Ubb, zinc and ring finger 2 and ubiquitin-conjugating enzyme were the hub genes identified only in CNIED. The research based on Comparative Toxicogenomics Database showed that as a hub gene, Ubb is closely related to Alzheimer disease or cognition impairment.[59] Research has shown that zinc can induce ubiquitin conjugation in cultured hippocampal neurons.[60] A cGMP-hydrolyzing phosphodiesterase, PDE9A, was recently identified as a novel interactor and substrate of neuralized E3 ubiquitin protein ligase 1.[61] Prolonged PDE9 was detected in human corpus cavernosum; and PDE9 inhibition amplified the NO-cGMP-mediated cavernosal responses, and may be of therapeutic value for CNIED.[62]

Differences in the above discussed ED models from Fisher 344 and Sprague Dawley strain might explain the variations in the aforementioned critical biomarkers. It was reported that inbred Fisher 344 rats were more metabolically sensitive than outbred Sprague Dawley strain.[63] That may be the reason that Fisher 344 strain was chosen for DMED model.[9] Furthermore, some research demonstrated that different rat strains respond to injury differently, and thus in preclinical neurotrauma studies, strain influence is an important consideration during evaluation of outcomes.[64] An important question is: do these variable critical biomarkers also occur in humans?

As we mentioned before, it is exceedingly difficult to obtain penile tissue specimens from patients with ED. Still, there have been a few studies of human tissues.[65] GO and KEGG pathway enrichment analysis of hub DEGs in human's ED samples showed that extracellular exosome in CC and collagen fibril organization in BP had the same downward trend compared with our present data.[65]

Related to pathway-associated biomarkers, some research tried to identify and map potential novel genes,[66,67] probe the relationship between the DEGs and altered or dysregulated pathways,[68,69] and to predict and prioritize pathway-associated biomarkers.[70] Based on NOS-cGMP-PDE5 pathway, the critical biomarkers identified in this study may provide some help on ED treatment from above points. On the other hand, the presented results were rather preliminary and the possible screening must be validated by analytical approaches and larger groups before any valid conclusion can be made.

5. Conclusions

Our analyses indicated that some critical biomarkers different and common in DMED and CNIED showed their relationships with 3 subtypes of NOS, cGMP, and PDE5 activities. According to the previous studies by Dr Rajfer and Dr Ferrini, the effect of iNOS was confirmed specifically in aging-related ED.[45] Our

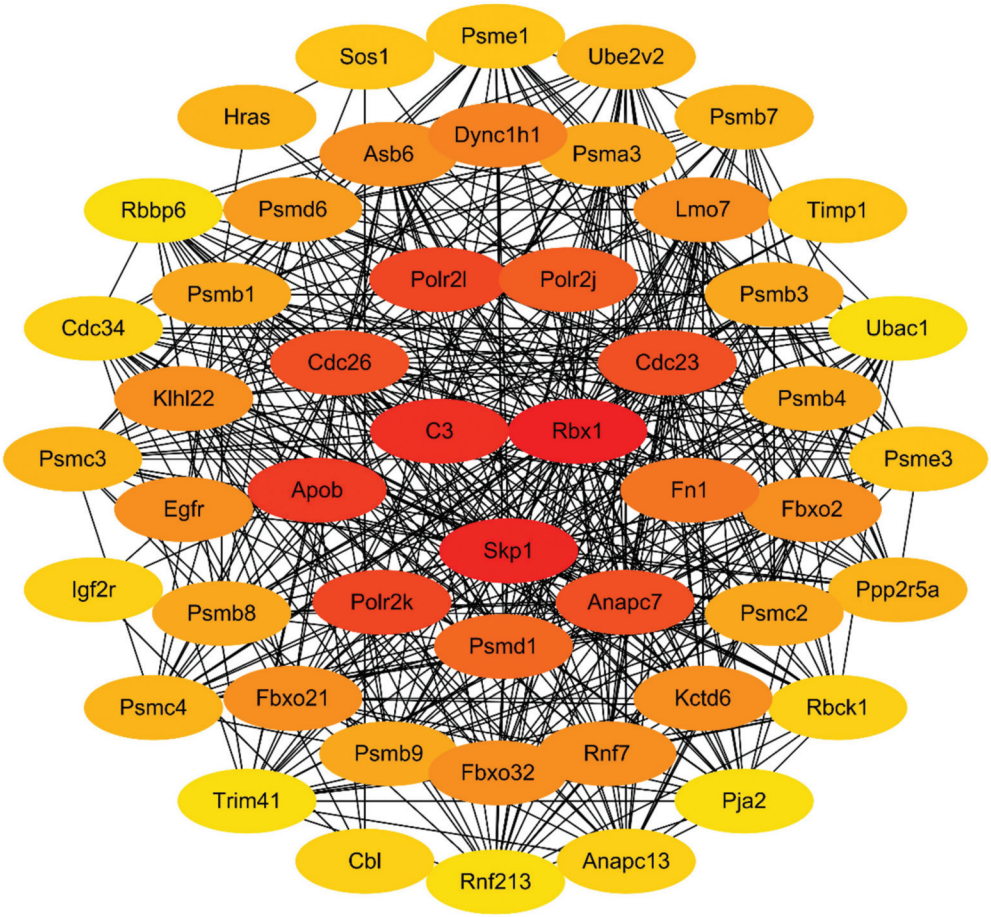

Figure 5. PPI analysis of the top 50 DEGs in GSE2457. $P < .05$, |logFC (fold change)| > 0, and the interactions with an average score of greater than 0.9 were chosen as the cutoff for statistical significance. DEGs = differentially expressed genes, PPI = protein-protein interaction.

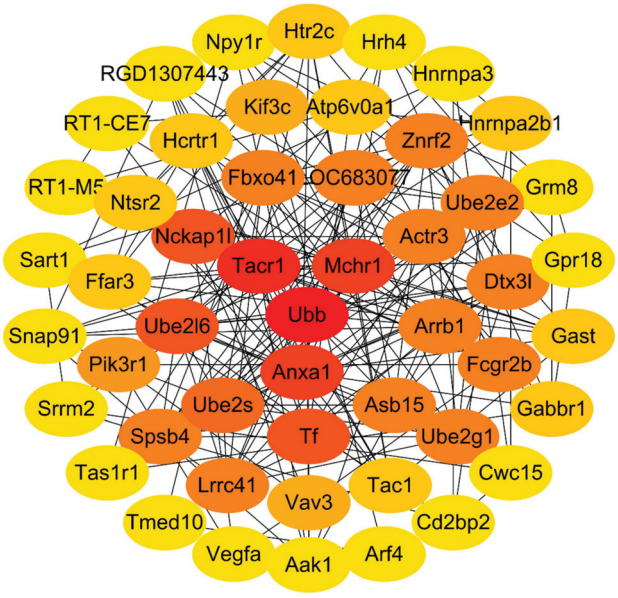

Figure 6. PPI analysis of the top 50 DEGs in GSE31247. $P < .05$, |logFC (fold change)| > 0, and the interactions with an average score of greater than 0.9 were chosen as the cutoff for statistical significance. DEGs = differentially expressed genes, PPI = protein-protein interaction.

current bioinformatics analysis showed that aging process was a common enriched functions and pathways in DMED and CNIED. The intervention between dysregulated iNOS and critical biomarkers, and how to improve iNOS activity to treat DMED and CNIED clinically should receive more attention in our future research.

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References

[1] Hatzimouratidis K, Amar E, Eardley I, et al. Guidelines on male sexual dysfunction: erectile dysfunction and premature ejaculation. Eur Urol 2010;57:804–14.
[2] Wu Y, Yang C, Meng F, et al. Nerve growth factor improves the outcome of type 2 diabetes-induced hypotestosteronemia and erectile dysfunction. Reprod Sci 2019;26:386–93.
[3] Mulhall JP, Klein EA, Slawin K, Henning AK, Scardino PT. A randomized, double-blind, placebo-controlled trial to assess the utility of tacrolimus (FK506) for the prevention of erectile dysfunction following bilateral nerve-sparing radical prostatectomy. J Sex Med 2018;15:1293–9.
[4] Chung E, De Young L, Brock GB. Investigative models in erectile dysfunction: a state-of-the-art review of current animal models. J Sex Med 2011;8:3291–305.
[5] Sun X, Luo LH, Feng L, Li DS, Zhong KZ. B cell lymphoma-2-modified bone marrow-derived mesenchymal stem cells transplantation for the treatment of diabetes mellitus-induced erectile dysfunction in a rat model. Urol Int 2017;98:358–66.
[6] Haney NM, Talwar S, Akula PK, et al. Insulin-like growth factor-1-loaded polymeric poly(lactic-co-glycolic) acid microspheres improved erectile function in a rat model of bilateral cavernous nerve injury. J Sex Med 2019;16:383–93.
[7] Yilmaz E, Kaya-Sezginer E, Yilmaz-Oral D, Cengiz T, Bayatli N, Gur S. Effects of hydrogen sulphide donor, sodium hydrosulphide treatment on the erectile dysfunction in L-NAME-induced hypertensive rats. Andrologia 2019;51:e13240.
[8] Brien SE, Smallegange C, Gofton WT, Heaton JPW, Adams MA. Development of a rat model of sexual performance anxiety: effect of behavioural and pharmacological hyperadrenergic stimulation on APO-induced erections. Int J Impot Res 2002;14:107–15.
[9] Sullivan CJ, Teal TH, Luttrell IP, Tran KB, Peters MA, Wessells H. Microarray analysis reveals novel gene expression changes associated with erectile dysfunction in diabetic rats. Physiol Genomics 2005; 23:192–205.
[10] Wei AY, Luo XG, Yang Y, He SH, Zhang T, Liu Y. Microarray data analysis of genes related with erectile dysfunction in diabetic rats. Nan Fang Yi Ke Da Xue Xue Bao 2011;31:694–7.
[11] Kam SC, Lee SH, Jeon JH, et al. Gene expression profile comparison in the penile tissue of diabetes and cavernous nerve injury-induced erectile dysfunction rat model. Investig Clin Urol 2016;57:286–97.
[12] Udhaya Kumar S, Thirumal Kumar D, Bithia R, et al. Analysis of differentially expressed genes and molecular pathways in familial hypercholesterolemia involved in atherosclerosis: a systematic and bioinformatics approach. Front Genet 2020;11:734.
[13] Wan J, Jiang S, Jiang Y, et al. Corrigendum: Data mining and expression analysis of differential lncRNA ADAMTS9-AS1 in prostate cancer. Front Genet 2020;11:361.
[14] Kumar S U, Rajan B, Kumar D T, et al. Involvement of essential signaling cascades and analysis of gene networks in diabesity. Genes (Basel) 2020;11:1256.
[15] Fu D, Zhang B, Yang L, Huang S, Xin W. Development of an immune-related risk signature for predicting prognosis in lung squamous cell carcinoma. Front Genet 2020;11:978.
[16] Burnett AL. The role of nitric oxide in erectile dysfunction: implications for medical therapy. J Clin Hypertens (Greenwich) 2006;8:53–62.
[17] Otunctemur A, Ozbek E, Sahin S, et al. Low serum insulin-like growth factor-1 in patients with erectile dysfunction. Basic Clin Androl 2016;26:1.
[18] Zhou ZY, Cheng SP, Huang H, et al. Decrease of the insulin-like growth factor-1 bioavailability in spontaneously hypertensive rats with erectile dysfunction. Andrologia 2016;48:824–8.
[19] Barrett T, Wilhite SE, Ledoux P, et al. NCBI GEO: archive for functional genomics data sets–update. Nucleic Acids Res 2013;41:D991–5.
[20] Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009;4:44–57.
[21] The Gene Ontology ConsortiumThe gene ontology resource: 20 years and still going strong. Nucleic Acids Res 2019;47:D330–8.
[22] Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 2020;28:27–30.
[23] Kanehisa M, Sato Y, Furumichi M, Morishima K, Tanabe M. New approach for understanding genome variations in KEGG. Nucleic Acids Res 2019;47:D590–5.
[24] Szklarczyk D, Morris JH, Cook H, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 2017;45:D362–8.
[25] Gonzalez-Cadavid NF, Rajfer J. The two phases of the clinical validation of preclinical translational mechanistic research on PDE5 inhibitors since Viagra's advent. A personal perspective. Int J Impot Res 2019;31:57–60.
[26] Morelli A, Vignozzi L, Filippi S, Mancina R, Maggi M. Erectile dysfunction: molecular biology, pathophysiology and pharmacological treatment. Minerva Urol Nefrol 2005;57:85–90.
[27] Andersson KE. PDE5 inhibitors-pharmacology and clinical applications 20 years after sildenafil discovery. Br J Pharmacol 2018;175:2554–65.
[28] Lopushnyan NA, Chitaley K. Genetics of erectile dysfunction. J Urol 2012;188:1676–83.
[29] Garcia LA, Hlaing SM, Gutierrez RA, et al. Sildenafil attenuates inflammation and oxidative stress in pelvic ganglia neurons after bilateral cavernosal nerve damage. Int J Mol Sci 2014;15:17204–20.
[30] Ferrini MG, Moon J, Rivera S, Rajfer J, Gonzalez-Cadavid NF. Amelioration of diabetes-induced cavernosal fibrosis by antioxidant and anti-transforming growth factor-β1 therapies in inducible nitric oxide synthase-deficient mice. BJU Int 2012;109:586–93.
[31] Takanami K, Sakamoto H. The gastrin-releasing peptide receptor (grpr) in the spinal cord as a novel pharmacological target. Curr Neuropharmacol 2014;12:434–43.
[32] Ito T, Oti T, Takanami K, et al. A sexually dimorphic peptidergic system in the lower spinal cord controlling penile function in non-human primates. Spinal Cord 2018;56:57–62.
[33] Muniz JJ, Lacchini R, Belo VA, et al. Circulating matrix metalloproteinases and their endogenous inhibitors in patients with erectile dysfunction. Int J Impot Res 2012;24:38–43.
[34] Chow BS, Chew EG, Zhao C, Bathgate RAD, Hewitson TD, Samuel CS. Relaxin signals through a RXFP1-pERK-nNOS-NO-cGMP-dependent pathway to up-regulate matrix metalloproteinases: the additional involvement of iNOS. PLoS One 2012;7:e42714.
[35] Pu XY, Zheng XG, Zhang Y, et al. Higher expression of mRNA and protein of insulin-like growth factor binding protein-3 in old rat penile tissues: implications for erectile dysfunction. J Sex Med 2011;8:2181–90.
[36] Hotta Y, Kataoka T, Kimura K. Testosterone deficiency and endothelial dysfunction: nitric oxide, asymmetric dimethylarginine, and endothelial progenitor cells. Sex Med Rev 2019;7:661–8.
[37] Seo DY, Lee SR, Kwak HB, et al. Exercise training causes a partial improvement through increasing testosterone and eNOS for erectile function in middle-aged rats. Exp Gerontol 2018;108:131–8.
[38] Zhu J, Zhang W, Ou N, et al. Do testosterone supplements enhance response to phosphodiesterase 5 inhibitors in men with erectile dysfunction and hypogonadism: a systematic review and meta-analysis. Transl Androl Urol 2020;9:591–600.
[39] Yamashita T, Takahashi Y, Takakura Y. Possibility of exosome-based therapeutics and challenges in production of exosomes eligible for therapeutic application. Biol Pharm Bull 2018;41:835–42.
[40] Li M, Lei H, Xu Y, et al. Exosomes derived from mesenchymal stem cells exert therapeutic effect in a rat model of cavernous nerves injury. Andrology 2018;6:927–35.
[41] Wang J, Mi Y, Wu S, et al. Exosomes from adipose-derived stem cells protect against high glucose-induced erectile dysfunction by delivery of corin in a streptozotocin-induced diabetic rat model. Regen Ther 2020;14:227–33.
[42] Yohannes E, Chang J, Tar MT, Davies KP, Chance MR. Molecular targets for diabetes mellitus-associated erectile dysfunction. Mol Cell Proteomics 2010;9:565–78.
[43] Zhang J, Wang YB, Ma CG, et al. Icarisid II, a PDE5 inhibitor from Epimedium wanshanense, increases cellular cGMP by enhancing NOS in diabetic ED rats corpus cavernosum tissue. Andrologia 2012;44:87–93.
[44] Zhang J, Li AM, Liu BX, et al. Effect of icarisid II on diabetic rats with erectile dysfunction and its potential mechanism via assessment of AGEs, autophagy, mTOR and the NO-cGMP pathway. Asian J Androl 2013;15:143–8.
[45] Ferrini MG, Gonzalez-Cadavid NF, Rajfer J. Aging related erectile dysfunction-potential mechanism to halt or delay its onset. Transl Androl Urol 2017;6:20–7.

[46] Clavijo RI, Miner MM, Rajfer J. Erectile dysfunction and essential hypertension: the same aging-related disorder? Rev Urol 2014;16:167–71.
[47] Shelton JB, Rajfer J. Androgen deficiency in aging and metabolically challenged men. Urol Clin North Am 2012;39:63–75.
[48] Guzik TJ, Cosentino F. Epigenetics and immunometabolism in diabetes and aging. Antioxid Redox Signal 2018;29:257–74.
[49] Jiating L, Buyun J, Yinchang Z. Role of metformin on osteoblast differentiation in type 2 diabetes. Biomed Res Int 2019;9203934.
[50] Dubey H, Gulati K, Ray A. Alzheimer's disease: a contextual link with nitric oxide synthase. Curr Mol Med 2020;20:505–15.
[51] Deng HY, Feng JR, Zhou WH, et al. Olfactory sensitivity is related to erectile function in adult males. Front Cell Dev Biol 2020;8:93.
[52] Özmen S, Dülger S, Çoban S, et al. Olfactory and erectile dysfunction association in smoking and non-smoking men. Physiol Behav 2016; 160:1–5.
[53] Nehra A, Kulaksizoglu H. Combination therapy for erectile dysfunction: where we are and what's in the future. Curr Urol Rep 2002;3:467–70.
[54] Shidara H, Hotta K, Oka K. Compartmentalized cGMP responses of olfactory sensory neurons in caenorhabditis elegans. J Neurosci 2017; 37:3753–63.
[55] Gao X, Liu H, He B, Fu Z. Resistance to streptozotocin-induced autoimmune diabetes in absence of complement C3: myeloid-derived suppressor cells play a role. PLoS One 2013;8:e66334.
[56] King BC, Kulak K, Krus U, et al. Complement component C3 is highly expressed in human pancreatic islets and prevents β cell death via ATG16L1 interaction and autophagy regulation. Cell Metab 2019; 29:202–10.
[57] Xu B, Wang L, Zhan H, et al. Investigation of the mechanism of complement system in diabetic nephropathy via bioinformatics analysis. J Diabetes Res 2021;2021:5546199.
[58] Tang S, Wang X, Deng T, Ge H, Xiao X. Identification of C3 as a therapeutic target for diabetic nephropathy by bioinformatics analysis. Sci Rep 2020;10:13468.
[59] Wang X, Wang L. Screening and identification of potential peripheral blood biomarkers for Alzheimer's disease based on bioinformatics analysis. Med Sci Monit 2020;26:e924263.
[60] Sun KJ, Zhu L, Wang HD, et al. Zinc as mediator of ubiquitin conjugation following traumatic brain injury. Brain Res 2013;1506: 132–41.
[61] Taal K, Tuvikene J, Rullinkov G, et al. Neuralized family member NEURL1 is a ubiquitin ligase for the cGMP-specific phosphodiesterase 9A. Sci Rep 2019;9:7104.
[62] da Silva FH, Pereira MN, Franco-Penteado CF, De Nucci G, Antunes E, Claudino MA. Phosphodiesterase-9 (PDE9) inhibition with BAY 73-6691 increases corpus cavernosum relaxations mediated by nitric oxide-cyclic GMP pathway in mice. Int J Impot Res 2013;25:69–73.
[63] Boyce G, Shoeb M, Kodali V, et al. Using liquid chromatography mass spectrometry (LC-MS) to assess the effect of age, high-fat diet, and rat strain on the liver metabolome. PLoS One 2020;15:e0235338.
[64] Reid WM, Rolfe A, Register D, Levasseur JE, Churn SB, Sun D. Strain-related differences after experimental traumatic brain injury in rats. J Neurotrauma 2010;27:1243–53.
[65] Hui J, Liu R, Zhang H, He S, Wei A. Screening and identification of critical biomarkers in erectile dysfunction: evidence from bioinformatic analysis. PeerJ 2020;8:e8653.
[66] Kumar SU, Kumar DT, Siva R, Doss CGP, Zayed H. Integrative bioinformatics approaches to map potential novel genes and pathways involved in ovarian cancer. Front Bioeng Biotechnol 2019;7:391.
[67] Peng Y, Wu D, Li F, Zhang P, Feng Y, He A. Identification of key biomarkers associated with cell adhesion in multiple myeloma by integrated bioinformatics analysis. Cancer Cell Int 2020;20:262.
[68] Kumar SU, Saleem A, Kumar DT, et al. A systemic approach to explore the mechanisms of drug resistance and altered signaling cascades in extensively drug-resistant tuberculosis. Adv Protein Chem Struct Biol 2021;127:343–64.
[69] Udhaya Kumar S, Thirumal Kumar D, Siva R, et al. Dysregulation of signaling pathways due to differentially expressed genes from the B-cell transcriptomes of systemic lupus erythematosus patients-a bioinformatics approach. Front Bioeng Biotechnol 2020;8:276.
[70] Mishra S, Shah MI, Kumar SU, et al. Network analysis of transcriptomics data for the prediction and prioritization of membrane-associated biomarkers for idiopathic pulmonary fibrosis (IPF) by bioinformatics approach. Adv Protein Chem Struct Biol 2021;123:241–73.