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Case Study

A genome-wide association study to identify candidate genes for erectile dysfunction

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Abstract

Erectile dysfunction (ED) can be caused by different diseases and controlled by several genetic networks. In this study, to identify the genes related to ED, the expression profiles of normal and ED samples were investigated by the Gene Expression Omnibus (GEO) database. Seventeen genes were identified as associated genes with ED. The protein and nucleic acid sequences of selected genes were retrieved from the UCSC database. Selected genes were diverse according to their physicochemical properties and functions. Category function revealed that selected genes are involved in pathways related to humans some diseases. Furthermore, based on protein interactions, genes associated with the insulin pathway had the greatest interaction with the studied genes. To identify the common *cis*-regulatory elements, the promoter site of the selected genes was retrieved from the UCSC database. The Gapped Local Alignment of Motifs tool was used for finding common conserved motifs into the promoter site of selected genes. Besides, INSR protein as an insulin receptor precursor showed a high potential site for posttranslation modifications, including phosphorylation and N-glycosylation. Also, in this study, two Guanine-Cytosine (GC)-rich regions were identified as conserved motifs in the upstream of studied genes which can be involved in regulating the expression of genes associated with ED. Also, the conserved binding site of miR-29-3p that is involved in various cancers was observed in the 3′ untranslated region of genes associated with ED. Our study introduced new genes associated with ED, which can be good candidates for further analyzing related to human ED.

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Introduction

Sexual issues in marital life are among the most important issues in life, as there is a two-way relationship between sexual dysfunction and marital discord, and about half of all divorce cases are due to sexual problems [1, 2]. Studies have shown that the prevalence of sexual problems during life is 68% in women and 54% in men [3, 4]. These statistics determine the need for timely and accurate treatment of these problems for the treatment team. Sexual problems are generally divided into three major groups: sexual identity disorders, sexual dysfunction and sexual perversions. Among sexual dysfunctions, erectile dysfunction (ED) has the highest prevalence [5, 6]. The main thing about people with ED is that they are not able to have successful sex because of the loose penis and poor erection. But these people are not considered patients and their impotence is not a disease. It is a sign of diseases such as diabetes or heart disease [7–9].

Diseases that cause ED include diabetes [10, 11], heart disease [12, 13], metabolic syndrome [14], high blood pressure [15], aging [16, 17], obesity [18], excessive alcohol consumption [19], thyroid dysfunction [20], postoperative benign prostate cancer [21], prostate cancer [22], sex hormone dysfunction [23], chronic renal failure [24], dysfunction of the pituitary gland [25], the hypothalamus in the brain and testicles [26], neurogenic diseases [27], multiple sclerosis [28], Parkinson's [29], spinal cord injury and tumor [30], trauma to the pelvis [31], pelvic surgery [32], pelvic radiation and chemotherapy [32–34], increased blood prolactin [35] and primary testicular failure due to infection, cancer or genetic [36–38].

ED can occur in both primary and secondary forms. Primary ED refers to men who have never had an erection or sexual intercourse in their lives. Secondary ED is very common. Men who have had normal sex before are then told they have become incapacitated, but it is not clear to what extent they have become incapacitated [23, 39, 40]. Short-term disability can sometimes occur chronically or gradually in the context of the aforementioned organic diseases. Weakness in transient sexual power occurs most often in adolescence when a man is unable to perform well during his first sexual intercourse despite all his tastes [8, 30].

ED also occurs in men who are under severe stress due to work or emotional problems, and as soon as the stress is relieved, they can have successful sexual intercourse again [36, 41]. Sometimes ED is so insignificant and so frightening in men that it is very distressing in their relationships and doubles the problem. Women need to be very sensitive about this and should not expect too much from their husbands. Spouses' patience and understanding are very effective in treating ED. On the other hand, the man should talk and consult with his wife about this so that he considers the woman as his supporter. If a man tries to solve this problem alone, it usually does not work [42, 43]. Although transient ED is not a concern, permanent ED cannot be cured with professional help. Sexual dysfunction can sometimes occur in the long run or in some cases suddenly. In both cases, the couple should not be negligent about medical care, because if left untreated, it can weaken the foundations of married life and even cause its disintegration and ultimately lead to a weakening of the man's morale and self-esteem [36, 44].

In many investigations about the relationship between certain gene expressions and ED, animal models of diabetic mice have been studied. Here are some reports. In 2013, Lacchini *et al.* determined the VEGF polymorphism and its relationship with ED. By studying 126 patients, they reported three polymorphisms of the VEGF gene promoter [2578C> A (rs699947), −1154G> A (rs1570360) and −634G> C (rs2010963)]. Finally, it was pointed out that genotype 1155AA has a significant relationship with ED [45]. In the same year, Liu *et al.* [46] reported that the reduction of VEGF gene expression is related to ED. They used a mouse model of diabetes for this purpose. Then the relationship between ED and tumor necrosis factor alpha (TNF-α) [47], GNB3 [48], IGFBP-3, [49], FGF2 [50], VEGF [51], DDAH2 [52], hNGFβ [53], NOS3 expression genes [54], Ad-COMP-Ang1 [55] and HMGCS2 [56](12) have been studied. In 2013, Zhang *et al.* [57] conducted a meta-analysis and found no significant relationship between angiotensin converting enzyme (ACE) gene expression and ED. In 2014, Chen *et al.* [58] reported that ED is significantly associated with the expression of PTAFR, IL27, CD37, CD40, IL7R, PSMB9 and CXCR3 genes. Kovanecz *et al.* [59], Kam *et al.* [60], Pan *et al.* [61] and Vishnubalaji *et al.* [62] used microarray technology to check the expression of ED-related genes. In 2015, Dai *et al.* [63] revealed the eNOS G894T gene polymorphism has no significant relationship with the ED. In 2015, Pan *et al.* [61] showed that some of long noncoding RNAs or lncRNAs effect on ED. Also, in 2018, the relationship between ED and GNB3 C825T, eNOS T-786C and eNOS G894T gene polymorphism was studied by Ben Khedher *et al.* [64]. In 2019, Segura *et al.* studied the relationship between ED and eNOS gene polymorphisms at T786C, 4VNTR and G894 T positions [65]. Mostaza *et al.* [66] studied the relationship between ED and PCSK9 gene polymorphism. Experiments have shown that reducing the expression of the LEGRNA MEG3 gene can reduce ED [67].

In this study, we attempt to analyze a new set of genes associated with ED. The selected genes were studied based on the biochemical characteristics, function, interaction pathways, conserved motifs in the promoter regions and their posttranslation/transcription modifications.

Materials and methods

Identification of gene associated with ED

To identify the new genes related to ED, the expression profiles of normal and ED samples were investigated using the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). Seventeen genes that, compared to control, showed significant differential expression were selected as genes associated with ED. The sequences (protein and nucleic acid) of selected genes were retrieved from the UCSC database (https://genome.ucsc.edu/). Furthermore, the sequence of selected genes was analyzed based on the biochemical traits, including isoelectric point (PI), molecular weight (MW), instability index and grand average of hydropathy (GRAVY) by ProtParam tool of Expasy database [68] (https://web.expasy.org/protparam/).

Functional category of selected genes

The protein sequences of selected genes were analyzed using the BlastKOALA tool (https://www.kegg.jp/blastkoala/) to

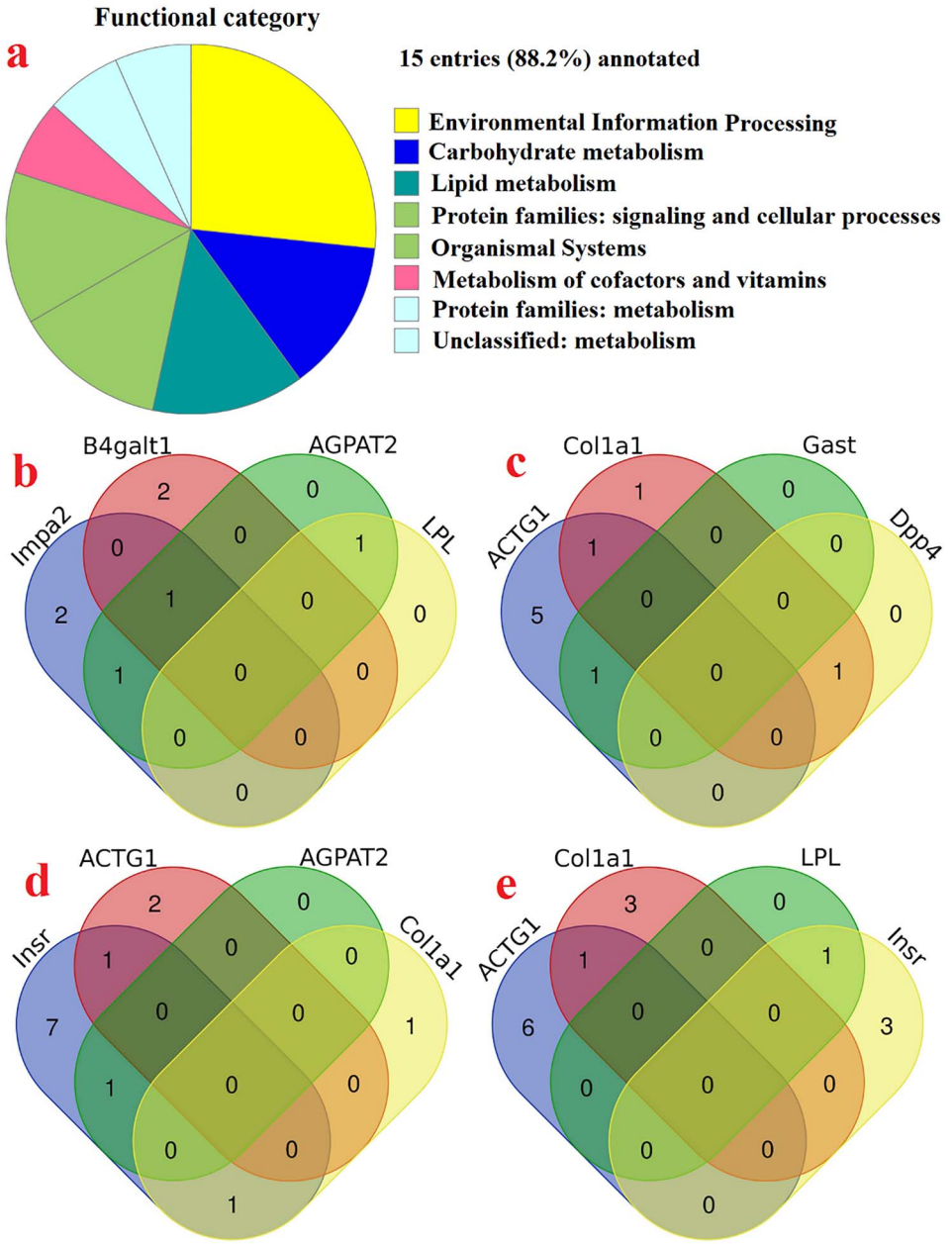

Figure 1. Functional category of studied genes (**A**), Venn diagrams based on metabolic (**B**), organismal systems (**C**), signaling (**D**) and human diseases (**E**).

determine the function category and identify the common pathways. Besides, Venn diagrams of common pathways were drawn using the Bioinformatics and Evolutionary Genomics tool (http://bioinformatics.psb.ugent.be/webtools/Venn/).

Identification of conserved motifs in promoter site

To identify the common *cis*-regulatory elements, the promoter site (1500 bp upstream of transcription start site) of selected genes was retrieved from the UCSC database. The Gapped Local Alignment of Motifs (GLAM2) tool was used [69] for finding common conserved motifs into the promoter site of the selected genes.

Identification of microRNA targets

Target Scan Human (http://www.targetscan.org/vert_72/) was applied to identify the conserved site for microRNA (miRNA) families that are broadly conserved among vertebrates.

Prediction the N-glycosylation and phosphorylation sites

The NetNGlyc 1.0 server (http://www.cbs.dtu.dk/services/NetNGlyc/) was used to predict the potential N-glycosylation sites into the protein sequence of selected genes [70]. Also, phosphorylation sites were predicted using the NetPhos 3.1 server (http://www.cbs.dtu.dk/services/NetPhos/) [71].

Table 1. The properties of selected genes related to human ED

Gene name	Gene Acc.	Chromosome	Length (aa)	MW (kDa)	PI	Instability index	GRAVY	N-glycosylation site
SMR3B	NM_006685.4	Chr.04	79	8.18	9.62	Unstable	−0.415	–
LPL	NM_000237.3	Chr.08	475	53.16	8.37	Unstable	−0.321	2 (284, 386)
AGPAT2	NM_006412.4	Chr.09	278	30.91	9.21	Unstable	0.310	1 (59)
B4galt1	NM_001497.3	Chr.09	398	43.92	8.88	Unstable	−0.206	1 (113)
Sdc3	NM_014654.4	Chr.01	442	45.49	4.61	Unstable	−0.231	–
Pdk4	NM_002612.4	Chr.07	411	46.47	6.19	Unstable	−0.191	2 (81, 187)
Sln	NM_003063.3	Chr.11	31	3.76	8.34	Stable	1.065	1 (11)
Lox	NM_002317.7	Chr.05	417	46.94	8.36	Unstable	−0.719	3 (81, 97, 144)
Retsat	NM_017750.4	Chr.02	610	66.82	8.54	Unstable	0.030	–
Slc41a3	NM_001164475.1	Chr.03	507	54.76	7.97	Unstable	0.599	–
Dpp4	NM_001935.4	Chr.02	766	88.27	5.67	Unstable	−0.340	8 (85, 92, 150, 219, 229, 263, 281, 321)
G0s2	NM_015714.4	Chr.01	103	11.32	9.73	Unstable	−0.137	–
Impa2	NM_014214.3	Chr.18	288	31.32	6.15	Stable	0.014	–
Col1a1	NM_000088.3	Chr.17	1464	138.91	5.60	Stable	−0.786	1 (1365)
Gast	NM_000805.5	Chr.17	101	11.39	5.08	Unstable	−0.770	–
Insr	NM_000208.4	Chr.19	1382	156.33	5.83	Unstable	−0.359	18 (43, 52, 105, 138, 242, 282, 364, 424, 445, 541, 621, 633, 651, 698, 769, 782, 920, 933)
ACTG1	NM_001614	Chr.17	375	41.79	5.31	Stable	−0.199	1 (12)

Protein–protein interactions

Protein–protein interactions (PPIs) of the studied genes were evaluated using the string v11 tool [72]. Besides, the gene ontology (GO) analysis for molecular function enrichment was done based on protein interactions.

Results

Physiochemical properties of selected genes

The physicochemical properties of 17 genes associated with ED are shown in Table 1. The table lists the gene name, gene ID, protein length, MW, PI, instability index and prediction of N-glycosylation site. Results illustrated that the selected genes are distributed on chromosomes 1, 2, 3, 4, 5, 7, 8, 9, 11, 17, 18 and 19. The length of protein sequence varied from 31 to 1464 aa, with SLN being the smallest protein while COL1A1 being the largest protein. Besides, the MW of selected genes ranged from 3.76 to 156.33 kDa. Furthermore, the PI ranged from 4.61 to 9.73; SDC3 was predicted as an acidophilic protein, while G0s2 was predicted as an alkaline protein. Based on the protein instability index, most of the studied proteins (13/17) were unstable. Also, most proteins showed negative GRAVY revealing hydrophilic properties.

Functional category of selected genes

The selected genes were classified based on their function using the BlastKOALA tool. The selected genes were separated into seven groups, including environmental information processing, carbohydrate metabolism, lipid metabolism, signaling and cellular processes, organismal systems, metabolism of cofactors and vitamins and metabolism (Figure 1A). Also, Venn diagrams of studied genes were designed based on common pathways. Impa2, B4galt1 and AGPAT2 gene are involving in metabolic pathways (Figure 1B). Besides, ACTG1, Col1a1, Gast and Dpp4 are jointly involved in organismal systems such as platelet activation, gastric acid secretion and protein digestion and absorption (Figure 1C, Supplementary Table S1 available online at https://academic.oup.com/bib). Furthermore, the pathways related to environmental information processing, including the Rap1 signaling pathway, phospholipase D signaling pathway and PI3K-Akt signaling pathway, are identified as the common signaling pathways between Insr, AGPAT2, ACTG1 and Col1a1 gene (Figure 1D, Supplementary Table S1 available online at https://academic.oup.com/bib). Also, human diseases such as proteoglycans in cancer and Alzheimer's are identified as the common pathways that ACTG1, Col1a1, LPL and Insr genes are involved (Figure 1E, Supplementary Table S1 available online at https://academic.oup.com/bib).

Protein–protein interaction

PPIs play a key role in most of the biological processes in living organisms, consist of DNA transcription and replication, enzyme-adjusted metabolic reactions, protein transport, signaling transduction, cell cycle regulation and protein degradation. In this study, we used the STRING v11 [72] to construct a protein interaction network of studied proteins and the other proteins in human (Figure 2), and the predicted interactions were based on gene neighborhood, gene fusions, gene co-occurrence, text-mining, co-expression and proteins homology. There were no significant interactions among studied proteins. However, IGF1, INS, IRS1 and PTPN1 were identified as important connecter proteins that can affect the interaction between the selected genes associated with ED. Also, INS protein showed high interaction with the other proteins, indicating the key role of this protein. The GO annotations of the interacted proteins based on molecular function revealed the momentous engagement of them in insulin-like growth factor (IGF) receptor binding, insulin receptor binding, protease binding, identical protein binding, platelet-derived growth factor binding, signaling receptor binding, growth factor binding and hormone activity (Table 2).

Identification of *cis*-regulatory elements related to ED

To identify common conserved motifs in the upstream of selected genes, the promoter site (1500 bp of upstream the start

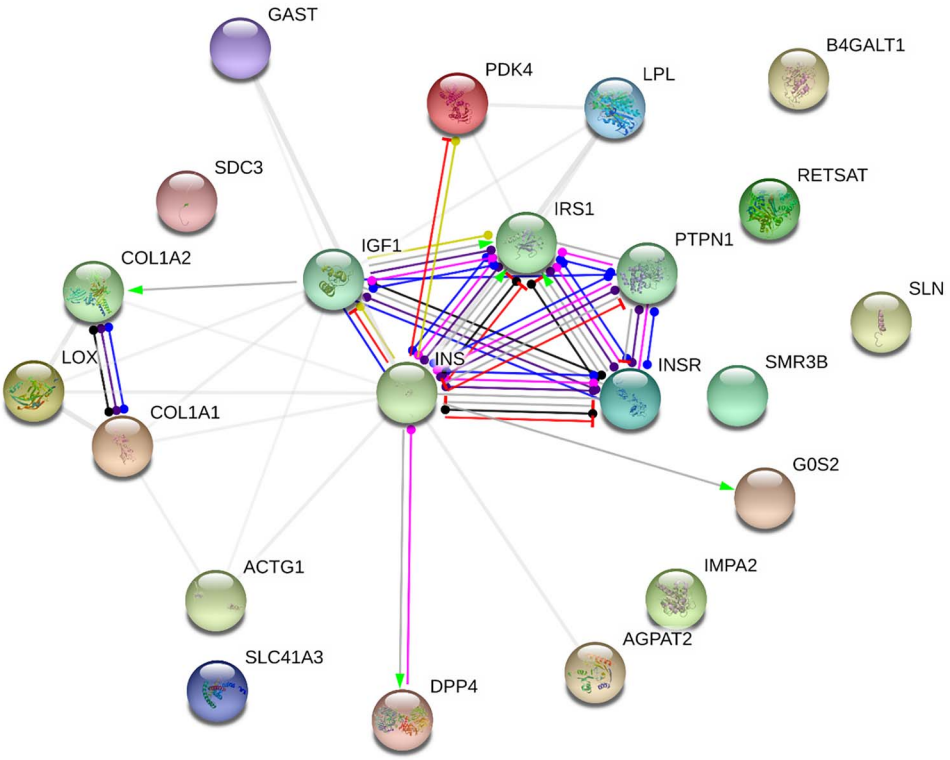

Figure 2. PPI between selected genes.

Table 2. Molecular function enrichment (FDR < 0.01) based on GO analysis of genes of Figure 2

GO-term	Description	Count in the gene set	FDR
GO:0005159	IGF receptor binding	4 of 16	1.00e-06
GO:0005158	Insulin receptor binding	4 of 23	1.80e-06
GO:0002020	Protease binding	4 of 135	0.00094
GO:0042802	Identical protein binding	9 of 1754	0.0028
GO:0048407	Platelet-derived growth factor binding	2 of 11	0.0033
GO:0005102	Signaling receptor binding	8 of 1513	0.0045
GO:0019838	Growth factor binding	3 of 126	0.0091
GO:0005179	Hormone activity	3 of 123	0.0091

codon) was screened using the GLAM2 tool. Two GC-rich regions were identified as conserved motifs in the upstream of studied genes, which can be involved in regulating the expression of the selected genes (Figure 3). The consensus sequence GGG (A/C) GGG was observed in the promoter site of most studied genes.

Prediction the posttranslation modification

Posttranscriptional modification such as phosphorylation and glycosylation can affect the stability and activity of proteins. In this study, the potential phosphorylation and glycosylation sites were predicted into the amino sequence of the selected genes associated with ED (Table 1, Figure 4). According to the result of predicted N-glycosylation sites, Insr and Dpp4 were identified as N- hyperglycosylated proteins that may be mostly glycosylated (Table 1). Also, according to the results of the predicted phosphorylation, Insr, Dpp4, Col1a1 and Sdc3 were more phosphorylated (Figure 4).

Prediction of the target site of miRNA

The transcript sequences of selected genes were screened to find the conserved site for miRNA families that were broadly conserved among vertebrates. The results revealed that 12 of 17 selected genes that were associated with ED were targeted by diverse miRNA families of vertebrates (Figure 5). The binding sites of miR-29-3p were observed in the transcript sequence of five genes, while the transcript sequence of four genes was targeted by miR-27-3p and miR-15-5p/16-5p/195-5p/424-5p/497-5p. Also, the binding sites of miR-33-5p, miR-138-5p, miR-143-3p, miR-124-3p/506-3p, miR-103-3p/107 and miR-133a-3p.2/133b were observed in the transcript sequence of studied genes.

Discussion

Sexual dysfunction is any type of complication that disrupts the sexual response cycle. The sexual response cycle is a set of body changes and emotions experienced during sexual intercourse. The sexual response cycle includes excitement, plateau,

NAME	START		END	STRAND
LPL	1075	gggtgggga tg ..... gggtgcgggg . tgagtgaggga	1106	+
AGPAT2	1495	gggcgggggc gc ..... ggggggaggagcgcgcggcaggg	1458	-
B4galt1	1485	gggcgggggc cc ..... ggggggcgggggccgggcgcgggg	1443	-
Sdc3	1440	gggcggaac. ..... ggaggagggcccgggggcgggg	1410	-
Pdk4	912	gtgccggtc tc ..... ggggggcggtg . ggggtgggggc	955	+
SLN	364	atgaggaaa tg ..... ggggtatgag . gaatggaggga	426	+
LOX	1116	gggaggg tgct ..... ggggg . tgagagggggggcgggg	1085	-
Retsat	1451	gggcggagc ca ..... agaggctgagcgcagtcggagc	1490	+
Slc41a3	161	gggagggac ag ..... agggggcagagccaggtgggtgg	99	-
Dpp4	1424	gtgcgggggc cc ..... ggggggagggg . aaagggcgcgc	1382	-
G0s2	1399	gggaggaaga . ..... agaggagggggaaaaggagggggg	1368	-
Impa2	1111	gggcgggggc tg ..... agggggcgggggccaggtacgggg	1207	+
Col1a1	1461	gggaggaacc cc ..... aggggggaggag . gagggaaggga	1341	-
Gast	1356	gggcgggggc ag ..... agggtagggggcgggggtgggggg	1443	+
Insr	1372	gcgcgggggc cc ..... ggaggcgggggaggcgggcgggg	1411	+
ACTG1	1303	gggcgggggc cg ..... ggaggcggtgcgggctgggggc	1338	+

Figure 3. The position and sequence of conserved motifs in the promoter sequence of studied genes.

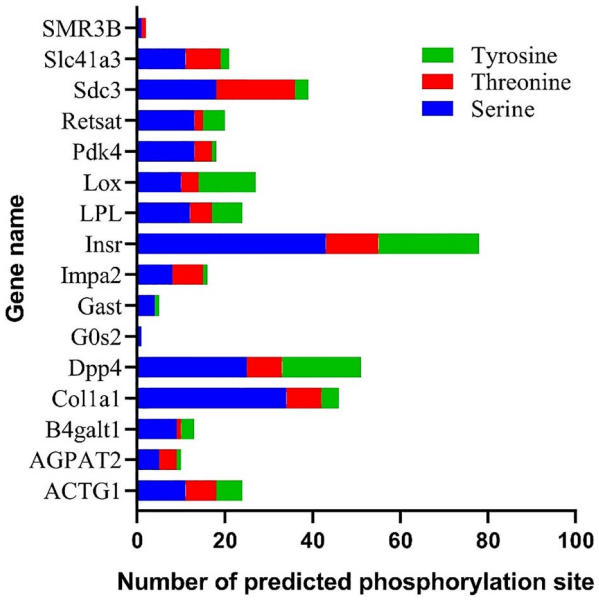

Figure 4. The prediction of phosphorylation sites into the amino sequence of studied genes.

orgasm and resolution. This cycle begins with the desire and then arousal phase, then the stimulation reaches the stabilization phase, and in the next phase, the person experiences orgasm and reaches the orgasm phase; and finally, the subsidence is experienced. Any problem that arises in one of these stages leads to dissatisfaction and sometimes even disappointment and frustration. All of these steps depend on the expression of a series of genes [73, 74].

ED is also related to several physiological factors. For a successful erection to take place, it is necessary that several processes have already taken place or that the processes take place at the same time. Physiological processes such as the different levels of hormones involved, the biosynthesis of these hormones and the efficiency of these hormones are important in the formation of a successful erection. All of these physiological processes related to erection are related to the expression of genes for which there are reports, and much research is being done to increase knowledge in this regard [75, 76].

The ED is a male sexual dysfunction that can associate with other diseases such as depression, diabetes, cardiovascular disease and metabolic syndrome [36, 77]. Also, several genetic factors are related to ED. In this study, 17 genes that showed differential expression between the normal and ED samples were selected as genes associated with ED. The selected genes were analyzed based on bioinformatics tools to reveal new aspects of their functions, interactions and regulatory systems. According to the results, the selected genes showed high variation based on function, MW, PI, protein length, location and GRAVY value. Besides, the most selected genes were predicted unstable proteins indicating that the stability of the enzymes is low in the involved cellular reactions [78]. In addition, GRAVY value as a solubility index [79] revealed that the most studied proteins have negative GRAVY value, indicating that they are more hydrophilic [80]. Also, in this study, two GC-rich conserved motifs were detected in the promoter site of studied genes affecting the expression patterns by activating and suppressing the transcription of studied genes in response to stresses.

In addition, the selected genes were functionally grouped in environmental information processing, carbohydrate metabolism, lipid metabolism, signaling, cellular processes, organismal systems, metabolism of cofactors and vitamins and metabolism. Also, ACTG1 and Col1a1 are involved in proteoglycans in cancer, and LPL and Insr are involved in Alzheimer's. It seems that some diseases such as cancer can cause ED. For instance, prostate cancer reduces sexual desire and causes ED [81]. Also, the correlation was observed between ED and Alzheimer's disease

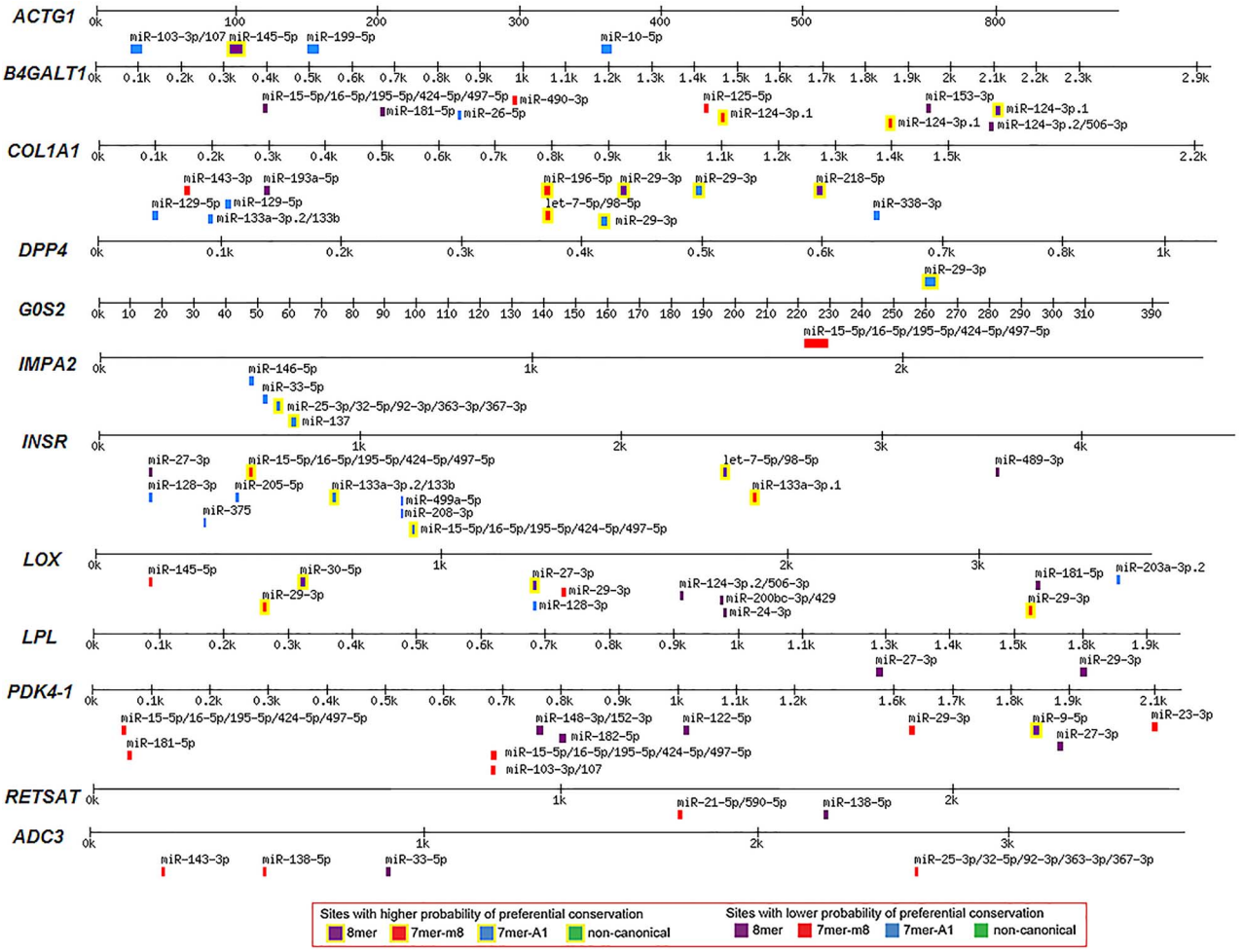

Figure 5. The position of miRNA targets in transcript sequences of the studied genes.

[82], and ED is common in patients with Alzheimer's disease and Parkinson's disease [83]. Bovijn *et al.* [84] completed a genome-wide association study of ED in 6175 case subjects among 223 805 European men, and identified one locus at 6q16.3 (lead variant rs57989773, OR 1.20 per C-allele; p¼5.7131014) located between MCHR2 and SIM1. Their analysis suggested SIM1 confers ED risk through hypothalamic dysregulation. These results provide insights into the biological underpinnings and the causes of ED and may help order the development of future therapies for this common disorder.

According to the results of the interaction network of selected genes and the other related genes, IGF receptor binding and insulin receptor binding were identified as significant GO annotations based on molecular function. ED is commonly associated with diabetes, and the correlation between insulin and ED is observed among diabetic patients [85, 86]. In diabetic rats, transferring the insulin-like growth factor-1 (IGF-1) gene could increase erectile function [85]. Furthermore, there is a link between testosterone levels and insulin resistance in a population of men with ED [87]. Also, some conditions such as reduced libido and premature ejaculation are linked with diabetic ED [88].

Posttranslational modifications play critical roles in regulating protein function [89]. In this study, INSR protein as an insulin receptor precursor showed a high potential site for posttranslation modifications, including phosphorylation and N-glycosylation. Phosphorylation is involved in key processes such as PPIs, regulating cell signaling, cell division and protein stability [78, 90]. Besides, glycosylation regulates the stability of a protein and may modify the MW of target proteins [80, 91]. In addition, posttranslational modifications such as GlcNAcylation/phosphorylation are involved in diabetes and Alzheimer's disease [92]. These results confirmed a relationship between diabetes and Alzheimer's with ED. In this regard, posttranslation modifications such as phosphorylation and N-glycosylation may play key important roles as regulator systems.

miRNAs are the group of noncoding RNAs controlling the expression of genes after transcription and involving in diverse biological and molecular processes [93]. In this study, the conserved binding site for putative miRNA families, including miR-29-3p, miR-27-3p and miR-15-5p/16-5p/195-5p/424-5p/497-5p, was observed in the 3′ untranslated region (UTR) of genes associated with ED. MiR-29-3p family is involved in various cancers [93, 94] and downregulated in tumor tissues, including lung cancer [95], stomach cancer [96], glioblastoma [97], bladder cancer [98], prostate cancer [99], ovarian cancer [100] and renal carcinoma [101]. In this study, the binding site of miR-29-3p was observed in five genes associated with ED. Besides, the binding site of miR-27-3p was detected in four genes related to ED. MiR-27-3p family is associated with metabolisms related to diseases such as liver cancer [102]. According to results, selected genes

associated with ED can be regulated by the same miRNAs which affect the related pathways and metabolisms.

Conclusion

In this study, 17 genes associated with human ED were analyzed using available bioinformatics tools. Our results revealed that selected genes are also related to human diseases such as Alzheimer's disease, diabetes and cancers. Also, the relationships between ED and diabetes were observed. In addition, miR-29-3p was identified as a regulator element related to ED. Our study introduced a network of genes which probably associates with ED, and they can be good candidates for further analyzing related to human ED.

Key Points

- Genes related to ED are involved in pathways related to human diseases such as diabetes and Alzheimer's disease.
- Based on protein interactions, genes associated with the insulin pathway had the greatest interaction with the studied genes.
- The INSR protein, which is the precursor of the insulin receptor, showed a high potential site for posttranslational modifications, including phosphorylation and N-glycosylation.
- The conserved binding site of miR-29-3p was observed in the 3′ UTR of genes associated with ED.
- Our research has identified new genes related to ED. These genes may be good candidates for further analysis related to human ED.

Supplementary data

Supplementary data are available online at *Briefings in Bioinformatics*.

Authors' contributions

Software was contributed by D.K. and P.H.; supervision was contributed by J.Z. and M.K.; writing, review and editing were contributed by E.K., K.A. and D.K.; investigation was contributed by E.K. and P.H.; validation was contributed by B.M. and Y.M. and H.K. was the advisor. All authors have read and agreed to the published version of the manuscript.

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Compliance with ethical standards

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

This article does not contain any studies with human participants.

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