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

Guangying Wang, MPharma, Dayue Shen, BPharm, Xilan Zhang, BPharm, Monica G. Ferrini, MD, Yuanping Li, BPharm, Hui Liao, MMEd

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, Ub = ubiquitin B.

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

Editor: Nejat Mahdieh.

This study was supported by grant (No. S220190007) from the Provincial Special Supporting Fund Scientific Research Project of Shanxi Provincial People’s Hospital and (No. 201903D421061) from the Key R & D Project of Shanxi Province. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

The authors declare there are no studies on human subjects, human data or tissue, or animals in this manuscript.

The authors have no conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

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.

© 2021 the Author(s). Published by Wolters Kluwer Health, Inc.

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and build upon the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

How to cite this article: Wang G, Shen D, Zhang X, Ferrini MG, Li Y, Liao H. Comparison of critical biomarkers in 2 erectile dysfunction models based on GEO and NOS-cGMP-PDE5 pathway. Medicine 2021;100:41(e27508).

Received: 28 March 2021 / Received in final form: 7 August 2021 / Accepted: 25 September 2021
http://dx.doi.org/10.1097/MD.00000000000027508
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-I (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 logFC (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.
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 reveal 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
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 matrix, 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 (Ub) 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 \(|\log FC\) (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.
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**

| 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:0007006 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:0005020 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:0018529 Sarcoplasm 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:0009898 Cell surface                                                          | 41                | 5.374      | 4.72E-04  |
| GO:0005989 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:00044325 Ion channel binding                                                 | 13                | 1.704      | 2.51E-03  |
| GO:00042802 Identiﬁcal protein binding                                          | 39                | 5.111      | 3.10E-03  |

(continued)
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. The research showed that nNOS expression in the penile dorsal nerves of CNIED model and in the cavernous tissues of DMED 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, and nNOS might be their common target.

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

| 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:0075686                                | 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 endochond | 4         | 0.524      | 5.22E-03 |
| KEGG_PATHWAYx                             |                                      |                   |            |          |
| mo00190                                   | Oxidative phosphorylation            | 34                | 4.456      | 2.83E-16 |
| mo05010                                   | Alzheimer disease                    | 34                | 4.456      | 4.30E-13 |
| mo04932                                   | Non-alcoholic fatty liver di         | 30                | 3.932      | 1.71E-11 |
| mo05012                                   | Parkinson disease                   | 29                | 3.801      | 1.90E-11 |
| mo05016                                   | Huntington disease                  | 33                | 4.325      | 4.88E-11 |
| mo04974                                   | Protein digestion and absorption     | 20                | 2.621      | 2.82E-09 |
| mo03050                                   | Proteasome                          | 13                | 1.704      | 3.15E-07 |
| mo01100                                   | Metabolic pathways                  | 88                | 11.533     | 1.09E-06 |
| mo04260                                   | Cardiac muscle contraction           | 15                | 1.966      | 3.29E-06 |
| mo04261                                   | 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 up-regulated item in CNIED (refer to Table 4).
| Term Description | Count in gene set | Gene ratio | P value |
|------------------|-------------------|------------|---------|
| Nucleoplasm      | 143               | 15.426     | 1.95E-12|
| Cell-cell adherens junction | 42            | 4.531 | 2.23E-12|
| Nucleus          | 303               | 32.686     | 6.50E-11|
| Cytoplasm        | 315               | 33.981     | 1.88E-09|
| Extracellular exosome | 181           | 19.525 | 5.25E-09|
| Membrane         | 143               | 15.426     | 6.37E-06|
| Cell junction    | 40                | 4.315      | 1.02E-05|
| Nuclear chromatin| 26                | 2.805      | 1.50E-05|
| Intracellular membrane-bounded organelle | 57           | 6.149 | 1.54E-05|
| Neuron projection| 38                | 4.099      | 2.13E-05|
| Focal adhesion   | 38                | 4.099      | 2.54E-05|
| Lateral plasma membrane | 11          | 1.187 | 1.33E-04|
| Nucleolus        | 60                | 6.472      | 1.69E-04|
| Synapse          | 29                | 3.128      | 1.72E-04|
| Axon             | 33                | 3.560      | 2.31E-04|
| Neuronal cell body | 44            | 4.746 | 3.28E-04|
| Cell-cell junction | 21           | 2.265 | 3.72E-04|
| Cell body        | 14                | 1.510      | 4.21E-04|
| MHC class I protein complex | 7         | 0.755 | 4.39E-04|
| Cytoskeleton     | 24                | 2.589      | 6.14E-04|
| Protein binding  | 144               | 15.534     | 1.01E-14|
| Cadherin binding involved in cell-cell adhesion | 33        | 3.560 | 1.28E-08|
| Chromatin binding | 46            | 4.962 | 1.60E-06|
| Identical protein binding | 58         | 6.257 | 3.94E-06|
| Glutathione binding | 7             | 0.755 | 8.46E-05|
| DNA binding      | 79                | 8.522      | 8.63E-05|
| RNA polymerase II core promoter proximal region sequence-specific DNA binding | 34        | 3.668 | 9.92E-05|
| Poly(A) RNA binding | 82            | 8.846 | 1.04E-04|
| Receptor binding | 34                | 3.668      | 2.20E-04|
| Protein complex binding | 33        | 3.560 | 6.81E-04|
| Nucleosome binding | 6             | 0.647 | 8.81E-04|
| RNA polymerase II activating transcription factor binding | 8         | 0.863 | 1.26E-03|
| ATP binding      | 91                | 9.817      | 1.50E-03|
| Transcription factor activity, sequence-specific DNA binding | 54        | 5.825 | 2.10E-03|
| Protein phosphatase binding | 12         | 1.294 | 3.01E-03|
| Protein kinase binding | 35        | 3.776 | 3.05E-03|
| Enzyme binding   | 32                | 3.452      | 3.12E-03|
| Actin binding    | 23                | 2.481      | 3.56E-03|
| Sequence-specific DNA binding | 42        | 4.531 | 4.44E-03|
| Transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding | 23        | 2.481 | 4.51E-03|
| Positive regulation of transcription, DNA-templated | 56        | 6.041 | 3.29E-07|
| Negative regulation of transcription from RNA polymerase II promoter | 67        | 7.228 | 4.98E-07|
| Cell-cell adhesion | 29            | 3.128 | 6.63E-07|
| Transcription, DNA-templated | 68        | 7.335 | 1.66E-06|
| Positive regulation of transcription from RNA polymerase II promoter | 81        | 8.738 | 4.33E-06|
| Negative regulation of transcription, DNA-templated | 49        | 5.286 | 1.11E-05|
| Response to estradiol | 25            | 2.697 | 2.50E-06|
| miRNA processing | 19                | 2.050      | 2.93E-06|
| Negative regulation of anokis | 7           | 0.755 | 3.62E-05|
| Response to drug | 47                | 5.070      | 4.04E-05|
| Response to organic cyclic compound | 29        | 3.128 | 8.34E-05|
| Cell adhesion    | 28                | 3.020      | 1.02E-04|
| Fatty acid beta-oxidation | 10          | 1.079 | 2.41E-04|
| Regulation of fatty acid oxidation | 4          | 0.431 | 3.96E-04|
| Liver development | 18            | 1.942 | 4.55E-04|
| Peptidyl-tyrosine autoprophosphorylation | 9          | 0.971 | 4.56E-04|
| Lung development | 17                | 1.834      | 4.57E-04|
| 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<sup>6</sup> |                                |                   |            |          |
| me04520  | Adherens junction              | 16                | 1.726      | 4.61E-06 |
| me05168  | Herpes simplex infection      | 25                | 2.697      | 4.16E-04 |
| me04612  | Antigen processing and presentation | 15 | 1.079 | 6.62E-03 |
| me00071  | Fatty acid degradation        | 10                | 1.079      | 3.09E-03 |
| me00480  | Glutathione metabolism        | 16                | 1.726      | 5.23E-03 |
| me04068  | FoxO signaling pathway        | 11                | 1.187      | 6.31E-03 |
| me04940  | Type  II diabetes mellitus    | 15                | 1.618      | 5.24E-04 |
| me01212  | Fatty acid metabolism         | 9                 | 0.971      | 6.91E-03 |
| me03320  | PPAR signaling pathway        | 11                | 1.187      | 6.92E-03 |
| me04152  | AMPK signaling pathway        | 15                | 1.618      | 7.37E-03 |

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

### 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<sup>7</sup> | Extracellular space   | 53                | 11.134     | 1.11E-04 |
| GO:0005615    | Extracellular space                | 124               | 26.050     | 1.98E-04 |
| GO:0005886    | Proteinaceous extracellular matrix | 15                | 3.151      | 2.42E-03 |
| GO:0005887    | Protease inhibitor activity        | 8                 | 1.891      | 2.08E-02 |
| GO:0005925    | 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:0005794    | Golgi apparatus                    | 29                | 6.092      | 2.91E-02 |
| GOTERM_MF_DIRECT<sup>7</sup> | G-protein coupled receptor activity | 57               | 11.975     | 3.43E-03 |
| GO:0004984    | GTP binding                        | 18                | 3.782      | 1.06E-02 |
| GO:0005525    | Protein complex binding            | 17                | 3.571      | 1.71E-02 |
| GO:0008201    | Heparin binding                    | 9                 | 1.891      | 2.48E-02 |
| GO:0005200    | Neuronal ribonucleoprotein granule | 6                 | 1.261      | 2.71E-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<sup>7</sup> | Cell adhesion                     | 16                | 3.361      | 1.49E-03 |
| GO:0001525    | Angiogenesis                       | 13                | 2.731      | 1.51E-03 |
| GO:0005911    | Detection of chemical stimulus     | 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)
Aging-related disorders have been reported such as ED, androgen response to oxidative stress in BP caught our attention. Many regulated response to hormone, osteoblast differentiation and phosphorylation (the second gene ration) in KEGG, and down-regulation), Alzheimer disease (the second gene ration), oxidative DMED, down-regulated metabolic pathways (the highest gene ration), but it showed the highest gene ration in DMED. Also in Wnt/β-catenin pathway, and promoted osteogenic differentiation of osteoblasts. 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. In smoking men the reduction of olfactory acuity could adversely affect sexuality. On the other hand, penile erection occurs in response to visual, olfactory and tactile stimuli initiated within the brain and/or on the periphery. It was reported that cGMP had essential and distinctive functions in olfactory sensation and adaptation. 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 suggested that identification of C3 might be a therapeutic target for DN. We will pay attention to the possibility of C3 as a tissue of diabetic ED rats, and also increased smooth muscle cell/collagen fibril proportions. 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. Current studies suggested strong correlations between low testosterone, metabolic syndrome and aging. Oxidative stress, balance between superoxide and NO, and deficiency of testosterone led to metabolic impairment and accelerated aging. 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. 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. In osteoblast-like cells, iNOS and eNOS stimulated by metformin inhibited the GSK3β/Wnt/β-catenin pathway, and promoted osteogenic differentiation of osteoblasts.
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. 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. Research has shown that zinc can induce ubiquitin conjugation in cultured hippocampal neurons. A cGMP-hydrolyzing phosphodiesterase, PDE9A, was recently identified as a novel interactor and substrate of neuralized E3 ubiquitin protein ligase 1. Prolonged PDE9 was detected in NO-cGMP-mediated cavernosal responses, and may be of human corpus cavernosum; and PDE9 inhibition amplified the GO:0009986 Glutamate binding 3 1.034 1.54E-02

GOTERM_MF_DIRECT†

GO:0036748 Acetylation 4 1.379 1.55E-02

GO:0032616 Protein K13-linked ubiquitination 4 1.379 1.55E-02

GO:0006182 cGMP biosynthetic process 3 1.034 2.53E-02

GO:0023347 Peptide bond formation 3 1.034 2.53E-02

GO:0009725 Response to hormone† 6 2.069 1.22E-02

GO:0009135 Beta-oxidation 2 0.690 4.07E-02

GO:0005769 Extracellular region, cGMP=P 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 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).

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. Our outcomes. 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. 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.

Related to pathway-associated biomarkers, some research tried to identify and map potential novel genes, probe the relationship between the DEGs and altered or dysregulated pathways, and to predict and prioritize pathway-associated biomarkers. 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.
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.

Acknowledgments
The authors thank Dr Jacob Rajfer for his help throughout the writing process.

Author contributions
Data curation: Guangying Wang, Dayue Shen, Xilan Zhang, Yuanping Li.
Formal analysis: Dayue Shen.
Funding acquisition: Hui Liao.
Methodology: Guangying Wang, Yuanping Li, Hui Liao.
Software: Guangying Wang, Xilan Zhang, Yuanping Li.
Supervision: Monica G Ferrini.
Validation: Guangying Wang, Dayue Shen, Xilan Zhang, Monica G Ferrini, Yuanping Li, Hui Liao.
Visualization: Guangying Wang, Dayue Shen, Xilan Zhang, Monica G Ferrini, Yuanping Li, Hui Liao.
Writing – original draft: Hui Liao.
Writing – review & editing: Monica G Ferrini, Hui Liao.

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 transplanation 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 (lactic-co-glycolic) acid microspheres improved erectile function in a rat model of bilateral cavernous nerve injury. J Sex Med 2019;16:183–93.

[7] Yilmaz E, Kaya-Sezginer E, Yilmaz-Oral D, Cengiz T, Bayart T, Gür S. Effects of hydrogen sulphide donor, sodium hydrosulphide treatment on the erectile function in L-NAME-induced hypertensive rats. Andrologia 2019;51:e13240.

[8] Brien SE, Smallengane C, Gofion 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;13:219–205.

[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, Bithra 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 IncRNA ADAMTS9-AS1 in prostate cancer. Front Genet 2020;11:361.

[14] Kumar SU, 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] Oruçtemur A, Ozbek E, Sahn S, et al. Low serum insulin-like growth factor-1 in patients with erectile dysfunction. Basic Clin Androl 2016;26:61.

[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, Willsie SE, Ledoux P, et al. NCBI GEO: archive for functional genomics data sets–update. Nucleic Acids Res 2013;41:D991–5.

[20] Huang DA, 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 Consortium. The gene ontology resource: 20 years and still going strong. Nucleic Acids Res 2019;47:D330–8.
[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] Oztun 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] Sludara H, Hotta K, Oka K. Compartamentalized cGMP responses of olfactory sensory neurons in caenorhabditis elegans. J Neurosci 2017;37:3753–63.

[55] 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.

[56] 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.

[57] 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.

[58] 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.

[59] Sun KJ, Zhu L, Wang HD, et al. Zinc as mediator of ubiquitin conjugation following traumatic brain injury. Brain Res 2013;1506:132–41.

[60] Taal K, Tutikeme J, Rullinkov G, et al. Neuralized family member NEURL1 is a ubiquitin ligase for the cGMP-specific phosphodiesterase 9A. Sci Rep 2019;9:7104.

[61] 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.

[62] Boyce G, Shob 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.

[63] Reid WM, Rolfe A, Register D, Levassor JE, Churn SB, Sun D. Strain-related differences after experimental traumatic brain injury in rats. J Neurotrauma 2010;27:1243–53.

[64] 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.

[65] 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.

[66] 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.

[67] 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.

[68] 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.

[69] 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.