Original Article

Identification of key genes and pathways in type 2 diabetes mellitus and vitamin C metabolism through bioinformatics analysis

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Background and Objectives: Type 2 diabetes mellitus (T2DM) is a major global public health problem. Vitamin C (VC) can improve metabolic dysfunctions associated with T2DM. To establish an association between T2DM and VC metabolism, it is necessary to investigate the biological mechanisms of T2DM and VC. Therefore, the aim of this study was to elucidate the underlying pathways and co-expression networks in T2DM and VC using bioinformatics analysis. **Methods and Study Design:** Data on 15 microarrays about T2DM were downloaded from the Gene Expression Omnibus (GEO) and analyzed for genes using the GEO2R online tool. VC–metabolism associated genes were obtained from the Comparative Toxicogenomics Database (CTD). Differentially expressed genes (DEGs) about T2DM and VC metabolism were identified using the jvenn online software. GO annotation and KEGG pathways for DEGs were enriched using DAVID. STRING and Cytoscape were used to construct PPI network and to predict the interaction relationships between T2DM-associated and VC–metabolism associated DEGs. **Results:** We identified 160 DEGs about T2DM and VC from the GEO and CTD. GO, KEGG and PPI network analysis suggested that DEGs might participate in crucial biological processes and pathways, such as negative regulation of apoptotic process, removal of superoxide radicals, and PERK-mediated unfolded protein response, insulin resistance, the TNF signaling pathway, and the FoxO signaling pathway. **Conclusions:** These findings could significantly improve the understanding of the mechanisms underlying impact of VC on T2DM. However, further research is needed to validate our findings.

Key Words: type 2 diabetes mellitus, vitamin C, differentially expressed genes, bioinformatics analysis, pathways

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a life-long metabolic disorder characterized by hyperglycemia due to a relative defect in insulin action and secretion. In 2019, the International Diabetes Federation estimated that 463 million adults aged 20–79 years worldwide have diabetes, and 700.2 million adults aged 20–79 years will be living with diabetes by 2045.[1] T2DM is one of the most common diabetes types, and accounts for approximately 90% of all diabetes cases worldwide.[2] T2DM and its complications affect health and quality of life in numerous patients. T2DM has become a major global public health problem, and it represents a drain on health systems, individuals, families, and societies.

Micronutrients are very important in the development of T2DM and its complications.[3] Vitamin C (VC) is an essential water-soluble micronutrient derived from fruit, vegetables, and glucose metabolism.[4] Observational studies suggest that VC is associated with T2DM.[5] In addition, VC can improve glycemic control and reduce the incidence of diabetic complications in patients with T2DM.[6,7] However, the molecular mechanisms of VC in T2DM are still not fully elucidated. Bioinformatics, a powerful tool of molecular biology and information technology, has been used to reveal signaling pathways and co-expression networks linked to micronutrients in certain diseases. The interactive molecular mechanisms among micronutrients

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and diseases have been explored by using bioinformatics, and progresses the prevention of T2DM.

In the present study, we downloaded the original gene-expression profiles of T2DM from the National Center for Biotechnology Information–Gene Expression Omnibus (GEO) database and the genes of VC metabolism from the Comparative Toxicogenomics Database (CTD). Common genes among VC metabolism–associated genes and T2DM-associated genes were screened using the jvenn software. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID). STRING and Cytoscape software were used to construct the protein–protein interaction (PPI) network and molecular interactions between T2DM-associated and VC-associated differentially expressed genes (DEGs). In conclusion, the biological functions and key signaling pathways of T2DM-associated and VC-associated DEGs were investigated, and our study provides accurate and reliable biomarkers for the early diagnosis, individualized prevention and treatment of T2DM.

METHODS

T2DM-associated genes

GEO DataSets is a public functional genomics data repository, and that can provide answers to queries, experiments available for download, and stores curated gene-expression profiles. In this study, data on 15 microarrays were searched on May 1, 2021, by using the key words "type 2 diabetes mellitus" and "Homo sapiens" [porgn:__txid9606] and "series" and "expression profiling by array". Finally, the genes of patients with T2DM and healthy individuals were analyzed using the GEO2R online tool. The key genes in T2DM were extracted from the gene-expression profiles by using adj $p<0.05$ and $|\log2 \text{ fold change (FC)}| \geq 1$ as the cutoff criterion. This study was approved by Qingdao Municipal Centers for Disease Control and Prevention.

VC metabolism–associated genes

The CTD (http://ctd.mdibl.org/) is a robust, high-quality database that aims to curate information on environmental exposures affecting human health and the pathogenesis of diseases induced by chemicals or gene affected by chemicals.8 VC metabolism–associated genes were obtained from the CTD by searching using the keyword "vitamin C" on May 1, 2021. Expressed VC metabolism–associated genes were considered as key genes in this study.

Identification of common genes among VC metabolism-associated genes and T2DM-associated genes

Common genes of T2DM-associated and VC metabolism–associated were screened using the jvenn online software (http://www.bioinformatics.com.cn/static/others/jvenn/index.html).

Enrichment analysis

DAVID (v.6.8), an online gene functional classification tool, provides systematic and comprehensive biological functional annotation information and biological information behind large-scale gene or protein lists. GO annotation and KEGG pathway enrichment analysis of common genes among VC metabolism–associated genes and T2DM-associated genes were performed using DAVID. GO annotation mainly characterized gene function by specifically annotating and categorizing a gene product's biological processes (BPs), molecular functions (MFs), and cellular components (CCs). GO annotation was enriched using DAVID, and $p<0.05$ and false discovery rate (FDR) <0.05 were considered statistically significant. KEGG pathway analysis mainly explored the major metabolic and signal transduction, and $p<0.05$ was considered statistically significant.

Construction of PPI network

STRING (https://string-db.org, v. 11.0) is an online database of comprehensive annotated and predicted protein interactions, including direct and indirect PPIs. Cytoscape is free software for analyzing, visualizing and modeling PPI networks. STRING and Cytoscape were used to construct the PPI network and to predict the interaction relationships of common genes among T2DM-associated genes and VC metabolism–associated genes.

RESULTS

Identification of common genes among VC metabolism-associated genes and T2DM-associated genes

A total of 15 microarray data from T2DM and healthy participants were analyzed in GEO DataSets, and details of T2DM-associated microarrays are presented in Table 1. The sociodemographic and other characteristics of the study population are presented in Table S1. After GEO2R online tool analysis, GEO DataSets identified 12 201 T2DM-associated genes by using adj $p<0.05$ and $|\log2\text{fold change (FC)}| \geq 1$ (Table S2). The CTD database showed that 311 VC metabolism–associated genes were identified (Table S2). Finally, 160 intersection genes of T2DM-associated and VC-associated, shown in Table 2, were chosen by using jvenn online tool.

Enrichment analysis

To explore the potentially common biological functions of T2DM-associated and VC metabolism–associated genes, GO annotation analysis was conducted to obtain 29 terms in BPs, 22 terms in CCs and 18 terms in MFs. For the BPs, the common genes were primarily associated with the negative regulation of apoptotic process, removal of superoxide radicals and PERK-mediated unfolded protein response. For the CCs, the common genes were associated with extracellular matrix, extracellular spaces and extracellular exosome. For the MFs, the common genes were associated with protein binding, enzyme binding, glucose transmembrane transporter activity, and oxidoreductase activity (Table 3). KEGG pathway analysis revealed significantly enriched T2DM-associated and VC metabolism–associated common genes in the tumor necrosis factor (TNF), the HIF-1 signaling pathway, and FoxO signaling pathway (Table 4).

PPI network construction

Using minimum required interaction score set at 0.700

Table 1. Details of T2DM-associated microarray datasets from the GEO database

GEO accession	Organism/tissue	Age	Sex (M/F)	N(M/F)	Non-T2DM (n)	T2DM (n)	Platform
GSE15653[34]	Skeletal Muscle	52-69			10	7	Affymetrix Human Genome U133 Plus 2.0 Array
GSE29221[35]	Skeletal Muscle	49–60	M	24/0	12	12	Illumina HumanHT-12 V3.0 expression beadchip
GSE55650[36]	Skeletal Muscle	48–70	M/F	8/4	11	12	Affymetrix Human Genome U133 Plus 2.0 Array
GSE26168[37]							
GPL6883	Blood	21–70	M	24	15	9	Illumina HumanRef-8 v3.0 expression beadchip
GSE9006[38]							
GPL96	Peripheral blood mononuclear cells	2–18	M/F	16/20	24	12	Affymetrix Human Genome U133A Array
GPL97	Peripheral blood mononuclear cells	2–18	M/F	16/20	24	12	Affymetrix Human Genome U133B Array
GSE105167[39]	Blood (Circulating Leukocytes)	30–64	M/F	35/39	7	4	Illumina HumanHT-12 V4.0 expression beadchip
GSE20966[40]	Pancreases	52–79	M/F	13/7	10	10	Affymetrix Human X3P Array
GSE76896[41,42]	Pancreatic islet	22–85	M/F	96/88	130	55	Affymetrix Human Genome U133 Plus 2.0 Array
GSE76895[41,42]	Pancreatic islet	24–84	M/F	48/35	47	36	Affymetrix Human Genome U133 Plus 2.0 Array
GSE76894[41,42]	Pancreatic islets	24–85	M/F	52/51	84	19	Affymetrix Human Genome U133 Plus 2.0 Array
GSE29231[35]	Visceral adipose	37–85	F	24	12	12	Illumina HumanHT-12 V3.0 expression beadchip
GSE29226[35]	Subcutaneous Adipose	48–65	F	24	12	12	Illumina HumanHT-12 V3.0 expression beadchip
GSE78721[43-45]	Adipocytes and infiltration macrophages		M/F	56/74	62	68	Affymetrix Human Gene Expression Array
GSE71416[46]	Omental adipose tissue		M/F	4/15	6	14	Affymetrix Human Genome U133 Plus 2.0 Array
GSE156249[47]	Skeletal Muscle	53-65	M	50	36	14	Affymetrix Human Gene 1.1 ST Array

T2DM: type 2 diabetes mellitus; GEO: Gene Expression Omnibus.

Table 2. Identification of common genes among VC metabolism-associated genes and T2DM-associated genes

Num.	Gene ID	Gene symbol	Interaction
1	19	ABCA1	ATP binding cassette subfamily A member 1
2	176	ACAN	aggrecan
3	38	ACAT1	acetyl-CoA acetyltransferase 1
4	112	ADCY6	adenylate cyclase 6
5	22859	ADGRL1	adhesion G protein-coupled receptor L1
6	27161	AGO2	argonaute RISC catalytic component 2
7	205	AK4	adenylate kinase 4
8	231	AKR1B1	aldo-keto reductase family 1 member B
9	1646	AKR1C2	aldo-keto reductase family 1 member C2
10	301	ANXA1	annexin A1
11	335	APOA1	apolipoprotein A1
12	337	APOA4	apolipoprotein A4
13	338	APOB	apolipoprotein B
14	351	APP	amyloid beta precursor protein
15	397	ARHGDIB	Rho GDP dissociation inhibitor beta
16	10092	ARPC5	actin related protein 2/3 complex subunit 5
17	440	ASNS	asparagine synthetase (glutamine-hydrolyzing)
18	11947	ATP5B	ATP synthase, H+ transporting mitochondrial F1 complex, beta subunit
19	523	ATP6V1A	ATPase H+ transporting V1 subunit A
20	627	BDNF	brain derived neurotrophic factor
21	632	BGLAP	bone gamma-carboxyglutamate protein
22	332	BIRC5	baculoviral IAP repeat containing 5
23	658	BMPR1B	bone morphogenetic protein receptor type 1B
24	841	CASP8	caspase 8
25	101093891	CAT	catalase
26	6347	CCL2	C-C motif chemokine ligand 2
27	595	CCND1	cyclin D1
28	962	CD48	CD48 molecule
29	1012	CDH13	cadherin 13
30	64405	CDH22	cadherin 22
31	1026	CDKN1A	cyclin dependent kinase inhibitor 1A
32	1051	CEBPB	CCAAT enhancer binding protein beta
33	1277	COL1A1	collagen type I alpha 1 chain
34	1280	COL2A1	collagen type II alpha 1 chain
35	1410	CRYAB	crystallin alpha B
36	10675	CSPG5	chondroitin sulfate proteoglycan 5
37	1513	CTSK	cathepsin K
38	2919	CXCL1	C-X-C motif chemokine ligand 1
39	3627	CXCL10	C-X-C motif chemokine ligand 10
40	6372	CXCL6	C-X-C motif chemokine ligand 6
41	79901	CYBRD1	cytochrome b reductase 1
42	1583	CYP11A1	cytochrome P450 family 11 subfamily A member 1
43	1543	CYP1A1	cytochrome P450 family 1 subfamily A member 1
44	1544	CYP1A2	cytochrome P450 family 1 subfamily A member 2
45	1548	CYP2A6	cytochrome P450 family 2 subfamily A member 6
46	1601	DAB2	DAB adaptor protein 2
47	1735	DIO3	iodothyronine deiodinase 3
48	1848	DUSP6	dual specificity phosphatase 6
49	1910	EDNRB	endothelin receptor type B
50	8894	EIF2S2	eukaryotic translation initiation factor 2 subunit beta
51	1973	EIF4A1	eukaryotic translation initiation factor 4A1
52	54859	ELP6	elongator acetyltransferase complex subunit 6
53	2192	FBLN1	fibulin 1
54	2232	FDXR	ferredoxin reductase
55	2321	FLT1	fms related receptor tyrosine kinase 1
56	79672	FN3KRP	fructosamine 3 kinase related protein
57	2353	FOS	Fos proto-oncogene, AP-1 transcription factor subunit
58	2539	G6PD	glucose-6-phosphate dehydrogenase
59	11337	GABARAP	GABA type A receptor-associated protein
60	2670	GFAP	glial fibrillary acidic protein
61	2717	GLA	galactosidase alpha
62	2785	GNG3	G protein subunit gamma 3
63	2786	GNG4	G protein subunit gamma 4
64	2817	GPC1	glypican 1
65	2879	GPX4	glutathione peroxidase 4
66	2896	GRN	granulin precursor

T2DM: type 2 diabetes mellitus; VC: vitamin C.

Table 2. Identification of common genes among VC metabolism-associated genes and T2DM-associated genes

Num.	Gene ID	Gene symbol	Interaction
67	2946	GSTM2	glutathione S-transferase mu 2
68	9446	GSTO1	glutathione S-transferase omega 1
69	3002	GZMB	granzyme B
70	9709	HERPUD1	homocysteine inducible ER protein with ubiquitin like domain 1
71	3141	HLCS	holocarboxylase synthetase
72	3162	HMOX1	heme oxygenase 1
73	3283	HSD3B1	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1
74	3303	HSPA1A	heat shock protein family A (Hsp70) member 1A
75	3309	HSPA5	heat shock protein family A (Hsp70) member 5
76	3315	HSPB1	heat shock protein family B (small) member 1
77	3480	IGF1R	insulin like growth factor 1 receptor
78	3549	IHH	Indian hedgehog signaling molecule
79	10320	IKZF1	IKAROS family zinc finger 1
80	22914	KLRK1	killer cell lectin like receptor K1
81	9817	KEAP1	kelch like ECH associated protein 1
82	3958	LGALS3	galectin 3
83	4017	LOXL2	lysyl oxidase like 2
84	56925	LXN	latexin
85	4133	MAP2	microtubule associated protein 2
86	5594	MAPK1	mitogen-activated protein kinase 1
87	4233	MET	MET proto-oncogene, receptor tyrosine kinase
88	4257	MGST1	microsomal glutathione S-transferase 1
89	4489	MT1A	metallothionein 1A
90	4502	MT2A	metallothionein 2A
91	4504	MT3	metallothionein 3
92	4629	MYH11	myosin heavy chain 11
93	83988	NCALD	neurocalcin delta
94	57447	NDRG2	NDRG family member 2
95	9603	NFE2L3	nuclear factor, erythroid 2 like 3
96	1482	NKX2-5	NK2 homeobox 5
97	4826	NNAT	neuronatin
98	4843	NOS2	nitric oxide synthase 2
99	4846	NOS3	nitric oxide synthase 3
100	50507	NOX4	NADPH oxidase 4
101	1728	NQO1	NAD(P)H quinone dehydrogenase 1
102	10062	NR1H3	nuclear receptor subfamily 1 group H member 3
103	9315	NREP	neuronal regeneration related protein
104	4915	NTRK2	neurotrophic receptor tyrosine kinase 2
105	4968	OGG1	8-oxoguanine DNA glycosylase
106	5066	PAM	peptidylglycine alpha-amidating monooxygenase
107	26025	PCDHGA12	protocadherin gamma subfamily A, 12
108	5106	PCK2	phosphoenolpyruvate carboxykinase 2, mitochondrial
109	5121	PCP4	Purkinje cell protein 4
110	64714	PDIA2	protein disulfide isomerase family A member 2
111	5037	PEBP1	phosphatidylethanolamine binding protein 1
112	5198	PFAS	phosphoribosylformylglycinamidine synthase
113	26227	PHGDH	phosphoglycerate dehydrogenase
114	5295	PIK3R1	phosphoinositide-3-kinase regulatory subunit 1
115	5315	PKM	pyruvate kinase M1/2
116	5376	PMP22	peripheral myelin protein 22
117	5460	POU5F1	POU class 5 homeobox 1
118	5468	PPARG	peroxisome proliferator activated receptor gamma
119	5551	PRF1	perforin 1
120	5627	PROS1	protein S
121	5721	PSME2	proteasome activator subunit 2
122	5744	PTHLH	parathyroid hormone like hormone
123	5901	RAN	RAN, member RAS oncogene family
124	85397	RGS8	regulator of G protein signaling 8
125	6281	S100A10	S100 calcium binding protein A10
126	6277	S100A6	S100 calcium binding protein A6
127	6285	S100B	S100 calcium binding protein B
128	6383	SDC2	syndecan 2
129	23480	SEC61G	SEC61 translocon subunit gamma
130	5055	SERPINB2	serpin family B member 2
131	6440	SFTPC	surfactant protein C
132	6472	SHMT2	serine hydroxymethyltransferase 2

T2DM: type 2 diabetes mellitus; VC: vitamin C.

Table 2. Identification of common genes among VC metabolism-associated genes and T2DM-associated genes (cont.)

Num.	Gene ID	Gene symbol	Interaction
133	4891	SLC11A2	solute carrier family 11 member 2
134	10864	SLC22A7	solute carrier family 22 member 7
135	9963	SLC23A1	solute carrier family 23 member 1
136	9962	SLC23A2	solute carrier family 23 member 2
137	6513	SLC2A1	solute carrier family 2 member 1
138	6514	SLC2A2	solute carrier family 2 member 2
139	6517	SLC2A4	solute carrier family 2 member 4
140	29988	SLC2A8	solute carrier family 2 member 8
141	6622	SNCA	synuclein alpha
142	6649	SOD3	superoxide dismutase 3
143	6272	SORT1	sortilin 1
144	6663	SOX10	SRY-box transcription factor 10
145	6664	SOX11	SRY-box transcription factor 11
146	6657	SOX2	SRY-box transcription factor 2
147	6667	SP1	Sp1 transcription factor
148	6670	SP3	Sp3 transcription factor
149	6671	SP4	Sp4 transcription factor
150	121340	SP7	Sp7 transcription factor
151	6750	SST	somatostatin
152	6818	SULT1A3	sulfotransferase family 1A member 3
153	9066	SYT7	synaptotagmin 7
154	64216	TFB2M	transcription factor B2, mitochondrial
155	7178	TPT1	tumor protein, translationally-controlled 1
156	7200	TRH	thyrotropin releasing hormone
157	57761	TRIB3	tribbles pseudokinase 3
158	706	TSPO	translocator protein
159	7297	TYK2	tyrosine kinase 2
160	7448	VTN	vitronectin

T2DM: type 2 diabetes mellitus; VC: vitamin C.

(high confidence), STRING database was used to collect function-related PPI data from the 160 common genes (Figure 1). PPI network model was constructed and 119 targets were successfully established in the PPI network, which had 237 edges, with average protein node degree of 160 and local clustering coefficient of 0.359. The PPI network had featured a significant difference ($p<0.001$) (Figure 2).

GeneMANIA analysis

Among the 123 targets and their interacting proteins, there were 60.21% of input showing co-expression; 7.91%, co-localization; 15.27%, genetic interactions; 6.62%, physical interactions; 3.56%, shared protein domains; 2.45%, predicted and 3.95%, pathway by using GeneMANIA (Figure 3, Table S3). As a result of Molecular Complex Detection (MCODE) and GeneMANIA, a total of 8 clusters of modules were visualized (Figure 3), and the top 3 significant clusters consisted of 10 nodes and 22 edges (Figure 3 Module 1), 5 nodes and 7 edges (Figure 3 Module 2) and 5 nodes and 7 edges (Figure 3 Module 3), respectively.

DISCUSSION

The prevalence of T2DM is still increasing worldwide, but strategies for diagnosis and treatment of T2DM are far from satisfactory.[1] A systematic review and meta-analysis of 15 randomized controlled trials reported that VC intake of ≥200 mg/day significantly reduced glucose concentrations in individuals with T2DM and in interventions with a duration of ≥30 days.[9] There is evidence that VC supplementation at 1000 mg/day for 90 days can improve fasting plasma glucose, glycated hemoglobin, and insulin concentration in patients with T2DM.[10] Chronic inflammation and oxidative stress play a pivotal role in high glucose concentration in long-standing T2DM. Generally, T2DM progression causes a chronic inflammatory process, which worsens chronic inflammation and causes a vicious circle that further aggravates oxidative stress.[11] Several studies have reported that VC is the most well-known anti-oxidant that has beneficial effects in T2DM prevention.[12] Kim et al.[13] revealed that high VC dietary consumption improved anti-oxidation and anti-glycation through regulation of anti-inflammatory processes. Clarification of the potential role of VC in T2DM is critical; however, no study has been conducted on the molecular pathways of VC in T2DM. Hence, we used bioinformatics analysis to uncover the underlying molecular mechanisms of VC in T2DM.

This study used bioinformatics to screen 12 201 T2DM-associated and 311 VC metabolism–associated genes in the GEO and CTD, respectively. Finally, 160 common T2DM-associated and VC metabolism–associated genes were analyzed through GO enrichment and KEGG pathway analysis. The common genes functions in GO were negative regulation of apoptotic process, removal of superoxide radicals, PERK-mediated unfolded protein response, and insulin resistance. T2DM is characterized by insulin resistance, a relative deficiency in insulin secretion, and inappropriately regulated glucagon secretion,[14] which depends on both mass and function

Table 3. GO analysis of common genes among VC metabolism-associated genes and T2DM-associated genes

Category ID	Term	Count	Genes	*p* value	FDR
BP					
GO:0042493	response to drug	20	ABCA1, CDKN1A, ANXA1, OGG1, MGST1, APOA1, AK4, FOS, PFAS, DUSP6, COL1A1, CCND1, BGLAP, SST, CAT, CYP1A1, TSPO, PPARG, PAM, SNCA	<0.001	<0.001
GO:0001666	response to hypoxia	15	NOS2, SLC11A2, TRH, MT3, SOD3, SLC2A8, LOXL2, PKM, CAT, CYP1A1, NOX4, CCL2, HMOX1, CRYAB, PAM	<0.001	<0.001
GO:0055114	oxidation-reduction process	24	NQO1, PDIA2, G6PD, NOS2, GPX4, GSTO1, NOS3, HSD3B1, MGST1, DIO3, AKR1B1, AKR1C2, CYBRD1, SOD3, LOXL2, CYP2A6, CYP11A1, CYP1A2, CYP1A1, FDXR, NOX4, PHGDH, PAM, SNCA	<0.001	<0.001
GO:0043066	negative regulation of apoptotic process	21	NQO1, CDKN1A, ANXA1, SERPINB2, HSPA5, OGG1, IHH, HSPB1, ASNS, PIK3R1, SOX10, MT3, IGF1R, DAB2, EDNRB, CAT, BIRC5, NKX2-5, CRYAB, TPT1, SNCA	<0.001	<0.001
GO:0032355	response to estradiol	11	COL1A1, NQO1, CASP8, ANXA1, CCND1, OGG1, CYP1A2, CAT, IHH, CRYAB, PAM	<0.001	<0.001
GO:0045471	response to ethanol	10	NQO1, G6PD, CASP8, CCND1, BGLAP, OGG1, CAT, CCL2, TRH, SLC2A4	<0.001	<0.001
GO:0008203	cholesterol metabolic process	8	ABCA1, APP, CYP11A1, CAT, FDXR, APOA1, APOA4, APOB	<0.001	0.001
GO:0019430	removal of superoxide radicals	5	NQO1, NOS3, APOA4, MT3, SOD3	<0.001	0.001
GO:0032496	response to lipopolysaccharide	11	CXCL6, CXCL10, CASP8, CYP1A2, MGST1, CYP1A1, CXCL1, FOS, APOB, PCK2, SNCA	<0.001	0.001
GO:0007568	aging	11	NQO1, EDNRB, OGG1, CAT, CYP1A1, TSPO, CCL2, FOS, CRYAB, LOXL2, SNCA	<0.001	0.001
GO:0071222	cellular response to lipopolysaccharide	9	ABCA1, CXCL10, CEBPB, KLRK1, EDNRB, NOS2, TSPO, CCL2, NR1H3	<0.001	0.002
GO:0071276	cellular response to cadmium ion	5	MT1A, OGG1, CYP1A2, HMOX1, MT3	<0.001	0.002
GO:0010468	regulation of gene expression	8	SOX2, EIF4A1, COL2A1, SORT1, CYP1A2, PHGDH, PTHLH, POU5F1	<0.001	0.006
GO:0019852	L-ascorbic acid metabolic process	4	SLC23A2, GSTO1, SLC23A1, SLC2A1	<0.001	0.008
GO:0036499	PERK-mediated unfolded protein response	4	HSPA5, ASNS, CCL2, HERPUD1	<0.001	0.020
GO:0043524	negative regulation of neuron apoptotic process	8	NTRK2, CEBPB, BDNF, HMOX1, CCL2, BIRC5, MT3, SNCA	<0.001	0.028
GO:0001501	skeletal system development	8	COL1A1, ACAN, COL2A1, BGLAP, IHH, SOX11, BMPR1B, PTHLH	<0.001	0.032
GO:0009409	response to cold	5	CXCL10, CASP8, PPARG, TRH, FOS	<0.001	0.032
GO:0030335	positive regulation of cell migration	9	COL1A1, DAB2, FLT1, HSPA5, CDH13, MAPK1, PIK3R1, ELP6, IGF1R	<0.001	0.032
GO:0043627	response to estrogen	6	CCND1, BGLAP, HMOX1, MAPK1, APOA1, PPARG	<0.001	0.032
GO:0045893	positive regulation of transcription, DNA-templated	15	NR1H3, SOX11, FOS, TFB2M, MT3, SOX2, COL1A1, DAB2, SP1, SP3, NFE2L3, MAPK1, PPARG, NKX2-5, RAN	<0.001	0.032
GO:0060291	long-term synaptic potentiation	5	NTRK2, MAPK1, S100B, GFAP, SNCA	<0.001	0.034
GO:0055093	response to hyperoxia	4	COL1A1, CDKN1A, CAT, CYP1A1	0.001	0.038
GO:0070837	dehydroascorbic acid transport	3	SLC23A1, SLC2A1, SLC2A2	0.001	0.038
GO:0051412	response to corticosterone	4	CDKN1A, CCND1, TRH, FOS	0.001	0.042
GO:0007584	response to nutrient	6	ABCA1, NQO1, PKM, SST, APOA1, PPARG	0.001	0.044
GO:0008202	steroid metabolic process	5	CYP2A6, CYP1A1, TSPO, AKR1C2, SULT1A3	0.001	0.044
GO:1904659	glucose transmembrane transport	4	SLC2A1, SLC2A2, SLC2A4, SLC2A8	0.001	0.044
GO:0032869	cellular response to insulin stimulus	6	SP1, CCL2, TRIB3, PPARG, SLC2A4, PIK3R1	0.001	0.048

T2DM: type 2 diabetes mellitus; VC: vitamin C; BP: biological process; MF: molecular functions; CC: cellular components.

Table 3. GO analysis of common genes among VC metabolism-associated genes and T2DM-associated genes (cont.)

Category ID	Term	Count	Genes	*p* value	FDR
CC					
GO:0005829	cytosol	65	EIF4A1, APP, CDKN1A, SLC2A1, PEBP1, KEAP1, AKR1B1, HSPB1, SLC2A4, PFAS, SOX2, MT2A, CASP8, CCND1, SEC61G, ARHGDIB, PHGDH, G6PD, GPX4, GSTO1, FN3KRP, APOA1, APOA4, TYK2, FOS, DUSP6, POU5F1, PCP4, MT1A, PKM, CAT, S100A6, BIRC5, PSME2, TRIB3, PPARG, CRYAB, ATP6V1A, PIK3R1, NDRG2, NCALD, GABARAP, HLCS, HMOX1, MAPK1, MYH11, APOB, SULT1A3, SNCA, NTRK2, NQO1, GSTM2, NOS2, SORT1, NOS3, ASNS, GZMB, ARPC5, EIF2S2, MT3, SYT7, GFAP, AGO2, RAN, HSPA1A	<0.001	<0.001
GO:0070062	extracellular exosome	58	EIF4A1, APP, SLC23A1, PROS1, SLC2A1, PEBP1, AKR1B1, HSPB1, SLC2A4, PFAS, LGALS3, ARHGDIB, TSPO, PHGDH, G6PD, ANXA1, GPX4, GSTO1, APOA1, APOA4, TYK2, PKM, CAT, S100A6, PSME2, CDH13, CD48, CRYAB, ATP6V1A, GRN, LXN, SHMT2, FBLN1, AK4, NDRG2, NCALD, ACAT1, VTN, ATP5B, GNG4, GPC1, MAPK1, MYH11, APOB, S100A10, PCK2, NQO1, GSTM2, HSPA5, CYBRD1, ARPC5, SOD3, SYT7, DAB2, GLA, PAM, TPT1, RAN	<0.001	<0.001
GO:0005615	extracellular space	37	APP, CXCL6, GRN, FLT1, PROS1, IHH, AKR1B1, HSPB1, FBLN1, CXCL1, PTHLH, LOXL2, VTN, LGALS3, BGLAP, GPC1, CTSK, CCL2, HMOX1, APOB, SNCA, ANXA1, SERPINB2, SFTPC, APOA1, APOA4, S100B, MT3, SOD3, COL1A1, CXCL10, COL2A1, SST, CAT, CDH13, PAM, TPT1	<0.001	<0.001
GO:0048471	perinuclear region of cytoplasm	24	ABCA1, APP, CDKN1A, NOS2, SORT1, BDNF, SLC11A2, AKR1B1, SLC2A4, NDRG2, S100B, MT3, GABARAP, MT2A, MT1A, S100A6, NOX4, CCL2, CDH13, HMOX1, PPARG, PAM, HSPA1A, SNCA	<0.001	<0.001
GO:0031012	extracellular matrix	14	EIF4A1, HSPA5, HSPB1, FBLN1, SOD3, LOXL2, COL1A1, ACAN, LGALS3, VTN, ATP5B, COL2A1, PKM, RAN	<0.001	<0.001
GO:0005737	cytoplasm	73	EIF4A1, APP, SLC23A2, SLC23A1, KEAP1, AKR1B1, SLC2A2, HSPB1, IKZF1, PFAS, SOX2, LGALS3, MT2A, CASP8, CCND1, ARHGDIB, TSPO, RGS8, G6PD, ANXA1, SERPINB2, GSTO1, SLC11A2, SOX11, TYK2, DUSP6, POU5F1, MT1A, PKM, S100A6, BIRC5, PSME2, CDH13, CRYAB, CEBPB, LXN, SHMT2, PIK3R1, NREP, NDRG2, PTHLH, MAP2, HLCS, BGLAP, NNAT, MAPK1, NKX2-5, APOB, SULT1A3, SNCA, NQO1, GSTM2, NOS2, NOS3, BDNF, GZMB, AKR1C2, ARPC5, S100B, EIF2S2, MT3, SOD3, GFAP, SP1, SP4, SP3, AGO2, NFE2L3, SP7, GLA, TPT1, RAN, HSPA1A	<0.001	<0.001
GO:0005796	Golgi lumen	8	ACAN, VTN, BGLAP, GPC1, PROS1, SDC2, CSPG5, SOD3	<0.001	0.001
GO:0005886	plasma membrane	57	APP, SLC23A2, FLT1, SLC23A1, PROS1, PRF1, IHH, SLC2A1, SLC2A2, HSPB1, TRH, SLC2A4, IGF1R, SLC2A8, LGALS3, EDNRB, RGS8, ANXA1, SERPINB2, SLC11A2, DIO3, APOA1, PKM, CAT, CDH13, TRIB3, CD48, MET, ATP6V1A, SDC2, PIK3R1, GABARAP, ADCY6, ATP5B, GNG3, KLRK1, GNG4, GPC1, CDH22, HMOX1, APOB, SNCA, ABCA1, HSPA5, SORT1, NOS3, CYBRD1, MT3, SYT7, PCDHGA12, DAB2, SP3, PMP22, ADGRL1, BMPR1B, PAM, SLC22A7	<0.001	0.006
GO:0016020	membrane	36	SLC23A2, EIF4A1, SLC23A1, PRF1, SLC2A1, SLC2A2, PIK3R1, SLC2A4, ADCY6, IGF1R, HERPUD1, LOXL2, LGALS3, ATP5B, CCND1, SEC61G, ARHGDIB, HMOX1, SNCA, G6PD, HSPA5, SLC11A2, GZMB, TYK2, FOS, SYT7, CAT, AGO2, ADGRL1, PSME2, CSPG5, CD48, BMPR1B, PAM, SLC22A7, RAN	<0.001	0.008

T2DM: type 2 diabetes mellitus; VC: vitamin C; BP: biological process; MF: molecular functions; CC: cellular components.

Table 3. GO analysis of common genes among VC metabolism-associated genes and T2DM-associated genes (cont.)

Category ID	Term	Count	Genes	*p* value	FDR
GO:0005576	extracellular region	29	APP, CXCL6, PROS1, PRF1, FBLN1, CXCL1, TRH, PTHLH, VTN, ACAN, CTSK, CCL2, APOB, SNCA, ANXA1, SERPINB2, BDNF, SFTPC, APOA1, APOA4, S100B, SOD3, COL1A1, CXCL10, COL2A1, SST, CSPG5, MET, GLA	<0.001	0.008
GO:0005739	mitochondrion	25	ATP6V1A, SHMT2, OGG1, MGST1, AK4, ACAT1, ATP5B, CASP8, HLCS, CYP11A1, FDXR, TSPO, MAPK1, SNCA, PCK2, HSPA5, GPX4, GZMB, TFB2M, PKM, CAT, CYP1A1, NOX4, CRYAB, HSPA1A	0.001	0.013
GO:0009986	cell surface	14	APP, ANXA1, HSPA5, SORT1, SDC2, SLC11A2, APOA1, APOA4, ATP5B, KLRK1, CSPG5, CRYAB, MET, PAM	0.001	0.021
GO:0005791	rough endoplasmic reticulum	5	APP, BGLAP, CCL2, MT3, SNCA	0.001	0.021
GO:0005788	endoplasmic reticulum lumen	8	COL1A1, PDIA2, COL2A1, HSPA5, BGLAP, APOA1, APOA4, APOB	0.001	0.028
GO:0043209	myelin sheath	7	ATP6V1A, ATP5B, PKM, HSPA5, PHGDH, CRYAB, GFAP	0.002	0.035
GO:0045121	membrane raft	8	ABCA1, APP, EDNRB, CASP8, GPC1, CD48, SLC2A4, S100A10	0.002	0.035
GO:0005925	focal adhesion	11	DAB2, FLT1, ANXA1, HSPA5, CAT, HSPB1, CDH13, MAPK1, NOX4, ARPC5, HSPA1A	0.002	0.035
GO:0043204	perikaryon	6	BGLAP, MAPK1, CCL2, RGS8, CRYAB, PAM	0.002	0.035
GO:0005901	caveola	5	NOS3, SLC2A1, CDH13, HMOX1, MAPK1	0.002	0.035
GO:0043231	intracellular membrane-bounded organelle	13	APP, G6PD, GRN, GZMB, S100B, IGF1R, DAB2, CYP1A2, CAT, CYP1A1, TSPO, PPARG, APOB	0.004	0.047
GO:0005764	lysosome	8	CTSK, SLC11A2, CAT, GLA, GABARAP, SYT7, GFAP, SNCA	0.004	0.047
GO:0031904	endosome lumen	3	APP, PRF1, APOB	0.004	0.047
MF					
GO:0005515	protein binding	118	APP, EIF4A1, SLC23A1, PRF1, KEAP1, PEBP1, IKZF1, IGF1R, LOXL2, SOX2, LGALS3, MT2A, EDNRB, CCND1, ARHGDIB, FDXR, RGS8, PDIA2, G6PD, SLC11A2, SOX10, PCP4, MT1A, S100A6, PSME2, TRIB3, LXN, SHMT2, SDC2, MGST1, NREP, PIK3R1, NDRG2, GABARAP, VTN, ATP5B, KLRK1, MAP2, HLCS, HMOX1, MYH11, APOB, NKX2-5, SULT1A3, S100A10, PCK2, ABCA1, GSTM2, HSPA5, CYBRD1, ASNS, ARPC5, EIF2S2, MT3, SYT7, GFAP, COL1A1, CXCL10, DAB2, SP1, SP4, SP3, AGO2, CYP1A1, NFE2L3, NOX4, CSPG5, BMPR1B, SLC22A7, PAM, CDKN1A, FLT1, OGG1, SLC2A1, HSPB1, SLC2A4, HERPUD1, CASP8, SEC61G, CTSK, TSPO, ANXA1, GSTO1, SFTPC, APOA1, APOA4, FOS, TYK2, POU5F1, PKM, BIRC5, PPARG, CD48, CRYAB, MET, CEBPB, GRN, NCALD, ADCY6, ACAN, GNG3, GNG4, MAPK1, SNCA, NQO1, NOS2, SORT1, NOS3, NR1H3, GZMB, S100B, SOD3, PMP22, ADGRL1, GLA, TPT1, RAN, HSPA1A	<0.001	<0.001
GO:0019899	enzyme binding	17	APP, GSTM2, HSPA5, SORT1, PEBP1, APOA1, ACAT1, CYP2A6, CCND1, HLCS, CYP1A2, CAT, CYP1A1, BIRC5, HMOX1, PPARG, HSPA1A	<0.001	<0.001
GO:0042803	protein homodimerization activity	23	NTRK2, GSTM2, G6PD, CEBPB, ANXA1, NOS2, MGST1, ASNS, APOA4, S100B, ACAT1, KLRK1, HLCS, SP1, CAT, S100A6, BIRC5, CDH13, HMOX1, NKX2-5, CRYAB, GLA, S100A10	<0.001	<0.001
GO:0042802	identical protein binding	23	NQO1, APP, G6PD, SHMT2, SFTPC, SLC2A1, APOA1, HSPB1, FBLN1, S100B, SOX10, GFAP, IGF1R, COL1A1, VTN, COL2A1, CASP8, BIRC5, PSME2, MAPK1, PPARG, CRYAB, SNCA	<0.001	<0.001
GO:0005507	copper ion binding	7	GPC1, APOA4, MT3, SOD3, PAM, LOXL2, SNCA	<0.001	0.001
GO:0020037	heme binding	9	CYP2A6, NOS2, NOS3, CYP11A1, CYP1A2, CAT, CYP1A1, HMOX1, NOX4	<0.001	0.003

T2DM: type 2 diabetes mellitus; VC: vitamin C; BP: biological process; MF: molecular functions; CC: cellular components.

Table 3. GO analysis of common genes among VC metabolism-associated genes and T2DM-associated genes (cont.)

Category ID	Term	Count	Genes	*p* value	FDR
GO:0055056	D-glucose transmembrane transporter activity	4	SLC2A1, SLC2A2, SLC2A4, SLC2A8	<0.001	0.003
GO:0005509	calcium ion binding	19	ANXA1, HSPA5, PROS1, PRF1, IHH, FBLN1, S100B, SYT7, NCALD, ACAN, PCDHGA12, BGLAP, CDH22, S100A6, CDH13, PAM, TPT1, S100A10, SNCA	<0.001	0.007
GO:0008134	transcription factor binding	11	CEBPB, CCND1, SP1, KEAP1, MAPK1, PPARG, FOS, PIK3R1, NKX2-5, SOX10, POU5F1	<0.001	0.014
GO:0017127	cholesterol transporter activity	4	ABCA1, APOA1, APOA4, APOB	<0.001	0.014
GO:0005355	glucose transmembrane transporter activity	4	SLC2A1, SLC2A2, SLC2A4, SLC2A8	<0.001	0.014
GO:0033300	dehydroascorbic acid transporter activity	3	SLC23A1, SLC2A1, SLC2A2	0.001	0.019
GO:0015485	cholesterol binding	5	ABCA1, TSPO, NR1H3, APOA1, APOA4	0.001	0.019
GO:0016491	oxidoreductase activity	9	GSTO1, NOS3, CYP1A2, CYP1A1, FDXR, AKR1B1, NOX4, AKR1C2, SNCA	0.001	0.019
GO:0008201	heparin binding	8	CXCL6, VTN, CXCL10, APP, LXN, CCL2, APOB, SOD3	0.001	0.024
GO:0044212	transcription regulatory region DNA binding	9	SOX2, SP1, NR1H3, PPARG, IKZF1, FOS, NKX2-5, POU5F1, SNCA	0.001	0.025
GO:0046870	cadmium ion binding	3	NOS3, SLC11A2, MT3	0.001	0.035
GO:0005102	receptor binding	11	ABCA1, CXCL10, APP, GSTM2, ANXA1, NOS2, CAT, CCL2, CXCL1, GLA, HSPA1A	0.002	0.046

T2DM: type 2 diabetes mellitus; VC: vitamin C; BP: biological process; MF: molecular functions; CC: cellular components.

Table 4. KEGG pathway analysis of common genes among VC metabolism-associated genes and T2DM-associated genes

ID	Term	Count	Genes	*p* value	FDR
hsa04066	HIF-1 signaling pathway	9	CDKN1A, FLT1, NOS2, NOS3, SLC2A1, HMOX1, MAPK1, PIK3R1, IGF1R	<0.001	0.022
hsa05230	Central carbon metabolism in cancer	7	G6PD, PKM, SLC2A1, SLC2A2, MAPK1, PIK3R1, MET	<0.001	0.035
hsa00980	Metabolism of xenobiotics by cytochrome P450	7	GSTM2, CYP2A6, GSTO1, CYP1A2, MGST1, CYP1A1, AKR1C2	0.001	0.035
hsa05200	Pathways in cancer	16	CDKN1A, NOS2, SLC2A1, PIK3R1, FOS, ADCY6, IGF1R, GNG3, CASP8, EDNRB, CCND1, GNG4, BIRC5, MAPK1, PPARG, MET	0.001	0.035
hsa04068	FoxO signaling pathway	9	CDKN1A, CCND1, CAT, MAPK1, SLC2A4, PIK3R1, GABARAP, PCK2, IGF1R	0.001	0.035
hsa04668	TNF signaling pathway	8	CXCL10, CEBPB, CASP8, MAPK1, CCL2, CXCL1, FOS, PIK3R1	0.001	0.035
hsa04931	Insulin resistance	8	NOS3, SLC2A1, SLC2A2, NR1H3, TRIB3, SLC2A4, PIK3R1, PCK2	0.002	0.035
hsa05204	Chemical carcinogenesis	7	GSTM2, CYP2A6, GSTO1, CYP1A2, MGST1, CYP1A1, SULT1A3	0.002	0.035

T2DM: type 2 diabetes mellitus; KEGG: Kyoto Encyclopedia of Genes and Genomes; VC: vitamin C.

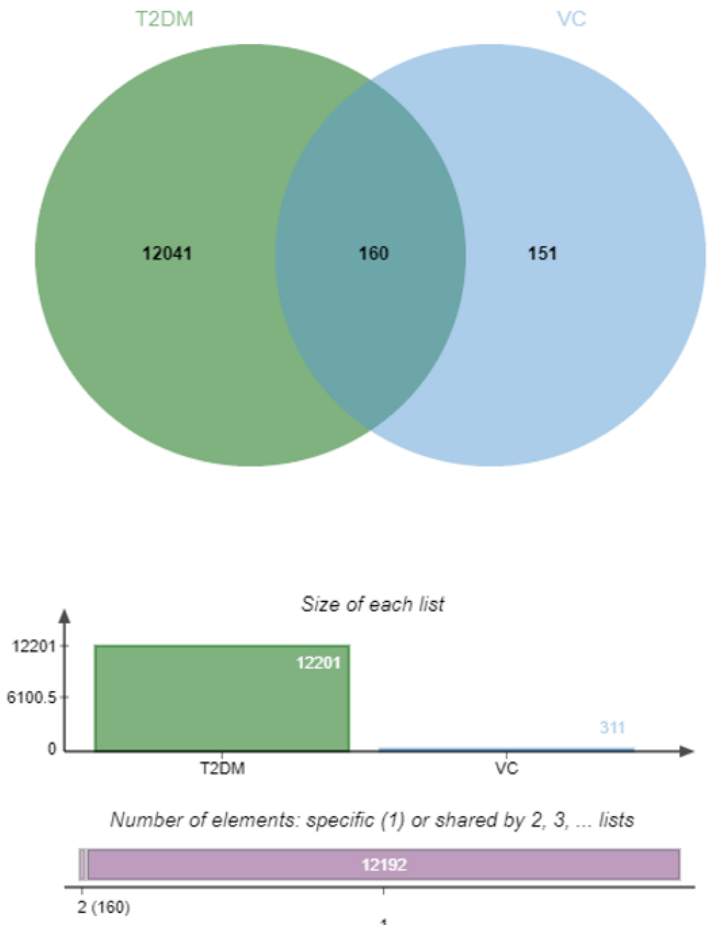

Figure 1. T2DM-associated and VC metabolism–associated DEGs.

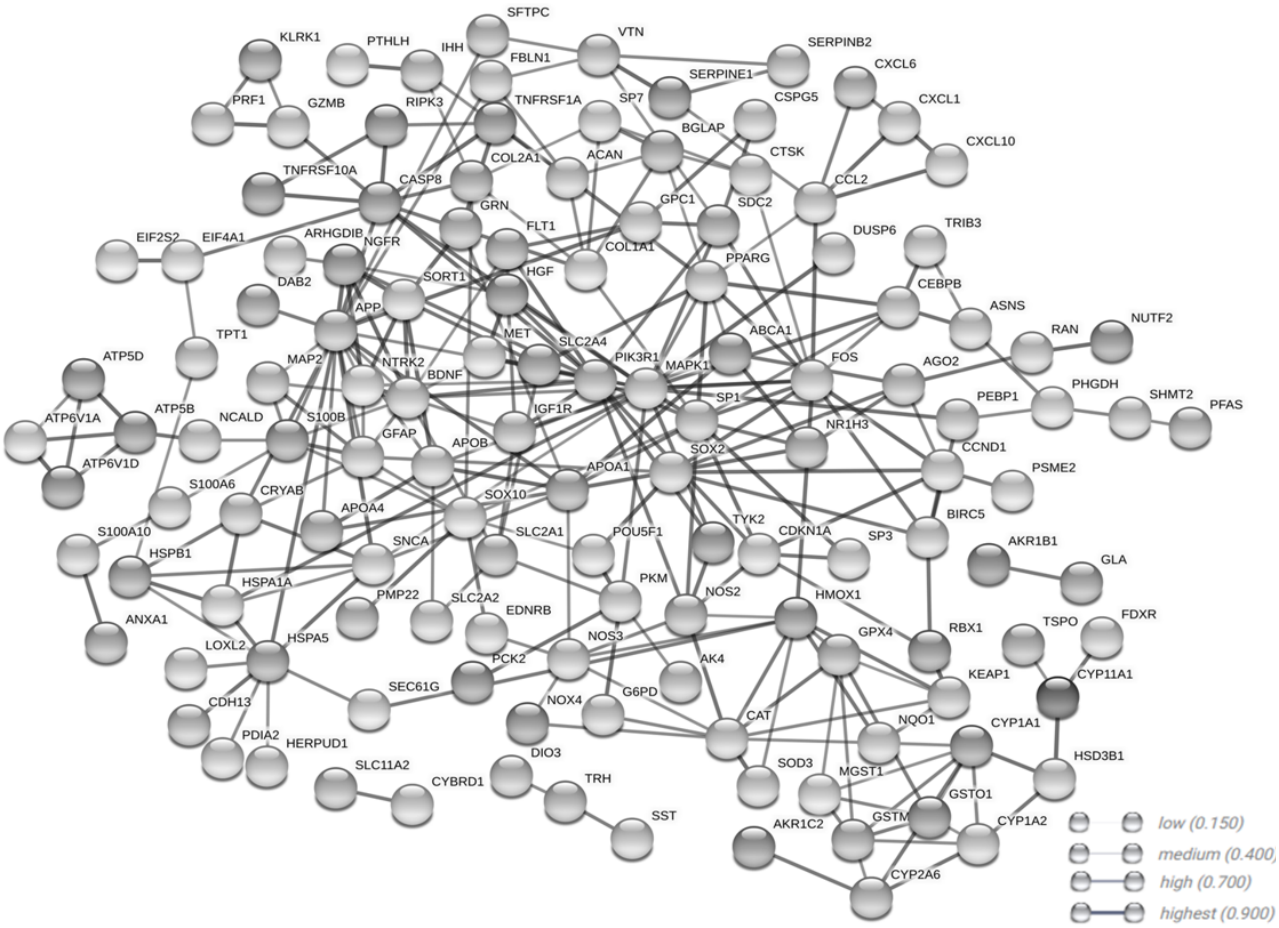

Figure 2. PPI of common genes in T2DM. (Network statistics: number of nodes = 160, and number of edges = 237).

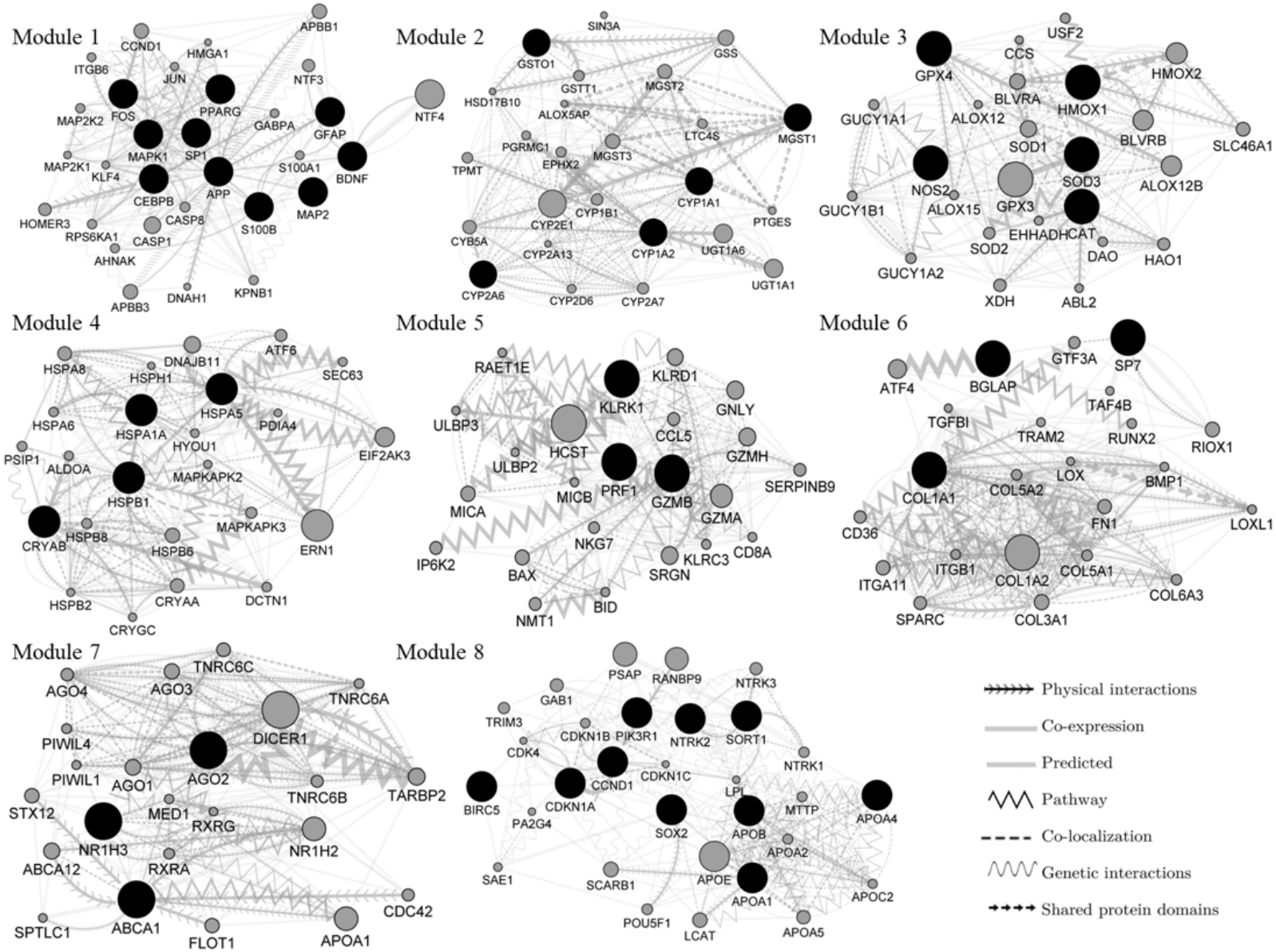

Figure 3. PPI of common genes among VC metabolism-associated genes and T2DM-associated genes using GeneMANIA. (Black nodes represent common genes, and connecting colors indicate different correlations. Genes in black circles were query terms, and those in grey circles show genes associated with query genes.).

of β-cells.[15] When mild or tolerable concentration of the unfolded protein response (UPR) is activated in β-cells, the UPR transducers PERK are essential to maintaining β-cells homeostasis.[16] The identified common genes are involved in the TNF signaling pathway and the HIF-1 signaling pathway. These two pathways play crucial roles in T2DM pathogenesis,[17,18] whereas VC is affected dramatically by pro-inflammatory cytokines such as TNF-α.[19] VC regulated glucose and insulin by decreasing the concentration of HIF-1α.[20]

The PPI network showed that T2DM-associated and VC metabolism–associated common genes were associated with GO terms and KEGG pathways. Endothelial dysfunction plays a key role in deleterious events in diabetes,[21] FoxO transcription factor plays an important role in regulating endothelial cell morphogenesis and vascular homeostasis, meanwhile, serve a vital purpose in endothelial dysfunction.[22,23] VC has been shown to participate in several important functions for preventing endothelial dysfunction, including endothelial proliferation, inhibition apoptosis, scavenge of radical species, explaining the beneficial actions of VC in T2DM.[24] Insulin resistance and hyperglycemia result in specific immune responses that exacerbate the inflammatory state, including impaired cytokine production,[25,26] neutrophil dysfunction,[27] macrophage dysfunction,[28] and natural killer cell dysfunction.[29] VC contributes to immune defense by modulating cytokine production,[30] enhancing neutrophil chemotactic ability,[31] facilitating macrophages clearance,[32] and enhancing natural killer cell differentiation.[33]

Conclusion

In summary, our study analyzed T2DM-associated and VC metabolism–associated common genes by using the GEO and CTD databases. We discovered a number of enriched GO, KEGG pathways and PPI networks. The findings could significantly improve the understanding of the mechanisms underlying effect of VC on T2DM. However, further research is needed to validate our findings.

AUTHOR DISCLOSURES

The authors declare that they have no conflicts of interest.

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