Identification of key gene biomarkers and pathways related to Dent disease in CLCN5 knockout mice by bioinformatics analysis

Zhijian Lin
Beijing University of Chinese Medicine https://orcid.org/0000-0001-5092-068X
Yu Wang
Beijing University of Chinese Medicine
Fanfan Guo
Beijing University of Chinese Medicine
Bing Zhang (zhangb@bucm.edu.cn)
https://orcid.org/0000-0001-6391-5142

Research article

Keywords: Dent disease; CLC-5 transporter gene knockout; bioinformatics analysis; differentially expressed genes (DEGs)

Posted Date: September 23rd, 2019

DOI: https://doi.org/10.21203/rs.2.14810/v1

Abstract

Background Dent disease is an X-linked inherited renal disease that occurs almost exclusively in males. Abnormal CLC-5 function might play a role in the development of Dent disease, but the genetic interaction changes and biomarkers in Dent disease are not fully understood. The aim of this study was to explore the potential key gene biomarkers and pathways related to Dent disease in CLCN5 knockout mice model.Methods The gene expression profile GSE10162 was analyzed differentially expressed genes (DEGs), between 3 samples of CLC-5 transporter gene knockout mouse model of Dent disease and 3 samples from wild type mouse. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were applied for the enriched pathway by the online tool DAVID. A protein-protein interaction (PPI) network of DEGs was constructed to find the hub genes by STRING, and visualized with Cytoscape software.Results Three samples from were incorporated into this study. A total of 500 DEGs were filtered, consisting of 231 upregulated genes and 269 downregulated genes. GO analysis indicated that the up-regulated DEGs were significantly enriched in the regulation of transcription form RNA, regulation of cell proliferation, and ion transport, whereas down-regulated genes were mainly enriched in oxidation-reduction process, and metabolic process. KEGG analysis demonstrated that the DEGs were enriched in the metabolic pathways, neuroactive ligand-receptor interaction, nicotine addiction, morphine addiction, fatty acid elongation, TNF signaling pathway, calcium signaling pathway, and cAMP signaling pathway. PPI network analysis found 17 hub genes with greater than 10 degrees of connectivity. The hub genes might participate in TNF signaling pathway, fat digestion and absorption, and enrich in lipid metabolic process, regulation of blood pressure, cellular response to hypoxia, positive regulation of angiogenesis, positive regulation of developmental growth, and positive regulation of cytosolic calcium ion concentration.Conclusions Our study suggests that Apob, Lep, C3, Cxcl1, Acly and Mmp9 may play key roles in the progression of Dent disease. Blood lipid profiles and calcium levels might be potential prognostic biomarkers for Dent disease.

Background

Dent disease, a rare an X-linked disorder of proximal renal tubular dysfunction[1], was first described in 1964. It is characterized by low-molecular-weight proteinuria (LMWP), hypercalciuria, nephrocalcinosis, kidney stones, renal failure, and rickets. The exact prevalence of Dent disease is unknown; to date, >250 families have been described [2]. Dent disease is inherited in an X-linked manner. The father of an affected male will not have the disease nor will he be hemizygous for the pathogenic variant. Molecular genetic testing is often used for Dent disease diagnosis; include single-gene testing, use of a multigene panel, and more comprehensive genomic testing. Patients with Dent disease often carry mutations in genes encoding the Cl-/H+ exchanger CLC-5 and/or inositol polyphosphate 5-phosphatase (OCRL1) [3,4]. About 60% of patients with Dent disease are found to have a CLCN5 mutation, 15% have an OCRL mutation, and in the remaining 25% a mutation cannot be identified[5,6]. Gene mutations in Dent disease have been identified at least 20 sites in the CLC-5 channel sequence, which might lead to the formation of nonfunctional channels[7]. The various types of loss-of-function were found since the mutation of the

OCRL gene with nearly 200 different genetic alterations[8]. But, the physiologic function of CLC-5 and OCRL1 is not well known, and also, the pathway and mechanism of these genes are not known [7]. Moreover, the phenotype of Dent disease varies and the phenotype can overlap with other kidney disease, which can make diagnosis difficult [9]. Thus, the disease is likely under-diagnosed or diagnosed in late stag [10]. So far, the biochemical effects of these mutations and the pathogenic mechanism are not fully understood [11].

Bioinformatics is a multidisciplinary field that can provide useful methods to identify and analyze the associations and molecular mechanisms among key genes and central signaling pathways [12]. Gene expression profiling analysis is a useful method with broad clinical application for identifying gene changes, from molecular diagnosis to pathological classification [13]. In this study, we downloaded an original microarray dataset containing CLCN5 Knockout samples and wild type mouse samples from the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/). Then, we screened the gene expression profiles of CLCN5 Knockout samples and wild type mouse samples in order to identify the differentially expressed genes (DEGs) and hub genes through a DEG interaction network. We subsequently performed the functional enrichment analyses, including Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and protein-protein interaction (PPI) network, by online software. After analyzing the biological functions and pathways, we further explored the potential biomarkers for diagnosis and prognosis by survival analysis. This study may offer better insight into potential molecular mechanisms for Dent disease, allowing the exploration of novel therapeutic strategies.

Results

Identification of DEGs in Dent Disease

A GSE10162 data profile was downloaded from NCBI GEO with 21723 genes in the microarray data. GEO2R was used to identify the DEGs from the expression profile data P value <0.05. A total of 500 DEGs in the proximal tubule tissues of CLCN5 knockout mice compared with wild type mice, including 231 upregulated DEGs and 269 downregulated DEGs (**Supplement Table 1**). Volcano plot was generated to show the correlation between DEGs (**Figure 1**). Top 10 up-regulated DEGs were *Slc10a2, Tmprss11g, March2, LOC102639518, BC018473, Ear12///Ear3///Ear2///Ear1, Ankrd1, Gdpd3, Pde4b, and Acly*. Top 10 down-regulated DEGs were *Cyp4a14, Lmod1, Gm8096///Phgdh, Rnase4, B3gnt3, Hmgcs2, 9130214F15Rik, Gm31210, Smarce1,* and *4930579H20Rik*, and the cluster heat map of the top 20 genes is shown in **Figure 2**.

GO analysis and signaling pathway enrichment of DEGs in Dent Disease

All DEGs were uploaded to the DAVID website to analyze the GO terms enrichment. The GO term analysis included the MF, BP and CC group respectively. The results were considered statistically significant if P <0.05 and gene counts ≥10. The GO enrichment analysis of the up-regulated and down-regulated differential genes is shown in **Table 1 and Figure 3**. The up-regulated genes were mainly enriched in negative regulation of transcription from RNA polymerase II promoter (ontology: Biological Process, BP), dendrite (ontology: Cellular Component, CC), and protein binding (ontology: Molecular Function, MF); the down-regulated genes were mainly enriched in oxidation-reduction process (ontology: BP), extracellular region (ontology: CC) and oxidoreductase activity (ontology: MF).

KEGG pathway analysis of DEGs

A KEGG pathway analysis of the DEGs was also conducted with the online DAVID database, and the results of significantly enriched pathway (P value <0.05) are shown in **Table 2**. The results of the pathway enrichment analysis indicated that the pathways of the DEGs were mainly enriched in eight pathways: Metabolic pathways, Neuroactive ligand-receptor interaction, Nicotine addiction, Morphine addiction, Fatty acid elongation, TNF signaling pathway, Calcium signaling pathway, and cAMP signaling pathway.

Integration of protein-protein interaction (PPI) network and hub gene Analysis

The STRING online database was used to identify the interactions and key genes of the DEGs and to dissect the PPI networks. The results were downloaded and analyzed using Cytoscape software. There were 119 nodes and 692 edges in the PPI network, which represented proteins and interactions (**Figure 4**). The top 17 hub genes were screened according to their degree values and the identified hub genes in were Apob, Lep, C3, Cxcl1, Acly, Mmp9, Hmgcs2, Sox2, Edn1, Hmox1, Plk1, Fabp1, Ftcd, Ttr, Cyp4a14, Mchr1, and Spp2, as shown in **Table 3**. The hub genes were again analyzed by DAVID. GO analysis showed that the 17 hub genes enriched in lipid metabolic process, regulation of blood pressure, cellular response to hypoxia, positive regulation of angiogenesis, positive regulation of developmental growth, and positive regulation of cytosolic calcium ion concentration. KEGG pathway analysis showed that the 17 hub genes might participate in the TNF signaling pathway, fat digestion and absorption.

Discussion

Dent disease is a chronic kidney disorder that occurs almost exclusively in males. Genetics plays a strong role in urinary stone pathogenesis [14]. Patients with Dent's disease often carry mutations in genes of CLCN5 or OCRL1 that are located on chromosome [2]. A gene mutation can affect different genes the cell that influence physiological, morphological and pathological variation [15]. Previous studies indicate that Dent's disease may be associated with aminoaciduria, phosphaturia, glycosuria, uricosuria, kaliuresis,

and impaired urinary acidification, and is often complicated by rickets or osteomalacia [4, 16]. The clinical diagnosis of Dent's disease is based on urinary LMWP, hypercalciuria, and at least one of the following: nephrocalcinosis, kidney stones, hematuria, hypophosphataemia, or renal insufficiency. The clinical diagnosis is confirmed by genetic tests of the identification of mutation in either CLCN5 or OCRL1[17,18]. The CLC-5 chloride channel knock-out mouse was established as an animal model for Dent's disease [19, 20]. This mouse model had elevated serum 1alpha,25-dihydroxyvitamin D3, alkaline phosphatase, osteocalcin, and urinary deoxypyridinoline that similar with the serum parameters of Dent disease patients [21, 22].

In this study, the original microarray analysis showed that hundreds of genes are expressed differentially in the proximal tubule samples of the Dent related CLCN5 knockout mice compared with the wild-type mice, 8-week-old[23]. A total of 500 DEGs were identified from dataset of GSE10162 by GEO2R analysis. These DEGs were then subjected to BP, CC and MF enrichment analysis. The up-regulated genes were mainly enriched in negative regulation of transcription from RNA polymerase II promoter (ontology: BP), dendrite (ontology: CC), and protein binding (ontology: MF), and the down-regulated genes were mainly enriched in oxidation-reduction process (ontology: BP), extracellular region (ontology: CC) and oxidoreductase activity (ontology: MF). KEGG pathway analysis showed the down-regulated genes were mainly enriched in metabolic pathways and fatty acid elongation, and the up-regulated genes were mainly enriched in neuroactive ligand-receptor interaction, nicotine addiction, and morphine addiction, TNF signaling pathway, calcium signaling pathway, and cAMP signaling pathway and oxidation-reduction process.

In addition to the pathway discussed above, the candidate hub genes were identified through PPI network construction and analysis, and ranking the hub genes by degree of connectivity. Our results indicated that 17 hub genes had significant correlations with Dent disease. The 17 hub genes enriched in lipid metabolic process, regulation of blood pressure, cellular response to hypoxia, positive regulation of angiogenesis, positive regulation of developmental growth, and positive regulation of cytosolic calcium ion concentration. KEGG pathway analysis showed that the 17 hub genes might participate in the TNF signaling pathway, fat digestion and absorption. The first hub genes are Apob and Lep, which serve as critical function in lipid metabolism. Apob gene encodes the main apolipoprotein of chylomicrons and low density lipoproteins [24]. Lep gene encodes a protein that is secreted by white adipocytes into the circulation and plays a major role in the regulation of energy homeostasis, such as the regulation of energy balance and body weight control [25, 26]. The gene C3, a Protein Coding gene, plays a central role in the activation of the complement system. There were some research indicated that C3 had relationship with acylation stimulating protein, which can stimulate triglyceride (TG) synthesis and glucose transport in adipocytes [27, 28]. The Cxcl1 gene encoded protein is a secreted growth factor that signals through the G-protein coupled receptor and CXC receptor 2. The Acly gene encodes the primary enzyme (ATP citrate lyase), which responsible for the synthesis of cytosolic acetyl-CoA in many tissues. The Mmp9 gene is involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and

metastasis. Based on gene function description of the top five ranking hub genes, we could find them playing important roles in lipid metabolism.

These results suggested that these DEGs are involved in the lipid metabolism, calcium signaling pathway and. Dent disease has multiple defects attributed to proximal tubule malfunction including LMWP, hypercalciuria, aminoaciduria, phosphaturia, glycosuria, and nephrolithiasis[29], but symptoms of Dent's disease vary by patient. Some patients have typical symptoms such as kidney stones, nephrocalcinosis, hypercalciuria; and some patients exhibit other typical symptoms of nephrotic syndrome, including hypoproteinemia, hyperlipemia, or edema [3]. Scheinman SJ's study indicated that inactivation of CLCN5 can be found in the setting of hypercalciuria[18]. However, Dent's disease is often underdiagnoses since the mild clinical and biochemical signs, and unobvious of the X-linked inheritance[30].

Some limitations exiting in our studies should be mentioned. There is only one available dataset from CLCN5 knockout mouse model in online GEO database. In this study, we conducted bioinformatics analysis with GO and KEGG pathway enrichment, and PPI network construction. All these analysis was based on the gene expression data from the comparison between 3 CLCN5 knockout mouse samples and 3 wild type mouse samples. In DEG identification, we identified 500DEGs with P value <0.01 and a |log2 fold change (FC)| ≥1 as the thresholds, and 63 DEGs with adjust P value <0.05 and a |log2 fold change (FC)| ≥1 as the thresholds. Comparing to these two methods, the 63 DEGs were also ranked in the top 63 list DEGs screened by p value. It is acceptable to use the p value instead adjust p value to identify the DEGs, since the small number of sample size. Multi-central and large sample studies on Dent disease expression profiles were needed for validation of our findings in the future. Integrating more omics data such as epigenetic or epidemiological data would help illustrate the genetic, epigenetic, and environmental factors for Dent disease. Moreover, the lack of experimental validation is another limitation of our research. And further experimental research is needed to verify these findings with a larger sample size.

Conclusions

The present study, we presumed these key genes and pathways identified by a series of bioinformatics analyses on DEGs between CLCN5 knockout mouse samples and wild type mouse samples. The results showed that several pathways are altered and numerous hub genes. The hub genes and the enriched pathways indicated lipid and calcium might be importantly associated with the pathogenesis of Dent disease. According to the study, down-regulation of Apob and Mmp9, up-regulation of Lep, C3, Cxcl1 and Acly might be considered as biomarkers or therapeutic targets for Dent disease. The blood lipid profiles and urinary calcium may be valuable as prognostic markers for Dent disease patients. This study provided new insight for the understanding of molecular mechanisms in Dent disease. However, further experiments are required to confirm and validate these predicted results.

Methods

Microarray data information

The aim of this study was to explore the potential key gene biomarkers and pathways related to Dent disease in CLCN5 knockout mice model from public database. Raw gene expression data of GSE10162 used in the present study was downloaded from the National Center of Biotechnology Information Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE10162). The original gene expression profile was contributed by Wright JM et al which included 6 mouse kidney proximal tubule samples from 3 wild type (GSM256956, GSM256957,) and 3 CLCN5 knockout mice (GSM256958 GSM256959, GSM256960, GSM256961) [23]. This dataset comprised from and was generated from renal proximal tubule samples using the platform [Mouse430_2] Mouse Genome 430 2.0 Array (Affymetrix, Santa Clara, CA). The microarray of GeneChip initial expression analysis was performed using the GCOS 1.2 software (Affymetrix, Santa Clara, CA). The 3'-to-5' ratio for GAPDHMur was confirmed to be <2 for all six chips used in the analysis[23].

Data preprocessing and identification of DEGs

GEO2R is an interactive online analysis tool based on R language that allows users to compare two or more groups of samples in a GEO series in order to identify genes that are differentially expressed across experimental conditions [31]. The 6 mouse kidney proximal tubules samples were classified into two groups that had similar expression patterns. We analyzed the raw data by GEO2R (www.ncbi.nlm.nih.gov/geo/geo2r/). Statistically significant DEGs were defined with *P* value <0.01 and a |log2 fold change (FC)| ≥1 as the thresholds described in the references[32-34].

Gene Function Enrichment Analysis of DEGs

Gene Ontology (GO) enrichment analysis of DEGs was implemented via a common functional annotation tool DAVID (https://david.ncifcrf.gov/). All genes in the genome were used as the enrichment background. *P* values were calculated based on accumulative hypergeometric distribution, and q-values were calculated using the Benjamini-Hochberg procedure to account for multiple testing. GO enrichment analysis was performed in order to distinguish the biological attributes, such as molecular function (MF), biological process (BP), and cellular component (CC) of important DEGs. The results were considered statistically significant if P values < 0.05 and gene counts ≥10. For each given gene list, pathway and process enrichment analyses were performed using the following ontology sources: Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. The results were considered statistically significant if *P* values < 0.05.

Protein-Protein Interaction (PPI) Network Construction and hub gene Analysis

The PPI network was constructed using the Search Tool for the Retrieval of Interacting Genes online database (STRING Version: 11.0, https://string-db.org/) [35], a biological predictive web resource including numerous proteins and known interactive functions. A combined score of >0.4 was designated as the cutoff standard. Genes with degrees >10 were selected as hub genes and sorted according to the ranking order of connectivity degree. The PPI network was visualized by the Cytoscape software (Version 3.7.1) (The Cytoscape Consortium, New York, NY), and module of PPI network was screened by the cytoHubba in Cytoscape.

Abbreviations

DEGs: Differentially Expressed Genes

GO: Gene Ontology

KEGG: Kyoto Encyclopedia of Genes and Genomes

DAVID: Database for Annotation, Visualization and Integrated Discovery software

PPI: Protein-Protein Interaction

STRING: Search Tool for the Retrieval of Interacting Genes/Proteins

LMWP: Low-Molecular-Weight Proteinuria

BP: Biological Process

MF: Molecular Function

CC: Cellular Component.

Declarations

Ethical approval and consent to participate

Not applicable. This article does not contain any studies with human participants or animals performed by any of the authors. We performed all bioinformatic procedures based online public database.

Consent for publication

Not applicable.

Availability of data and material

The gene expression profile GSE10162, used and/or analyzed during the current study, was downloaded from the largest fully public online free database (The GEO database - NCBI - NIH,

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE10162).

Competing interests

The authors declare that they have no competing interests.

Funding

In this study, the design of the study, the analysis, the interpretation of data and in writing the manuscript were supported in part by the National Major Scientific and Technological Special Project for “Significant New Drugs Development” (Grant No. 2017ZX09301024) and National Natural Science Foundation of China (Grant No. 81403152).

Author information

Affiliations

School of Chinese Materia Medica and Research Center for Chinese Medicine Pharmacovigilance and Rational Drug Use, Beijing University of Chinese Medicine, Beijing, 100029, People’s Republic of China

Zhijian Lin, Bing Zhang

School of Chinese Materia Medica and Research Center for Chinese Medicine Pharmacovigilance and Rational Drug Use, Beijing University of Chinese Medicine, Beijing, 100029, People’s Republic of China

Yu Wang, Fanfan Guo

Contributions

ZJ LIN performed the data analysis and wrote the manuscript and paper submission. Y WANG performed double check of the data analysis and revised the manuscript draft. FF GUO and B ZHANG revised the manuscript. All authors have read and approved the manuscript.

Corresponding authors

Correspondence to Bing ZHANG.

Acknowledgments

We would like to thank Yue LI (School of Public Health University of Minnesota) for her comments on the manuscript structure and language checking.

References

1. van Berkel Y, Ludwig M, van Wijk JAE, Bokenkamp A. Proteinuria in Dent disease: a review of the literature. *Pediatr Nephrol.* 2017;32(10):1851-1859.
2. Devuyst O, Thakker RV. Dent's disease. *Orphanet journal of rare diseases.* 2010;5:28.
3. Zhang Y, Fang X, Xu H, Shen Q. Genetic Analysis of Dent's Disease and Functional Research of CLCN5 Mutations. *DNA and Cell Biology.* 2017;36(12):1151-1158.
4. Anglani F, D'Angelo A, Bertizzolo LM, et al. Nephrolithiasis, kidney failure and bone disorders in Dent disease patients with and without CLCN5 mutations. *Springerplus.* 2015;4:492.
5. Zhang H, Wang F, Xiao H, Yao Y. Dent disease: Same CLCN5 mutation but different phenotypes in two brothers in China. *Intractable Rare Dis Res.* 2017;6(2):114-118.
6. Lloyd SE, Pearce SH, Fisher SE, et al. A common molecular basis for three inherited kidney stone diseases. *Nature.* 1996;379(6564):445-449.
7. Mo L, Xiong W, Qian T, Sun H, Wills NK. Coexpression of complementary fragments of ClC-5 and restoration of chloride channel function in a Dent's disease mutation. *Am J Physiol Cell Physiol.* 2004;286(1):C79-89.
8. Festa BP, Berquez M, Gassama A, et al. OCRL deficiency impairs endolysosomal function in a humanized mouse model for Lowe syndrome and Dent disease. *Hum Mol Genet.* 2019;28(12):1931-1946.
9. Ludwig M, Utsch B, Monnens LA. Recent advances in understanding the clinical and genetic heterogeneity of Dent's disease. *Nephrol Dial Transplant.* 2006;21(10):2708-2717.
10. Gambaro G, Vezzoli G, Casari G, Rampoldi L, D'Angelo A, Borghi L. Genetics of hypercalciuria and calcium nephrolithiasis: from the rare monogenic to the common polygenic forms. *American journal of kidney diseases : the official journal of the National Kidney Foundation.* 2004;44(6):963-986.
11. Wang X, Anglani F, Beara-Lasic L, et al. Glomerular Pathology in Dent Disease and Its Association with Kidney Function. *Clinical Journal of the American Society of Nephrology.* 2016;11(12):2168-2176.
12. Diniz WJS, Canduri F. REVIEW-ARTICLE Bioinformatics: an overview and its applications. *Genetics and Molecular Research.* 2017;16(1).
13. Medeiros F, Rigl CT, Anderson GG, Becker SH, Halling KC. Tissue handling for genome-wide expression analysis: a review of the issues, evidence, and opportunities. *Arch Pathol Lab Med.* 2007;131(12):1805-1816.
14. Lieske JC, Wang XL. Heritable traits that contribute to nephrolithiasis. *Urolithiasis.* 2019;47(1):5-10.
15. Ishak. GENE MUTATIONS, GENETIC DISEASE AND PHARMACOGENETIC GENES DISORDER. *International Journal of Research in Ayurveda and Pharmacy.* 2010;1.
16. Wrong OM, Norden AG, Feest TG. Dent's disease; a familial proximal renal tubular syndrome with low-molecular-weight proteinuria, hypercalciuria, nephrocalcinosis, metabolic bone disease, progressive renal failure and a marked male predominance. *QJM : monthly journal of the Association of Physicians.* 1994;87(8):473-493.

17. Frishberg Y, Dinour D, Belostotsky R, et al. Dent's disease manifesting as focal glomerulosclerosis: Is it the tip of the iceberg? *Pediatr Nephrol.* 2009;24(12):2369-2373.
18. Scheinman SJ, Cox JP, Lloyd SE, et al. Isolated hypercalciuria with mutation in CLCN5: relevance to idiopathic hypercalciuria. *Kidney Int.* 2000;57(1):232-239.
19. Gunther W, Piwon N, Jentsch TJ. The ClC-5 chloride channel knock-out mouse - an animal model for Dent's disease. *Pflugers Arch.* 2003;445(4):456-462.
20. Wang SS. Mice lacking renal chloride channel, CLC-5, are a model for Dent's disease, a nephrolithiasis disorder associated with defective receptor-mediated endocytosis. *Human Molecular Genetics.* 2000;9(20):2937-2945.
21. Silva IV, Cebotaru V, Wang H, et al. The ClC-5 knockout mouse model of Dent's disease has renal hypercalciuria and increased bone turnover. *J Bone Miner Res.* 2003;18(4):615-623.
22. Cebotaru V, Kaul S, Devuyst O, et al. High citrate diet delays progression of renal insufficiency in the ClC-5 knockout mouse model of Dent's disease. *Kidney Int.* 2005;68(2):642-652.
23. Wright J, Morales MM, Sousa-Menzes J, et al. Transcriptional adaptation to Clcn5 knockout in proximal tubules of mouse kidney. *Physiol Genomics.* 2008;33(3):341-354.
24. Schaefer EJ, Geller AS, Endress G. The biochemical and genetic diagnosis of lipid disorders. *Curr Opin Lipidol.* 2019;30(2):56-62.
25. Boyapati R, Chintalapani S, Ramisetti A, Salavadhi SS, Ramachandran R. Evaluation of Serum Leptin and Adiponectin in Obese Individuals with Chronic Periodontitis. *Contemp Clin Dent.* 2018;9(Suppl 2):S210-S214.
26. Sarac S, Atamer A, Atamer Y, et al. Leptin levels and lipoprotein profiles in patients with cholelithiasis. *J Int Med Res.* 2015;43(3):385-392.
27. Baldo A, Sniderman AD, St-Luce S, et al. The adipsin-acylation stimulating protein system and regulation of intracellular triglyceride synthesis. *J Clin Invest.* 1993;92(3):1543-1547.
28. Murray I, KÖHl J, Cianflone K. Acylation-stimulating protein (ASP): structure–function determinants of cell surface binding and triacylglycerol synthetic activity. *Biochemical Journal.* 1999;342(1):41-48.
29. Tang X, Brown MR, Cogal AG, et al. Functional and transport analyses of CLCN5 genetic changes identified in Dent disease patients. *Physiol Rep.* 2016;4(8).
30. Tosetto E, Ghiggeri GM, Emma F, et al. Phenotypic and genetic heterogeneity in Dent's disease–the results of an Italian collaborative study. *Nephrol Dial Transplant.* 2006;21(9):2452-2463.
31. Barrett T, Wilhite SE, Ledoux P, et al. NCBI GEO: archive for functional genomics data sets—update. *Nucleic Acids Research.* 2012;41(D1):D991-D995.
32. Dalman MR, Deeter A, Nimishakavi G, Duan ZH. Fold change and p-value cutoffs significantly alter microarray interpretations. *BMC Bioinformatics.* 2012;13 Suppl 2:S11.
33. Tang F, He Z, Lei H, et al. Identification of differentially expressed genes and biological pathways in bladder cancer. *Mol Med Rep.* 2018;17(5):6425-6434.

34. Liu Y, Hua T, Chi S, Wang H. Identification of key pathways and genes in endometrial cancer using bioinformatics analyses. *Oncol Lett.* 2019;17(1):897-906.
35. Szklarczyk D, Franceschini A, Wyder S, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res.* 2015;43(Database issue):D447-452.

Tables

Table 1: Gene ontology analysis of DEGs in Dent disease

Expression	Category	Term	Count	P Value
Up-regulation	BP_DIRECT	GO:0000122~negative regulation of transcription from RNA polymerase II promoter	14	0.0171
	BP_DIRECT	GO:0007275~multicellular organism development	17	0.0274
	BP_DIRECT	GO:0008284~positive regulation of cell proliferation	11	0.0282
	BP_DIRECT	GO:0006811~ion transport	11	0.0433
	CC_DIRECT	GO:0030425~dendrite	14	0.0005
	CC_DIRECT	GO:0005737~cytoplasm	81	0.0012
	CC_DIRECT	GO:0005783~endoplasmic reticulum	24	0.0020
	CC_DIRECT	GO:0030424~axon	11	0.0021
	CC_DIRECT	GO:0005887~integral component of plasma membrane	21	0.0031
	CC_DIRECT	GO:0005576~extracellular region	27	0.0084
	CC_DIRECT	GO:0016020~membrane	79	0.0139
	CC_DIRECT	GO:0042995~cell projection	13	0.0289
	MF_DIRECT	GO:0005515~protein binding	58	0.0009
	MF_DIRECT	GO:0042803~protein homodimerization activity	16	0.0093
	MF_DIRECT	GO:0043565~sequence-specific DNA binding	12	0.0398
	MF_DIRECT	GO:0003700~transcription factor activity, sequence-specific DNA binding	15	0.0424
Down-regulation	BP_DIRECT	GO:0055114~oxidation-reduction process	14	0.0127
	BP_DIRECT	GO:0008152~metabolic process	11	0.0133
	CC_DIRECT	GO:0005576~extracellular region	31	0.0019
	CC_DIRECT	GO:0005615~extracellular space	26	0.0067
	CC_DIRECT	GO:0005829~cytosol	26	0.0444
	MF_DIRECT	GO:0016491~oxidoreductase activity	14	0.0054
	MF_DIRECT	GO:0046982~protein heterodimerization activity	11	0.0263

Notes: BP, biological process. CC, cellular component. MF, molecular function.

Table 2. KEGG pathway enrichment analysis of the DEGs

Pathway	ID	Gene Count	P Value	Genes
* Metabolic pathways	mmu01100	23	0.0014	ETNPPL, CYP24A1, COX11, FUT7, PIK3C2A, HEXA, ACOT1, ASNS, GCH1, ACOT3, FDFT1, MTHFD2, GAD2, HMGCS2, UGT2B34, AGPAT9, PKLR, CYP4A31, PHGDH, B3GNT3, PSAT1, CYP4A14, MTMR7
*Fatty acid elongation	mmu00062	3	0.0231	ELOVL1, ACOT1, ACOT3
# Neuroactive ligand-receptor interaction	mmu04080	10	0.0021	LEP, MCHR1, CRHR2, PTGER1, ADRB2, GABRA6, GRIK3, HTR4, BDKRB2, GABRQ
# Nicotine addiction	mmu05033	4	0.0073	SLC32A1, GABRA6, CACNA1A, GABRQ
# Morphine addiction	mmu05032	5	0.0139	SLC32A1, GABRA6, PDE4B, CACNA1A, GABRQ
# TNF signaling pathway	mmu04668	5	0.0234	CXCL1, VCAM1, SOCS3, MMP9, EDN1
# Calcium signaling pathway	mmu04020	6	0.0339	PTGER1, ADRB2, HTR4, RYR2, BDKRB2, CACNA1A
# cAMP signaling pathway	mmu04024	6	0.0470	ADRB2, ROCK1, PDE4B, HTR4, RYR2, ARAP3

Note: * represents the down-regulated gene pathway; # represents the up-regulated gene pathway

Table 3. Top 17 hub genes with higher degree of connectivity

Rank	Gene Name	Description	Degree	Type
1	Apob	apolipoprotein B	24	down
1	Lep	leptin	24	up
3	C3	complement 3	19	up
4	Cxcl1	chemokine (C-X-C motif) ligand 1	17	up
5	Acly	ATP citrate lyase	16	up
5	Mmp9	matrix metallopeptidase 9	16	up
7	Hmgcs2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	15	down
7	Sox2	SRY (sex determining region Y)-box 2	15	down
9	Edn1	endothelin 1	14	up
9	Hmox1	heme oxygenase 1	14	down
11	Plk1	polo like kinase 1	13	down
12	Fabp1	fatty acid binding protein 1	12	down
12	Ftcd	formiminotransferase cyclodeaminase	12	up
12	Ttr	transthyretin	12	down
15	Cyp4a14	cytochrome P450, family 4, subfamily a, polypeptide 14	11	down
15	Mchr1	melanin-concentrating hormone receptor 1	11	up
15	Spp2	secreted phosphoprotein 2	11	Down

Figures

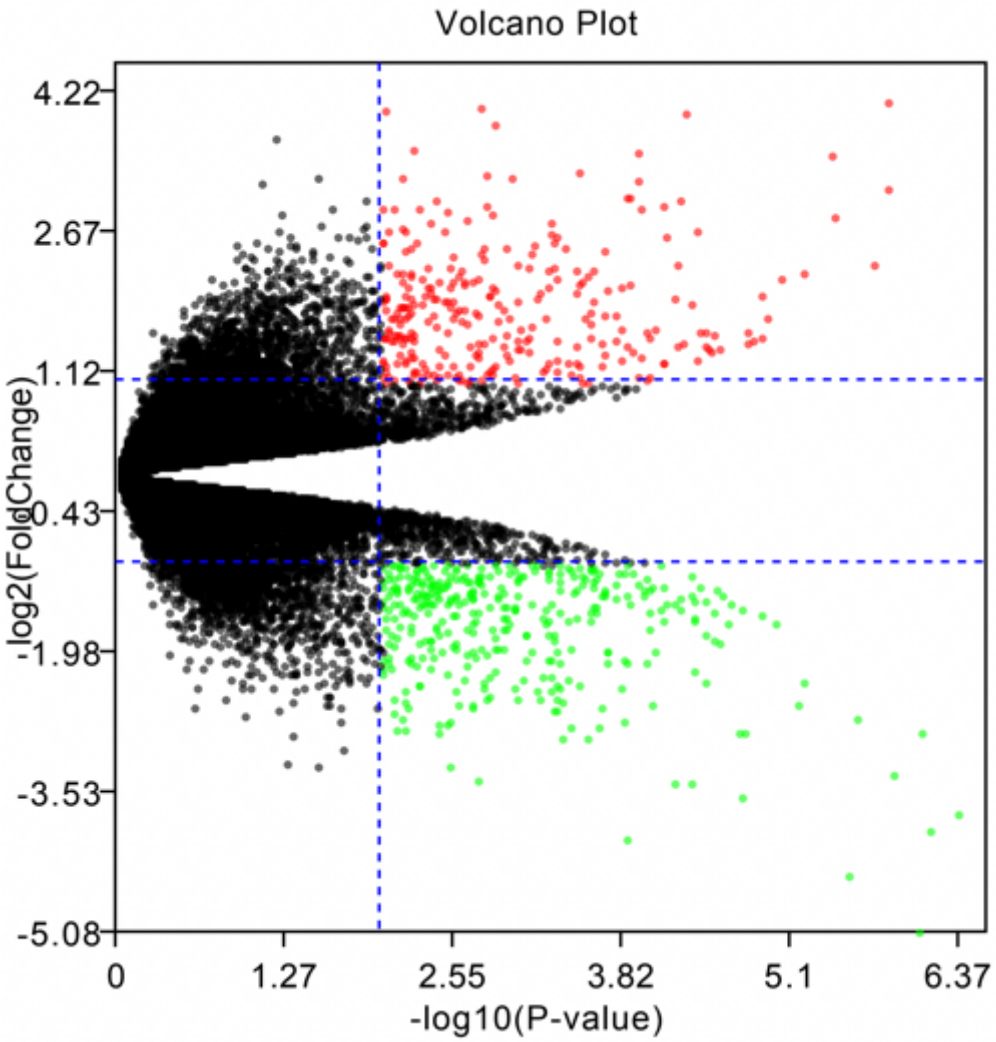

Figure 1

Volcano plot of differentially expressed genes. Red, green and black denote up-regulated, down-regulated and nonsignificant expressed genes, respectively

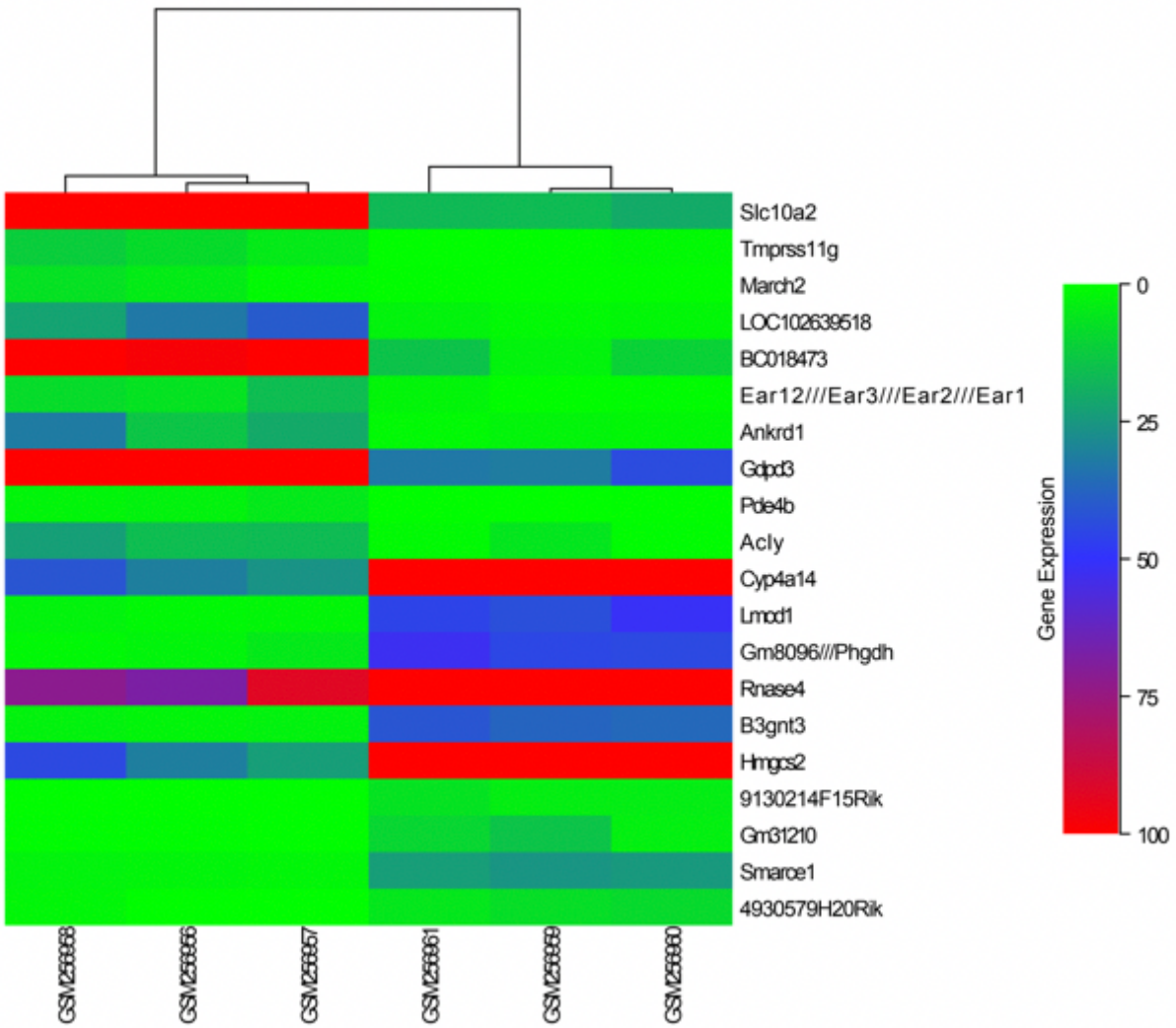

Figure 2

The heat maps of the top 20 DEGs. Each row represents a gene, and each column represents a sham control or a CLCN5 knockout mouse. Each colored square shows the relative change of a single gene in a single mouse. The red color (score=100) indicates highest and green color (score=0) indicates lowest expression levels.

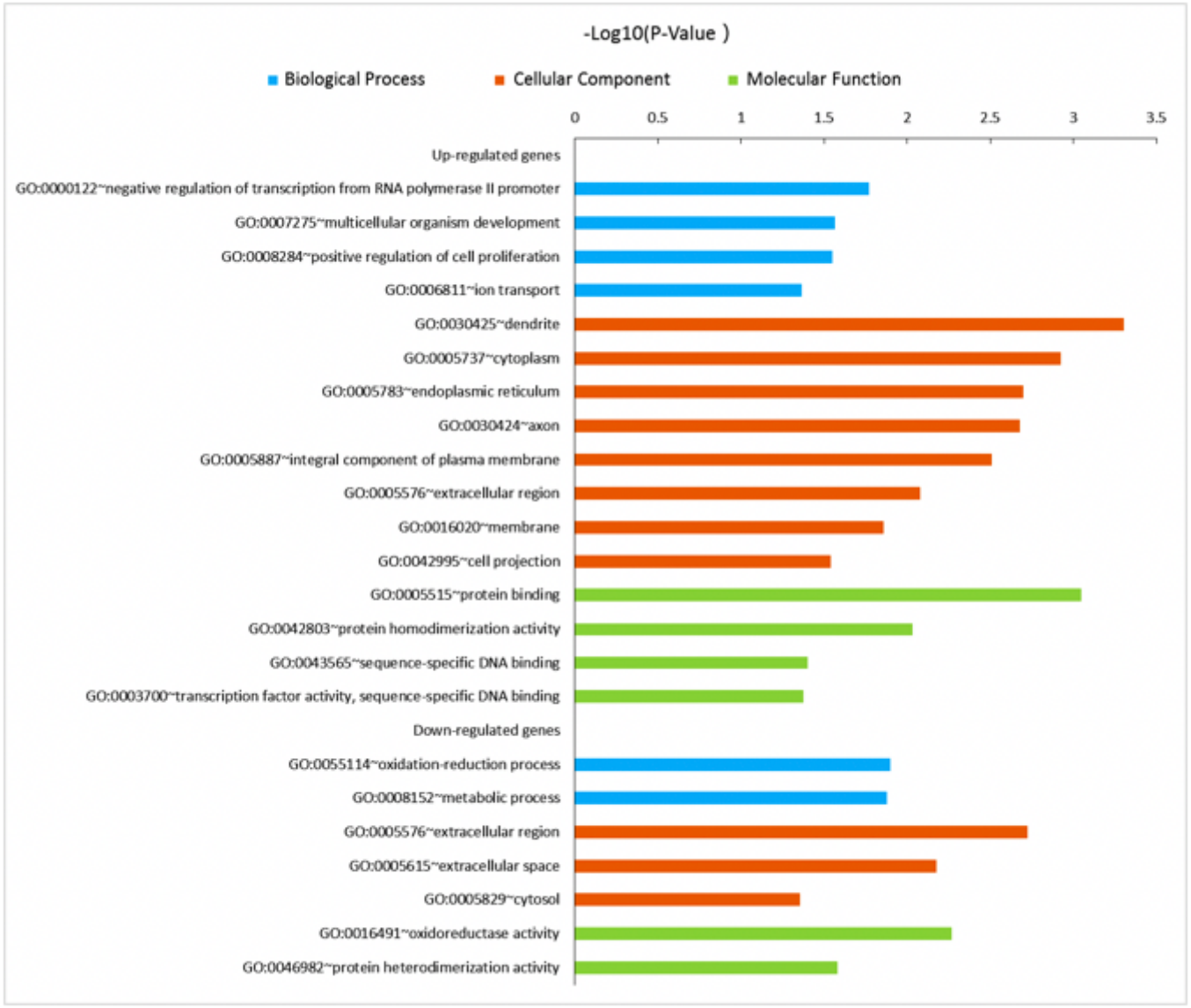

Figure 3

Gene ontology (GO) annotation and enrichment analysis of 500 DEGs. The horizontal axis shows the negative log 10 of the P-Value, while the vertical axis represents biological process, molecular function, and cellular component, respectively.

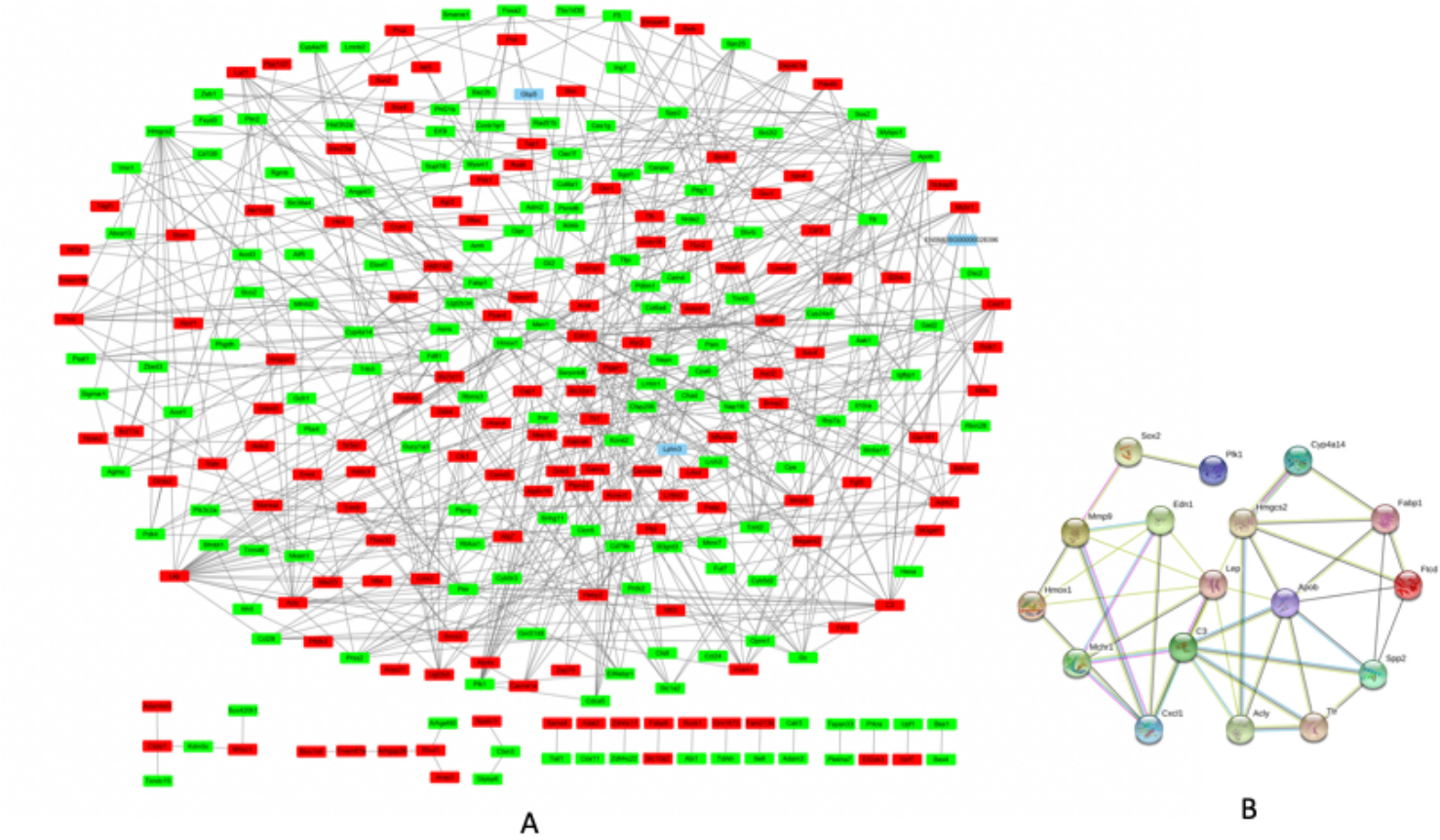

Figure 4

Protein-protein interaction network of DEGs and hub genes. (A): The PPI network for Dent disease based on overall DEGs constructed by Cytoscape. (B): the PPI network of top 17 hub genes with high connectivity degree constructed by STRING. Notes: Red indicates the upregulated DEGs. Green indicates the downregulated DEGs. Abbreviations: DEGs, differentially expressed genes; PPI, protein–protein interaction.

Supplementary Files

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

- FinalSupplementarymaterialTables0824.docx