Bioinformatics Analysis of Next Generation Sequencing Data Identifies Molecular Biomarkers Associated With Type 2 Diabetes Mellitus

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ABSTRACT

BACKGROUND: Type 2 diabetes mellitus (T2DM) is the most common metabolic disorder. The aim of the present investigation was to identify gene signature specific to T2DM.

METHODS: The next generation sequencing (NGS) dataset GSE81608 was retrieved from the gene expression omnibus (GEO) database and analyzed to identify the differentially expressed genes (DEGs) between T2DM and normal controls. Then, Gene Ontology (GO) and pathway enrichment analysis, protein-protein interaction (PPI) network, modules, miRNA (micro RNA)-hub gene regulatory network construction and TF (transcription factor)-hub gene regulatory network construction, and topological analysis were performed. Receiver operating characteristic curve (ROC) analysis was also performed to verify the prognostic value of hub genes.

RESULTS: A total of 927 DEGs (461 were up regulated and 466 down regulated genes) were identified in T2DM. GO and REACTOME results showed that DEGs mainly enriched in protein metabolic process, establishment of localization, metabolism of proteins, and metabolism. The top centrality hub genes *APP, MYH9, TCTN2, USP7, SYNPO, GRB2, HSP90AB1, UBC, HSPA5*, and *SQSTM1* were screened out as the critical genes. ROC analysis provides prognostic value of hub genes.

CONCLUSION: The potential crucial genes, especially *APP, MYH9, TCTN2, USP7, SYNPO, GRB2, HSP90AB1, UBC, HSPA5*, and *SQSTM1*, might be linked with risk of T2DM. Our study provided novel insights of T2DM into genetics, molecular pathogenesis, and novel therapeutic targets.

KEYWORDS: bioinformatics analysis, differentially expressed genes, hub genes, Type 2 diabetes mellitus, pathway enrichment analysis

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Introduction

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder and is characterized primarily by a decrease in insulin secretion, typically accompanied by insulin resistance.[1] Globally, it is predicted that 25 million adults (20-79 years) have diabetes, projected to reach 629 million by 2045 and is the ninth leading cause of death.[2,3] T2DM is mainly associated with macrovascular complications include stroke, coronary artery disease and peripheral arterial disease, and microvascular complications include diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy, and non-vascular diabetes complications include nonalcoholic fatty liver disease, psychiatric disease, obesity, cancer, cognitive impairment, infections, and disability.[4] There are several important risk factors for T2DM, such as age, sex, family history of diabetes, hypertension, obesity, abdominal obesity, stress in the workplace or home, a sedentary lifestyle, smoking, insufficient fruit and vegetable consumption, physical activity, genetic, and environmental causes.[5] Our understanding of the occurrence and development mechanism of T2DM has been greatly improved; however, the cause and potential molecular mechanism of T2DM are still unclear.[6] Therefore, it is necessary to identify key genes and pathways for understanding the molecular mechanism and discovering potential biomarkers for T2DM.

In recent decades, more and more researchers have devoted themselves to exploring the potential mechanisms for progression of T2DM. Recent investigation have shown that key biomarkers, such as *HHEX, CDKN2A/B*, and *IGF2BP2*,[7] *CDKAL1* and *HHEX/IDE*,[8] *ADIPOQ, PPAR-γ*, and *RXR-α*,[9] ABCC8 and *KCNJ11*,[10] *TCF7L2, SLC30A8, PCSK1*, and *PCSK2*[11] were involved in the T2DM. Recent studies have shown that signaling pathway, including *PI3K/AKT*-and *AMPK* signaling pathway,[12] mTOR signaling pathway,[13] insulin signaling pathway,[14] *AGE/RAGE/JNK, STAT3/SCOS3*, and *RAS* signaling pathway,[15] and *ERK* signaling pathway[16] were involved in progression of T2DM. Therefore, it is of great

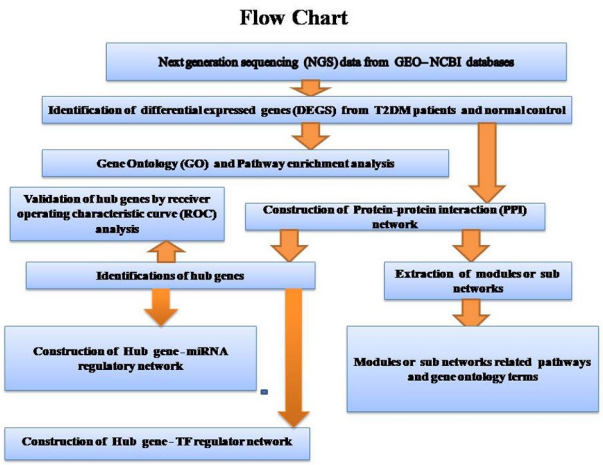

Figure 1. Research design flow chart.

practical significance to explore the genes and signaling pathways of T2DM on islet cells.

RNA sequencing technology can rapidly detect gene expression on a global basis and are particularly useful in screening for differentially expressed genes (DEGs) in diseases.[17] RNA sequencing technology allows the investigation of gene expression in a high throughput manner with high sensitivity, specificity and repeatability. Significant amounts of data have been produced via the use of RNA sequencing and the majority of such data has been uploaded and stored in public databases. Indeed, some researchers found key genes and pathways in T2DM by integrated bioinformatics analysis.[18-23] However, the comparative analysis of DEGs across a range of independent investigation might yield only a relatively limited amount of useful data with regard to T2DM advancement. The disadvantages of these single investigations might be overcome by NGS analysis, as this approach would make it possible to analyze the signaling pathways and interaction networks linked with the identified DEGs. This knowledge might help in elucidating the molecular mechanisms underlying T2DM and its associated complications.

In the present investigation, next generation sequencing (NGS) dataset was downloaded from the Gene Expression Omnibus (GEO) (GEO, http://www.ncbi.nlm.nih.gov/geo/)[24]: GSE81608.[25] DEGs were identified in T2DM. We then carried out gene ontology (GO), REACTOME pathway enrichment analysis, protein-protein interaction (PPI) network analysis, module analysis, miRNA-hub gene regulatory network, and TF-hub gene regulatory network analysis to elucidate the underlying molecular mechanisms. Finally, hub genes were validated by receiver operating characteristic curve (ROC). Collectively, the findings of the present investigation highlighted crucial genes and signaling pathways that might contribute to the pathology of T2DM and associated complications. The research flowchart of this investigation was shown in Figure 1. These may provide a basis for the advancement of future diagnostic, prognostic and therapeutic tools for T2DM.

Materials and Methods

Data resources

NGS dataset GSE81608[25] was downloaded from the GEO database. The platform used for NGS data was the GPL16791 Illumina HiSeq 2500 (*Homo sapiens*). The GSE81608 dataset contained data from 1600 samples, including 949 T2DM samples (single human islet cells), and 651 healthy control samples (single human islet cells).

Identification of DEGs

Limma package in R software[26] is a tool to identify DEGs by comparing samples from GEO series. Limma package in R software was used to search for in messenger RNAs (mRNAs; DEGs) that were differentially expressed between T2DM and healthy control samples. The cutoff criteria were an adjusted *P*-value of $<.05$, whereas the logFC value were >0.181 for up regulated genes and <-0.27 for down regulated genes. DEG of this dataset was visualized with volcano map and hierarchical clustering heat map. The volcano plot was drawn using ggplot2 package in R software. Hierarchical clustering heat maps of DEG expression (up regulated genes and down regulated genes) were visualized with gplots package in R software.

GO and REACTOME pathway enrichment analysis of DEGs

GO enrichment analysis (http://geneontology.org/)[27] implements the annotation of biological processes (BP), cellular components (CC) and molecular functions (MF) of DEGs. REACTOME (https://reactome.org/)[28] is a database that stores large amounts of data on genomics, biological pathways, signaling pathways, diseases, drugs, and chemicals. The present investigation used Database for g:Profiler (http://biit.cs.ut.ee/gprofiler/)[29] to perform GO and REACTOME pathway enrichment analysis. $P<.05$ was considered to indicate a statistically significant difference.

Construction of the PPI network and module analysis

The IID interactome database (http://iid.ophid.utoronto.ca/) may be searched for associations between known and predicted proteins, and is commonly used to predict PPI information in molecular biology.[30] Cytoscape 3.8.2 (http://www.cytoscape.org/)[31] was used to visualize the results from the PPI network In this investigation, node degree,[32] betweenness centrality,[33] stress centrality,[34] and closeness centrality,[35] which constitutes a fundamental parameter in network theory, was adopted to evaluate the nodes in a network. The node degree betweenness centrality, stress centrality and closeness centrality methods were calculated using Cytoscape plugin Network Analyzer. Module analysis on the PPI network results was performed

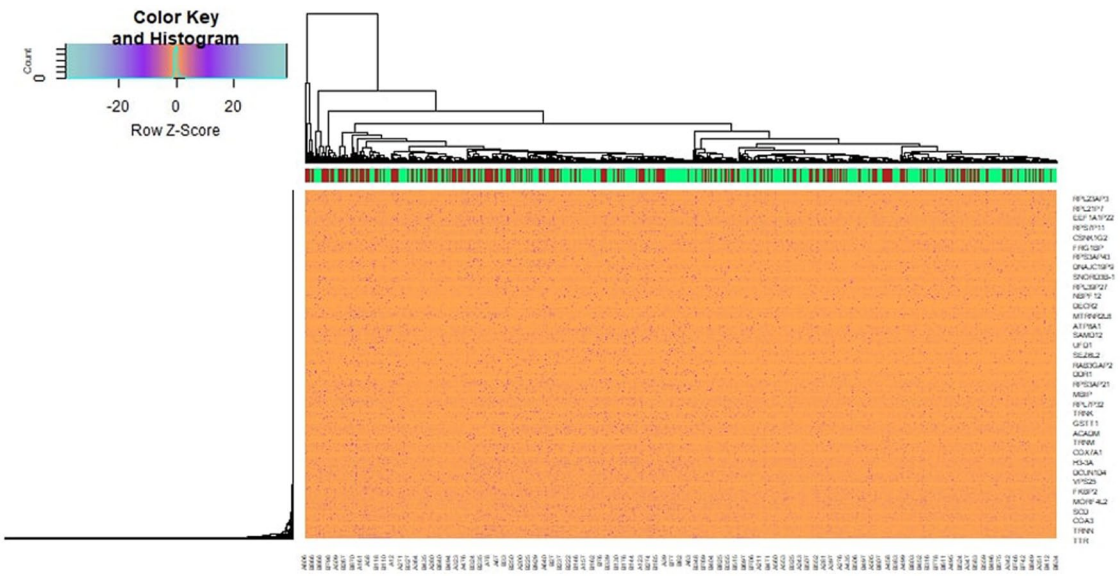

Figure 2. Heat map of differentially expressed genes. Legend on the top left indicate log fold change of genes. (A1-A651 = normal control samples; B1-B949 = T2DM samples).

using the PEWCC1[36] clustering algorithm that comes with Cytoscape. Module analysis might be used to find out more connected gene groups. In addition, the module analysis were further analyzed for GO and pathway enrichment analysis.

MiRNA-hub gene regulatory network construction

Prediction of miRNA-hub genes was performed by miRNet database (https://www.mirnet.ca/).[37] According to the regulatory interaction, miRNA-hub gene regulatory network was constructed based on miRNet by Cytoscape 3.8.2 software.[31]

TF-hub gene regulatory network construction

Prediction of TF-hub genes was performed by NetworkAnalyst database (https://www.networkanalyst.ca/).[38] According to the regulatory interaction, TF-hub gene regulatory network was constructed based on NetworkAnalyst by Cytoscape 3.8.2 software.[31]

Validation of hub genes by receiver operating characteristic curve (ROC) analysis

ROC curve analysis was performed to evaluate the sensitivity and specificity of the hub genes for T2DM diagnosis using the R package "pROC."[39] An area under the curve (AUC) value was determined and used to label the ROC effect. GEO datasets were used in ROC analysis. AUC > 0.8 indicated that the model had a good fitting effect.[40]

Results

Identification of DEGs

A total of 927 genes were identified to be differentially expressed between T2DM and normal control samples with

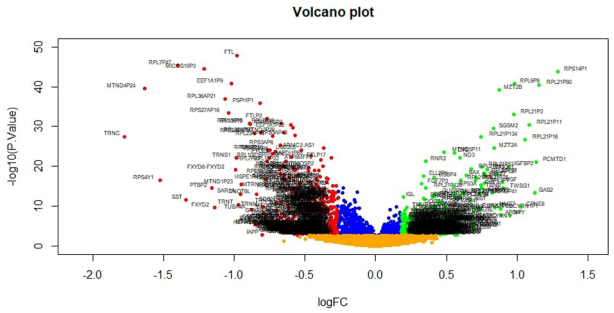

Figure 3. Volcano plot of differentially expressed genes. Genes with a significant change of more than two-fold were selected. Green dot represented up regulated significant genes and red dot represented down regulated significant genes.

the threshold of adjusted *P*-value of <.05, and logFC value were >0.181 for up regulated genes and <−0.27 for down regulated genes. Among these DEGs, 461 were up regulated and 466 down regulated genes in T2DM compared with normal control samples and DEGs are listed in Supplemental Table S1. A heat map (Figure 2) and a volcano plot (Figure 3) for the identified DEGs was generated.

GO and REACTOME pathway enrichment analysis of DEGs

To identify the pathways which had the most significant involvement with the genes identified, up regulated and down regulated genes were submitted into g:Profiler for GO terms are listed in Supplemental Table S2 and REACTOME pathway enrichment analysis are listed in Supplemental Table S3. GO enrichment analysis revealed that in BP terms, the up regulated genes were mainly enriched in protein metabolic process and positive regulation of biological process. Down regulated genes were mainly enriched in establishment of

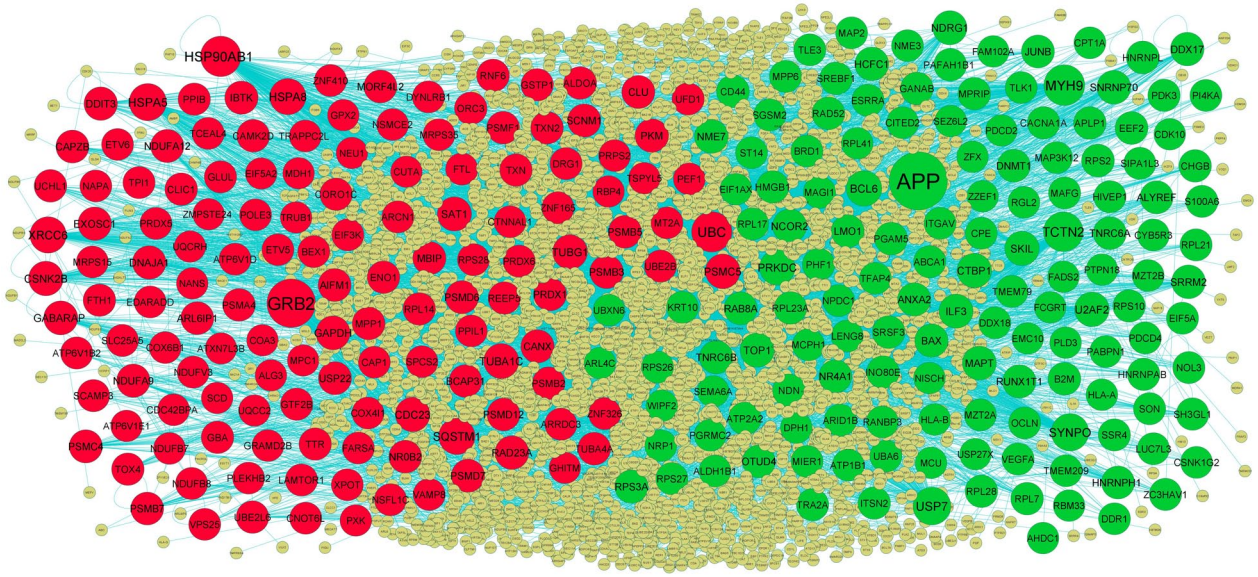

Figure 4. PPI network of DEGs. The PPI network of DEGs was constructed using Cytoscap. Up regulated genes are marked in green; down regulated genes are marked in red. Big node represents nod with more number of interactions and small node represents nod with least number of interactions.

localization and cellular metabolic process. In CC terms, up regulated genes were mainly enriched in intracellular anatomical structure and endomembrane system, whereas down regulated genes were mainly enriched in cytoplasm and intracellular anatomical structure. In MF terms, up regulated genes were mainly enriched in heterocyclic compound binding and protein binding, whereas down regulated genes were mainly enriched in catalytic activity and protein binding. REACTOME pathway enrichment analysis demonstrated that up regulated genes were significantly enriched in metabolism of proteins, and *NR1H3* and *NR1H2* regulate gene expression linked to cholesterol transport and efflux. Down regulated genes were significantly enriched in the metabolism and the citric acid (TCA) cycle and respiratory electron transport.

Construction of the PPI network and module analysis

Following the analysis based on the PPI networks, 4424 nodes and 8670 edges were identified in Cytoscape (Figure 4). The genes with higher scores were the hub genes, as the genes of higher node degree, betweenness centrality, stress centrality, and closeness centrality might be linked with T2DM. The top hub genes include *APP, MYH9, TCTN2, USP7, SYNPO, GRB2, HSP90AB1, UBC, HSPA5*, and *SQSTM1*, and topological properties of each hub genes in PPI network is given in Table 1. A total of 2 modules were selected through PEWCC1 analysis, and module 1 had nodes 98 and edges 117 (Figure 5A) and module 2 had nodes 81 and edges 248 (Figure 5B). Enrichment analysis demonstrated that modules 1 and 2 might be linked with RNA polymerase II transcription, intracellular anatomical structure, metabolism of proteins, protein metabolic process, positive regulation of biological process, metabolism, immune system, establishment of localization, cytoplasm, neutrophil degranulation, cellular metabolic process, intracellular anatomical structure, and protein binding.

MiRNA-hub gene regulatory network construction

The hub genes of the DEGs in T2DM were performed by online databases miRNet. Based on the miRNAs, a miRNA-hub gene regulatory network was constructed with 2630 nodes (miRNA: 2345 and hub gene: 285) and 20765 interaction pairs (Figure 6). *PRKDC* was the gene targets of 163 miRNAs (eg, *hsa-mir-142-5p*), *MYH9* was the gene targets of 126 miRNAs (eg, *hsa-mir-181b-3p*), *APP* was the gene targets of 125 miRNAs (eg, *hsa-mir-216b-5p*), *ILF3* was the gene targets of 107 miRNAs (eg, *hsa-mir-3157-3p*), *SKIL* was the gene targets 91 of miRNAs (eg, *hsa-mir-1294*), *HSPA8* was the gene targets of 116 of miRNAs (eg, *hsa-mir-3661*), *HSP90AB1* was the gene targets of 103 of miRNAs (eg, *hsa-mir-200a-3p*), *SQSTM1* was the gene targets of 94 of miRNAs (eg, *hsa-mir-520d-5p*), *HSPA5* was the gene targets of 88 of miRNAs (eg, *hsa-mir-573*), and *GRB2* was the gene targets of 65 of miRNAs (eg, *hsa-mir-1291*), and topological properties of each hub genes and miRNAs in miRNA-hub gene regulatory network are listed in Table 2.

TF-hub gene regulatory network construction

The hub genes of the DEGs in T2DM were performed by online databases NetworkAnalyst. Based on the TFs, a TF-hub gene regulatory network was constructed with 477 nodes (TF: 192 and hub gene: 285) and 8507 interaction pairs (Figure 7). *BCL6* was the gene targets of 60 TFs (eg, *NOTCH1*), *MYH9* was the gene targets of 53 TFs (eg, *PPARD*), *NCOR2* was the gene targets of 50 TFs (eg, *HIF1A*), *APP* was the gene targets of 45 TFs (eg, *SMARCA4*), *NDRG1* was the gene targets of 44

Table 1. Topology table for up and down regulated genes.

REGULATION	NODE	DEGREE	BETWEENNESS	STRESS	CLOSENESS
Up	APP	674	0.277552	48171576	0.396397
Up	MYH9	231	0.067132	12987566	0.351087
Up	TCTN2	203	0.074204	9822804	0.31559
Up	USP7	156	0.048917	10011292	0.341756
Up	SYNPO	148	0.019807	5325396	0.315343
Up	BCL6	99	0.03129	4924434	0.316788
Up	PRKDC	97	0.02396	5704444	0.339213
Up	U2AF2	77	0.016653	3792650	0.316448
Up	SKIL	72	0.021529	2766174	0.31763
Up	CTBP1	72	0.018919	3933722	0.30334
Up	TOP1	66	0.012706	3485932	0.318522
Up	NDRG1	65	0.014232	2582316	0.327508
Up	NCOR2	62	0.014748	2563224	0.327727
Up	ILF3	62	0.010796	3729048	0.325197
Up	MAPT	59	0.011654	2038018	0.335304
Up	SNRNP70	56	0.013306	2811964	0.337737
Up	NME7	54	0.009971	2818468	0.302345
Up	HCFC1	54	0.011189	4114590	0.285724
Up	SRRM2	53	0.008517	2842260	0.304719
Up	DDX17	48	0.010257	2309956	0.34835
Up	RUNX1T1	45	0.007377	1300474	0.298327
Up	TNRC6B	44	0.005765	1772074	0.294513
Up	RPS3A	43	0.008663	1580346	0.314044
Up	BAX	43	0.010013	1360956	0.305287
Up	ANXA2	42	0.006725	1415220	0.32746
Up	RAB8A	38	0.009675	2880488	0.28541
Up	ALYREF	37	0.006723	3260410	0.299114
Up	OTUD4	36	0.009901	1095992	0.306175
Up	TNRC6A	36	0.006285	1066772	0.298629
Up	LMO1	36	0.007004	1983992	0.281738
Up	NR4A1	35	0.007386	1366096	0.293516
Up	DNMT1	35	0.007153	1311716	0.318247
Up	JUNB	34	0.007201	2171380	0.289501
Up	HNRNPH1	34	0.004039	1126254	0.310822
Up	HNRNPL	33	0.002406	639204	0.306684
Up	EEF2	32	0.00508	1312314	0.31811

(Continued)

Table 1. (Continued)

REGULATION	NODE	DEGREE	BETWEENNESS	STRESS	CLOSENESS
Up	RPS2	32	0.005007	944774	0.30743
Up	PGAM5	32	0.004601	1343578	0.295695
Up	CD44	31	0.005854	812632	0.311523
Up	B2M	31	0.005781	1559068	0.265885
Up	RAD52	30	0.004293	852988	0.296289
Up	ATP2A2	30	0.007054	1516120	0.312823
Up	SREBF1	30	0.007025	2186330	0.278526
Up	PAFAH1B1	29	0.005215	2372866	0.276438
Up	NDN	29	0.007043	1329464	0.296547
Up	SRSF3	28	0.003437	1013722	0.303174
Up	APLP1	27	0.006221	892288	0.276818
Up	GANAB	27	0.004094	990330	0.298347
Up	RPL7	26	0.003381	933236	0.310757
Up	MPRIP	26	0.001472	342632	0.295873
Up	INO80E	25	0.005901	1721260	0.272453
Up	CACNA1A	24	0.010596	1171628	0.281863
Up	CHGB	24	0.006952	765394	0.299337
Up	RPL28	24	0.002372	585818	0.299074
Up	ITSN2	24	0.003281	939596	0.286093
Up	RPS10	23	0.004922	897446	0.326132
Up	RPL23A	23	0.003289	505840	0.311962
Up	PHF1	22	0.003983	1034682	0.277426
Up	HNRNPAB	22	0.001844	495324	0.3108
Up	SH3GL1	22	0.003033	986094	0.270008
Up	HMGB1	21	0.002117	776702	0.290681
Up	PDK3	19	0.002946	742934	0.275937
Up	ARID1B	19	0.004	716712	0.264217
Up	ABCA1	18	0.003966	549762	0.275216
Up	ESRRA	18	0.002502	368532	0.276023
Up	TRA2A	18	0.001473	605004	0.285005
Up	MAGI1	18	0.00426	637328	0.277722
Up	VEGFA	17	0.004134	836256	0.248525
Up	ZC3HAV1	17	0.001292	489318	0.274227
Up	HIVEP1	16	0.001767	378796	0.2718
Up	SIPA1L3	16	0.001056	218866	0.285465
Up	MPP6	16	0.002628	324224	0.275972
Up	RPL17	16	0.001736	515376	0.297165

(Continued)

Table 1. (Continued)

REGULATION	NODE	DEGREE	BETWEENNESS	STRESS	CLOSENESS
Up	SSR4	16	0.001986	441016	0.283617
Up	RPS27	15	0.002603	522416	0.297905
Up	SON	15	4.63E-04	263370	0.282512
Up	PDCD4	15	0.001508	323916	0.276732
Up	TMEM79	15	0.004325	604778	0.229337
Up	MAFG	14	0.002447	382134	0.252123
Up	SGSM2	14	0.002229	327358	0.278544
Up	NOL3	14	6.71E-04	214960	0.264091
Up	S100A6	14	9.74E-04	248722	0.280861
Up	MZT2A	14	2.68E-04	75218	0.276801
Up	RPL21	14	0.001091	579282	0.289843
Up	MCPH1	14	8.71E-04	244188	0.255857
Up	LUC7L3	13	0.001174	368838	0.272386
Up	DDX18	13	0.001648	394268	0.31722
Up	TLE3	13	0.002428	330598	0.309193
Up	EIF5A	13	0.002018	470016	0.279742
Up	LENG8	13	0.001599	412736	0.24473
Up	OCLN	12	0.001253	314884	0.263462
Up	PABPN1	12	0.001338	370074	0.285705
Up	BRD1	11	0.001735	432360	0.250156
Up	KRT10	11	5.29E-04	212650	0.28389
Up	RPS26	11	6.46E-04	207408	0.291102
Up	TMEM209	11	0.002671	362732	0.281917
Up	TLK1	11	0.001007	270716	0.252627
Up	NPDC1	11	7.46E-04	230746	0.250595
Up	ST14	11	0.001157	228876	0.261871
Up	UBA6	10	0.00209	401360	0.269055
Up	MAP3K12	10	0.00226	336206	0.272201
Up	MAP2	10	0.001373	254464	0.319327
Up	ITGAV	10	0.00199	532090	0.270371
Up	CPE	10	0.001881	615956	0.227778
Up	TFAP4	10	7.77E-04	213056	0.26184
Up	MIER1	10	0.0015	272476	0.264123
Up	CPT1A	9	5.45E-04	93620	0.276438
Up	RGL2	9	6.06E-04	170470	0.251865
Up	CYB5R3	9	9.29E-04	184034	0.256748
Up	DDR1	9	0.001327	189372	0.266558

(Continued)

Table 1. (Continued)

REGULATION	NODE	DEGREE	BETWEENNESS	STRESS	CLOSENESS
Up	ATP1B1	9	0.001955	400240	0.267073
Up	NME3	9	0.001643	194338	0.301808
Up	MZT2B	9	1.10E-04	28768	0.258413
Up	ALDH1B1	9	9.67E-04	319144	0.277583
Up	PI4KA	9	7.40E-04	268444	0.277879
Up	CSNK1G2	9	0.001421	396030	0.252931
Up	CITED2	9	0.002327	530770	0.263431
Up	RANBP3	9	0.001499	304686	0.236853
Up	NRP1	8	8.45E-04	148358	0.258398
Up	PGRMC2	8	4.66E-04	87082	0.262275
Up	HLA-B	5	0	0	0.210049
Up	HLA-A	4	7.39E-05	16916	0.249408
Up	EIF1AX	1	0	0	0.28389
Up	ARL4C	1	0	0	0.28389
Up	RPL41	1	0	0	0.28389
Up	UBXN6	1	0	0	0.28389
Up	CDK10	1	0	0	0.267235
Up	DPH1	1	0	0	0.266639
Up	AHDC1	1	0	0	0.259871
Up	SEZ6L2	1	0	0	0.191166
Up	PDCD2	1	0	0	0.222239
Up	PTPN18	1	0	0	0.285576
Up	NISCH	1	0	0	0.285576
Up	RBM33	1	0	0	0.285576
Up	WIPF2	1	0	0	0.285576
Up	MCU	1	0	0	0.225479
Up	ZZEF1	1	0	0	0.241075
Up	FCGRT	1	0	0	0.210049
Up	EMC10	1	0	0	0.239898
Up	PLD3	1	0	0	0.239898
Up	FADS2	1	0	0	0.239898
Up	FAM102A	1	0	0	0.212614
Up	SEMA6A	1	0	0	0.222463
Up	ZFX	1	0	0	0.232008
Up	USP27X	1	0	0	0.21869
Down	GRB2	431	0.172947	23419584	0.399693
Down	HSP90AB1	225	0.07414	15655320	0.364663

(Continued)

Table 1. (Continued)

REGULATION	NODE	DEGREE	BETWEENNESS	STRESS	CLOSENESS
Down	UBC	224	0.075316	15138294	0.356148
Down	HSPA5	144	0.05737	10318564	0.363554
Down	SQSTM1	123	0.036494	6909982	0.339317
Down	XRCC6	121	0.034438	8751842	0.335228
Down	HSPA8	120	0.03154	6567910	0.365537
Down	TUBA1C	91	0.023847	3670590	0.328896
Down	TUBG1	81	0.017666	3231832	0.324267
Down	PSMC5	80	0.015146	3450624	0.332456
Down	CSNK2B	77	0.019998	5358078	0.32002
Down	CDC23	75	0.021091	6346650	0.302076
Down	SAT1	69	0.01963	4672960	0.296746
Down	GAPDH	63	0.012495	2722934	0.342046
Down	GABARAP	62	0.01438	2298584	0.299175
Down	RAD23A	56	0.009202	1854168	0.312778
Down	PKM	51	0.009281	2445062	0.320972
Down	DNAJA1	51	0.008274	2322170	0.320763
Down	PSMB3	50	0.005865	1322226	0.308051
Down	EXOSC1	50	0.01119	2195338	0.294709
Down	DDIT3	48	0.01172	2529152	0.297345
Down	NDUFA9	47	0.013121	3147348	0.291102
Down	CANX	46	0.01141	2049224	0.314089
Down	IBTK	45	0.008641	1736696	0.290891
Down	SCNM1	45	0.006692	2252600	0.281594
Down	PSMC4	42	0.003699	829472	0.31331
Down	NDUFA12	42	0.004926	813880	0.250837
Down	MBIP	42	0.01045	2311340	0.274601
Down	PSMD7	41	0.002834	722504	0.302179
Down	USP22	41	0.011629	3407576	0.279884
Down	PSMD6	40	0.001629	559916	0.281863
Down	CLU	40	0.00841	1216580	0.309431
Down	AIFM1	39	0.005419	1729814	0.299966
Down	MORF4L2	39	0.011082	2538970	0.275662
Down	PRDX1	38	0.005407	1341464	0.312403
Down	ARL6IP1	37	0.012555	1641598	0.244635
Down	CAPZB	36	0.004358	1050292	0.311742
Down	PSMD12	34	0.001447	422448	0.305161
Down	PSMB7	34	0.001154	364868	0.289577

(Continued)

Table 1. (Continued)

REGULATION	NODE	DEGREE	BETWEENNESS	STRESS	CLOSENESS
Down	BCAP31	34	0.013785	1768344	0.327824
Down	PSMB5	34	0.003403	666674	0.313199
Down	NR0B2	34	0.005798	1517314	0.302138
Down	TUBA4A	33	0.005178	1530624	0.307217
Down	RNF6	33	0.003643	1333208	0.262166
Down	MPP1	32	0.007264	815422	0.302924
Down	CAMK2D	32	0.008412	1997208	0.289805
Down	PSMF1	32	0.006012	1557236	0.280594
Down	ALDOA	31	0.004458	960392	0.311391
Down	PSMA4	30	0.002283	522348	0.297505
Down	PSMB2	30	0.003431	769210	0.296309
Down	UCHL1	28	0.00501	791348	0.328067
Down	TXN2	28	0.00596	1364924	0.272084
Down	ORC3	28	0.005692	1623144	0.284273
Down	CLIC1	27	0.004327	824568	0.304447
Down	UBE2L6	27	0.006391	1958890	0.274499
Down	GBA	26	0.004796	875116	0.282061
Down	ENO1	26	0.00367	1143078	0.306876
Down	RPL14	26	0.004161	1610674	0.298569
Down	FTH1	24	0.004084	777832	0.276783
Down	TXN	24	0.003003	482510	0.302717
Down	GSTP1	24	0.004232	1080546	0.295201
Down	REEP5	24	0.005234	1041918	0.267817
Down	TOX4	23	0.005025	1370332	0.276058
Down	TTR	23	0.003317	617718	0.272823
Down	CORO1C	23	0.001258	341072	0.296269
Down	GTF2B	22	0.004837	1919700	0.246228
Down	PPIB	21	0.002633	702460	0.286557
Down	UFD1	20	0.00202	299278	0.298952
Down	GLUL	20	0.003636	572524	0.266767
Down	SCAMP3	19	0.002153	497590	0.274738
Down	FTL	19	0.004867	440140	0.298006
Down	SLC25A5	19	0.001986	352068	0.310844
Down	UBE2B	19	0.002118	572844	0.260713
Down	ZNF326	19	0.002394	916478	0.295359
Down	LAMTOR1	19	0.004398	814628	0.252454
Down	ZNF410	18	0.004751	566192	0.302386

(Continued)

Table 1. (Continued)

REGULATION	NODE	DEGREE	BETWEENNESS	STRESS	CLOSENESS
Down	PRDX6	18	0.002313	594 114	0.280327
Down	DRG1	18	0.002816	810 156	0.295122
Down	ATP6V1B2	18	0.002914	816 976	0.270388
Down	ZNF165	18	0.003519	739 876	0.262493
Down	NDUFV3	18	0.001755	335 550	0.259292
Down	EIF3K	18	0.004959	1 105 612	0.27343
Down	NSFL1C	17	0.003491	339 954	0.287189
Down	ARCN1	17	0.002733	778 374	0.290204
Down	TPI1	17	0.001365	553 820	0.293848
Down	ARRDC3	17	0.001321	468 292	0.265518
Down	NDUFB8	16	0.001576	271 076	0.25175
Down	PRDX5	15	0.00314	356 484	0.304824
Down	RPS28	15	0.00171	509 708	0.276317
Down	TCEAL4	15	0.002744	249 400	0.281451
Down	VAMP8	15	0.003131	687 744	0.270437
Down	TRAPPC2L	14	0.004206	559 988	0.293516
Down	CNOT6L	14	0.002421	339 456	0.257721
Down	ATP6V1D	14	0.002838	320 770	0.28589
Down	DYNLRB1	14	0.001865	358 956	0.26184
Down	EDARADD	13	0.001601	322 314	0.258051
Down	PEF1	13	0.001577	414 800	0.268957
Down	FARSA	13	0.001697	508 804	0.2652
Down	POLE3	12	0.004074	517 276	0.286019
Down	COX4I1	12	0.002449	321 508	0.261561
Down	ETV5	12	0.003438	1 053 244	0.236334
Down	NAPA	12	0.002141	556 788	0.283217
Down	VPS25	12	0.002068	519 502	0.245014
Down	BEX1	12	0.001246	267 736	0.267979
Down	XPOT	11	0.001347	381 778	0.281774
Down	MT2A	11	0.001882	359 724	0.241562
Down	NDUFB7	11	0.001	155 788	0.238231
Down	MRPS35	11	0.00194	415 706	0.264107
Down	MRPS15	11	0.001587	179 036	0.276058
Down	PLEKHB2	11	0.001437	374 900	0.236057
Down	NSMCE2	11	0.001999	798 156	0.237464
Down	CTNNAL1	11	0.001959	302 074	0.253119
Down	PPIL1	11	0.001172	393 624	0.278019

(Continued)

Table 1. (Continued)

REGULATION	NODE	DEGREE	BETWEENNESS	STRESS	CLOSENESS
Down	PRPS2	10	0.001596	384080	0.259383
Down	COX6B1	10	5.69E-04	148866	0.236765
Down	ETV6	10	0.002363	207722	0.296408
Down	CAP1	10	5.76E-04	164992	0.273972
Down	SPCS2	10	0.001427	294448	0.282801
Down	GRAMD2B	10	0.002536	387376	0.231425
Down	CDC42BPA	10	0.001848	460620	0.252858
Down	ATP6V1E1	2	1.68E-05	7358	0.256421
Down	MPC1	1	0	0	0.230293
Down	TRUB1	1	0	0	0.28389
Down	NANS	1	0	0	0.267235
Down	MDH1	1	0	0	0.267235
Down	GHITM	1	0	0	0.266639
Down	COA3	1	0	0	0.207341
Down	NEU1	1	0	0	0.224518
Down	UQCC2	1	0	0	0.285576
Down	RBP4	1	0	0	0.214355
Down	CUTA	1	0	0	0.226925
Down	GPX2	1	0	0	0.228591
Down	PXK	1	0	0	0.230257
Down	TSPYL5	1	0	0	0.254722
Down	ALG3	1	0	0	0.239898
Down	ZMPSTE24	1	0	0	0.239898
Down	SCD	1	0	0	0.239898
Down	UQCRH	1	0	0	0.200544
Down	EIF5A2	1	0	0	0.232008
Down	ATXN7L3B	1	0	0	0.21869

TFs (eg, *SUZ12*), *UBC* was the gene targets of 64 TFs (eg, *TAF7L*), *HSP90AB1* was the gene targets of 49 TFs (eg, RUNX2), *TUBA1C* was the gene targets of 47 TFs (eg, *MITF*), *HSPA5* was the gene targets of 44 TFs (eg, *YAP1*), and *HSPA8* was the gene targets of 39 TFs (eg, *E2F1*), and topological properties of each hub genes and TFs in TF-hub gene regulatory network are listed in Table 2.

Validation of hub genes by receiver operating characteristic curve (ROC) analysis

Validated by ROC curves, we found that 10 hub genes had high sensitivity and specificity, including *APP* (AUC = 0.853), *MYH9* (AUC = 0.852), *TCTN2* (AUC = 0.881), *USP7* (AUC = 0.862), *SYNPO* (AUC = 0.893), *GRB2* (AUC = 0.850), *HSP90AB1* (AUC = 0.870), *UBC* (AUC = 0.865), *HSPA5* (AUC = 0.902), and *SQSTM1* (AUC = 0.875) (Figure 8). The hub genes might be biomarkers of T2DM and have positive implications for early medical intervention of the disease.

Discussion

Although there are various investigations on T2DM that have been conducted, the mortality of T2DM is still high. This might be due to the lack of valid biomarkers for detection of early stage T2DM and of valid treatment for T2DM. Therefore, molecular mechanisms of T2DM are necessary for scientists to

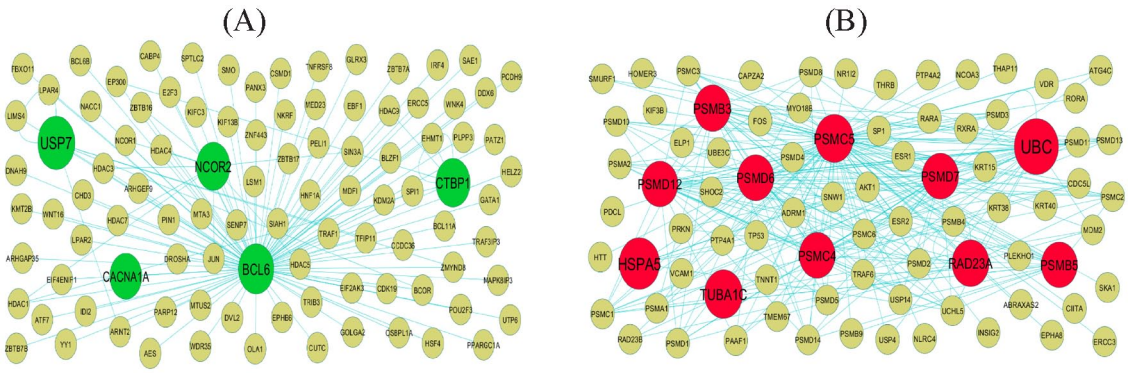

Figure 5. Modules of isolated from PPI of DEGs: (A) the most significant module was obtained from PPI network with 98 nodes and 117 edges for up regulated genes and (B) the most significant module was obtained from PPI network with 81 nodes and 248 edges for down regulated genes. Up regulated genes are marked in green; down regulated genes are marked in red.

Figure 6. MiRNA-hub gene regulatory network. The purple color diamond nodes represent the key miRNAs; up regulated genes are marked in green; down regulated genes are marked in orange.

find the treat and diagnosis method of T2DM. Because of the fast advancement of NGS technology, it is more convenient to find out the genetic modification of development of diseases. NGS facilitates us to examine the gene, the genetic modification in T2DM, which had been proved to be a better approach to find novel biomarkers in other metabolic diseases.

In the present investigation, we observed whether there were more beneficial genes which could be better biomarkers for the diagnosis, prognosis and therapeutic for T2DM. In order to find out the significant gene of T2DM, we analyzed the NGS data GSE81608 in Limma, where a total number of 927 DEGs were obtained between T2DM and normal control, comprising was 461 up regulated and 466 down regulated genes. *CTBP1*[41] and *TRNC*[42] are involved in the pathogenesis of T2DM. A previous study has demonstrated that *SST* (somatostatin) serves an essential role in obesity.[43] Therefore, the data suggest that the identified DEGs might participant in the development of T2DM and associated complications and contribute to T2DM treatment.

Then, databases including GO and REACTOME were selected to do gene enrichment analysis. Metabolism of proteins,[44] metabolism,[45] the citric acid (TCA) cycle and respiratory electron transport,[46] gluconeogenesis,[47] immune system,[48] heterocyclic compound binding,[49] protein binding,[50] establishment of localization,[51] cellular metabolic process,[52] cytoplasm,[53] and catalytic activity[54] were the GO terms and signaling pathways responsible for the advancement of T2DM. A previous study showed that *IGFBP2*,[55] *APOH* (apolipoprotein H),[56] *ANXA2*,[57] *BAX* (BCL2 associated X, apoptosis regulator),[58] *PCSK1N*,[59] *PDK4*,[60] *CPE* (carboxypeptidase E),[61] *OCLN* (occludin),[62] *CD44*,[63] *NDN* (necdin, MAGE family member),[64] *MLXIPL* (MLX interacting protein like),[65] *CD36*,[66] *SREBF1*,[67] *NR4A1*,[68] *PCSK2*,[69] *CHGB* (chromogranin B),[70] *PDK3*,[71] *PDCD4*,[72] *EIF5A*,[73] *NRP1*,[74] *ABCA1*,[75] *DNMT1*,[76] *MYH9*,[77] *HMGB1*,[78] *B4GALT5*,[79] *B2M*,[80] *MAP3K12*,[81] *KSR2*,[82] *NPY* (neuropeptide Y),[83] *CHGA* (chromogranin A),[84] *CD47*,[85] *DLK1*,[86] *PDK4*,[87] *CPE* (carboxypeptidase E),[61] *OCLN* (occludin),[62] *CXXC4*,[88] *PEMT* (phosphatidylethanolamine

Table 2. miRNA-target gene and TF-target gene interaction.

REGULATION	TARGET GENES	DEGREE	MICRORNA	REGULATION	TARGET GENES	DEGREE	TF
Up	PRKDC	163	hsa-mir-142-5p	Up	BCL6	60	NOTCH1
Up	MYH9	126	hsa-mir-181b-3p	Up	MYH9	53	PPARD
Up	APP	125	hsa-mir-216b-5p	Up	NCOR2	50	HIF1A
Up	ILF3	107	hsa-mir-3157-3p	Up	APP	45	SMARCA4
Up	SKIL	91	hsa-mir-1294	Up	NDRG1	44	SUZ12
Up	NCOR2	81	hsa-mir-4708-3p	Up	TOP1	41	CREM
Up	U2AF2	65	hsa-mir-196a-5p	Up	ILF3	41	DACH1
Up	NDRG1	60	hsa-mir-374a-5p	Up	MAPT	38	YAP1
Up	TOP1	45	hsa-mir-424-5p	Up	SKIL	37	GATA2
Up	CTBP1	45	hsa-mir-944	Up	PRKDC	36	NUCKS1
Up	BCL6	35	hsa-mir-4701-3p	Up	CTBP1	35	ELF1
Up	USP7	34	hsa-mir-93-5p	Up	U2AF2	34	KDM6A
Up	MAPT	23	hsa-mir-2278	Up	SYNPO	28	SMAD4
Up	SYNPO	15	hsa-mir-138-5p	Up	USP7	27	NANOG
Up	TCTN2	9	hsa-mir-34a-5p	Up	TCTN2	10	SRF
Down	HSPA8	116	hsa-mir-3661	Down	UBC	64	TAF7L
Down	HSP90AB1	103	hsa-mir-200a-3p	Down	HSP90AB1	49	RUNX2
Down	SQSTM1	94	hsa-mir-520d-5p	Down	TUBA1C	47	MITF
Down	HSPA5	88	hsa-mir-573	Down	HSPA5	44	YAP1
Down	GRB2	65	hsa-mir-1291	Down	HSPA8	39	E2F1
Down	SAT1	56	hsa-mir-301b-3p	Down	GAPDH	38	SOX11
Down	UBC	55	hsa-mir-9-5p	Down	GABARAP	38	HOXB4
Down	GAPDH	54	hsa-mir-5690	Down	GRB2	37	TFAP2A
Down	TUBA1C	53	hsa-mir-603	Down	CSNK2B	35	THAP11
Down	CDC23	51	hsa-mir-376a-5p	Down	PSMC5	33	STAT4
Down	TUBG1	32	hsa-mir-182-5p	Down	SQSTM1	30	EP300
Down	XRCC6	31	hsa-mir-618	Down	CDC23	27	TEAD4
Down	GABARAP	26	hsa-mir-34b-3p	Down	SAT1	25	SALL4
Down	PSMC5	10	hsa-mir-452-5p	Down	XRCC6	23	YY1
Down	CSNK2B	9	hsa-mir-149-3p	Down	TUBG1	22	SOX2

N-methyltransferase),[89] *FADS2*,[90] *RREB1*,[91] *HNRNPAB* (heterogeneous nuclear ribonucleoprotein A/B),[92] *CPT1A*,[93] *ALDH1B1*, [94] *ESRRA* (estrogen related receptor alpha),[95] *NISCH* (nischarin),[96] *SSTR3*,[97] *ND1*,[98] *NCOR2*,[99] *RBP4*,[100] *GSTP1*,[101] *CYB5A*,[102] *G6PC2*,[103] *DNAJC15*,[104] *TMED6*,[105] *PSMD6*,[106] *CLU* (clusterin),[107] *TTR* (transthyretin),[108] *TXN* (thioredoxin),[109] *LAMTOR1*,[110] *GLUL* (glutamate-ammonia ligase),[111] *NEU1*,[112] *HSPA8*,[113] *AP3S2*,[114] *COX4I1*,[115] *MT2A*[116] *MTCH2*,[117] *ESD* (esterase D),[118] *UBE2L6*,[119] *SCD* (stearoyl-CoA desaturase),[120] *MGST3*,[121] *NQO1*,[122] *NSMCE2*,[123] and *PRSS1*[124] played an important role in T2DM. Quintela et al,[125] Yuan et al,[126] Cacace et al,[127] Hao et al,[128] Beckelman et al,[129] Liu et al,[130] Sekiguchi et al,[131] Castillon et al,[132] O'Donnell-Luria et al,[133] Coupland et al,[134] Koufaris et al,[135] Qvist et al,[136] Richter et al,[137] Torres et al,[138] Jeong et al,[139] Bermejo-Bescós et al,[140] Ramon-Duaso et al,[141] Guilarte,[142]

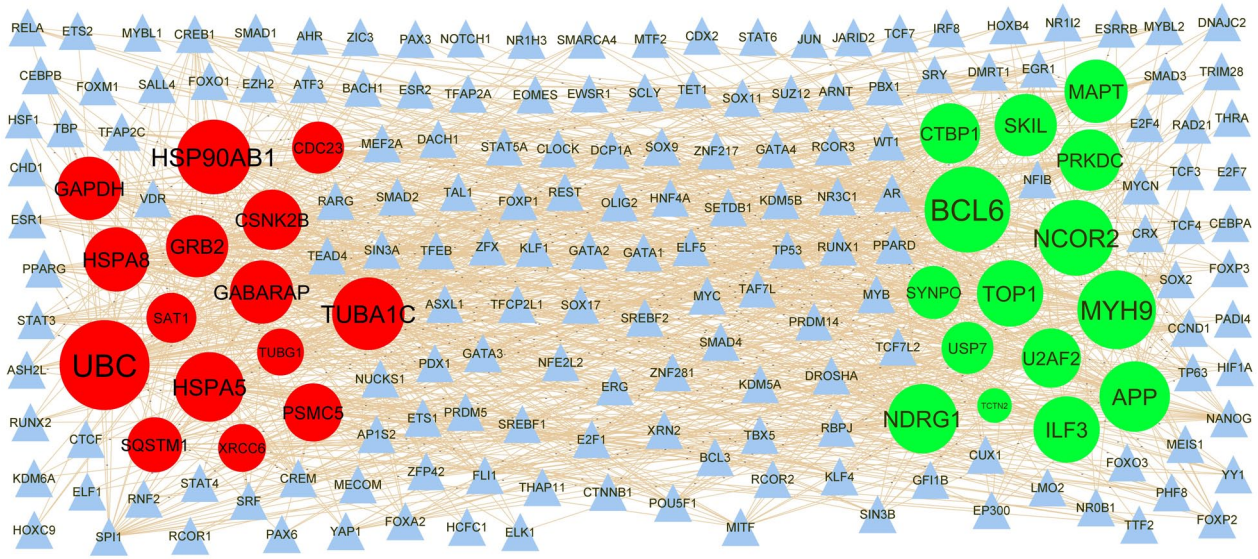

Figure 7. TF-hub gene regulatory network. The blue color triangle nodes represent the key TFs; up regulated genes are marked in green; down regulated genes are marked in red.

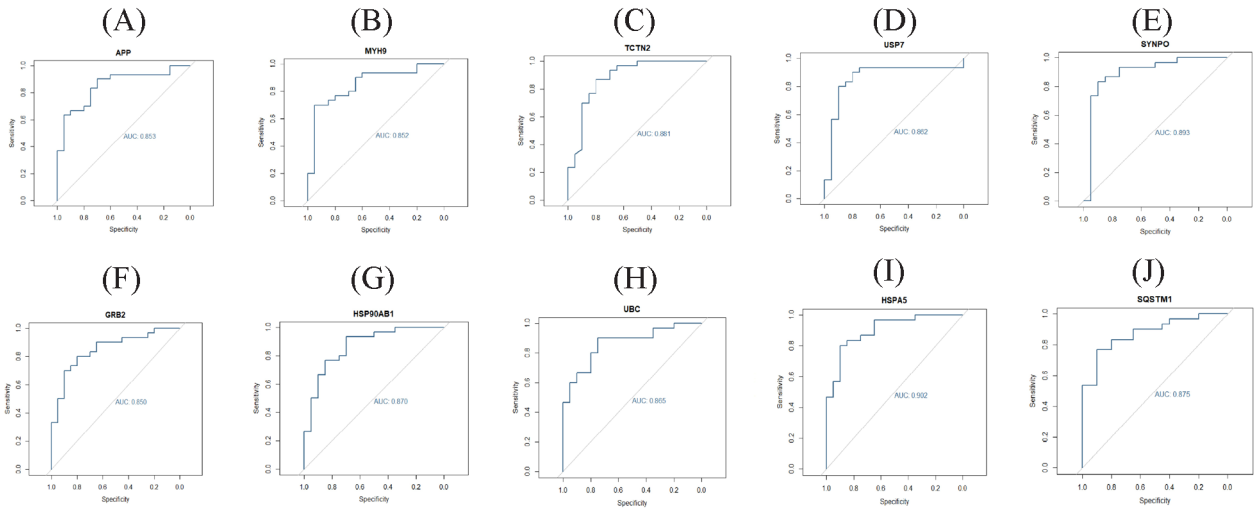

Figure 8. ROC curve validated the sensitivity, specificity of hub genes as a predictive biomarker for T2DM: (A) APP, (B) MYH9, (C) TCTN2, (D) USP7, (E) SYNPO, (F) GRB2, (G) HSP90AB1, (H) UBC, (I) HSPA5, and (J) SQSTM1.

Mukaetova-Ladinska et al,[143] Fazeli et al,[144] Butler et al,[145] Nackenoff et al,[146] Konyukh et al,[147] Hu et al,[148] Kaur et al,[149] Nakamura et al,[150] Liu et al,[151] Obara et al,[152] Herrmann et al,[153] Ozgen et al,[154] Masciullo et al,[155] Perrone et al,[156] Su et al,[157] Zhao et al,[158] Iqbal et al,[159] Gal et al,[160] Wang et al,[161] Stefanović et al,[162] Zahola et al,[163] Bik-Multanowski et al,[164] Mata et al,[165] Li et al,[166] Payton et al,[167] and Chai et al[168] indicated that *UBA6, TLA1, DPP6, USP7, EEF2, ITM2B, DPH1, PAK3, KMT2E, MAPT* (microtubule associated protein tau), *HCFC1, BRD1, TAOK2, PHF1, STMN2, APP* (amyloid beta precursor protein), *MBNL2, APLP1, MAP2, SRRM2 CST3, SRRM2, CST3, PLD3, SEZ6L2, DOC2A, PI4KA, GNAO1, TRA2A, MIDN* (midnolin), *HOOK3, MCPH1, SACS* (sacsin molecular chaperone), TUBA4A, ASAH1, ATP6V1B2, SVBP (small vasohibin binding protein), *AIFM1, UBC* (ubiquitin C), *IFI30, SCGN* (secretagogin, EF-hand calcium binding protein), *MTRNR2L12, GBA* (glucosylceramidase beta), *TXN2, NQO2*, and *PPIL1* were involved in the development and progression of cognitive impairment. *RPS3A*,[169] *PGAM5*,[170] *RPL7*,[171] *TLK1*,[172] *DDR1*,[173] *ILF3*,[174] *TNRC6A*,[175] *GGCX* (gamma-glutamyl carboxylase),[176] *S100A6*,[177] *LSAMP* (limbic system associated membrane protein),[178] *KCNA5*,[179] *LUC7L3*,[180] *ATAD3C*,[181] *SRSF3*,[182] *MCU* (mitochondrial calcium uniporter),[183] *ATP2A2*,[184] *GAA* (glucosidase alpha, acid),[185] *MAGI1*,[186] *WIPF2*,[187] *VAMP8*,[188] *UCHL1*,[189] *CLIC1*,[190] *PSMB5*,[191] *GRB2*,[192] *MPSTE24*,[193] *COX6B1*,[194] *SQSTM1*[195], *COTL1*[196], *CD63*[197], *NDUFB7*[198], *BEX1*[199] and *MTRNR2L8* [200] plays a major role in mediating cardiovascular diseases progression. *HLA-A*,[201] *VEGFA* (vascular endothelial growth factor A),[202] *RPS26*,[203] *BMP6*,[204] *HLA-B*,[205] *IER3IP1*,[206] *MT1E*,[207] *ACADM* (acyl-CoA dehydrogenase medium chain),[208] and *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase)[209] are

associated with progression of Type 1 diabetes mellitus. *PEMT* (phosphatidylethanolamine N-methyltransferase),[210] *INSM1*,[211] *BCL6*,[212] *RUNX1T1*,[213] *PGRMC2*,[214] *ARID1B*,[215] *CITED2*,[216] *KLF13*,[217] *PPT1*,[218] *ARRDC3*,[219] *HSPA5*,[220] *MDH2*,[221] and *COA3*,[222] have been previously reported to be a key biomarkers for the early detection of obesity. A previous study demonstrated that *IGFBP5*,[223] *PRDX6*,[224] *PKM (pyruvate kinase M1/2)*,[225] *PRDX1*,[226] and *USP22*[227] were more highly expressed in diabetic nephropathy. Durgin et al,[228] Zhang et al,[229] Hamada et al,[230] Gong et al,[231] Li et al,[232] Lin et al,[233] and Schweigert et al[234] suggested that *CYB5R3, CACNA1A, GLCCI1, CAP1, HSP90AB1, BLVRA* (biliverdinreductase A), and *CRIP1* were involved in the progression of hypertension. These results suggested that these, cognitive impairment, cardiovascular diseases, obesity, diabetic nephropathy and hypertension responsible genes might influence the development of T2DM through the altered expression. Therefore, these genes might involve in these GO terms and pathways are most likely to be important in the development of T2DM and T2DM associated complications.

By PPI network and module analysis, we identified the hub genes that might affect the origin or advancement of T2DM. *TCTN2, SYNPO* (synaptopodin), *PSMD12, PSMC4, TUBA1C, PSMC5, PSMD7*, and *RAD23A* might serve as a novel target for early diagnosis and specific therapy of T2DM and T2DM associated complications, and the related mechanisms need to be further investigation.

In addition, miRNA-hub gene regulatory network construction and TF-hub gene regulatory network were constructed. In addition, miRNA-mRNA networks were constructed. The roles of hub genes, miRNA and TF in the pathogenesis of T2DM are discussed. *Hsa-mir-142-5p*,[235] hsa-mir-1291,[236] *NOTCH1*,[237] *PPARD* (peroxisome proliferator-activated receptor delta),[238] *HIF1A*,[239] *RUNX2*,[240] and *E2F1*[241] levels are correlated with disease severity in patients with T2DM. Previous studies have demonstrated that *hsa-mir-216b-5p*[242] and *hsa-mir-200a-3p*[243] appears to be expressed in Type 1 diabetes. *Hsa-mir-1294*,[244] *SUZ12*,[245] and *YAP1*[246] were responsible for progression of cognitive impairment. *Hsa-mir-573*[247] and *SMARCA4*[248] were linked with progression of hypertension. Therefore, cognitive impairment and hypertension responsible biomarkers might be used as a diagnostic biomarker because of its essential role in the pathogenesis in early T2DM. Our study also suggests that *PRKDC* (protein kinase, DNA-activated, catalytic subunit), *SKIL* (SKI like proto-oncogene), *NDRG1, hsa-mir-181b-3p, hsa-mir-3157-3p, hsa-mir-3661, hsa-mir-520d-5p, TAF7L*, and *MITF* (Microphthalmia-associated transcription factor) are the novel biomarkers of the entire process of T2DM and T2DM associated complications development and might be used as the novel diagnostic biomarker for T2DM and T2DM associated complications.

The conduct of updating methods was calculated the classification work in which collected a higher score in efficiency, AUC, specificity, and sensitivity. As a result, 10 hub genes with AUC > 0.80 showed excellent diagnostic value for T2DM, and thus were considered as hub genes of T2DM, including *APP, MYH9, TCTN2, USP7, SYNPO, GRB2, HSP90AB1, UBC, HSPA5*, and *SQSTM1*. Through these analyses, we expect to provide novel insights into the molecular pathogenesis of T2DM and its associated complications and provide a more detailed molecular mechanism for the development of T2DM treatment. Although bioinformatics analysis has been performed in these present investigations, some limitations exist. Lacking of experimental validation of hub genes is a limitation of the study. In addition, we do not conduct in vitro and in vivo experiments of hub genes in T2DM. Corresponding experiments will be performed to verify in our future investigation, thus conversely testifying in bioinformatics analysis.

In conclusion, the present study identified 10 hub genes (*APP, MYH9, TCTN2, USP7, SYNPO, GRB2, HSP90AB1, UBC, HSPA5*, and *SQSTM1*) with crucial role in progression of T2DM; our results suggested these genes could add a new dimension to our understanding of the T2DM and might be served as potential biomarkers that will be assisting endocrinologist in developing novel therapeutic strategies for T2DM patients. However, there are some limitations in this study. Further larger clinical sample size and in-depth clinical experiments are needed to clarify the clear mechanism and warrant the prognostic value of these DEGs in T2DM.

Declarations

Ethics approval and consent to participate

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

Consent for publication

Not applicable.

Author contributions

Varun Alur: Methodology; Validation. Varshita Raju: Formal analysis; Validation. Basavaraj Vastrad: Writing – original draft; Writing – review & editing. Chanabasayya Vastrad: Investigation; Software. Satish Kavatagimath: Formal analysis; Resources. Shivakumar Kottureshetti: Resources; Supervision.

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Availability of data and materials

The datasets supporting the conclusions of this article are available in the GEO (Gene Expression Omnibus) (https://www.ncbi.nlm.nih.gov/geo/) repository [(GSE81608) (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE81608)].

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Informed consent

No informed consent because this study does not contain human or animals participants.

Ethics approval

Not applicable.

Trial registration

Not applicable.

Supplemental material

Supplemental material for this article is available online.

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