Identification of significant genes with invasive promotion in non-functional pituitary adenoma via bioinformatical analysis

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Research

Keywords: Invasive non-functional pituitary adenoma, Bioinformational analysis, PEGG pathway, Differentially expressed genes, GEO2R

Posted Date: January 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-146994/v1

Abstract

Background

Non-functional pituitary adenoma (NFPA) is a disease with a high incidence, which accounts for a large part of pituitary tumors and plays a pivotal role. While invasive NFPAs which have not any endocrinology manifestations and space-occupying symptoms at early stages account for about 30 percent of NFPAs. The purpose of the present academic work was to identify significant genes with invasive promotion and their underlying mechanisms.

Methods

Gene expression profiles of GSE51618 was available from GEO database. There are 4 non-invasive NFPA tissues, 3 invasive NFPA tissues and 3 normal tissues in the profile datasets. Differentially expressed genes (DEGs) between non-invasive NFPA tissues and invasive NFPA tissues were picked out by GEO2R online tool. There were total of 226 up-regulated genes and 298 down-regulated genes. Next, we made use of the Database for Annotation, Visualization and Integrated Discovery (DAVID) to analyze Kyoto Encyclopedia of Gene and Genome (KEGG) pathway, gene ontology (GO) and Kaplan Meier Plotter. Then protein-protein interaction (PPI) of these DEGs was visualized by Cytoscape with Search Tool for the Retrieval of Interacting Genes (STRING). There were total of 141 up-regulated genes and 171 down-regulated genes. Of PPI network analyzed by Molecular Complex Detection (MCODE) plug-in, all 141 up-regulated genes were selected.

Results

After reanalysis of GO, five genes (ATP2B3, ADCYAP1R1, PTGER2, FSHβ, HTR4) were found to significantly enrich in the cAMP signaling pathway, Neuroactive ligand-receptor interaction and Renin secretion via reanalysis of DAVID.

Conclusions

We have identified five significant up-regulated DEGs with invasive promotion in invasive NFPAs on the basis of integrated bioinformatical methods, which could be potential therapeutic targets for invasive NFPAs patients.

Background

Pituitary adenomas (PA) account for 10–15% of all intracranial tumors. Nearly 35% of adenomas exhibit aggressive biological phenotypes, including rapid proliferative activity and invasion of adjacent tissues.[1] Invasive NFPA is a common type of pituitary adenoma, as well as an incurable tumor, which is

characterized by tumor invasion of sphenoid and ethmoid sinus, upper slope, sellar bone and dura mater. Due to invasive NFPAs can grow in multiple directions, extensively invade the surrounding structures of the sella area, result in treatment of invasive tumor is very difficult and the recurrence rate of surgery is high. Besides, another threatening cause is that they have no obvious clinical manifestations in the process of tumor progression, and they will not be discovered until the patient's disease is serious when the tumor produces headaches, vision loss, visual field defects and other oppressive symptoms. After the operation, the patient can relieve the oppression on the optic chiasm and brain tissue and reduce symptoms, but the transsphenoidal approach has a 0.9% mortality rate, and the transcranial approach has a 2%-5% mortality rate.[2] This two types Surgery may have various complications including vision loss, hypothalamic injury, vascular injury, intracranial infection, oculomotor nerve palsy, cerebrospinal fluid rhinorrhea and other complications, which reduce the quality of life of patients. Therefore, to explore the biomarkers related to aggressiveness in NFPA, whether it is to predict the type of patient's tumor in advance to choose the treatment method or to target the treatment in the future, our research is very meaningful.

It is currently believed that the following five genes have direct or indirect effects on the aggressiveness of NFPA. Nucleophosmin P53 is a tumor suppressor gene, which is believed to play a key role in the invasion of NFPA after its mutation. [3] Martin believes that the Nm23 gene can inhibit tumor formation and invasion. In the long-term follow-up, it was found that Nm23 was negative or weakly positive in aggressive pituitary adenomas, and Nm23 was negatively correlated with aggressiveness.[4] FGF-2 is believed to be related to the growth and development of NFPA.[5] Guo H found that MMP-9 is a potential molecular marker.[6] Mastronardi found that Ki-67 is closely related to the aggressiveness of pituitary tumors.[7]

After more than ten years of development, gene chip technology has become a mature and reliable technology. It can quickly detect a large number of differentially expressed genes, and these data are then uploaded to a public database for further discovery.[8] Although chip data mining has been carried out in many diseases, there are still gaps in the invasiveness of NFPA. Therefore, the application of biological information methods can help us further study differential genes and potential mechanisms.

In this study, first, we chose GSE51618 from Gene Expression Omnibus (GEO). Second, we used GEO2R online tool to obtain the commonly differentially expressed genes (DEGs) in the datasets above. Third, the Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to analyze these DEGs including molecular function (MF), cellular component (CC), biological process (BP) and Kyoto Encyclopedia of Gene and Genome (KEGG) pathways. Fourth, we established protein-protein interaction (PPI) network and then applied Cytotype MCODE (Molecular Complex Detection) for additional analysis of the DEGs which would identify some core genes. Taken above, only 13 DEGs were qualified. Then we re-analyzed these 13 DEGs for KEGG pathway enrichment. Finally, five DEGs (ATP2B3, ADCYAP1R1, PTGER2, FSHβ, HTR4) were generated and significantly enriched in the cAMP signaling pathway,

Neuroactive ligand-receptor interaction and Renin secretion. In conclusion, the bioinformatic study of our study provides some additional useful biomarkers which could be related to invasive NFPA.

Methods

Microarray data information

NCBI-GEO is famous as a free public database of microarray/gene profile and we searched the gene expression profile of GSE51618 in non-invasive NFPA tissues and invasive NFPA tissues. Microarray data of GSE51618 was on account of GPL6480 Platforms (Expression profiling by array) which included 4 non-invasive NFPA tissues, 3 invasive NFPA tissues and 3 normal tissues.

Data processing of DEGs

DEGs between non-invasive NFPA specimen and invasive NFPA specimen were identified via GEO2R online tools with |log FC| > 2 and adjust P value < 0.05. Then, the raw data in TXT format were downloaded from the website. The DEGs with log FC < 0 was considered as down-regulated genes, and the DEGs with log FC > 0 was considered as an up-regulated gene.

Gene ontology and pathway enrichment analysis

Gene ontology analysis (GO) is a commonly used approach for defining genes and its RNA or protein product to identify unique biological properties of high-throughput transcriptome or genome data. KEGG is a public database dealing with genomes, diseases, biological pathways, drugs, and chemical materials. DAVID is an online bioinformatic tool which is designed to identify a large number of genes or proteins function. We use DAVID to visualize the DEGs enrichment of BP, MF, CC and pathways ($P<0.05$).

PPI network and module analysis

PPI information can be evaluated by the useful online tool, STRING (Search Tool for the Retrieval of Interacting Genes). Then, Cytoscape app was applied to examine the potential correlation between these DEGs (maximum number of interactors = 0 and confidence score ≥ 0.4). Besides, the MCODE plug-in in Cytoscape was used to check modules of the PPI network (degree cutoff = 2, max. Depth = 100, k-core = 2, and node score cutoff = 0.2).

Results

Identification of DEGs in invasive NFPAs

There are 4 non-invasive NFPA tissues, 3 invasive NFPA tissues and 3 normal tissues in our study. Via GEO2R online tools we extracted 1338 DEGs from GSE51618. Then, we used logFC < 0 to identify down-regulated genes and logFC > 0 to identify up-regulated genes. Results showed that a total of 524

commonly DEGS were detected, including 298 down-regulated genes and 226 up-regulated genes. (Table I).

DEGs gene ontology and KEGG pathway analysis in invasive NFPAs

All 524 DEGs were analyzed by DAVID software and results of GO analysis indicated that 1) for biological processes (BP), up-regulated DEGs were particularly enriched in retina layer formation, positive and negative positive regulation of transcription from RNA polymerase II promoter, cardiac muscle contraction, glutamate receptor signaling pathway, positive regulation of neuron projection development, vesicle-mediated transport, positive regulation of phosphorylation, endochondral ossification, regulation of establishment of protein localization to plasma membrane, neurofilament bundle assembly, positive regulation of vasoconstriction, JAK-STAT cascade, response to hydrostatic pressure, morphogenesis of an epithelial fold, skeletal system development, cell cycle arrest, camera-type eye photoreceptor cell differentiation, positive regulation of transcription, DNA-templated, muscle filament sliding, positive regulation of tyrosine phosphorylation of Stat3 protein, intracellular receptor signaling pathway, nervous system development, craniofacial suture morphogenesis, positive regulation of sodium ion transmembrane transporter activity, homophilic cell adhesion via plasma membrane adhesion molecules, response to activity, cytoskeleton organization, metanephric collecting duct development, chemical synaptic transmission, regulation of mitotic spindle organization, positive regulation of cyclin-dependent protein serine/threonine kinase activity involved in G1/S transition of mitotic cell cycle, neuron projection morphogenesis, response to ethanol, phosphatidylinositol-mediated signaling, positive regulation of GTPase activity, chondrocyte proliferation, interleukin-6-mediated signaling pathway, negative regulation of neuron migration, cell-cell signaling, axon development, keratinocyte development, locomotion, placenta blood vessel development, secretion by cell, seminiferous tubule development and down-regulated DEGs in learning, chloride transmembrane transport, regulation of cell migration, glycosaminoglycan biosynthetic process, chemical synaptic transmission, synaptic vesicle exocytosis, regulation of cardiac conduction, synapse assembly, ion transmembrane transport, cell surface receptor signaling pathway, cell migration, long-term synaptic potentiation, negative regulation of viral genome replication, positive regulation of gene expression, regulation of steroid biosynthetic process, paraxial mesoderm development, cellular response to hypoxia, C21-steroid hormone biosynthetic process, skeletal muscle acetylcholine-gated channel clustering, peptidyl-tyrosine phosphorylation. 2) for molecular function (MF), up-regulated DEGs were enriched in protein dimerization activity, structural constituent of muscle, actin binding, small GTPase binding, structural constituent of cytoskeleton, calmodulin binding, protein domain specific binding, RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding, cholesterol binding, growth factor activity, calcium ion binding, transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding, ion channel binding, steroid hormone receptor activity, RNA polymerase II core promoter sequence-specific DNA binding and down-regulated EDGs in transmembrane-ephrin receptor activity, calmodulin binding, metal ion binding, catalytic activity, receptor tyrosine kinase binding, retinoid binding, transferase activity, guanylate cyclase activity. 3) for GO cell component (CC), up-regulated DEGs were significantly enriched in synapse, plasma membrane, cell surface, neurofilament, axon, Z disc,

sarcolemma, endosome, cytoskeleton, TSC1-TSC2 complex, integral component of plasma membrane, protein complex, ruffle, extracellular region, cell junction, focal adhesion, endocytic vesicle, late endosome and down-regulated DEGs in integral component of plasma membrane, cell junction, perikaryon, postsynaptic membrane, neuronal cell body, neuromuscular junction, presynaptic membrane, dendritic spine, axon, integral component of membrane, neuronal cell body membrane, microtubule, lamellipodium, secretory granule, receptor complex, postsynaptic density, ruffle membrane, sarcoplasm, proteinaceous extracellular matrix, dendrite, cell surface, plasma membrane, external side of plasma membrane, guanylate cyclase complex, soluble. (TableII &Table III)

Protein-protein interaction network (PPI) and modular analysis

A total of 524 DEGs were imported into the DEGs PPI network complex, including 226 up-regulated genes and 298 down-regulated genes. There were total 312 of the 524 DEGs which were contained into the DEGs PPI network (Fig. 1a). Next, we used Cytotype MCODE for further analysis, which showed that 13 core genes including 5 up-regulated DEGs and 8 down-regulated DEGs were identified (Fig. 1b).

Analysis of core genes by the Kaplan Meier Plotter

Kaplan Meier Plotter (http://kmplot.com/analysis) was used to identify 13 core genes survival data. We found that all of them had a significantly worse survival ($P < 0.05$, Fig 2).

Analysis of 13 selected genes via KEGG pathway enrichment

To learn more about the possible pathway of these 13 selected DEGs, KEGG pathway enrichment was analyzed via DAVID ($P < 0.05$). Results showed that five genes (ATP2B3, PTGER2, ADCYAP1R1, HTR4, FSHB) enriched in the cAMP signaling pathway ($P = 2.12E-05$, Table 4 & Fig 3) , neuroactive ligand-receptor interaction ($P = 0.001821569$, Table IV & Fig 4) and renin secretion ($P = 0.06453993$, Table IV & Fig 5).

Discussion

According to the method described above, we have derived the five most critical genes (ATP2B3, PTGER2, ADCYAP1R1, HTR4, FSHB) from the profile dataset (GSE51618). They are all had a significance ($P < 0.05$) which could be considered as new effective targets to improve the prognosis of invasive NFPA patients.

ATPase Plasma Membrane Ca^{2+} Transporting 3 (ATP2B3), also known as SCAX1, CLA2 and PMCA3, the protein it encodes belongs to the P-type primary ion transport ATPase family, which is characterized by the formation of an aspartyl phosphate intermediate during the reaction cycle. These enzymes can transport divalent calcium ions from eukaryotic cells against large concentration gradients and play a key role in intracellular calcium homeostasis.[9] Mammalian plasma membrane calcium ATPase subtypes are encoded by at least 4 different genes, which are achieved by generating more than 20 PMCA variants to meet the needs of different cells and tissues.[10] This gene encodes plasma membrane calcium ATPase 3

subtype type. According to reports, p38MAPK can promote the migration and metastasis of BRAF mutant melanoma by inducing PMCA4 degradation.[11] PMCA2 is enriched in basal breast cancer. Silencing PMCA2 can reduce the proliferation of breast cancer cells, thereby silencing the isoforms PMCA1 and PMCA4.[12] In addition, PMCA is also related to diabetes. Overexpression of PMCA2 can lead to endoplasmic reticulum calcium consumption, which in turn leads to internal Plasma reticulum stress causes β-cell apoptosis.[13] In recent years, some scholars have discovered that PMCA can not only transport CA^{2+}, but also Ba^{2+}, Sr, Co and other elements.[14] The current research on PMCA mostly focuses on adrenal aldosterone secretion and cardiac conduction, especially hypertrophic cardiomyopathy[15] and cardiac-specific myocardial protection.[16] Traditionally, PMCA is considered to play a major role in maintaining intracellular CA^{2+}, and Na^{+}/Ca^{2+} exchanger (NCX) plays a synergistic role.[17] Therefore, PMCA changes in the expression pattern of different isoforms produced by different tissues and cells, which is considered as an important reason for the imbalance of Ca^{2+} homeostasis in cancer cells.[18]

ADCYAP1R1, also known as PACAPR, encodes a type I adenylate cyclase polypeptide receptor, which is a membrane-associated protein that has significant homology with the glucagon/secretin receptor family. This receptor mediates multiple biological effects of AC-activated polypeptide 1, and is positively correlated with AC. In addition, this is the receptor for PACAP-27 and PACAP-38. The activity of this receptor is mediated by the G protein that activates adenosine cyclase. It can regulate the release of corticotropin, luteinizing hormone, growth hormone, prolactin, epinephrine and catecholamines. It may be related to spermatogenesis and sperm motility, which can also cause smooth muscle relaxation and gastrointestinal secretion. According to reports, PACAP is closely related to the development and protection of the nervous system, which has a strong neuroprotective effect on acute ischemic neuronal cell death. It may be an attractive treatment option for the treatment of ischemic stroke.[19] The signal transduction dysfunction of PACAP is an important pathophysiological mechanism of some mental and neurological diseases, which can cause abnormal spinal formation.[20] There are also reports that PACAP may also play an important role in sweat glands by acting on PAC1 receptors in sweat glands. The author believes that this may be a new treatment option to combat sweating disorders.[21] PACAP may also be an important cause of anxiety. In chronic variable stress, the transcription of PACAP and its receptor CAP1R increases in male rats.[22] There is also evidence that PAC receptor may be the pathophysiological target of PACAP in migraine.[23] Changes in cognitive function are also related to PACAP. According to reports, PACAP levels in the cerebrospinal fluid, superior frontal gyrus, and middle temporal gyrus are negatively correlated with the degree of dementia.[24] In addition, PACAP has been revealed to have anti-aging effects. The loss of related signals involved in PACAP gene-deficient mice will cause the disorder of cartilage matrix components, and may change the articular cartilage, making it more prone to degeneration,[25] and age-related PACAP Abnormal signal regulation of angiogenesis can also lead to impaired angiogenesis and repair functions.[26] In summary, PACAP is closely related to neurodevelopment and protection, and may be involved in mediating the release of adrenal cortex

hormones, growth hormone, prolactin, epinephrine and catecholamines, as well as sweat gland secretion, cognitive function changes, vascular endothelium and cartilage anti-aging Both are closely related.

Prostaglandin E Receptor 2 (PTGER2), also known as Prostaglandin E2 Receptor EP2 Subtype. This gene encodes the prostaglandin E2 receptor, which is a metabolite of arachidonic acid and a G protein-coupled receptor. It has different biological activities in a variety of tissues and is related to a variety of physiological and pathological events, including tumor occurrence, invasion and metastasis, cell apoptosis. The expression of PGE2 receptor EP2 is widely distributed in humans. It is expressed in the arteries and arterioles of the human small intestine, lung, kidney, thymus, uterus and cerebral cortex,[27] and it is widely present in the brain and central nervous system.[28] PGE2 is closely related to desensitization. The difference is that EP2 does not undergo homology desensitization,[29] while the EP4 receptor is rapidly desensitized after PGE2 induction.[30] In addition, PEG2 can promote the expression of COX-2 through EP2.[31] It is reported that LEF-1 and TCF-4 are up-regulated by EP2 in the nucleus, which can lead to the up-regulation of COX-2.[32] The lack of EP2 may down-regulate the expression of p-Pl3K, p-AKT, and p-GSK-3β, so inhibiting EP2 may reduce the proliferation and invasion of cancer cells.[33–35] As the main inflammatory mediator of COX-2, PEG2 can induce a variety of pro-inflammatory factors,[36] which can promote cell proliferation, survival, angiogenesis, tumor invasion and migration.[37] In addition, the activation of EP2 can significantly induce tumor cells to express crude inflammatory factors,[38] so small molecule antagonists of EP2 can be used to reduce chronic inflammation in tumor tissues.[39] In summary, the PGE2 receptor EP2 is a very important research target, which does not undergo homology desensitization, and is related to tumor aggressiveness and chronic inflammation. At the same time, it is also involved in immune response, inducing angiogenesis, and promoting tumor drug resistance.

Follicle Stimulating Hormone Subunit Beta (FSHβ) is a protein-coding gene. All pituitary glycoprotein families are composed of an identical α subunit and a hormone-specific β subunit. The expression of this hormone-specific β subunit is mainly regulated by gonadotropin-releasing hormone (GnRH) and activin, in addition to feedback regulation by various steroid hormones.[40] 1) GnRH up-regulates FSHβ through AP-1 and Nur77. According to reports in the literature, AP-1 has several binding sites in FSH. Initially, two putative AP-1 binding sites were found in the sheep FSH promoter, and they were found to mediate part of the GnRH response in non-gonadotrophic cells.[41] Later, some scholars also found an AP-1 binding site at a similar position in the promoter of Chinook salmon FSH.[42] The mouse FSHβ gene promoter is different. It contains a complex half AP-1/NFY site. The specific mechanism may be that GnRH is partially mediated by CREB phosphorylation combined with AP-1/NFY site.[43] siRNA-mediated knockout can significantly reduce the GnRH effect.[44] Coincidentally, in αT3-1 cells, Nur77 was also found at the promoter of FSHβ, and it was proved to play an important role in regulating GnRH-induced de-suppression of FSHβ gene.[45] 2) The GnRH signaling pathway of FSHβ involves multiple pathways. c-Fos is a type of AP-1 protein. In humans, mice and sheep, the GnRH-mediated ERK1/2 pathway is the main pathway, and P38-MAPK is the secondary pathway, which upregulates c-Fos to regulate FSHβ.[46] In addition, GnRH phosphorylates MEF2D through ERK5 to activate the Nur77 promoter activity, and

phosphorylated Nur77 can transactivate various target genes, ultimately increasing the promoter activity and mRNA level of mouse FSH.[47] 3) Regulated by activin. Activin is a member of the TGF superfamily, which activates the transcription of FSHβ gene in rodents through the SMAD protein pathway, and SMAD interacts with various transcription factors and steroid receptors including Pitx protein.[48] 4) Activin and GnRH can also work together to regulate FSHβ.

5-Hydroxytryptamine Receptor 4 (HTR4) is a member of the serotonin receptor family and a G protein-coupled receptor that stimulates cAMP production in response to serotonin. The gene product is a glycosylated transmembrane protein, which plays a role in the release of various neurotransmitters in the peripheral and central nervous systems. The receptors are mainly located in the substantia nigra striatum, midbrain limbic system and smooth muscle.[49] Current research on HTR4 focuses on heart activity and lung function. It has been reported that HTR4 is localized in cardiac mitochondria and regulates mitochondrial activity and cell function.[50] In addition, HTR4 also plays an important role in the pathogenesis of airflow obstruction in COPD patients.[51]

Numerous studies have proved that these five genes were related to various types of cancer's invasion and promotion. However, very few studies have been reported about these five genes in invasive NFPAs after we searched in PubMed website. Therefore, the data in our study could provide useful information for our future study in invasive NFPAs.

Conclusions

In summary, our bioinformatics analysis study identified five DEGs (ATP2B3, ADCYAP1R1, PTGER2, FSHβ, HTR4) between non-invasive NFPA tissues and invasive NFPA tissues on the base of GSE51618 microarray datasets. Results showed that these five genes could play important roles in the future. Anyway, these data can provide many useful information into the potential bio-markers and biological mechanisms of invasive NFPAs.

Declarations

Ethics approval and consent to participate

In our manuscript, we used database GSE51618 to analyse DEGs. There are no human participants, human organizations or animal studies involved in our study, so ethical approval is not required.

Consent for publication

There are no individual person's data in any form involved in our study.

Availability of data and material

All the original data are from the GSE database of NCBI website. We use the online tool GEO2R to get the differential genes. The data were imported into DAVID website for gene enrichment analysis. In addition, we also used STRING website for PPI network analysis to visualize the protein connection. All the data can be found and shared.

Competing interests

The authors declare that they have no competing interests.

Funding

This project was completed with the help of funds:

1. National Natural Science Foundation of China (No. 81502141)
2. Postdoctoral Foundation of Anhui (2019B322)

Authors' contributions

Wang AS processed and analyzed the dataset (GSE51618), and used Cytoscape software to generate visual images. Xu H and Zeng MH helped process all the images and tables. Professor Wang F guided the thinking of this article and helped review the manuscript and prepare to collect patient specimens for the next experiment.

Acknowledgements

We are very grateful to the GeenMedical website for its help in finding high-quality literature.

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Tables

Due to technical limitations, table 1, 2, 3, 4 is only available as a download in the Supplemental Files section.

Figures

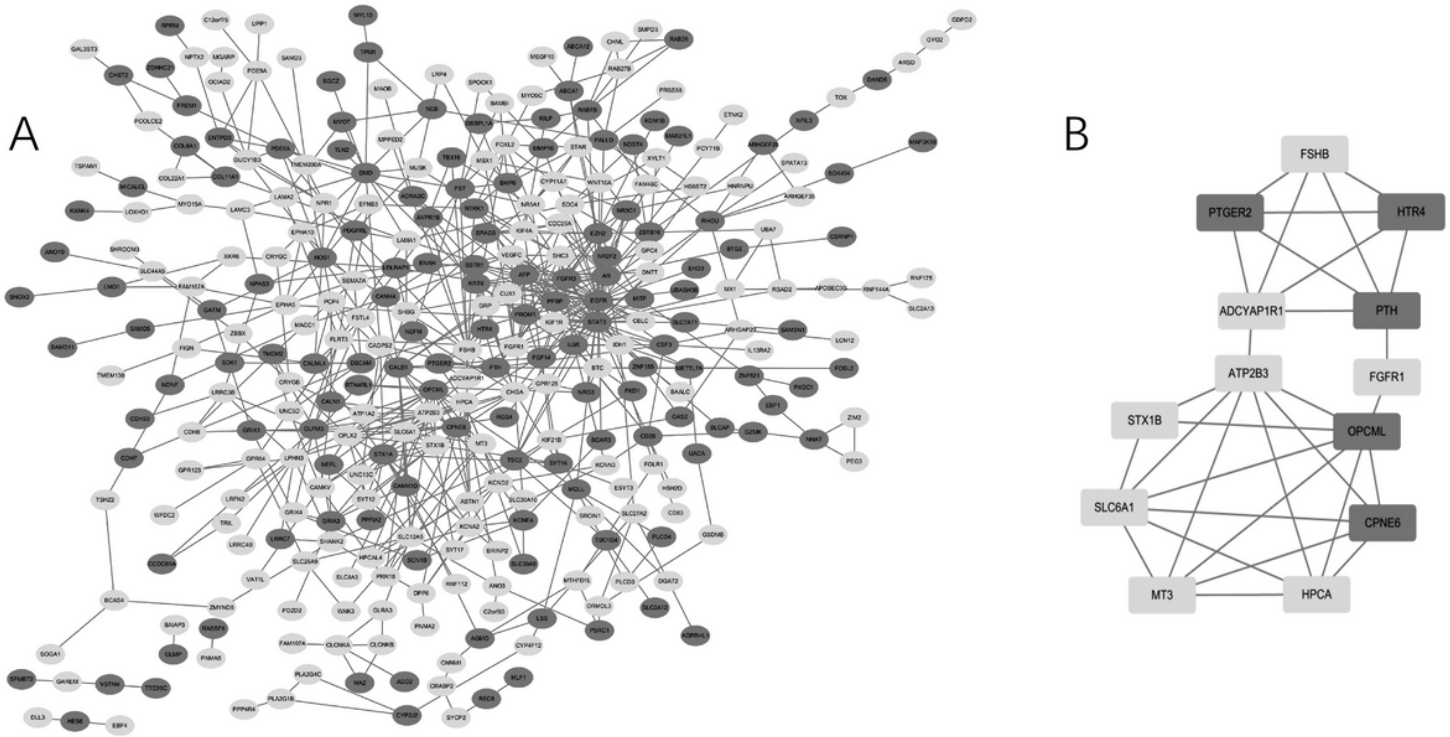

Figure 1

Common DEGs PPI network constructed by STRING online database and Module analysis. A There are a total of 524 DEGs in the DEGs' project. The nodes mean proteins and the edges mean the interaction of proteins (light colored circles mean down-regulated DEGs and dark colored circles mean up-regulated DEGs). B Module analysis via Cytoscape software (degree cutoff = 2, node score cutoff = 0.2, k-core = 2, and max. Depth = 100)

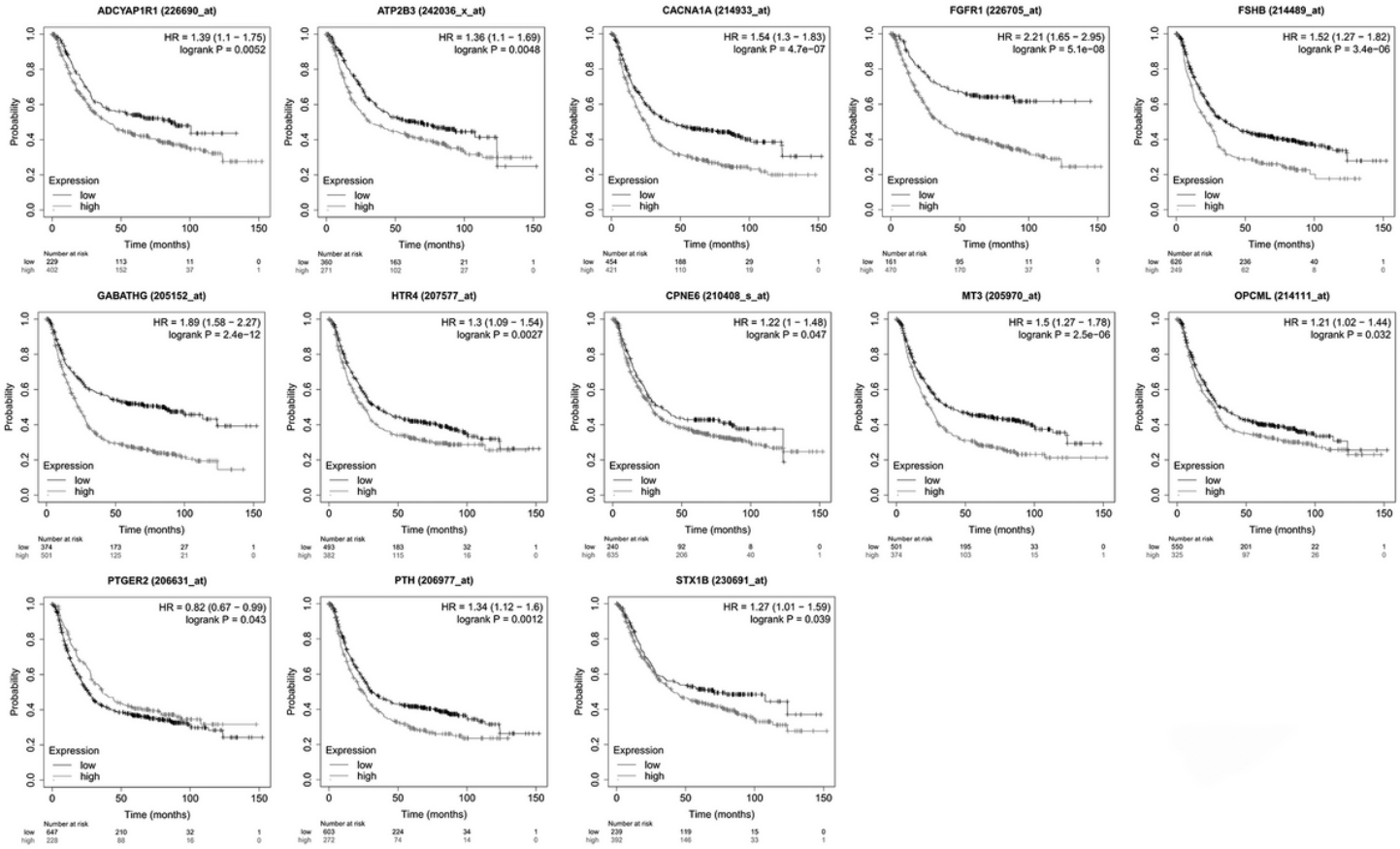

Figure 2

The prognostic information of the 13 core genes. Kaplan meier plotter online tools were used to identify the prognostic information and all of them have a significantly worse survival rate ($P < 0.05$)

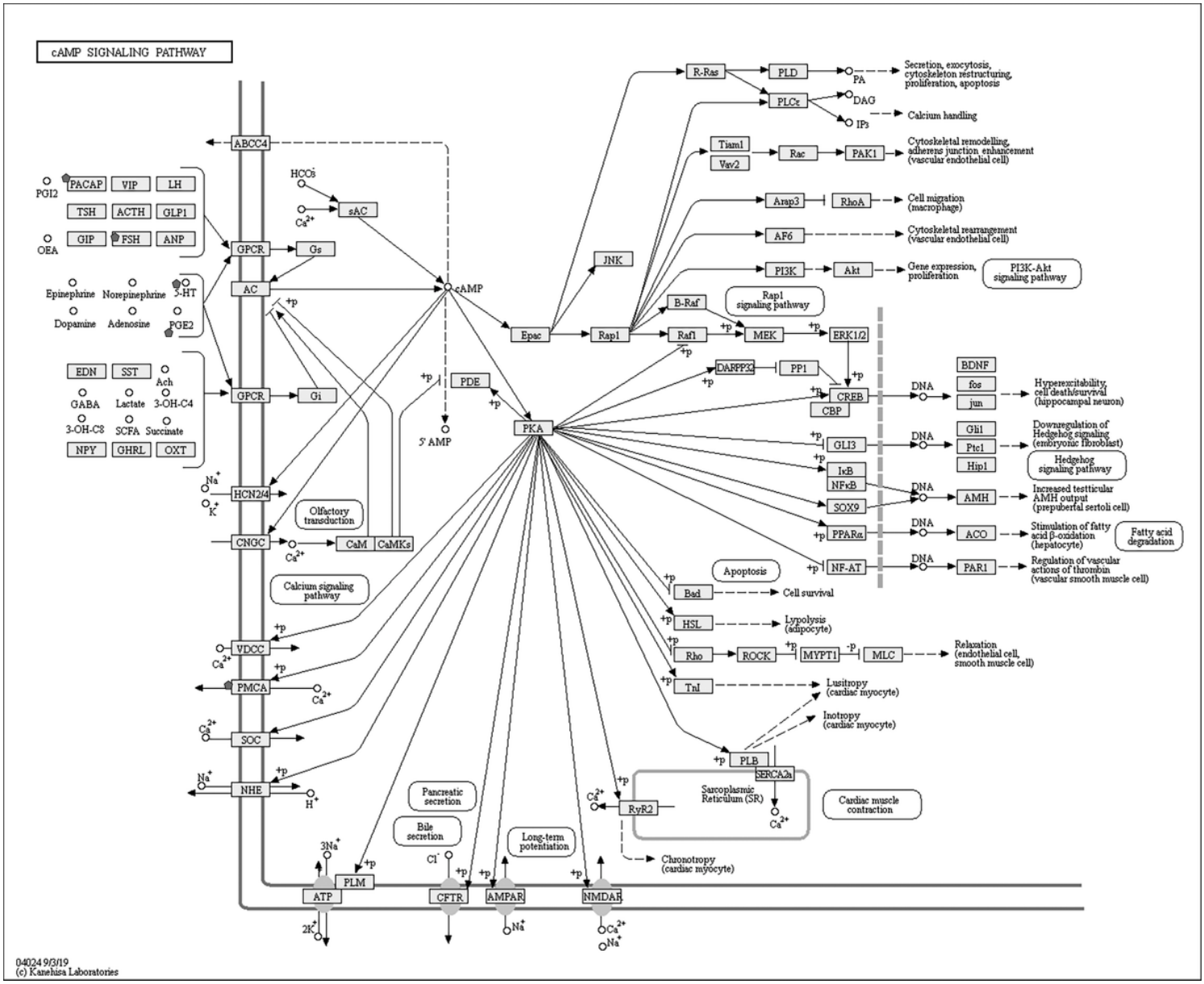

Figure 3

Re-analysis of 13 selected genes by KEGG pathway enrichment. Five genes (ATP2B3, PTGER2, ADCYAP1R1, HTR4, FSHB) were significantly enriched in the cAMP signaling pathway. PACAP means ADCYAP1R1. PMCA means ATP2B3.

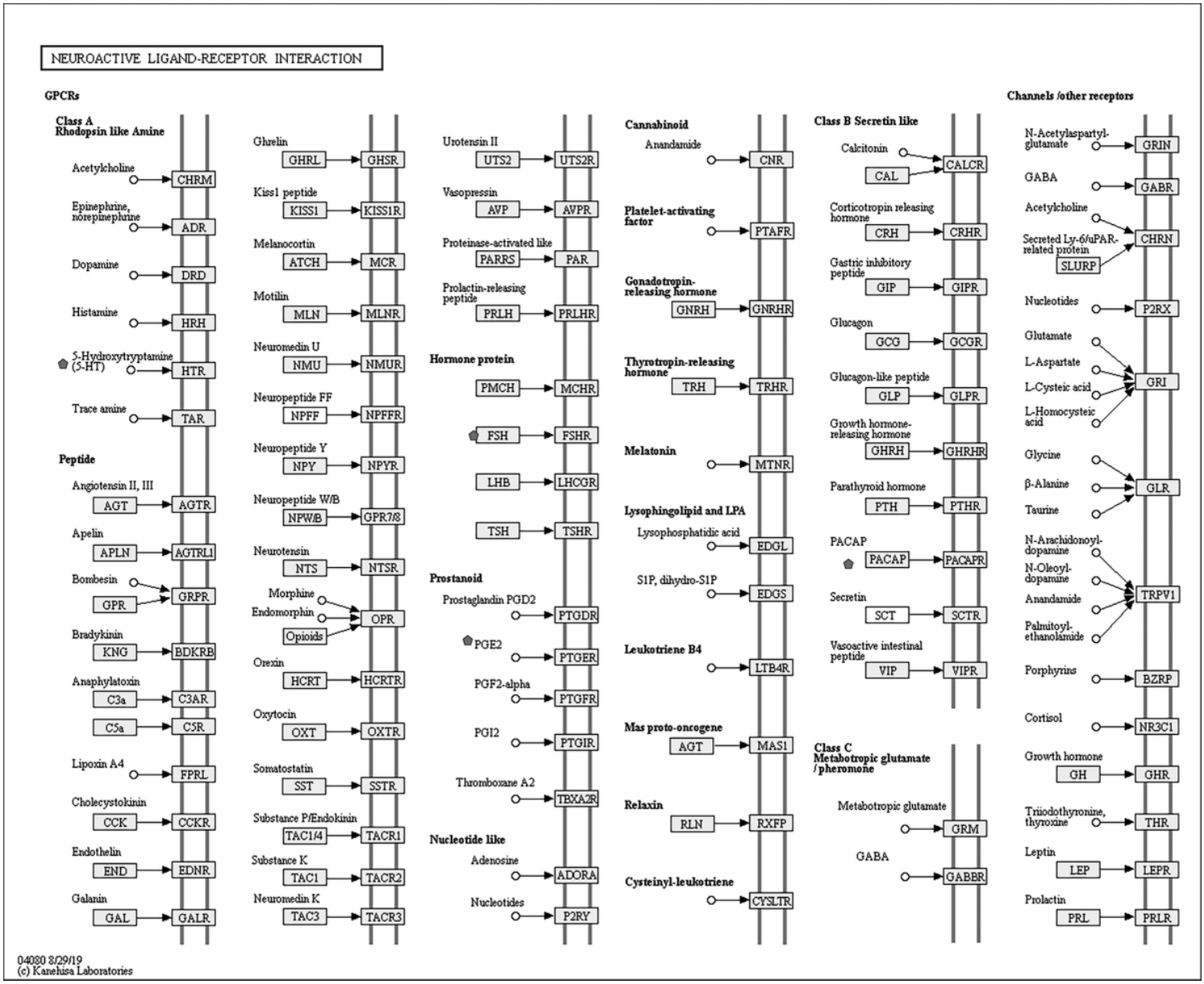

Figure 4

Re-analysis of 13 selected genes by KEGG pathway enrichment. Four genes (PTGER2, ADCYAP1R1, HTR4, FSHB) were significantly enriched in the Neuroactive ligand-receptor interaction.

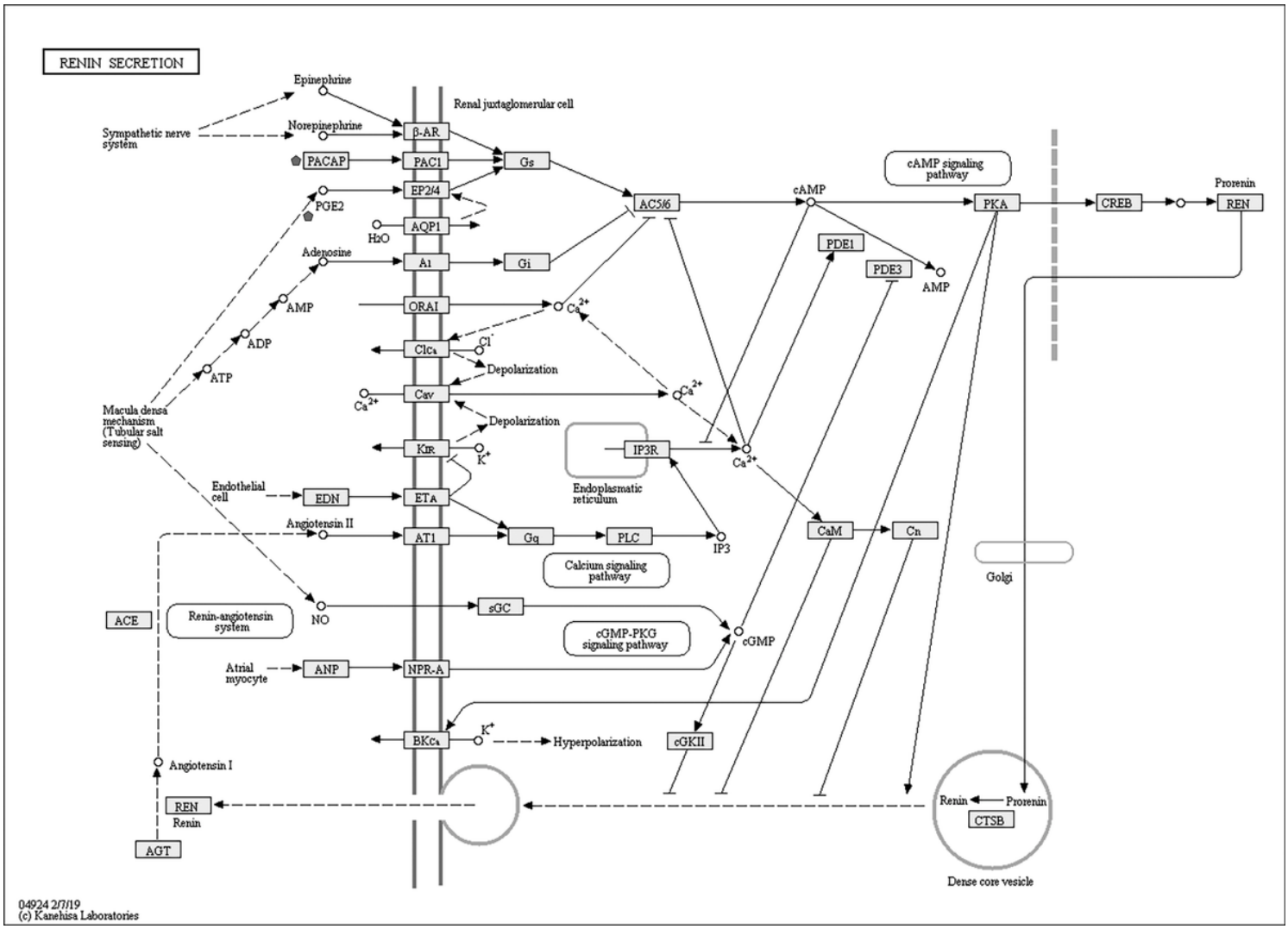

Figure 5

Re-analysis of 13 selected genes by KEGG pathway enrichment. Two genes (PTGER2, ADCYAP1R1) were significantly enriched in the Renin secretion.

Supplementary Files

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

- Table1.xlsx
- Table2.xlsx
- Table3.xlsx
- Table4.xlsx