

A Bioinformatics Investigation into the Pharmacological Mechanisms of Sodium-Glucose Co-transporter 2 Inhibitors on Diabetes Mellitus and Heart Failure based on Network Pharmacology

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Abstract

Background

Diabetes mellitus (DM) is a major risk factor for the development of heart failure (HF). Sodium-glucose co-transporter 2 (SGLT2) inhibitors have been demonstrated consistent benefits in the reduction of hospitalization for HF in patients with DM. However, the pharmacological mechanism is not clear. To investigate the mechanisms of SGLT2 inhibitors on HF and DM, we performed target prediction and network analysis by network pharmacology method.

Material/Methods

We selected targets of SGLT2 inhibitors according to SwissTargetPrediction and DrugBank databases and collected therapeutic targets on HF and DM from the Human Gene (GeneCards) and Human Mendelian Inheritance (OMIM) databases. The “Drug-Target” and “Drug-Target-Disease” networks were constructed by using Cytoscape_v3.6.1. Then the protein-protein interaction (PPI) was analyzed by using the String database. Gene Ontology (GO) biological functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were performed to investigate by using Bioconductor tool for analysis.

Results

There were 125 effective targets among SGLT2 inhibitors, HF and DM. Through further screening and analyzing, 33 core targets were obtained, such as SRC, MAPK1, NARS, MAPK3 and EGFR. And it is predicted that Rap1 signaling pathway, MAPK signaling pathway, EGFR tyrosine kinase inhibitor resistance, AGE-RAGE signaling pathway in diabetic complications and other signaling pathways were involved in the treatment of HF and DM by SGLT2 inhibitors.

Conclusions

Our study elucidated the possible mechanisms of SGLT2 inhibitors from a systemic and holistic perspective based on pharmacological networks. The key targets and pathways will provide new insights for further research on the pharmacological mechanism of SGLT2 inhibitors in the therapy of HF and DM.

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia due to partial or complete insulin deficiency and/or insulin resistance, which affects 1–2% of the population worldwide[1]. Heart failure (HF) is a substantial but frequently overlooked complication of DM[2]. Several studies showed that the incidence of HF is 2–5 times higher in diabetic patients than in those without DM[3]. Furthermore, diabetic patients with HF have longer HF-related hospital stays, more frequent HF-related readmissions and higher risk for cardiovascular mortality than patients with HF but without DM[4–6]. However, treating patients with these concomitant diseases can be challenging. Some drugs have been recommended for the treatment of HF and DM, such as metformin and sulphonylureas. But they are insufficient in the

therapy of HF and DM. For instance, metformin alone is often not enough to keep glycemia under control, which is not well improved the condition of patients with HF and DM[7]. Sulphonylureas are commonly prescribed in DM but associated with weight gain and hypoglycemia which are detrimental in heart failure [8, 9]. Therefore, there is a compelling impetus to explore the potential strategies to reduce the risk of HF in patients with DM.

Sodium-glucose co-transporter 2 (SGLT2) inhibitors, a class of glucose-lowering therapies, including dapagliflozin, canagliflozin, empagliflozin and ertugliflozin, have been approved by the US Food and Drug Administration for the treatment of type 2 diabetes mellitus[10]. SGLT2 inhibitors can inhibit proximal renal tubular sodium and glucose reabsorption to increase the urinary excretion of glucose, thereby reducing blood sugar[11]. Data to date suggest this agent appears to moderately reduce the risk of major adverse cardiovascular events beyond simply reducing plasma glucose levels[10]. Particularly, most attention has focused on the pleiotropic effects of SGLT2 inhibitors on cardiac function and their potential benefits with regards to heart failure and mortality rates. Three large cardiovascular outcomes trials, the EMPAREG OUTCOME trial, the CANVAS Program 30, and the DECLARE-TIMI 58 trial, have demonstrated the significant and consistent reduction in heart failure events and other cardiovascular events with SGLT2 inhibitors[12–14]. Based on these composite data, SGLT2 inhibitors represent an important new therapeutic approach for the prevention of heart failure in at-risk patients with diabetes mellitus. However, the therapeutic targets and mechanism of SGLT2 inhibitors acting on HF in patients with DM have not been revealed, which needs to be further explored and analyzed.

With the development of high-throughput sequencing and computer technology, huge and brand-new bio-information networks have emerged. Network pharmacology, one of the bio-information networks, aims to construct a multi-level network through various database searches, high-throughput omics data analysis and computer simulations to analyze the relationship of medicines, diseases and targets[15]. Compared with traditional experimental pharmacology methods, network pharmacology is based on a comprehensive system, which is relatively efficient to explore the target and pathway relationships between drugs and diseases. Therefore, we applied network pharmacology analysis to systematically excavate the action targets of SGLT2 inhibitors on HF and DM to establish the network of protein interactions to analyze the biological pathways involved, which will lay a good foundation for further in-depth exploration of the mechanism of SGLT2 inhibitors acting on HF and DM. The study flowchart of this network pharmacology-based study of SGLT2 inhibitors is shown in Fig. 1.

Methods

1. Prediction of SGLT2 inhibitors related targets

The chemical structures of four SGLT2 inhibitors, namely as canagliflozin, dapagliflozin, empagliflozin, and ertugliflozin, were obtained using Pubchem (<https://pubchem.ncbi.nlm.nih.gov/>), which is an open chemistry database with 96,502,248 compositions of which 3,151,393 have been tested. Then SwissTargetPrediction (<http://www.swisstargetprediction.ch/>), a tool for target prediction according to 2-

dimensional and 3-dimensional similarity measures with known ligands, was selected to predict potential targets for four SGLT2 inhibitors by putting their chemical structures into this platform [16]. Additionally, SGLT2 inhibitors related genes were also collected from DrugBank (<https://www.drugbank.ca/>), which is a unique bioinformatics and chemical informatics database, containing 11,628 drugs and related chemical information, drug targets, protein data, and so on [17]. And with further correction and transformation by the retrieval of Universal Protein Resource (UniProt, <http://www.uniprot.org/>), all the SGLT2 inhibitors related genes were normalized into consistent symbols for subsequent analysis.

2. The prediction of known therapeutic targets on HF and DM

With the keywords of “heart failure”, “diabetes”, and “diabetes mellitus”, target genes related to HF and DM were found in GeneCards (<https://www.genecards.org>), and OMIM (<https://www.omim.org>) databases. The GeneCards database includes more than 7000 human genes, and each gene has an approved gene symbol. And the OMIM database is a knowledge base of human genes and hereditary diseases. The two methods have a good reference for the collection of disease targets. According to the targets of SGLT2 inhibitors and diseases, the repeated targets of the two were screened by Excel, and their intersection targets of SGLT2 inhibitors were obtained. According to their intersection, we get the Venn diagram on the website (<https://bioinfogp.cnb.csic.es/tools/venny/>).

3. Construction of the Network Model

The four SGLT2 inhibitors and their corresponding targets and the intersection targets of diseases and drugs were sorted out and input into Cytoscape_v3.6.1 to construct the following networks: (1) network between four SGLT2 inhibitors (canagliflozin, dapagliflozin, empagliflozin, and ertugliflozin) and their corresponding targets; (2) network between four SGLT2 inhibitors, intersection targets, and diseases (HF and DM). Cytoscape is a kind of software which can express the interaction between protein and protein, protein and DNA or gene efficiently and can visualize network relationships[18].

4. Screening of Core Targets of SGLT2 inhibitors in HF and DM Treatment

The STRING (<https://string-db.org>) dataset is now one of the largest PPI datasets, including co-expression data, biomedical literature data, high-throughput data, and genomic back-ground data [19]. In the platform, “Multiple proteins” was selected and the organism was selected as “Homo sapiens.” The intersection targets of SGLT2 inhibitors, HF and DM were then imported to construct the protein-protein interaction network. In order to ensure the high confidence of information, the scoring condition was set to >0.90, and the isolated proteins in the figure were hidden. The file was exported in “TSV format,” and then, Cytoscape_v3.6.1 was used to analyze the topological properties of the PPI network. To detect the core targets in the common targets between drugs and diseases, the degree of targets were calculated using Cytohubba plugin based on Cytoscape[20].

5. Construction of Protein-Protein Interaction (PPI) Network of Core Targets

String online platform was used to build the PPI network of core targets. After selecting the multiple proteins module, the Gene Name list of target proteins was uploaded to the network, limiting the species to Homo sapiens. And the confidence score was set to >0.9. In the PPI diagram, each solid circle represents a gene, and the middle of the circle shows the structure of the protein, while the circles are connected by lines of different colors. Each line represents the biological process between protein and protein, including regulation of gene expression, signal transduction, cell migration and so on.

6. GO Enrichment Analysis and KEGG Pathway Enrichment Analysis

In order to better understand the potential biological process of core genes, KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (Gene Ontology) were analyzed for pathway functional enrichment using clusterProfiler software package on the R platform[21, 22]. The interaction network was constructed by using the Top-Go package of the R platform [23].

Results

1. Screening potential related targets of SGLT2 inhibitors

PubChem platform was used to acquire the molecular structure of dapagliflozin empagliflozin, canagliflozin and ertugliflozin. The details of their structure were shown in **Table 1**. After importing their structures into the SwissTargetPrediction database respectively for target matching and prediction, we screened the 213 targets with probability >0, of which 71 targets were in dapagliflozin, 65 in canagliflozin, 61 in ertugliflozin and 16 in empagliflozin, respectively. We also adopted the DrugBank database to find 37 targets on the four SGLT2 inhibitors, with 11 targets in dapagliflozin, 10 in empagliflozin, 8 in canagliflozin, 8 in ertugliflozin. By integrating and eliminating duplicate targets in the two databases, a total of 135 targets with potential effects of SGLT2 inhibitors were obtained. Then introducing them into Cytoscape_v3.6.1 software to analyze, we constructed a visualized drug-target network (Fig. 2). There are 139 nodes (135 targets, 4 drugs) and 252 edges shown in Fig. 2, where the pink nodes represent SGLT2 inhibitors, the blue nodes represent the drug targets (the predicted target) and the edges represent the interactions between the drugs and the targets. It reflects the potential mechanism of interaction between SGLT2 inhibitors and multitarget.

2. Construction and analysis of drug-target-disease network

After the repetition was removed, a total of 13523 targets related to HF and 15291 targets corresponding to DM were collected from the GeneCards database and the OMIM database. Among these, 125 common targets were shared between potential targets of SGLT2 inhibitors, known HF-related targets and DM-related targets by Draw Venn diagram online platform (Fig. 3). And 125 “drug-disease” common targets

were introduced into Cytoscape_v3.6.1 software to construct a visualized drug-target-disease network (Fig. 4). There are 131 nodes (125 targets, 4 drugs, 2 diseases) and 441 lines shown in the network. The purple nodes are the four SGLT2 inhibitors (dapagliflozin, empagliflozin, canagliflozin and ertugliflozin); the red nodes are HF and DM; the green nodes represent 125 common targets. In this network, the average target number of each SGLT2 inhibitor is 6.73. Thus, there is an interaction between one SGLT2 inhibitor and multiple targets in the treatment of HF and DM. With regard to the targets, the top three in the degree are SLC5A2, SLC5A1, and SLC2A1, well known as the sodium-dependent glucose cotransporter gene, which can interact with four SGLT2 inhibitors respectively. These targets connect the relationship between SGLT2 inhibitors and disease, which provides a better reference for exploring the mechanism of SGLT2 inhibitors in the treatment of HF and DM.

3. Core Targets of SGLT2 inhibitors on HF and DM

125 common targets were imported into STRING online platform, and a file in "TSV" format was exported. Then, it was imported into Cytoscape_v3.6.1 and the function of "Cytohubba" was used to calculate the degree of targets to find the core targets. 33 core targets of SGLT2 inhibitors in HF and DM treatment were screened (Fig. 5), among of which, red, orange, yellow and purple nodes represent the gradual decrease of degree value from large to small. The specific information of core targets is shown in Table 2.

4. Analysis of 33 core targets protein interaction network (PPI)

The STRING database platform was used to construct a network of target protein interactions, and the 33 core targets of SGLT2 inhibitors-HF-DM were imported. By selecting species as "Homo sapiens" and setting the combined score > 0.9 thresholds, the final protein-protein interaction network was obtained (Fig. 6). As can be seen from Fig. 6, there are a total of 33 solid circles of many colors, each circle represents a key target gene, and the center of the dot shows the protein structure of the target gene. And the statistical analysis was performed on each target gene to obtain the top targets with the number of adjacent genes ≥ 10 (shown in Fig. 7), revealing that SRC, MAPK1, NARS, MAPK3 and EGFR are the top 5 hub targets, which might be the key targets for SGLT2 inhibitors treatment of HF and DM.

5. GO biological function annotation and KEGG pathway enrichment analysis for targets

To study the mechanism of SGLT2 inhibitors on HF and DM more systematically, the 33 core target genes of the SGLT2 inhibitors-HF-DM intersection were introduced into R statistical programming language to analyze the GO biological function and KEGG signaling pathway. Filtering with P value = 0.05 and Q value = 0.05 as threshold values, we mainly selected the 10 biological processes of 33 core targets, as shown in Fig. 8. The results indicate that the targets mainly are associated with positive regulation of MAP kinase activity, positive regulation of protein serine/threonine kinase activity, regulation of MAP kinase activity, activation of protein kinase activity, regulation of phosphatidylinositol 3-kinase signaling,

response to reactive oxygen species, phosphatidylinositol 3-kinase signaling, muscle cell proliferation, cellular response to reactive oxygen species and positive regulation of reactive oxygen species metabolic process.

Based on the KEGG enrichment analysis of 33 key targets, we found that the top 15 signal paths with high confidence (P-value < 0.05) are selected for analysis in Fig. 9. According to the result, it is demonstrated that the core targets might affect some signaling pathways, such as Rap1 signaling pathway, MAPK signaling pathway, EGFR tyrosine kinase inhibitor resistance and AGE-RAGE signaling pathway in diabetic complications, which predicts that SGLT2 inhibitors might achieve treatment of HF and DM by regulating the aforementioned signal pathways.

Discussions

Heart failure (HF) is a major complication of diabetes mellitus (DM). It is necessary to prevent HF in patients with DM. In clinical studies, SGLT2 inhibitors were effective in treating HF among patients with DM, which aroused great interest and attention. However, the mechanism is not clear. Based on the network pharmacological analysis, this study explored the mechanism of SGLT2 inhibitors in the therapy of HF and DM. In the present study, four SGLT2 inhibitors (canagliflozin, dapagliflozin, empagliflozin, and ertugliflozin) were obtained to analyze the effects on HF and DM based on the network pharmacology analysis. According to the drugs-diseases target network and PPI network, the 33 core genes of the SGLT2 inhibitors acting on HF and DM were obtained from 125 common genes. Among the 33 core genes, the top 5 hub targets SRC, MAPK1, NRAS, MAPK3 and EGFR were screened according to the degree. The module analysis confirmed that SGLT2 inhibitors have the potential to influence varieties of biological pathways that play an important role in the pathogenesis of HF and DM. The key pathways were screened by KEGG analysis, mainly involved Rap1 signaling pathway, MAPK signaling pathway, EGFR tyrosine kinase inhibitor resistance and AGE-RAGE signaling pathway in diabetic complications, etc.

1. Further analysis of the core genes

Among the top 5 core genes, the first one, SRC, is a non-receptor tyrosine kinase that serves roles in numerous biological process including cell adhesion, cell cycle and cell migration. Haendeler et al. demonstrated that SRC kinases could be activated by Ang II, which plays an important role in Ang II-mediated processes, including the pathophysiology of cardiac hypertrophy and remodeling, vascular thickening and heart failure[24]. Meanwhile, Pandey et al. found that SRC activation would contribute to the alteration of non-myofibrillar tension, which will impact the baseline tension in fibrotic hearts after MI or in dilated cardiomyopathy[25]. And inhibition of SRC(c-Src) activation can decrease endogenous ROS production and increase ATP production in diabetic GK rat islets [26]. Safari-Alighiarloo et al. also identified that SRC is one of the key genes for type 1 diabetes through analysis of gene expression profiles[27]. SRC may be a potential target for HF and DM disease.

And the next two hub genes, MAPK1(mitogen-activated protein kinase 1) and MAPK3(mitogen-activated protein kinase 3), also named ERK1 and ERK2 respectively, are the members of the MAP kinase (MAPK) family. MAPK family act as an integration point for multiple biochemical signals and are involved in a wide variety of cellular processes such as proliferation, differentiation, transcription regulation and development. Bueno et al. indicated that the activation of ERK1/2 seems to be related to a beneficial form of myocardial hypertrophy, which may be advantageous to a failing or dilated myocardium[28]. Besides, stimulation by glucose can activate the ERK1/2 signaling pathway in rat islets, and blocking the activation of the ERK1/2 signaling pathway can reduce glucose-stimulated insulin secretion (GSIS)[29]. This suggests that MAPK1 and MAPK3 may be the potential targets in HF and DM.

Next, the following gene is, NRAS, an N-ras oncogene encoding a membrane protein that shuttles between the Golgi apparatus and the plasma membrane, works in various differentiation processes and signal transduction, involving the regulation of cell survival and growth, T cell activation and apoptosis. Katoh et al. has reported that NRAS was one of the representative targets on cardio-miR-214 that were upregulated in human heart failure, showing that NRAS might be associated with the progression of heart failure[30]. And it's observed that NRAS expression elevated with tumor advancement in diabetic rats owing to increased levels of IRS-1, leading to the activation of MAP kinase cascade[31]. Thus, NRAS has the potential to be the one target of HF and DM.

And the last one of top 5 hub gene is EGFR, epidermal growth factor receptor, a cell surface protein binding to epidermal growth factor, can regulate cell growth, proliferation, and survival, which involved in blood pressure regulation, neointimal hyperplasia, atherogenesis and reactive oxygen. Xu et al. revealed that the enhanced myogenic constriction of the mesenteric artery in heart failure might be related to the loss of plasmalemmal caveolae in mesenteric VSMCs, while the increased activity of EGFR and AT₁ – receptor was considered to be one of the mechanisms leading to this result[32]. Furthermore, EGFR transactivation also could mediate the Ang II to affect the pathophysiology of left ventricular (LV) hypertrophy[33]. And Belmadani et al. found that elevated EGFR phosphorylation contributed to resistance artery dysfunction in type 2 diabetes[34]. Zhang et al. concluded that inhibiting EGFR could slow the progression of diabetic nephropathy by decreasing endoplasmic reticulum stress and increasing autophagy[35]. The findings demonstrate that EGFR plays an irreplaceable role in HF and DM. Above all, the top 5 core genes of the present study based on network pharmacology are supported by previous studies.

2. Enrichment analysis based on core targets

GO functional enrichment and KEGG pathway analysis have illustrated the role of the SGLT2 inhibitors in the gene function and signaling pathway. According to the biological processes that mainly reflects the GO functional enrichment, these core targets are focused on kinase activity, primarily in positive regulation of MAP kinase activity, positive regulation of protein serine/threonine kinase activity, regulation of MAP kinase activity, activation of protein kinase activity and so on. In the enrichment of the KEGG pathway, Rap1 signaling pathway, MAPK signaling pathway, EGFR tyrosine kinase inhibitor resistance and AGE-RAGE signaling pathway in diabetic complications are the significant pathways.

Rap1 signaling pathway is implicated in a wide range of biological processes from cell proliferation and differentiation to cell adhesion[36].Rap1 has been proved to play a part in the regulation of integrin affinity, adhesion, and migration in postnatal neovascularization[37], mainly mediating in the angiogenesis pathway served an important role in cardiac hypertrophy. Furthermore, Rap1B can prevent excessive vascular leakage in early diabetes mellitus by inhibiting VEGF signal transduction. Through controlling telomere length, Rap1 can decrease the occurrence and development of diabetes-related cardiovascular disease [38]. Downregulation of Rap1B to reduce VEGF signal transduction can impede excessive vascular leakage in early diabetes mellitus [39]. At the same time, Rap1 also regulates MAP kinase (MAPK) activity in a manner highly dependent on the context of cell types[40].

Consistent with the core target results above, the MAPK pathway is also predicted to play a role in HF and DM. It's demonstrated that the MAPK signaling pathway cascade initiated in cardiomyocytes through activation of G protein-coupled receptors, receptor tyrosine kinases, and stress stimulation [41]. Zhang et al. informed that the MAPK signaling pathway could regulate cardiomyocyte apoptosis in mice with heart failure after MI, indicating that this pathway has a potential role in heart failure disease [42]. During the process of insulin resistance, there exists a chronic inflammatory response, which makes the MAPK pathway serve on the inflammatory response of type 2 diabetes, such as diabetic nephropathy and liver disease[43, 44].

EGFR tyrosine kinase inhibitor resistance, acts on EGFR tyrosine kinase, also identical with the core genes predicted based on the PPI. Previous studies had indicated that inhibition of EGFR activity protected against progressive DN in T1 DM and T2 DM [45]. And Zeng et al. inferred that EGFR inhibition reduced ROS production in the left ventricle and blunted hypertensive myocardial hypertrophy in spontaneously hypertensive rats [46]. Therefore, the EGFR tyrosine kinase inhibitor resistance may have a common effect to accelerate the progression of HF and DM.

AGE-RAGE signaling pathway, is a well-studied cascade in DM. It's found that the AGE-RAGE signaling pathway can directly mediate vascular calcification in diabetes[47]. Additionally, the pathway can also impact diabetic complications, as it leads to oxidative stress, increased inflammation, and enhanced extracellular matrix accumulation resulting in diastolic and systolic dysfunction[48]. Fukami et al. proved that the activation of the AGE-RAGE signaling pathway in diabetic complications could cause excessive production of advanced glycation end products to hurt cardiomyocyte, leading to HF[49], which pointed that the AGE-RAGE signaling pathway in diabetic complications predicted in the study may mediate the progression of heart failure, as the same as the results of pathway analysis in another study[50]. Therefore, our results suggest that the 4 signaling pathways might be involved in the mechanisms of SGLT2 inhibitors affecting HF and DM.

Network pharmacology is indeed considered to be a new method to study the relationship between drugs and diseases. In our study, the network pharmacology revealed the potential targets of SGLT2 inhibitors, as well as HF and DM related targets, and bioinformatics was used to study the main enrichment pathways. Based on the network pharmacology, our study predicted the potential therapeutic targets of

SGLT2 inhibitors on HF and DM, revealed its action on the main pathways through core genes, which explained the mechanisms of SGLT2-inhibitors on HF and DM and provided scientific evidence for SGLT2-inhibitors to HF and DM. However, the main limitation of this study is the lack of experimental verification. Consequently, it is of great significance to undertake pharmacological studies to research the relationship between SGLT2 inhibitors in HF and DM. Moreover, the validation of molecular levels of our findings is necessary for the future.

Conclusions

Taken all together, our study systematically predicted, screened and analyzed the targets and pathways that might play a vital role in the biological process, which elaborated the possible mechanisms of SGLT2 inhibitors on HF and DM. Most importantly, these results provide evidence and new insights for further researches on the pharmacological mechanism of SGLT2 inhibitors.

Declarations

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Conflict of Interest

The authors declare that there is no conflict of interest.

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 performed by any of the authors.

Consent for publication

All authors agree to public.

Data Availability

Author can confirm that all relevant data are included in the article and/or its supplementary information files.

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Not applicable.

Author Contributions

(I) Conception and design: Ziling Mai, Huanqiang Li, Yong Liu, Jiyan Chen; (II) Administrative support: Enzhao Chen, Shiqun Chen; (III) Collection and assembly of data: Zhubin Lun, Huanqiang Li, Liwei Liu, Wenguang Lai; (IV) Data analysis and interpretation: Ziling Mai, Guanzhong Chen, Enzhao Chen; (V) Manuscript writing: All authors; (VI) Final approval of manuscript: All authors.

References

1. Zimmet P, Alberti KG, Magliano DJ, Bennett PH. Diabetes mellitus statistics on prevalence and mortality: facts and fallacies. *Nature reviews Endocrinology*. 2016;12(10):616-22. doi:10.1038/nrendo.2016.105.
2. McMurray JJ, Gerstein HC, Holman RR, Pfeffer MA. Heart failure: a cardiovascular outcome in diabetes that can no longer be ignored. *The lancet Diabetes & endocrinology*. 2014;2(10):843-51. doi:10.1016/s2213-8587(14)70031-2.
3. Nichols GA, Gullion CM, Koro CE, Ephross SA, Brown JB. The incidence of congestive heart failure in type 2 diabetes: an update. *Diabetes care*. 2004;27(8):1879-84. doi:10.2337/diacare.27.8.1879.
4. Green JB, Bethel MA, Armstrong PW, Buse JB, Engel SS, Garg J et al. Effect of Sitagliptin on Cardiovascular Outcomes in Type 2 Diabetes. *The New England journal of medicine*. 2015;373(3):232-42. doi:10.1056/NEJMoa1501352.
5. Romero SP, Garcia-Egido A, Escobar MA, Andrey JL, Corzo R, Perez V et al. Impact of new-onset diabetes mellitus and glycemic control on the prognosis of heart failure patients: a propensity-matched study in the community. *International journal of cardiology*. 2013;167(4):1206-16. doi:10.1016/j.ijcard.2012.03.134.
6. Sarma S, Mentz RJ, Kwasny MJ, Fought AJ, Huffman M, Subacius H et al. Association between diabetes mellitus and post-discharge outcomes in patients hospitalized with heart failure: findings from the EVEREST trial. *European journal of heart failure*. 2013;15(2):194-202. doi:10.1093/eurjhf/hfs153.
7. Singh JS, Fathi A, Vickneson K, Mordi I, Mohan M, Houston JG et al. Research into the effect Of SGLT2 inhibition on left ventricular remodelling in patients with heart failure and diabetes mellitus (REFORM) trial rationale and design. *Cardiovascular diabetology*. 2016;15:97. doi:10.1186/s12933-016-0419-0.

8. Aquilante CL. Sulfonylurea pharmacogenomics in Type 2 diabetes: the influence of drug target and diabetes risk polymorphisms. *Expert review of cardiovascular therapy*. 2010;8(3):359-72. doi:10.1586/erc.09.154.
9. Tzoulaki I, Molokhia M, Curcin V, Little MP, Millett CJ, Ng A et al. Risk of cardiovascular disease and all cause mortality among patients with type 2 diabetes prescribed oral antidiabetes drugs: retrospective cohort study using UK general practice research database. *Bmj*. 2009;339:b4731. doi:10.1136/bmj.b4731.
10. Vaduganathan M, Januzzi JL, Jr. Preventing and Treating Heart Failure with Sodium-Glucose Co-Transporter 2 Inhibitors. *The American journal of cardiology*. 2019;124 Suppl 1:S20-s7. doi:10.1016/j.amjcard.2019.10.026.
11. Rieg T, Vallon V. Development of SGLT1 and SGLT2 inhibitors. *Diabetologia*. 2018;61(10):2079-86. doi:10.1007/s00125-018-4654-7.
12. Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N et al. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. *The New England journal of medicine*. 2017;377(7):644-57. doi:10.1056/NEJMoa1611925.
13. Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A et al. Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes. *The New England journal of medicine*. 2019;380(4):347-57. doi:10.1056/NEJMoa1812389.
14. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *The New England journal of medicine*. 2015;373(22):2117-28. doi:10.1056/NEJMoa1504720.
15. Hopkins AL. Network pharmacology. *Nature biotechnology*. 2007;25(10):1110-1. doi:10.1038/nbt1007-1110.
16. Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic acids research*. 2019;47(W1):W357-w64. doi:10.1093/nar/gkz382.
17. Wishart DS, Feunang YD, Marcu A, Guo AC, Liang K, Vázquez-Fresno R et al. HMDB 4.0: the human metabolome database for 2018. *Nucleic acids research*. 2018;46(D1):D608-d17. doi:10.1093/nar/gkx1089.
18. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome research*. 2003;13(11):2498-504. doi:10.1101/gr.1239303.
19. Zhang Y, Lin H, Yang Z, Wang J, Liu Y, Sang S. A method for predicting protein complex in dynamic PPI networks. *BMC bioinformatics*. 2016;17 Suppl 7(Suppl 7):229. doi:10.1186/s12859-016-1101-y.
20. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY. cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC systems biology*. 2014;8 Suppl 4(Suppl 4):S11. doi:10.1186/1752-0509-8-s4-s11.

21. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic acids research*. 2017;45(D1):D353-d61. doi:10.1093/nar/gkw1092.
22. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *Omics : a journal of integrative biology*. 2012;16(5):284-7. doi:10.1089/omi.2011.0118.
23. Alexa A, Rahnenführer J, Lengauer T. Improved scoring of functional groups from gene expression data by decorrelating GO graph structure. *Bioinformatics (Oxford, England)*. 2006;22(13):1600-7. doi:10.1093/bioinformatics/btl140.
24. Haendeler J, Berk BC. Angiotensin II mediated signal transduction. Important role of tyrosine kinases. *Regulatory peptides*. 2000;95(1-3):1-7. doi:10.1016/s0167-0115(00)00133-6.
25. Pandey P, Hawkes W, Hu J, Megone WV, Gautrot J, Anilkumar N et al. Cardiomyocytes Sense Matrix Rigidity through a Combination of Muscle and Non-muscle Myosin Contractions. *Developmental cell*. 2018;45(5):661. doi:10.1016/j.devcel.2018.05.016.
26. Mukai E, Fujimoto S, Sato H, Oneyama C, Kominato R, Sato Y et al. Exendin-4 suppresses SRC activation and reactive oxygen species production in diabetic Goto-Kakizaki rat islets in an Epac-dependent manner. *Diabetes*. 2011;60(1):218-26. doi:10.2337/db10-0021.
27. Safari-Alighiarloo N, Taghizadeh M, Tabatabaei SM, Shahsavari S, Namaki S, Khodakarim S et al. Identification of new key genes for type 1 diabetes through construction and analysis of protein-protein interaction networks based on blood and pancreatic islet transcriptomes. *Journal of diabetes*. 2017;9(8):764-77. doi:10.1111/1753-0407.12483.
28. Bueno OF, De Windt LJ, Tymitz KM, Witt SA, Kimball TR, Klevitsky R et al. The MEK1-ERK1/2 signaling pathway promotes compensated cardiac hypertrophy in transgenic mice. *The EMBO journal*. 2000;19(23):6341-50. doi:10.1093/emboj/19.23.6341.
29. Costes S, Broca C, Bertrand G, Lajoix AD, Bataille D, Bockaert J et al. ERK1/2 control phosphorylation and protein level of cAMP-responsive element-binding protein: a key role in glucose-mediated pancreatic beta-cell survival. *Diabetes*. 2006;55(8):2220-30. doi:10.2337/db05-1618.
30. Katoh M. Cardio-miRNAs and onco-miRNAs: circulating miRNA-based diagnostics for non-cancerous and cancerous diseases. *Frontiers in cell and developmental biology*. 2014;2:61. doi:10.3389/fcell.2014.00061.
31. Vairaktaris E, Spyridonidou S, Goutzanis L, Vylliotis A, Lazaris A, Donta I et al. Diabetes and oral oncogenesis. *Anticancer research*. 2007;27(6b):4185-93.
32. Xu Y, Henning RH, Sandovici M, van der Want JJ, van Gilst WH, Buikema H. Enhanced myogenic constriction of mesenteric artery in heart failure relates to decreased smooth muscle cell caveolae numbers and altered AT1- and epidermal growth factor-receptor function. *European journal of heart failure*. 2009;11(3):246-55. doi:10.1093/eurjhf/hfn027.
33. Kagiya S, Eguchi S, Frank GD, Inagami T, Zhang YC, Phillips MI. Angiotensin II-induced cardiac hypertrophy and hypertension are attenuated by epidermal growth factor receptor antisense.

- Circulation. 2002;106(8):909-12. doi:10.1161/01.cir.0000030181.63741.56.
34. Belmadani S, Palen DI, Gonzalez-Villalobos RA, Boulares HA, Matrougui K. Elevated epidermal growth factor receptor phosphorylation induces resistance artery dysfunction in diabetic db/db mice. *Diabetes*. 2008;57(6):1629-37. doi:10.2337/db07-0739.
 35. Zhang MZ, Wang Y, Pauksakon P, Harris RC. Epidermal growth factor receptor inhibition slows progression of diabetic nephropathy in association with a decrease in endoplasmic reticulum stress and an increase in autophagy. *Diabetes*. 2014;63(6):2063-72. doi:10.2337/db13-1279.
 36. Caron E. Cellular functions of the Rap1 GTP-binding protein: a pattern emerges. *Journal of cell science*. 2003;116(Pt 3):435-40. doi:10.1242/jcs.00238.
 37. Chrzanowska-Wodnicka M, Kraus AE, Gale D, White GC, 2nd, Vansluys J. Defective angiogenesis, endothelial migration, proliferation, and MAPK signaling in Rap1b-deficient mice. *Blood*. 2008;111(5):2647-56. doi:10.1182/blood-2007-08-109710.
 38. Cai Y, Kandula V, Kosuru R, Ye X, Irwin MG, Xia Z. Decoding telomere protein Rap1: Its telomeric and nontelomeric functions and potential implications in diabetic cardiomyopathy. *Cell cycle (Georgetown, Tex)*. 2017;16(19):1765-73. doi:10.1080/15384101.2017.1371886.
 39. Lakshmikanthan S, Sobczak M, Li Calzi S, Shaw L, Grant MB, Chrzanowska-Wodnicka M. Rap1B promotes VEGF-induced endothelial permeability and is required for dynamic regulation of the endothelial barrier. *Journal of cell science*. 2018;131(1). doi:10.1242/jcs.207605.
 40. Kay AM, Simpson CL, Stewart JA, Jr. The Role of AGE/RAGE Signaling in Diabetes-Mediated Vascular Calcification. *Journal of diabetes research*. 2016;2016:6809703. doi:10.1155/2016/6809703.
 41. Sugden PH, Clerk A. "Stress-responsive" mitogen-activated protein kinases (c-Jun N-terminal kinases and p38 mitogen-activated protein kinases) in the myocardium. *Circulation research*. 1998;83(4):345-52. doi:10.1161/01.res.83.4.345.
 42. Zhang Q, Lu L, Liang T, Liu M, Wang ZL, Zhang PY. MAPK pathway regulated the cardiomyocyte apoptosis in mice with post-infarction heart failure. *Bratislavske lekarske listy*. 2017;118(6):339-46. doi:10.4149/bl_2017_065.
 43. Bak EJ, Choi KC, Jang S, Woo GH, Yoon HG, Na Y et al. Licochalcone F alleviates glucose tolerance and chronic inflammation in diet-induced obese mice through Akt and p38 MAPK. *Clinical nutrition (Edinburgh, Scotland)*. 2016;35(2):414-21. doi:10.1016/j.clnu.2015.03.005.
 44. Zhao Y, Tang Z, Zhu X, Wang X, Wang C, Zhang W et al. TAB3 involves in hepatic insulin resistance through activation of MAPK pathway. *General and comparative endocrinology*. 2015;224:228-34. doi:10.1016/j.ygcen.2015.08.019.
 45. Li Z, Li Y, Overstreet JM, Chung S, Niu A, Fan X et al. Inhibition of Epidermal Growth Factor Receptor Activation Is Associated With Improved Diabetic Nephropathy and Insulin Resistance in Type 2 Diabetes. *Diabetes*. 2018;67(9):1847-57. doi:10.2337/db17-1513.
 46. Zeng SY, Yan QJ, Yang L, Mei QH, Lu HQ. Inhibition of the ROS-EGFR Pathway Mediates the Protective Action of Nox1/4 Inhibitor GKT137831 against Hypertensive Cardiac Hypertrophy via

Suppressing Cardiac Inflammation and Activation of Akt and ERK1/2. Mediators of inflammation. 2020;2020:1078365. doi:10.1155/2020/1078365.

47. Shah S, Brock EJ, Ji K, Mattingly RR. Ras and Rap1: A tale of two GTPases. *Seminars in cancer biology*. 2019;54:29-39. doi:10.1016/j.semcancer.2018.03.005.
48. Bodiga VL, Eda SR, Bodiga S. Advanced glycation end products: role in pathology of diabetic cardiomyopathy. *Heart failure reviews*. 2014;19(1):49-63. doi:10.1007/s10741-013-9374-y.
49. Fukami K, Yamagishi S, Okuda S. Role of AGEs-RAGE system in cardiovascular disease. *Current pharmaceutical design*. 2014;20(14):2395-402. doi:10.2174/13816128113199990475.
50. Tao YG, Huang XF, Wang JY, Kang MR, Wang LJ, Xian SX. Exploring Molecular Mechanism of Huangqi in Treating Heart Failure Using Network Pharmacology. *Evidence-based complementary and alternative medicine : eCAM*. 2020;2020:6473745. doi:10.1155/2020/6473745.

Tables

Due to technical limitations, table 1, 2 is only available as a download in the Supplemental Files section.

Figures

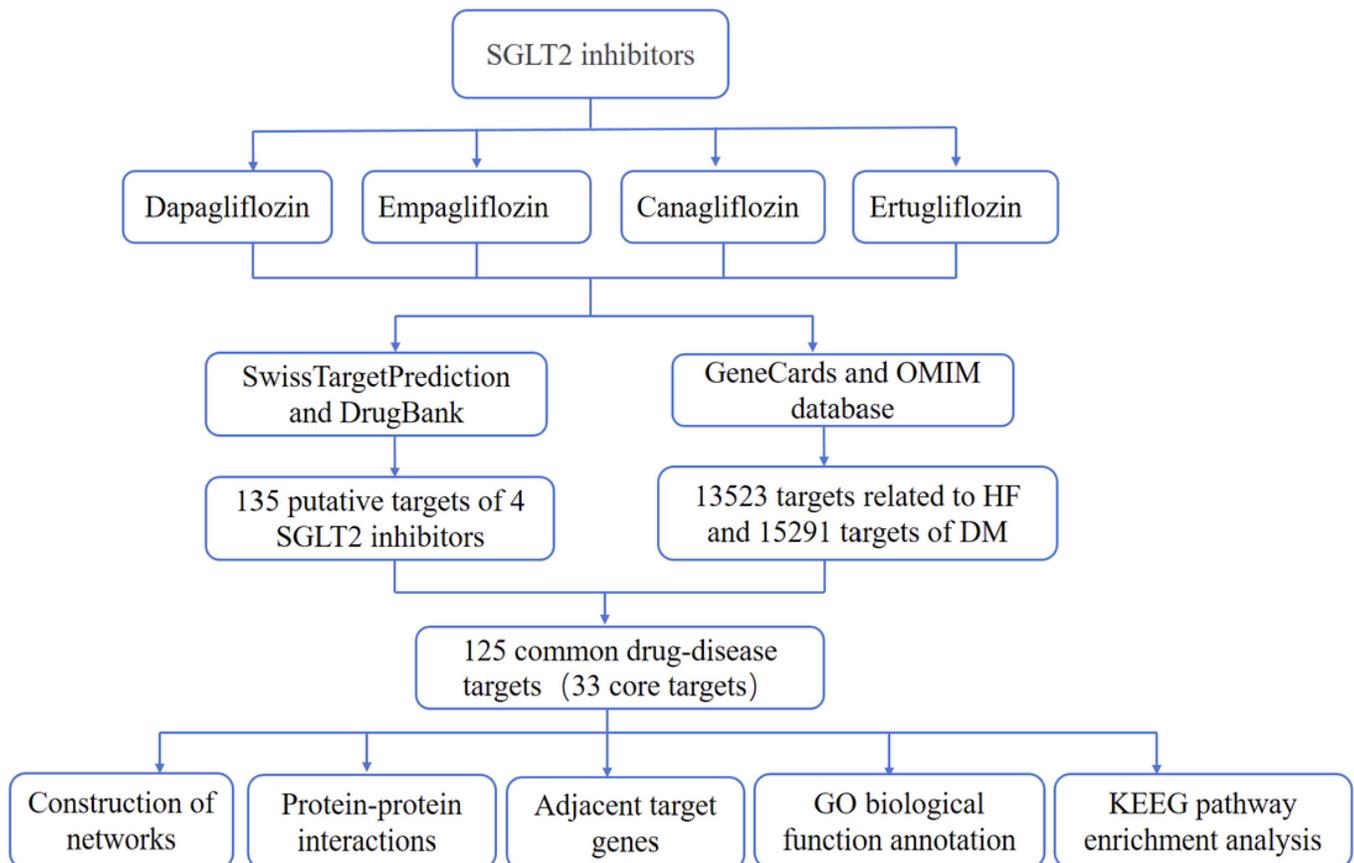


Figure 1

The workflow of the network pharmacology strategies for determining the pharmacological mechanisms of the SGLT2 inhibitors on HF and DM through cluster and pathway analysis.



Figure 2

Interaction network to indicate drug-target composed of 4 SGLT2 inhibitors (blue) and 136 targets (pink).

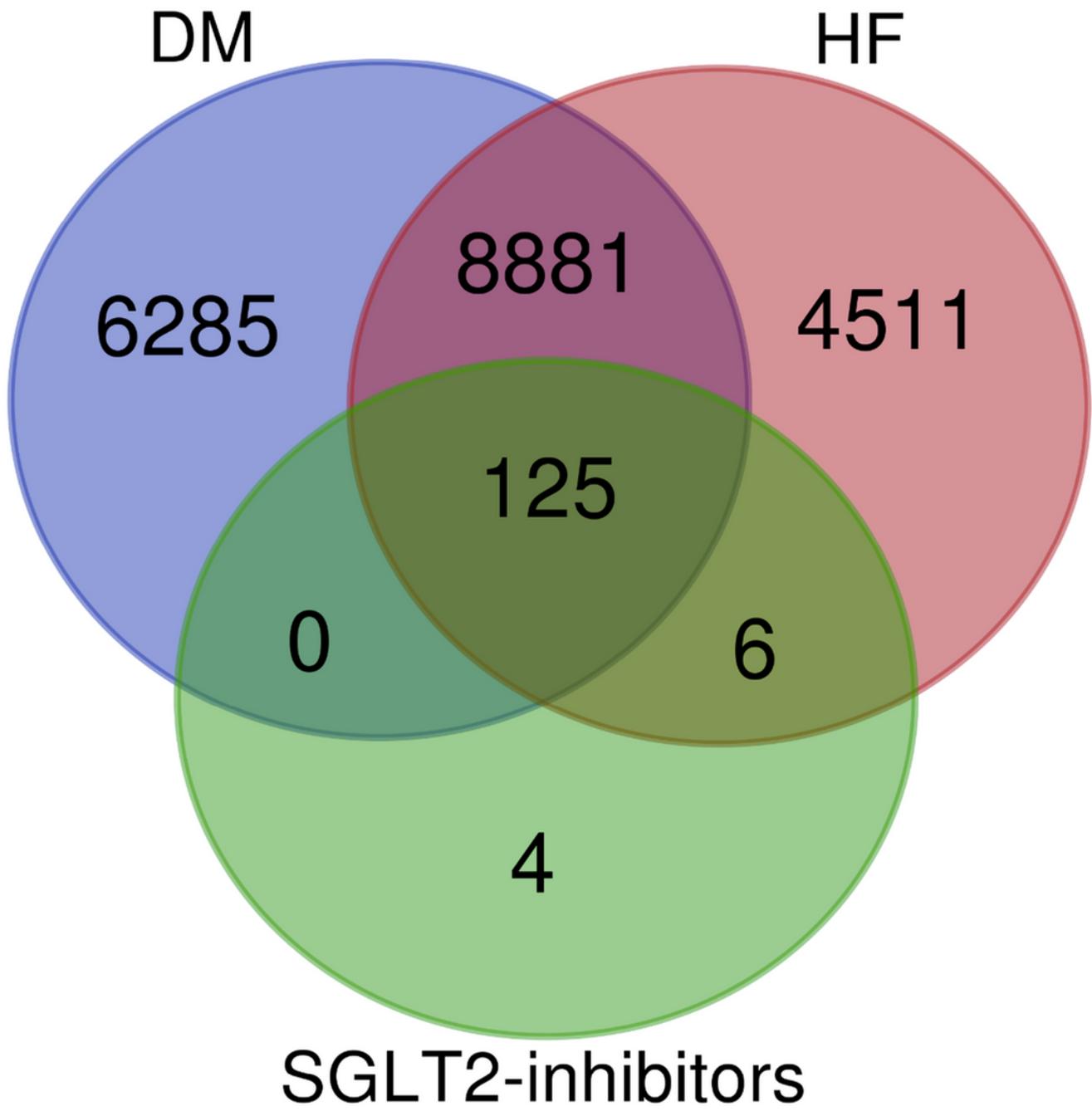


Figure 3

The numbers of SGLT2 inhibitors, HF and DM related targets were shown in the venn diagram. There were 13523 targets of HF (left), 15291 targets of DM (right), 135 targets of SGLT2 inhibitors (down) and 125 common targets between SGLT2 inhibitors, HF and DM (middle).

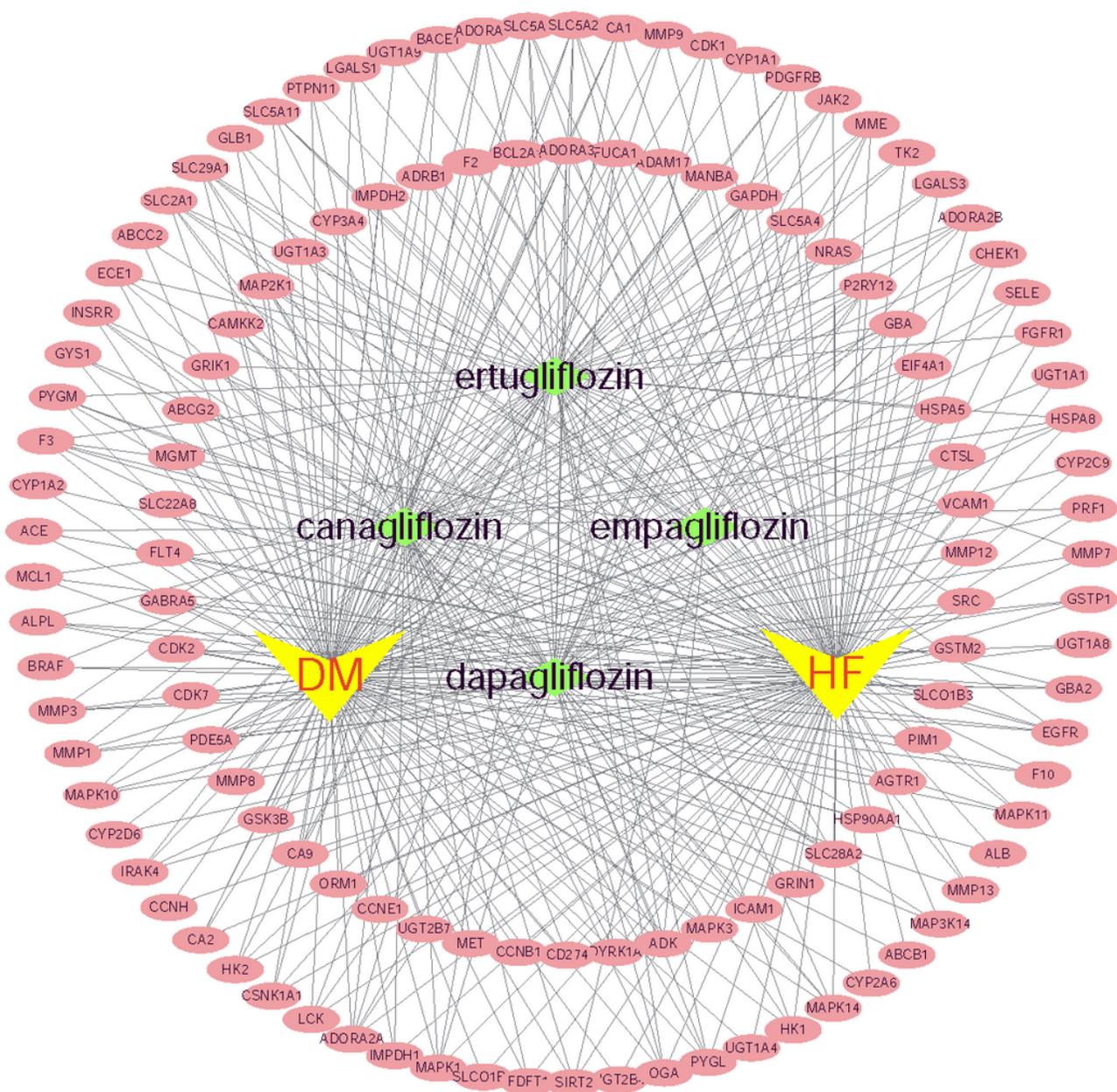


Figure 4

The drug-target-disease networks of SGLT2 inhibitors, HF and DM. The yellow nodes represent DM and HF; the green nodes represent four SGLT2 inhibitors; the pink nodes represent 125 common targets.

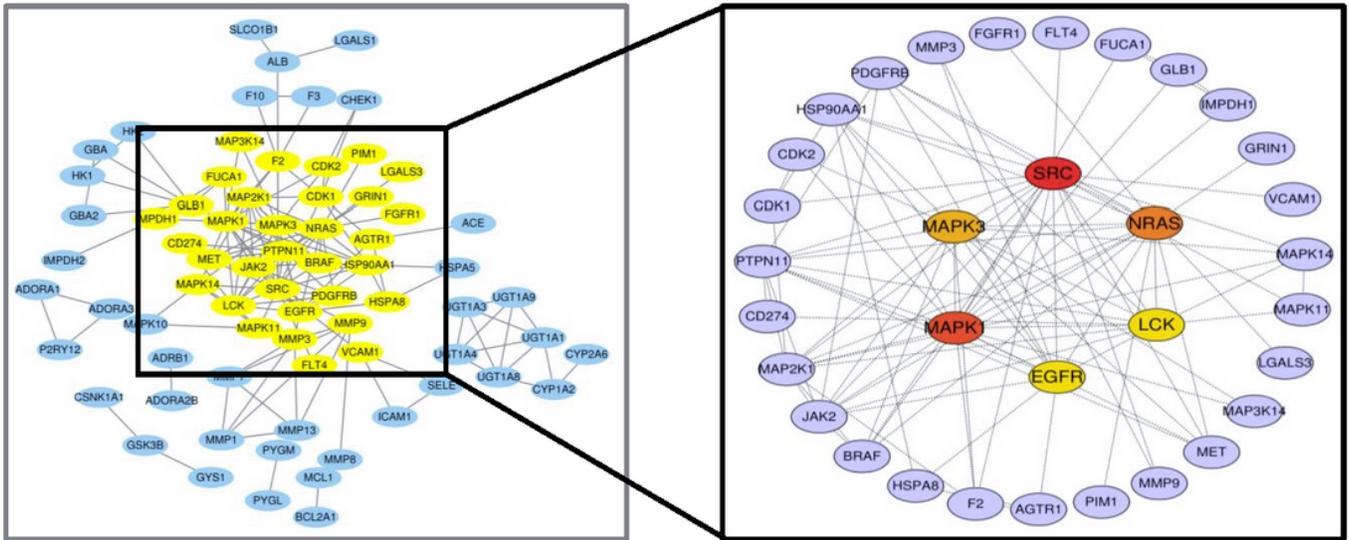


Figure 5

The process of screening core targets by Cytohubba according to degree value. From 125 common targets (left), 33 core targets (right) ranked in the front of the degree value were selected.

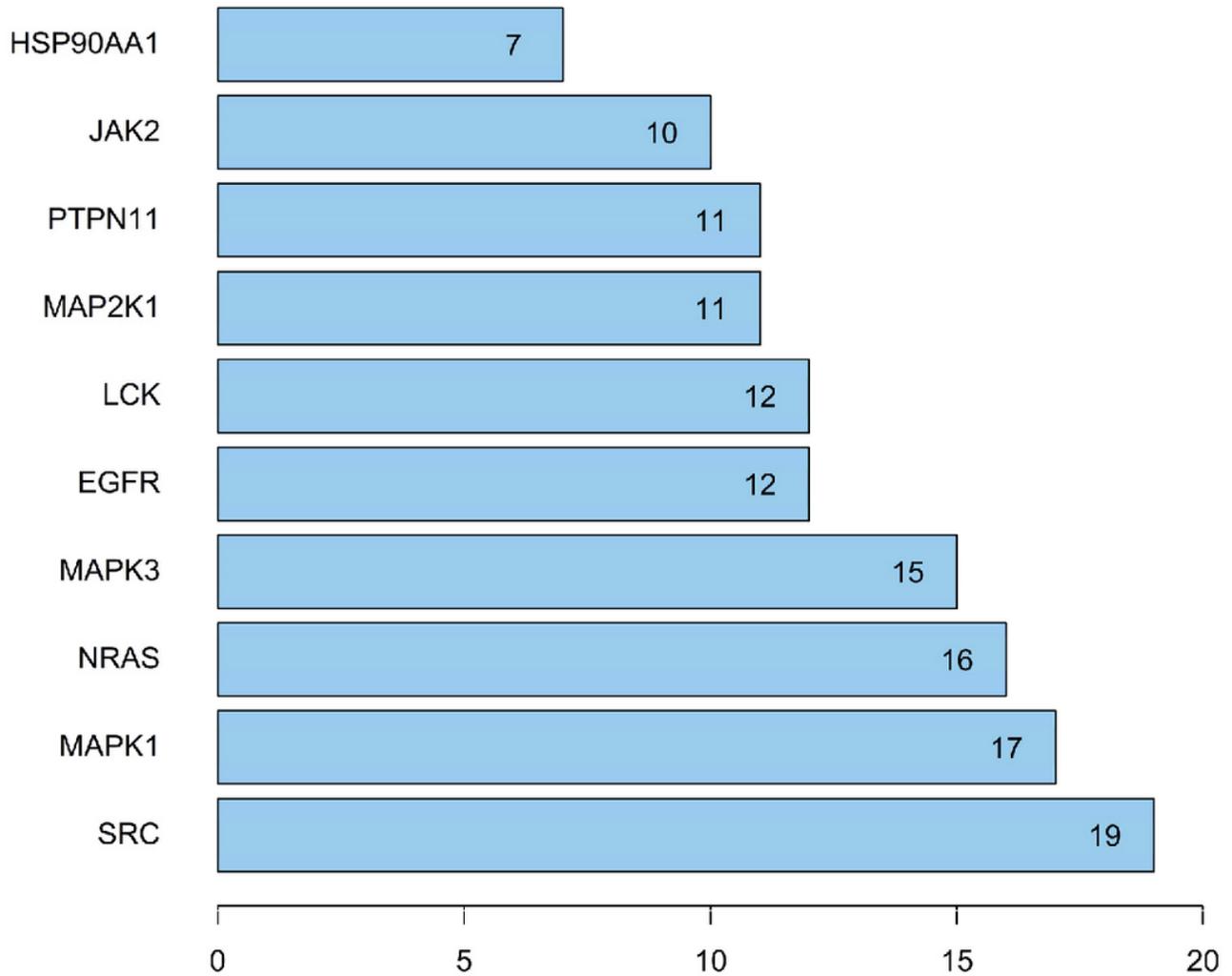


Figure 6

The protein-protein interaction (PPI) network based on 33 core targets of SGLT2 inhibitors on HF and DM. Network nodes represent different proteins. The structures in the nodes are the protein structures. Edges represent protein-protein associations, and the line thickness indicates the strength of data support.

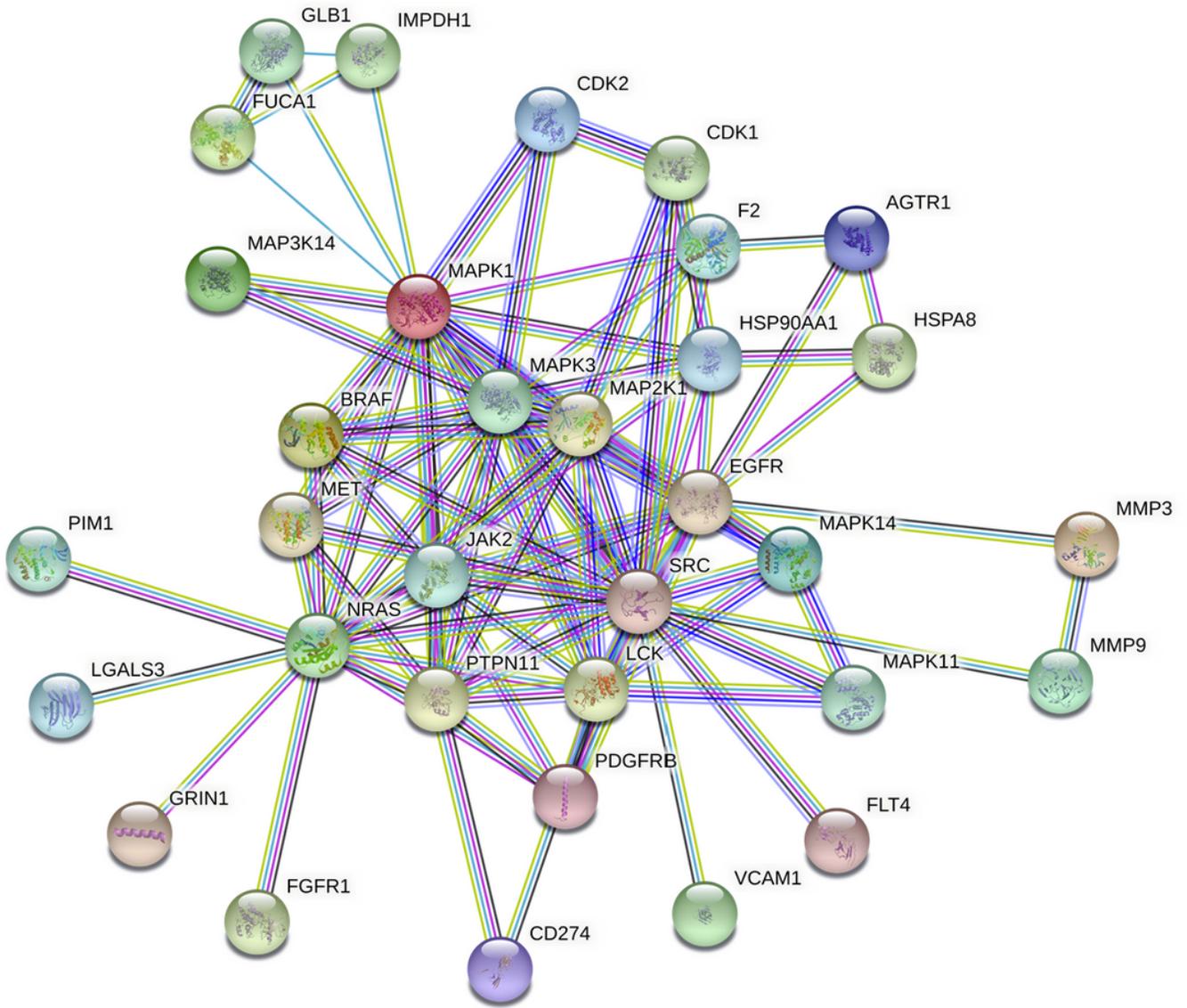


Figure 7

Protein interaction relationship histogram of SGLT2 inhibitors.

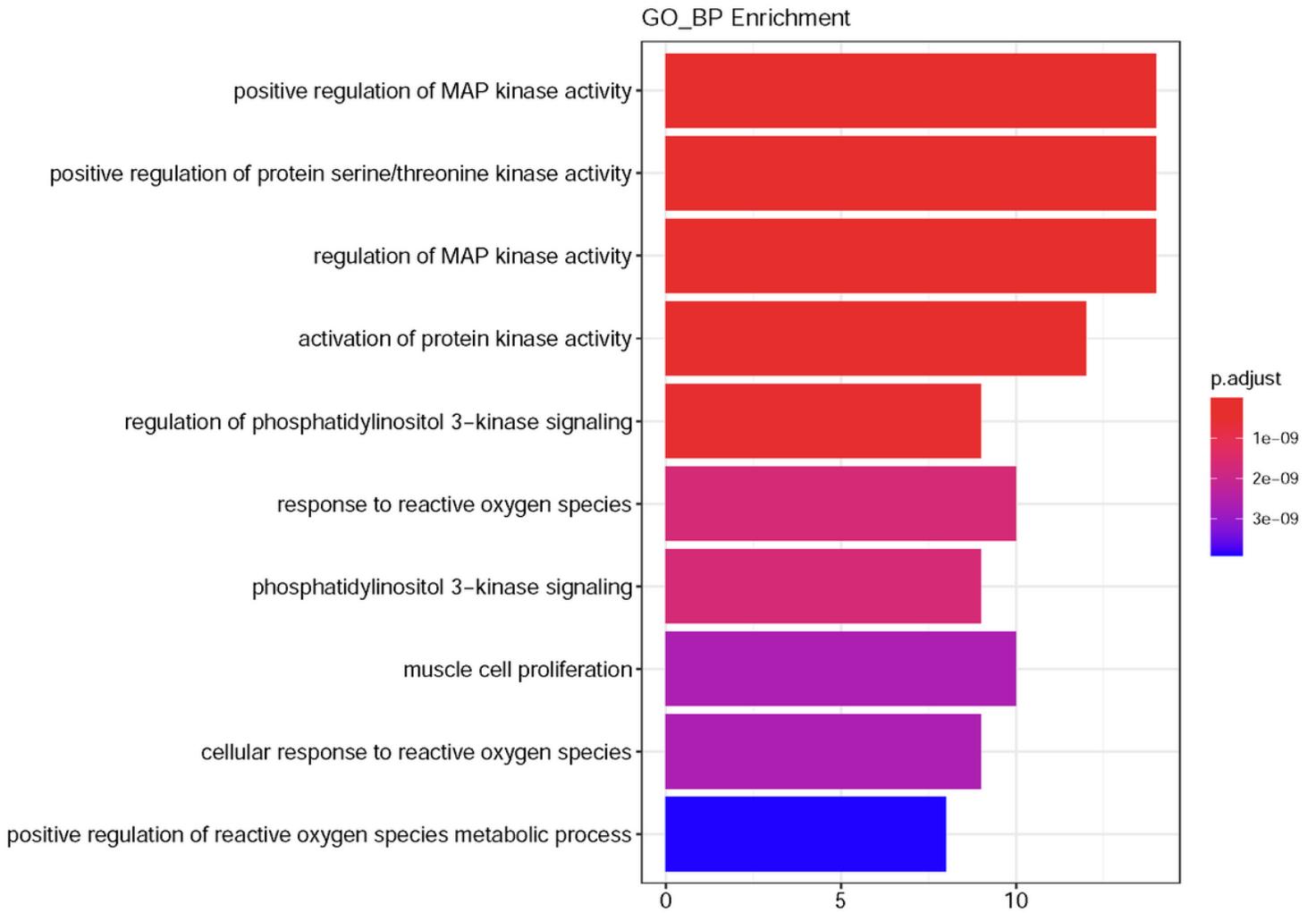


Figure 8

Enrichment analysis of Gene Ontology (GO) biological process of 33 core genes related to SGLT2 inhibitors on HF and DM.

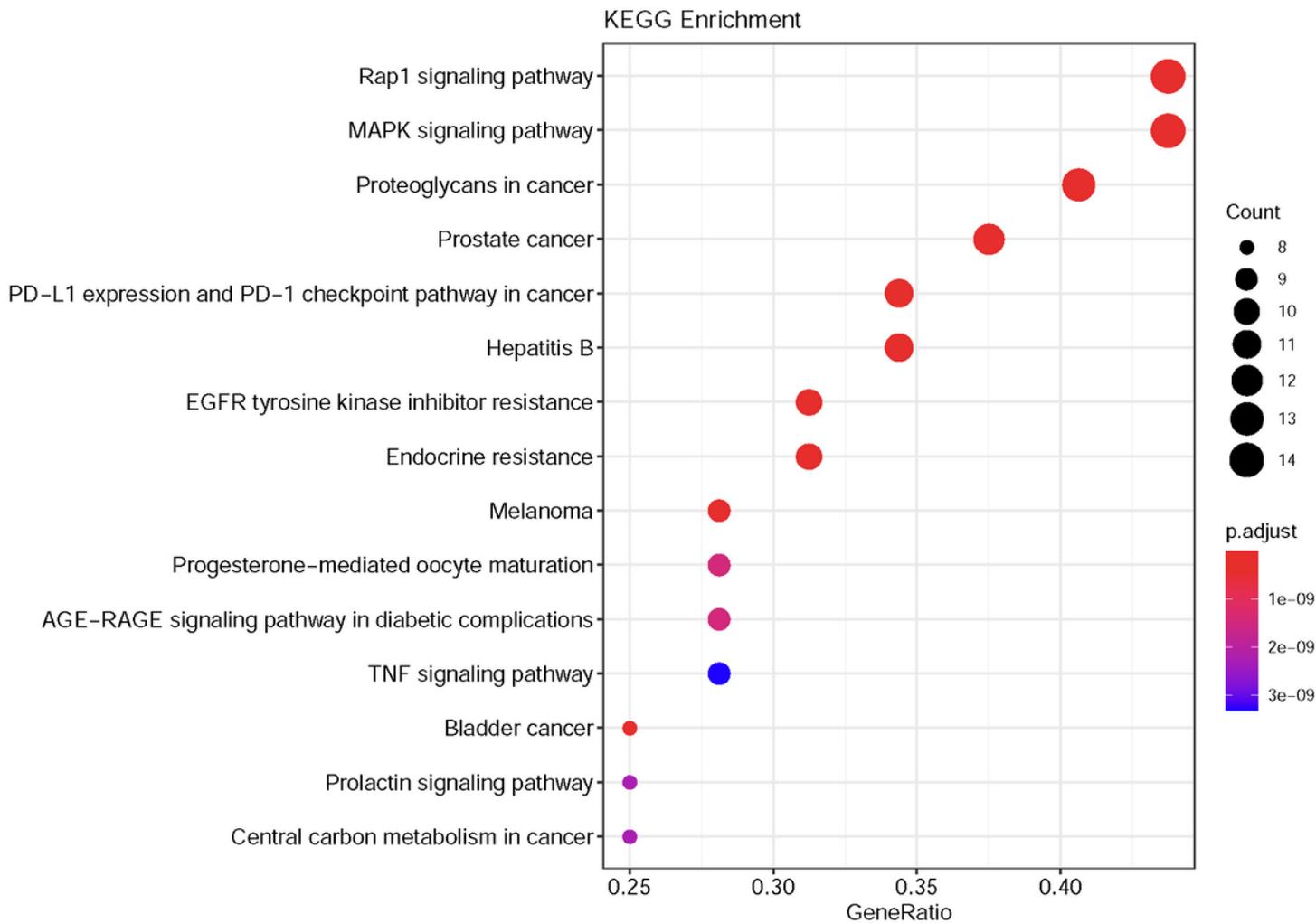


Figure 9

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway for 33 core targets of SGLT2 inhibitors on HF and DM.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1InformationonfourSGLT2inhibitorsfromPubChem.pdf](#)
- [Table2Specificinformationforcoretargets.pdf](#)
- [TableS1SwissTargetPredictionfourSGLT2inhibitors.csv](#)
- [TableS2DrugBankdatabasfourSGLT2inhibitors.csv](#)
- [TableS3GeneCardsheartfailure1.csv](#)
- [TableS4OMIMGeneHeartFailure.csv](#)
- [TableS5GeneCardsDiabete.csv](#)

- [TableS6MIMGeneDiabete.csv](#)
- [TableS7GEOBP.csv](#)
- [TableS8KEGG.csv](#)