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Original article

Cholesterol Metabolism Pathway, the Main Target of Coffee

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Abstract

Background and objectives: Coffee as a common drink for many people has been evaluated in the preset study due to its relationship with cancer risk or prevention, regulation of cholesterol level, and anti-oxidant properties. The dysregulated genes in liver of high-fat dieted mice which were treated with coffee were evaluated via network analysis to explore molecular mechanism of the event. **Methods:** Data were downloaded from gene expression omnibus (GEO) and the significant differentially expressed genes (DEGs) were analyzed via protein-protein interaction (PPI) network analysis by Cytoscape V.3.7.2. The Selected DEGs were enriched via gene ontology by ClueGO. Results of PPI network analysis and gene ontology enrichment were interpreted together to find the critical genes and pathways. **Results:** Hmgcr, Hmgcs1, Msmo1, Nsdhl, Lss, Fdps, Idi1, Mvd, Ppara, and Hsp90aa1 were identified as the central targeted genes while "cholesterol metabolism pathway" was introduced as the main affected pathway. **Conclusion:** Final analysis led to determine Hmgcr, Hmgcs1, Msmo1, Nsdhl, Lss, Fdps, Idi1, and Mvd as key dysregulated genes which are related to the most biological terms of "cholesterol metabolism pathway".

Keywords: cholesterol; coffee; gene expression; gene ontology; network analysis

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Introduction

It is reported that usual coffee consumption is accompanied with reduced risk of several diseases such as cancer. Caffeine, caffeic acid, and polyphenols are the compounds of coffee that play useful role in reduced risk of various types of cancers [1]. It is pointed that coffee consumption affects regulation of serum lipid profile [2]. Caffeine is evaluated as an effective factor which influences cholesterol metabolism via several proposed mechanisms [3,4]. Understanding the molecular mechanism of biological events has attracted attention of scientists in all fields of medicine, biology, and nutrition. Today genomics, proteomics, and bioinformatics are well-known methods to detect molecular mechanism of biological events. Combination of bioinformatics with the other omics methods has opened a new gate to explore details of molecular aspects of diseases and other induced conditions in human body [5-7].

Protein-protein interaction (PPI) network analysis is a method to screen large number of proteins or

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genes to identify the more important individuals among the queried ones. A network forms from nodes and their connections which are known as edges. Each element of the network has its properties thus it plays a different role relative to the other nodes in integrity of the network. The central nodes such as hubs and bottlenecks are the crucial nodes of the network and they may induce the main effects of network in the studied condition [8-10].

PPI network analysis as a useful tool is used to analyze many diseases and also has introduced many drug targets. Anticancer, anti-inflammatory, and anti-oxidative properties of several herbal compounds have been investigated via PPI network analysis. Effective role of cinnamon extract on ribosome function, evaluation of ghost pepper anticancer effect, and anti-stress effect of saffron are investigations that have been administrated via PPI network analysis [11-13].

Another well-known method to analyze molecular mechanism is gene ontology. Molecular function, biological processes, and biochemical pathways related to the studied genes provide valuable information about biological events [14]. In the present study, the top 250 dysregulated genes in the liver of high-fat dieted mice which were treated with coffee relative to the liver of high-fat dieted mice were downloaded from GEO. The significant DEGs were identified and evaluated via PPI network analysis and gene ontology. The critical DEGs and the important related pathway were determined.

Material and Methods

Ethical considerations

This project was approved by Shahid Beheshti University of Medical Sciences (IR.SBMU.RETECH.REC.1401.002).

Data collection

GSM1282827-9 related to the gene expression profiles of the liver of mice that were fed with a high-fat diet and GSM1282830-2 associated to the gene expression profiles of the liver of mice that were fed with a high-fat diet containing 2% coffee from GSE53131were downloaded from GEO (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=gse53131). Data were analyzed by GEO2R and the significant DEGs based on, p-value<0.001 and fold change >1.5 were determined. The DEGs with higher values of FC were selected among the repetitive DEGs. To evaluate the gene expression profiles "expression density" plot was illustrated.

The significant DEGs were evaluated via PPI network analysis by Cytoscape software [15]. The queried DEGs were included in STRING database from "protein query" option of STRING by Cytoscape software. The network was constructed and for making more connections between nodes of the network, suitable numbers of first neighbors from STRING were added to the queried DEGs and the network was reconstructed. The queried DEGs of main connected component of the reconstructed network were considered for more analysis.

The final candidate elements of the main connected component were included in ClueGO [16] application of Cytoscape to find the related biological terms. ClueGO setting was as: all sources of; ontologies/pathways, evidence; all, network specificity; medium, and $p \leq 0.05$. The biological terms were grouped based; Term p-value, term p-value corrected with Bonferroni step down, group p-value, and group p-value corrected with Bonferroni step down are less than 0.01. The important group of biological terms based on numbers of terms and the related genes was introduced.

The reconstructed PPI network was analyzed by "Network Analyzer" application of Cytoscape and 10 top nodes based on degree value were selected as hubs. The hubs and the related DEGs to the important group of biological terms were analyzed and screened to find the crucial dysregulated genes.

Results and Discussion

Numbers of 96 significant DEGs were identified among the 250 top dysregulated genes that were analyzed by GEO2R. Expression density plot for the six studied GSMs is presented in Figure 1.

The six curves follow a similar pattern and are comparable. Maximum FC (-2.822) belonged to complement factor D (adipsin) (CFD) which was down-regulated. Among the 96 queried DEGs, 82 individuals were recognized by STRING database.

As it is shown in Figure 2, 32 nodes remained as isolated and three paired nodes appeared. Figure 2 is presented to show the isolation of considerable numbers of the queried genes and illustration of poor interactions between the

nodes of network. The main connected component of network included 44 nodes. The network was reconstructed after adding 50 first neighbors from STRING database (Figure 3). Ten isolated nodes and one paired DEGs were reduced; therefore, 22 isolated nodes and 2 paired individuals appeared.

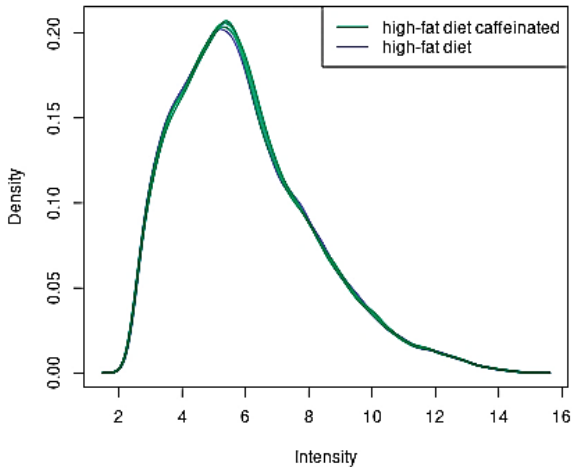

Figure 1. Expression density plot of six studied; the plotted curves follow a similar pattern

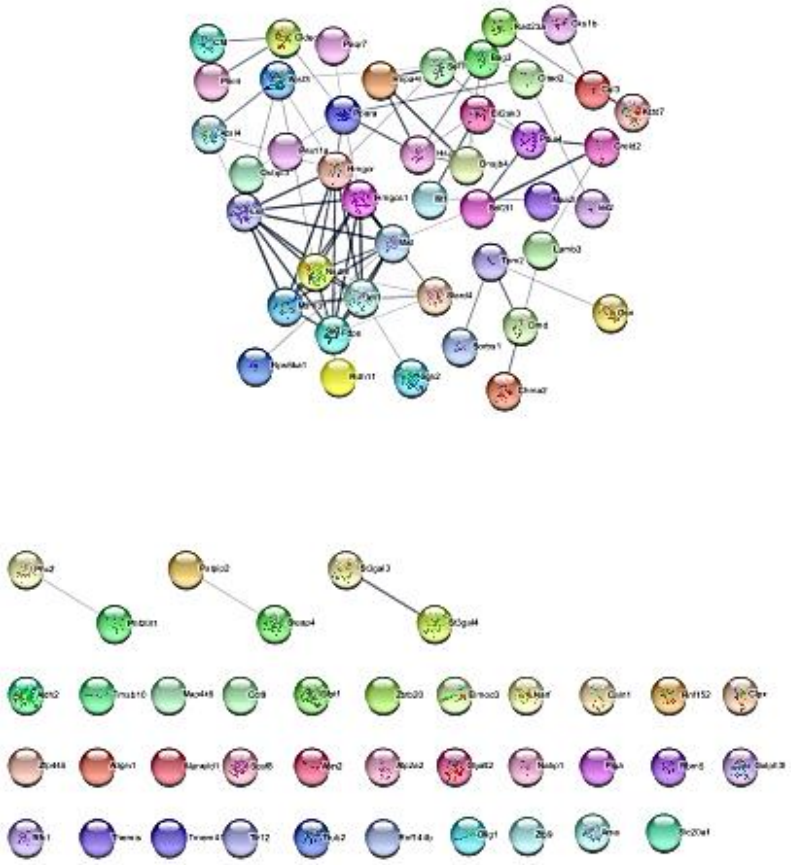

Figure 2. PPI network including 82 recognized queried DEGs that discriminate liver of mice with high-fat diet from liver of mice with high-fat diet containing 2% coffee

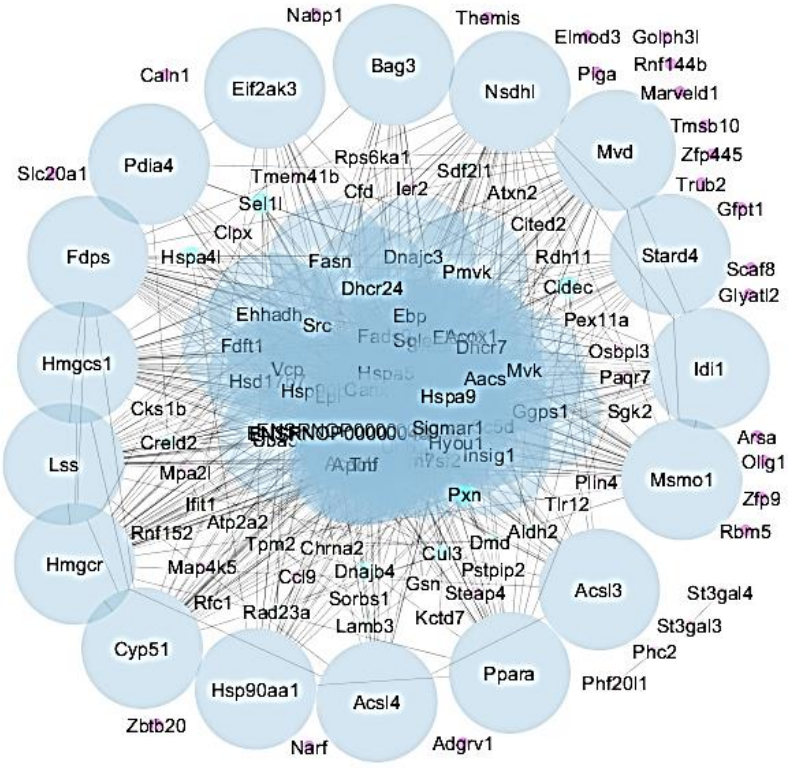

Figure 3. Reconstructed PPI network including 82 recognized queried DEGs plus 50 added first neighbors; to increase resolution of nodes of queried DEGs the added individuals are concentrated in the center of network

The main connected component including 106 nodes (56 queried DEGs and 50 added first neighbors) which were connected by 974 connections were formed. The 11 nodes which were characterized with degree value = 1 were considered as non- critical elements of the main connected component of the reconstructed network and were excluded from more investigations. The remained 45 DEGs were assessed via ClueGO which led to introducing 6 groups of biological terms (Figure 4). List of the biological terms of the important pathway (cholesterol metabolism pathway) and the related data is presented in Table 1. Seventy queried DEGs among the remained 45 DEGs were related to the biological terms of "cholesterol metabolism pathway" (Figure 5).

Top 10 nodes among the 56 queried DEGs from the main connected component of the reconstructed network based on degree value were selected as hubs. The hubs including Hmgcr, Hmgcs1, Msmo1, Nsdhl, Lss, Fdps, Idi1, Mvd, Ppara, and Hsp90aa1 are tabulated in the Table 2.

PPI network analysis as a useful method is applied to analyze and detect molecular mechanism of herbal medicine in pharmacognosy [17]. As it is shown in Figure 1, gene expression profiles of the studied samples are comparable. PPI network analysis revealed the queried DEGs were connected to each other poorly; however, adding first neighbors led to formation of a suitable network for analysis (Figures 2-3).

As it is depicted in Figure 4, 6 groups of biological terms are related to the queried nodes of the main connected component. The main group is "cholesterol metabolism pathway" which includes 41 biological terms (Figure 4 and Table 1). As it is reported in literature "cholesterol metabolism pathway" is an important pathway that is related to a set of diseases [18]. Association between dysregulation of cholesterol metabolism and nonalcoholic fatty liver disease (NAFLD) which is tied to disease severity and cardiovascular risks has been investigated by Min HK et al. [19]. Rezaei Tavirani et al. published data about contradictory effect of coffee consumption related to NAFLD) [20].

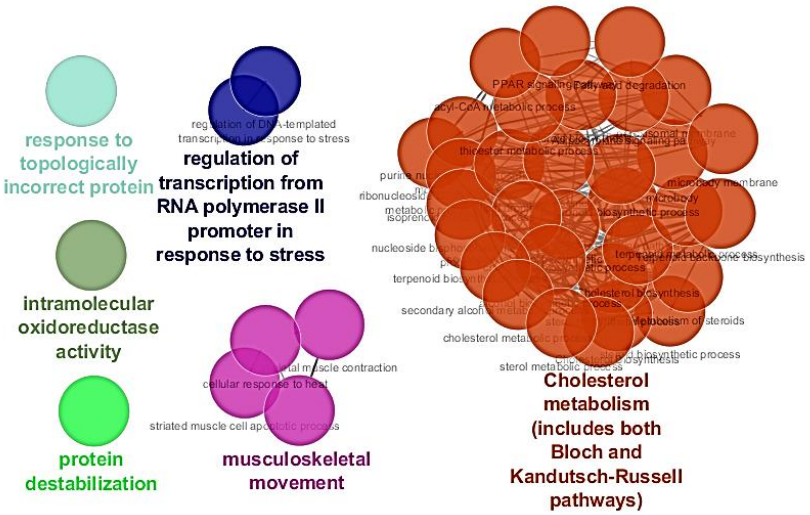

Figure 4. Six groups of biological terms which are related to the remained 45 DEGs of the main connected component; term p value, term p value corrected with Bonferroni step down, group p value, and group p value corrected with Bonferroni step down were less than 0.01

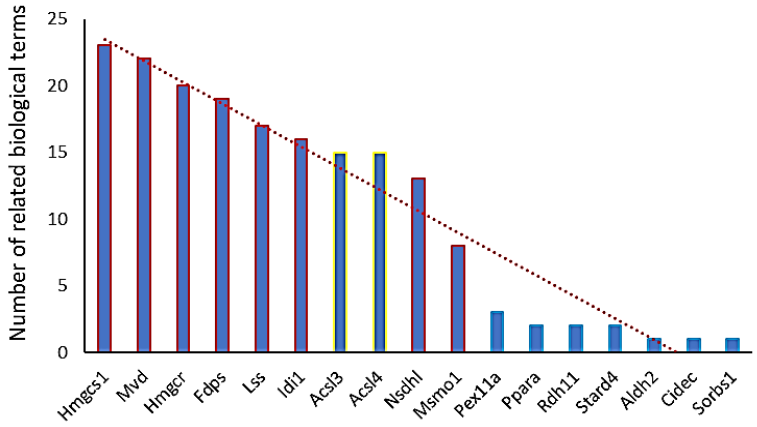

Figure 5. The queried DEGs Among the remained 45 DEGs which were related to biological terms of "cholesterol metabolism pathway" are presented. Red line is the trend line and the blue bars are not supported by this line. The blue bars with margined red lines are the DEGs that are common with hubs and supported by trend line, the bars with margined yellow lines are not hubs.

Among the 45 enriched DEGs, 17 genes were related to the "cholesterol metabolism pathway" (Table 1). It can be concluded that 17 DEGs among the queried genes played crucial role. Other finding indicated that 10 central nodes appeared as key dysregulated genes (Table 2). A simple comparison between Figure 5 and Table 2 indicated that except for Hsp90aa1 and Ppara, the other 8 hub nodes were common with the DEGs which were related to "cholesterol metabolism pathway" and are supported by the related trend line. Hsp90aa1 is the 10th hub that was ranked in the Table 2. Ppara is the other hub that was not supported by trend line in Figure 5; consequently, it was excluded from more investigations. Finally, eight genes including Hmgcr, Hmgcs1, Msmo1, Nsdhl, Lss, Fdps, Idi1, and Mvd were identified as the critical DEGs.

3-Hydroxy-3-Methylglutaryl-CoA Synthase 1(Hmgcs1) is the second top hub node which is related to 23 (the maximum numbers) of the biological terms. Cytoplasmic Hmgcs1 and mitochondrial Hmgcs2 are two subtypes of Hmgcs. Hmgcs1 plays a key role in cholesterol metabolism [21].

Table 1. List of biological terms of "cholesterol metabolism pathway" that are related to the 45 elements of the main connected component of the reconstructed network.

Gene ontology term	Associated genes found
Intramolecular oxidoreductase activity	[Creld2, Idi1, Pdia4]
Protein destabilization	[Cul3, Gsn, Rad23a]
Response to topologically incorrect protein	[Bag3, Cul3, Eif2ak3, Hsp90aa1, Hspa4l, Sdf2l1]
Regulation of DNA-templated transcription in response to stress	[Bag3, Cited2, Eif2ak3]
Regulation of transcription from RNA polymerase II promoter in response to stress	[Bag3, Cited2, Eif2ak3]
Musculoskeletal movement	[Atp2a2, Dmd, Hsp90aa1]
Cellular response to heat	[Atp2a2, Bag3, Hsp90aa1]
Skeletal muscle contraction	[Atp2a2, Dmd, Hsp90aa1]
Striated muscle cell apoptotic process	[Bag3, Hmgcr, Hsp90aa1]
Fatty acid degradation	[Acsl3, Acsl4, Aldh2]
Steroid biosynthesis	[Lss, Msmo1, Nsdhl]
Terpenoid backbone biosynthesis	[Fdps, Hmgcr, Hmgcs1, Idi1, Mvd]
PPAR signaling pathway	[Acsl3, Acsl4, Hmgcs1, Ppara, Sorbs1]
Adipocytokine signaling pathway	[Acsl3, Acsl4, Ppara]
Cholesterol biosynthesis	[Fdps, Hmgcr, Hmgcs1, Idi1, Lss, Msmo1, Mvd, Nsdhl]
Metabolism of steroids	[Fdps, Hmgcr, Hmgcs1, Idi1, Lss, Msmo1, Mvd, Nsdhl, Stard4]
Cholesterol Biosynthesis	[Fdps, Hmgcr, Hmgcs1, Idi1, Lss, Msmo1, Mvd, Nsdhl]
PPAR signaling pathway	[Acsl3, Acsl4, Ppara, Sorbs1]
Cholesterol metabolism (includes both Bloch and Kandutsch-Russell pathways)	[Acsl3, Acsl4, Fdps, Hmgcr, Hmgcs1, Idi1, Lss, Msmo1, Mvd, Nsdhl]
Thioester metabolic process	[Acsl3, Acsl4, Hmgcs1, Mvd]
Microbody	[Acsl3, Acsl4, Fdps, Hmgcr, Idi1, Mvd, Pex11a]
Lipid droplet	[Acsl3, Acsl4, Cidec, Lss, Nsdhl]
Isoprenoid metabolic process	[Fdps, Hmgcr, Hmgcs1, Idi1, Lss, Mvd, Rdh11]
Sterol metabolic process	[Fdps, Hmgcr, Hmgcs1, Idi1, Lss, Msmo1, Mvd, Nsdhl]
Alcohol biosynthetic process	[Fdps, Hmgcr, Hmgcs1, Idi1, Lss, Mvd, Nsdhl]
Secondary alcohol metabolic process	[Fdps, Hmgcr, Hmgcs1, Idi1, Lss, Mvd, Nsdhl]
Peroxisome	[Acsl3, Acsl4, Fdps, Hmgcr, Idi1, Mvd, Pex11a]
Steroid biosynthetic process	[Fdps, Hmgcr, Hmgcs1, Idi1, Lss, Msmo1, Mvd, Nsdhl, Stard4]
Isoprenoid biosynthetic process	[Fdps, Hmgcr, Hmgcs1, Idi1, Lss, Mvd]
Microbody membrane	[Acsl3, Acsl4, Hmgcr, Pex11a]
Terpenoid metabolic process	[Fdps, Hmgcs1, Lss, Rdh11]
Cholesterol metabolic process	[Fdps, Hmgcr, Hmgcs1, Idi1, Lss, Mvd, Nsdhl]
Sterol biosynthetic process	[Fdps, Hmgcr, Hmgcs1, Idi1, Lss, Msmo1, Mvd, Nsdhl]
Secondary alcohol biosynthetic process	[Fdps, Hmgcr, Hmgcs1, Idi1, Lss, Mvd, Nsdhl]
Peroxisomal membrane	[Acsl3, Acsl4, Hmgcr, Pex11a]
Terpenoid biosynthetic process	[Fdps, Hmgcs1, Lss]
Nucleoside bisphosphate metabolic process	[Acsl3, Acsl4, Hmgcr, Hmgcs1, Mvd]
Cholesterol biosynthetic process	[Fdps, Hmgcr, Hmgcs1, Idi1, Lss, Mvd, Nsdhl]
Purine nucleoside bisphosphate metabolic process	[Acsl3, Acsl4, Hmgcr, Hmgcs1, Mvd]
Acyl-CoA metabolic process	[Acsl3, Acsl4, Hmgcs1, Mvd]
Ribonucleoside bisphosphate metabolic process	[Acsl3, Acsl4, Hmgcr, Hmgcs1, Mvd]

Term p value, term p value corrected with Bonferroni step down, group p value, and group p value corrected with Bonferroni step down were less than 0.01

Table 2. Hub nodes among the 56 queried DEGs from the main connected component of the reconstructed network are presented.

No.	Query term	K	BC	CC	Stress	Clustering coefficient
1	Hmgcr	50	0.880	0.600	7598	0.520
2	Hmgcs1	42	0.140	0.515	2108	0.650
3	Msmo1	38	0.040	0.479	944	0.696
4	Nsdhl	37	0.040	0.469	720	0.691
5	Lss	36	0.020	0.465	532	0.722
6	Fdps	34	0.020	0.463	526	0.783
7	Idi1	32	0.040	0.461	526	0.780
8	Mvd	32	0.220	0.469	1158	0.744
9	Ppara	28	1.000	0.547	6988	0.471
10	Hsp90aa1	22	0.240	0.486	2014	0.537

K, BC, and CC refer to degree, betweenness centrality, closeness centrality, respectively

This function of Hmgcs1 is associated with several critical functions such as regulation of testosterone synthesis, risk of prostate cancer, and gastric cancer promotion [22-24]. It is reported that the enhanced total cholesterol and low-density lipoprotein cholesterol (LDL-C) in serum due to hypercholesteremia, increase the risk of promotion of atherosclerosis. Investigations have revealed that Hmgcs1 inhibition is associated with considerable reduction of total cholesterol and LDL-C in serum of the high-fat diet-induced hypercholesteremia mice [25].

HMG CoA reductase (Hmgcr) is the first top hub and is ranked as the third genes in Figure 5 which is related to 20 biological terms. This enzyme as like Hmgcs1 is a rate-limiting enzyme in cholesterol synthesis [26].

Mevalonate diphosphate decarboxylase (Mvd) is the second top gene in Figure 5 which is related to 22 biological terms. Mvd has appeared as the 8th hub in Table 2. Based on the published data, sterol regulatory element-binding protein 2 (Srebp2) transcription factor regulates cholesterol biosynthesis tightly via regulation of Hmgcs1, Hmgcr, Fdps, and Mvd as the related enzyme to cholesterol synthesis [27]. Farnesyl diphosphate synthase (FDPS) is the 6th hub and the 4th ranked gene in Figure 5 that is related to 19 biological terms of "cholesterol metabolism pathway".

Lanosterol synthase (Lss) is the fifth hub and ranked gene in Figure 5. Lss is related to the 17 biological terms. Published shows that mutations of Lss result in cholesterol deficiency- associated cataracts in rat [28]. Another key dysregulated gene is isopentenyl-diphosphate delta isomerase 1 (Idi1). Upregulation of Idi1 and downregulation of Hmgcs2; the enzymes of "super-pathway of cholesterol biosynthesis" in stretched atrial myocytes of mitral (HL-1 atrial myocytes) are reported. It should be mentioned that lipid over-expression is detected in the atrial myocytes of mitral regurgitation patients [29].

The last two key genes are Nsdhl and Msmo1. NAD(P) dependent steroid dehydrogenase-like (Nsdhl) is highlighted in breast cancer metastasis via "TGFβ signaling pathway" and "cholesterol biosynthesis" alteration [30]. Evaluation of gene expression pattern of preadipocytes and differentiated adipocytes of 3T3-L1 to explore regulation and mechanism of adipogenesis indicates that methyl sterol Monooxygenase 1 (Msmo1) (the related enzyme of "cholesterol synthesis pathway") is down-regulated in differentiated adipocytes [31].

Conclusion

PPI network analysis showed Hmgcr, Hmgcs1, Msmo1, Nsdhl, Lss, Fdps, Idi1, Mvd, Ppara, and Hsp90aa1 are the critical DEGs that are targeted by coffee. Considering gene ontology analysis and PPI network analysis, Hmgcr, Hmgcs1, Msmo1, Nsdhl, Lss, Fdps, Idi1, and Mvd were introduced as the key targeted genes.

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Author contributions

Mostafa Rezaei Tavirani, Zahra Razzaghi, Babak Arjmand and Reza Vafaee were involved in project design, data collection and analysis and approved the final draft of the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

References

[1] Nkondjock A. Coffee consumption and the risk of cancer: an overview. *Cancer Lett*. 2009; 277(2): 121–125.

[2] Chang HC, Nfor ON, Ho CC, Chen PH, Kung YY, Hsu SY, Tantoh DM, Liaw YC, Hsieh CF, Liaw YP. Changes in high-density lipoprotein cholesterol levels in relation to coffee consumption among Taiwanese adults. *J Multidiscip Healthc*. 2020; 13: 1427–1432.

[3] Hu S, Liu K, Luo H, Xu D, Chen L, Zhang L, Wang H. Caffeine programs hepatic SIRT1-related cholesterol synthesis and hypercholesterolemia via A2AR/cAMP/PKA pathway in adult male offspring rats. *Toxicology*. 2019; 418: 11–21.

[4] Lebeau PF, Byun JH, Platko K, Saliba P, Sguazzin M, MacDonald ME, Paré G, Steinberg GR, Janssen LG, Igdoura SA, Tarnopolsky MA, Chen SRW, Seidah NG, Magolan J, Austin RC. Caffeine blocks SREBP2-induced hepatic PCSK9 expression to enhance LDLR-mediated cholesterol clearance. *Nat Commun*. 2022; 13(1): 1–17.

[5] Ostrowski J, Wyrwicz LS. Integrating genomics, proteomics and bioinformatics in translational studies of molecular medicine. *Expert Rev Mol Diagn*. 2009; 9(6): 623–630.
[6] Doll S, Gnad F, Mann M. The case for proteomics and phospho-proteomics in personalized cancer medicine. *Proteomics Clin Appl*. 2019; 13(2): 1–10.
[7] Zhang T, Guo J, Gu J, Wang Z, Wang G, Li H, Wang J. Identifying the key genes and microRNAs in colorectal cancer liver metastasis by bioinformatics analysis and in vitro experiments. *Oncol Rep*. 2019; 41(1): 279–291.
[8] Athanasios A, Charalampos V, Vasileios T. Protein-protein interaction (PPI) network: recent advances in drug discovery. *Curr Drug Metab*. 2017; 18(1): 5–10.
[9] Vella D, Marini S, Vitali F, Di Silvestre D, Mauri G, Bellazzi R. MTGO: PPI network analysis via topological and functional module identification. *Sci Rep*. 2018; 8(1): 1–13.
[10] Safari Alighiarloo N, Taghizadeh M, Rezaei Tavirani M, Goliaei B, Peyvandi AA. Protein-protein interaction networks (PPI) and complex diseases. *Gastroenterol Hepatol Bed Bench*. 2014; 7(1): 17–31.
[11] Rezaei Tavirani M, Arjmand B, Razzaghi M, Ahmadzadeh A. 50S Ribosomal proteins family is the main target of cinnamon extract: a network analysis. *Res J Pharmacogn*. 2021; 8(2): 63–68.
[12] Zamanian Azodi M, Rezaei Tavirani M, Esmaeili S, Arjmand B, Jahani Sherafat S. Evaluation of anticancer effect of ghost pepper: a bioinformatics assessment. *Res J Pharmacogn*. 2021; 8(3): 77–82.
[13] Arjmand B, Khodadoost M, Razzaghi M, Ahmadzadeh A, Rezaei Tavirani S. Assessment of molecular mechanism of saffron anti-stress property. *Res J Pharmacogn*. 2021; 8(3): 25–31.
[14] Rezaei Tavirani M, Rezaei Tavirani M, Mansouri V, Rostami Nejad M, Rezaei Tavirani M. Protein-protein interaction network analysis for a biomarker panel related to human esophageal adenocarcinoma. *Asian Pac J Cancer Prev*. 2017; 18(12): 3357–3363.
[15] Ye J, Li L, Hu Z. Exploring the molecular mechanism of action of Yinchen Wuling powder for the treatment of hyperlipidemia, using network pharmacology, molecular docking, and molecular dynamics simulation. *Biomed Res Int*. 2021; Article ID 9965906.
[16] Mlecnik B, Galon J, Bindea G. Automated exploration of gene ontology term and pathway networks with ClueGO-REST. *Bioinformatics*. 2019; 35(19): 3864–3866.
[17] Zhang Y, Gu L, PuYang J, Liu M, Xia Q, Jiang W, Cao M. Systems bioinformatic approach to determine the pharmacological mechanisms of radix *Astragali* and radix *Angelicae* sinensis in idiopathic pulmonary fibrosis. *Pharmacogn Mag*. 2021; 17(76): 708–718.
[18] Xu H, Zhou S, Tang Q, Xia H, Bi F. Cholesterol metabolism: new functions and therapeutic approaches in cancer. *Biochim Biophys Acta Rev Cancer*. 2020; Article ID 188394.
[19] Min HK, Kapoor A, Fuchs M, Mirshahi F, Zhou H, Maher J, Kellum J, Warnick R, Contos MJ, Sanyal AJ. Increased hepatic synthesis and dysregulation of cholesterol metabolism is associated with the severity of nonalcoholic fatty liver disease. *Cell Metab*. 2012; 15(5): 665–674.
[20] Rezaei Tavirani M, Rezaei Tavirani M, Akbari Z, Hajimehdipoor H. Prediction of coffee effects in rats with healthy and NAFLD conditions based on protein-protein interaction network analysis. *Res J Pharmacogn*. 2019; 6(4): 7–15.
[21] Chen CC, Xie XM, Zhao XK, Zuo S, Li HY. Krüppel-like factor 13 promotes HCC progression by transcriptional regulation of HMGCS1-mediated cholesterol synthesis. *J Clin Transl Hepatol*. 2022; Article ID 00370.
[22] Shi X, Sun X, Xu P, Zhang C, Zhang M, Yuan X, Jiang J, Jin K, Chen C, Zuo Q, Zhang Y, Li B. HMGCS1 promotes male differentiation of chicken embryos by regulating the generate of cholesterol. *All Life*. 2021; 14(1): 577–587.
[23] Cheng Y, Meng Y, Li S, Cao D, Ben S, Qin C, Hua L, Cheng G. Genetic variants in the cholesterol biosynthesis pathway genes and risk of prostate cancer. *Gene*. 2021; Article ID145432.
[24] Wang IH, Huang TT, Chen JL, Chu LW, Ping YH, Hsu KW, Huang KH, Fang WL, Lee C, Chen CF, Liao CC, Hsieh RH, Yeh TC. Mevalonate pathway enzyme HMGCS1 contributes to gastric cancer progression. *Cancers*. 2020; 12(5): 1–22.

[25] Ma X, Bai Y, Liu K, Han Y, Zhang J, Liu Y, Hou X, Hao E, Hou Y, Bai G. Ursolic acid inhibits the cholesterol biosynthesis and alleviates high fat diet-induced hypercholesterolemia via irreversible inhibition of HMGCS1 in vivo. *Phytomedicine*. 2022: Article ID 154233.
[26] Xiang Z, Valenza M, Cui L, Leoni V, Jeong HK, Brilli E, Zhang J, Peng Q, Duan W, Reeves SA, Cattaneo E, Krainc D. Peroxisome-proliferator-activated receptor gamma coactivator 1 α contributes to dysmyelination in experimental models of Huntington's disease. *J Neurosci*. 2011; 31(26): 9544–9553.
[27] Kang H, Oh T, Bahk YY, Kim GH, Kan SY, Shin DH, Kim JH, Lim JH. HSF1 regulates mevalonate and cholesterol biosynthesis pathways. *Cancers*. 2019; 11(9): 1–19.
[28] Mori M, Li G, Abe I, Nakayama J, Guo Z, Sawashita J, Ugawa T, Nishizono S, Serikawa T, Higuchi K, Shumiya S. Lanosterol synthase mutations cause cholesterol deficiency-associated cataracts in the Shumiya cataract rat. *J Clin Invest*. 2006; 116(2): 395–404.
[29] Fang CY, Chen MC, Chang TH, Wu CC, Chang JP, Huang HD, Ho WC, Wang YZ, Pan KL, Lin YS, Huang YK, Chen CJ, Lee WC. Idi1 and Hmgcs2 are affected by stretch in HL-1 atrial myocytes. *Int J Mol Sci*. 2018; 19(12): 1–14.
[30] Chen M, Zhao Y, Yang X, Zhao Y, Liu Q, Liu Y, Hou Y, Sun H, Jin W. NSDHL promotes triple-negative breast cancer metastasis through the TGFβ signaling pathway and cholesterol biosynthesis. *Breast Cancer Res Treat*. 2021; 187(2): 349–362.
[31] Xin Y, Li C, Guo Y, Xiao R, Zhang H, Zhou G. RNA-Seq analysis reveals a negative role of MSMO1 with a synergized NSDHL expression during adipogenesis of 3T3-L1. *Biosci Biotechnol Biochem*. 2019; 83(4): 641–652.

Abbreviation

GEO: gene expression omnibus; PPI: protein-protein interaction; DEGs: differentially expressed genes; CFD: complement factor D; NAFLD: nonalcoholic fatty liver disease; K: degree; BC: betweenness centrality; CC: closeness centrality; LDL-C: low-density lipoprotein cholesterol