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Hub genes for early diagnosis and therapy of adamantinomatous craniopharyngioma

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Abstract

Background: Adamantinomatous craniopharyngioma (ACP) is a subtype of craniopharyngioma, a neoplastic disease with a benign pathological phenotype but a poor prognosis in the sellar region. The disease has been considered the most common congenital tumor in the skull. Therefore, this article aims to identify hub genes that might serve as genetic markers of diagnosis, treatment, and prognosis of ACP.

Methods: The procedure of this research includes the acquisition of public data, identification and functional annotation of differentially expressed genes (DEGs), construction and analysis of protein-protein interaction network, and the mining and analysis of hub genes by Spearman-rho test, multivariable linear regression, and receiver operator characteristic curve analysis. Quantitative real-time polymerase chain reaction was used to detect the level of mRNA of relative genes.

Results: Among 2 datasets, a total of 703 DEGs were identified, mainly enriched in chemical synaptic transmission, cell adhesion, odontogenesis of the dentin-containing tooth, cell junction, extracellular region, extracellular space, structural molecule activity, and structural constituent of cytoskeleton. The protein-protein interaction network was composed of 4379 edges and 589 nodes. Its significant module had 10 hub genes, and SYN1, SYP, and GRIA2 were significantly down-regulated with ACP.

Conclusion: In a word, we find out the DEGs between ACP patients and standard samples, which are likely to play an essential role in the development of ACP. At the same time, these DEGs are of great value in tumors' diagnosis and targeted therapy and could even be mined as biological molecular targets for diagnosing and treating ACP patients.

Abbreviations: ACP = adamantinomatous craniopharyngioma, DEGs = differentially expressed genes, GEO = Gene Expression Omnibus, GO = gene ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, MCODE = molecular complex detection, MNS = motor neurons, ROC = receiver operator characteristic, RT-qPCR = quantitative real-time polymerase chain reaction.

Keywords: adamantinomatous craniopharyngioma, biological information technology, biomarker, differentially expressed genes

1. Introduction

Craniopharyngioma is a neoplastic disease with a benign pathological phenotype but a poor prognosis in the sellar region.[1] The disease has been considered the most common congenital tumor in the skull. The prevalence of the disease is higher in Africa and the far east than in other regions except for Japan, and the tumor presents a bimodal distribution in age, namely 2 peaks in children and adults aged 40 to 50 years.[1] According to the central nervous system classification of craniopharyngioma

published by WHO in 2016, craniopharyngioma is classified into adamantinomatous craniopharyngioma (ACP) and papilloma craniopharyngioma.[2] The former is mainly found in children, while the latter is almost only seen in adults. Because craniopharyngioma locates deep and adjacent to the optic nerve, hypothalamus, basilar artery ring, and other essential structures, surgical treatment is complex, the mortality rate is high, and it is easy to relapse.

In recent years, bioinformatics technology has been widely used to explore the potential genetic targets of diseases and

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help us find the differentially expressed genes and possible pathways related to the occurrence and development of diseases.[3] Differentially expressed genes have been found and verified in many diseases and are potential targets for disease prediction and treatment. Bredemeier used the biological information method to identify the critical genes in the occurrence and development of breast cancer, and the results showed that KRT19, EPCAM, CDH1, and SCGB2A2 had significant expression differences.[4] Therefore, it is suggested that this gene should be regarded as a therapeutic target. Meanwhile, Feng et al[5] identified 2 lncRNAs, LOC146880 and ENST00000439577, which may promote the development and progression of lung cancer by analyzing gene expression and methylation microarray data. Therefore, bioinformatics technology has unique advantages in mining the differentially expressed genes between patients with the disease and regular patients and searching for the targeted genes related to the occurrence and development of disease.

Based on bioinformatics technology, this study combined spearman correlation analysis and multiple linear regression analysis to screen out the central genes significantly related to ACP. The results of this study may provide important targets for the clinical diagnosis and treatment of ACP and contribute to the clinical treatment and decision-making of ACP. It provides better help for the prognosis and survival of patients with ACP and more possibilities for future treatment of ACP.

2. Methods

2.1. Access to public data

Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo) is an open functional genomics database of high-throughput resources, including microarrays, gene expression data, and chips. Two expression profiling datasets (GSE94349 and GSE68015) were obtained from GEO. The probes would be transformed into the homologous gene symbol using the platform's annotation information. The GSE94349 dataset contained 24 ACP and 27 standard samples, and GSE68015 contained 15 ACP and 16 normal samples.

2.2. DEGs identified by limma package

The limma package screened the differentially expressed genes (DEGs) between ACP and normal samples. After setting up the differential experimental groups for 1 GEO series, the limma package could execute a command to compare the differential classifications to identify the DEGs. According to the method of Benjamini and Hochberg (false discovery rate), the tool could apply adjustment to the P values to obtain the adjusted P values (adj. P) and maintain 1 balance between the possibility of false positives and detection of statistically significant genes. If 1 probe set does not have the homologous gene, or if 1 gene has numerous probe sets, the data will be removed. The rule of statistical significance is that adj. P value ≤ .01 and log (Fold change, FC) ≥ 4 or ≤ –4. The Venn diagram was delineated by FunRich software.

2.3. Functional annotation of DEGs by GO and KEGG analyses

DAVID (https://david.ncifcrf.gov/home.jsp) (version 6.8), 1 online analysis tool suite with the function of Integrated Discovery and Annotation, mainly provides typical batch annotation and gene-gene ontology (GO) term enrichment analysis to highlight the most relevant GO terms associated with a given gene list. Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.kegg.jp/), 1 of the world's most commonly used biological information databases, aims to understand advanced functions and biological systems. From the molecular level, KEGG integrates many practical program database resources from high-throughput experimental technologies. GO is an ontology widely used in bioinformatics, which covers 3 aspects of biology, including cellular component, molecular function, and biological process. The DAVID online tool was implemented to perform GO and KEGG analyses of DEGs. The rule of statistical significance is that $P < .05$.

2.4. Construction and analysis of PPI network

After importing the common DEGs to the Search Tool for the Retrieval of Interacting Genes (http://string-db.org) (version 10.5), the online tool could predict and trace the protein-protein interaction (PPI) network. The analysis of interactions between various proteins might put forward some novel ideas into the pathophysiological mechanisms of the development of ACP. In this research, the Search Tool for the Retrieval of Interacting Genes database was used to construct the PPI network of DEGs, and the minimum required interaction score is medium confidence > 0.4.

2.5. The analysis and mining of hub genes

Based on the topology principles, the Molecular Complex Detection (MCODE) (version 1.5.1), a plug-in of Cytoscape, could discover the tightly coupled region. MCODE identified the most important module of the PPI network map. The criteria of MCODE analysis require that degree cut-off = 2, MCODE scores > 5, Max depth = 100, k-score = 2, and node score cut-off = 0.2. The hub genes were excavated when the degrees were set (degrees ≥ 10). Then, DAVID online tool was used to analyze the GO and KEGG pathway analyses for the hub genes. The clustering analysis of hub genes was performed using OmicShare (http://www.omicshare.com/tools), an available data analysis platform.

The Spearman-rho test was used for correlation analysis between ACP and relevant gene expression. Any test results reaching a liberal statistical threshold of $P < .2$ for each comparison were then entered into a multivariable linear regression model to identify independent predictive genes of ACP. Finally, we performed receiver operator characteristic (ROC) curve analysis to determine the ability of the hub genes to predict ACP. All statistical analyses were conducted using SPSS software (version 21.0; IBM, Armonk, NY). A P value of <0.05 was considered statistically significant.

2.6. Quantitative real-time polymerase chain reaction (RT-qPCR) assay

A total of 14 participants were recruited, including 7 control individuals and 7 ACP patients. After surgery, 7 ACP tumor samples from ACP patients and 7 control brain samples from control individuals were obtained. The research conformed to the Declaration of Helsinki and was authorized by the Human Ethics and Research Ethics Committees of the Zhejiang Cancer Hospital. Informed consent was obtained from all participants.

Total RNA was extracted from 7 ACP tumors and 7 control brain samples by the RNAiso Plus (Trizol) kit (Thermofisher, Massachusetts, United States, MA) and reverse transcribed to cDNA. RT-qPCR was performed using a Light Cycler® 4800 System (Roche Diagnostic Products Co., Basel, Switzerland) with specific GRIA2, SYN1, and SYP primers. Table 1 presents the primer sequences used in the experiments. The RQ values $(2–\Delta\Delta Ct$, where Ct is the threshold cycle) of each sample were calculated and are presented as fold changes in gene expression relative to the control group. GAPDH was used as an endogenous control.

3. Results

3.1. DEGs identified between standard and ACP samples

After analysis of the datasets (GSE94349 and GSE68015) with the limma package, the difference between ACP and standard samples could be presented in the volcano plots (Fig. 1A and B). Then these results were standardized, and DEGs (944 in GSE94349 and 764 in GSE68015) were distinguished. The Venn diagram could show that 703 genes were simultaneously contained in the 2 datasets (Fig. 1C).

3.2. Functional annotation of DEGs by GO and KEGG analyses

The results of GO analysis presented that variations in biological processes, cell component, and molecular function of DEGs were mainly enriched in chemical synaptic transmission, cell adhesion, epidermis development, extracellular matrix organization, odontogenesis of the dentin-containing tooth, cell junction, extracellular region, plasma membrane, extracellular space, axon, structural molecule activity, calcium ion binding, Gamma-aminobutyric acid-A (GABA-A) receptor activity, and structural constituent of the cytoskeleton (Table 2). Analysis of the KEGG pathway displayed that all DEGs were primarily enriched in retrograde endocannabinoid signaling, nicotine addiction, extracellular matrix-receptor interaction, morphine addiction, and GABAergic synapse (Table 2).

3.3. PPI and module networks construction and hub gene selection

The PPI network of DEGs was constructed (Fig. 2), and the most powerful module was obtained using Cytoscape (Fig. 3). A total of 10 genes (SNAP25, GRIA2, KCNJ9, SYN1, SLC32A1, SNCB, GRM5, GABRG2, SYP, and CDH1) were identified as hub genes with degrees ≥ 10 (Fig. 4A).

3.4. Hub gene analysis

The functional analyses of hub genes were analyzed using DAVID. Results showed that hub genes were mainly enriched in chemical synaptic transmission, neurotransmitter secretion, regulation of long-term neuronal synaptic plasticity, locomotory behavior, cell junction, presynaptic active zone, neuron projection, synaptic vesicle, calcium-dependent protein binding, retrograde endocannabinoid signaling, nicotine addiction, morphine addiction, and neuroactive ligand-receptor interaction (Table 3). The names, abbreviations, and functions for these hub genes are shown in Table 4. Hierarchical clustering showed that the hub genes could differentiate the ACP samples from normal ones (Fig. 4B and C). These hub genes showed the highest node score in the PPI network, suggesting that they might play essential roles in the occurrence or progression of ACP.

## 3.5. Correlation between ACP and hub genes expression

To ensure that the hub genes impacted ACP, we performed a further analysis of ACP and hub gene expression. Spearman correlation coefficient was used in the correlation analysis, and SNAP25 ($\rho = -0.702$, $P < .001$), GRIA2 ($\rho = -0.673$, $P < .001$), KCNJ9 ($\rho = -0.706$, $P < .001$), SYN1 ($\rho = -0.747$, $P < .001$), SLC32A1 ($\rho = -0.813$, $P < .001$), SNCB ($\rho = -0.848$, $P < .001$), GRM5 ($\rho = -0.680$, $P < .001$), GABRG2 ($\rho = -0.830$, $P < .001$), SYP ($\rho = -0.852$, $P < .001$), and CDH1 ($\rho = -0.865$, $P < .001$) were significantly correlated with ACP (Table 5). In the multivariate linear regression model, holding all other variables at any fixed value, the natural logarithmic ACP remained associated with GRIA2 ($\beta = 0.044$, $P = .041$), SYN1 ($\beta = 0.083$, $P = .017$), SYP ($\beta = -0.137$, $P < .001$), and CDH1 ($\beta = 0.154$, $P < .001$) (Table 5).

3.6. The hub genes could predict ACP sensitively and significantly by the ROC curve

We constructed receiver operator characteristic curves to identify accurate thresholds for hub genes predicting ACP. SYP was mostly associated with higher risk of ACP (area under the curve for ACP, 0.992; 95% confidence interval, 0.980–1.000; $P < .001$). The optimal diagnostic threshold of SYP for ACP was 5.672. (Table 6; Fig. 5A–K).

Table 1

Primers and their sequences for polymerase chain reaction analysis.

Primer	Sequence (5′–3′)
GRIA2-hF	CACCCCACATCGACAATTTGG
GRIA2-hR	GACGTGGAGTGTTCCGCAA
SYN1-hF	AGTTCTTCGGAATGGGGTGAA
SYN1-hR	CAAACTGCGGTAGTCTCCGTT
SYP-hF	CTCGGCTTTGTGAAGGTGCT
SYP-hR	CTGAGGTCACTCTCGGTCTTG
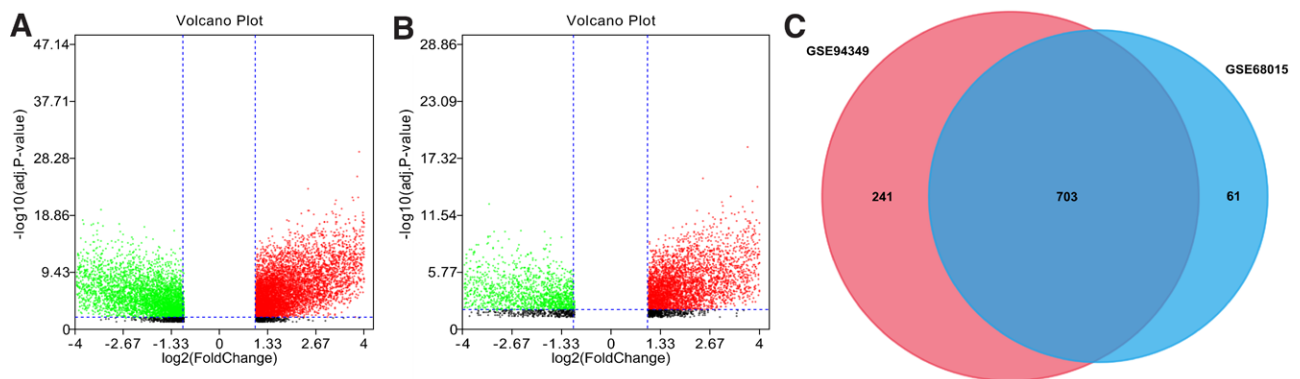
Figure 1. The identification of DEGs by limma package and Venn diagram. (A) The volcano plot presents the difference between ACP and normal samples after analysis of the datasets GSE94349 with limma package. (B) The volcano plot presents the difference between non-MM lung cancer and MM lung cancer tissues after analysis of the datasets GSE68015 with limma package. (C) The Venn diagram could show that 703 genes were contained in the GSE94349 and GSE68015 datasets simultaneously. ACP = adamantinomatous craniopharyngioma, DEGs = differentially expressed genes, MM = multiple myeloma.

GO and KEGG pathway enrichment analysis of DEGs in ACP samples.

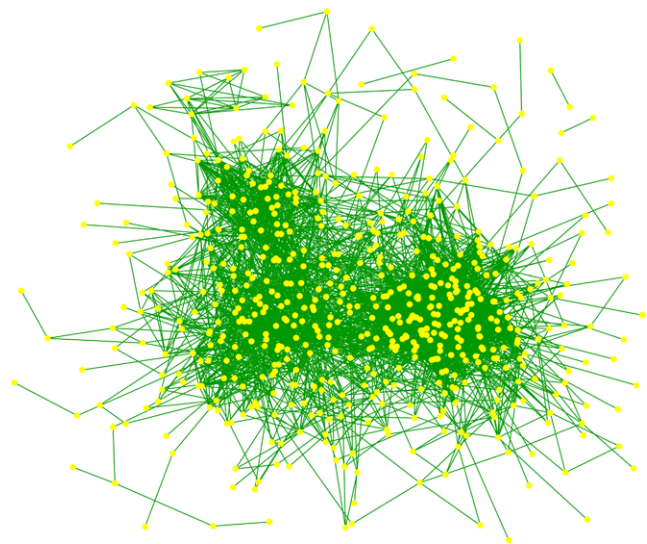| Term	Description	Count in gene set	FDR
GO:0007268	Chemical synaptic transmission	42	8.95E-15
GO:0007155	Cell adhesion	50	1.40E-09
GO:0008544	Epidermis development	20	7.22E-08
GO:0030198	Extracellular matrix organization	28	8.67E-07
GO:0042475	Odontogenesis of dentin-containing tooth	15	4.37E-06
GO:0030054	Cell junction	65	3.18E-19
GO:0005576	Extracellular region	121	4.93E-14
GO:0005886	Plasma membrane	222	3.54E-11
GO:0005615	Extracellular space	100	1.72E-10
GO:0030424	Axon	32	2.97E-08
GO:0005198	Structural molecule activity	35	2.77E-09
GO:0005509	Calcium ion binding	62	1.91E-08
GO:0004890	GABA-A receptor activity	8	0.002
GO:0005200	Structural constituent of cytoskeleton	16	0.005
hsa04723	Retrograde endocannabinoid signaling	23	6.94E-10
hsa05033	Nicotine addiction	15	1.23E-08
hsa04512	ECM-receptor interaction	17	1.58E-05
hsa05032	Morphine addiction	16	2.10E-04
hsa04727	GABAergic synapse	15	5.55E-04

ACP = adamantinomatous craniopharyngioma, DEGs = differentially expressed genes, ECM = extracellular matrix, FDR = false discovery rate, GO = gene ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes.

Figure 2. The PPI network presents the intricate relationships between DEGs. DEGs = differentially expressed genes, PPI = protein-protein interaction.

3.7. Results of RT-qPCR analysis

According to the analysis above, GRIA2, SYN1, and SYP were markedly down-regulated in ACP tumor samples. As presented in Figure 6, the relative expression levels of GRIA2, SYN1, and SYP were significantly lower in ACP samples compared with the control groups. The result demonstrated that GRIA2, SYN1, and SYP might be considered biomarkers for ACP.

4. Discussion

ACP is a congenital histologically benign but aggressive and invasive epithelial tumor of the saddle area. Current ACP treatment is still dominated by surgical resection. However, due to the complicated and essential anatomical relationships around tumor and tumor itself factors, the total surgical resection rate is low, operative mortality and incidence of severe complications and the postoperative recurrence rate are high,[6] and the complete resection rate reported in the literature was only 18% to 84%, the postoperative mortality was as high as 1.7% to 5.4%, and the 10-year recurrence rate of patients with total tumor resection was 0% to 62%. Long-term hypophysis and hypothalamus dysfunction are also complex problems in neurosurgery

for many years. Molecular biological targeted therapy for craniopharyngioma with BRAF mutation has become a research hotspot.[7] In 2016, gene blockers were used for the clinical treatment of patients with craniopharyngioma, and preliminary observation found that the tumor volume after treatment was significantly reduced.[8] Currently, phase II clinical trials are underway, and it still takes some time to use targeted molecular therapy widely. And there is a lack of molecular biological markers to predict the efficacy and prognosis of ACP patients.

Biological information technology can screen out the differentially expressed genes between tumor patients and normal individuals through the mining and utilization of gene databases and then use them as potential genetic, molecular markers for tumor diagnosis and prognosis.[9] At present, this technique has been used to screen differentially expressed genes in a variety of tumors and has broad application value.

We use bioinformatics technology to screen 2 gene expression data sets, the ACP and normal individuals screen out 703 differentially expressed genes, further analysis found the ten most significant hub genes, and through the GO, KEGG analysis to find out the function and pathway of this differential gene enrichment, to further explore the possible mechanism of these differentially expressed genes on disease. In spearman correlation analysis and multiple linear regression analysis, CDH1 was closely related to ACP, indicating that it might have significant statistical significance in the occurrence and development of ACP. However, in ROC analysis, the AUC expression was zero, indicating that the results had low authenticity and no application value. Therefore, it has no diagnostic value for the direction of this study. Finally, Spearman correlation, multiple linear regression, and ROC curve analysis were used to screen and identify the 3 hub genes (SYN1, SYP, and GRIA2) most valuable for ACP diagnosis. SYN1, SYP, and GRIA2 had significant statistical significance in the occurrence and development of ACP.

The SYN1 gene encodes a neuronal phosphoprotein that covers synaptic vesicles and binds to the cytoskeleton, which is thought to play an essential role in regulating the release of neurotransmitters.[10] Some scholars have found that SYN1 gene mutation causes changes in neuron development and nerve ending function and causes some diseases related to synaptic dysfunction, such as autism and epilepsy.[11] At the same time, it has been found that low expression of SYN1 may maintain malignant tumor proliferation and promote the occurrence and development of glioma.[12] Similar to these findings, we also found that this gene is highly expressed in normal individuals and low

Figure 3. The significant module network was identified from the PPI network. PPI = protein-protein interaction.

Figure 4. The mining and analysis of hub genes. (A) There 10 genes (SNAP25, GRIA2, KCNJ9, SYN1, SLC32A1, SNCB, GRM5, GABRG2, SYP, and CDH1) were identified as hub genes with degrees ≥10. (B) Hierarchical clustering showed that the hub genes could differentiate the ACP samples from the normal ones in the datasets GSE94349. (C) Hierarchical clustering showed that the hub genes could differentiate the ACP samples from the normal ones in the datasets GSE68015. Upregulation of genes is marked in red; downregulation is observed in green. ACP = adamantinomatous craniopharyngioma.

Table 3

GO and KEGG pathway enrichment analysis of hub genes.

Term	Description	Count in gene set	P value	FDR
GO:0007268	Chemical synaptic transmission	5	4.85E-06	0.005
GO:0007269	Neurotransmitter secretion	3	3.21E-04	0.333
GO:0048169	Regulation of long-term neuronal synaptic plasticity	2	.010	9.535
GO:0007626	Locomotory behavior	2	.044	37.410
GO:0030054	Cell junction	6	1.15E-06	0.001
GO:0048786	Presynaptic active zone	3	8.74E-05	0.088
GO:0043005	Neuron projection	4	1.72E-04	0.172
GO:0008021	Synaptic vesicle	3	8.87E-04	0.885
GO:0048306	Calcium-dependent protein binding	2	.031	24.422
hsa04723	Retrograde endocannabinoid signaling	5	6.42E-07	5.61E-04
hsa05033	Nicotine addiction	3	4.87E-04	0.425
hsa05032	Morphine addiction	3	.003	2.168
hsa04080	Neuroactive ligand-receptor interaction	3	.022	17.477

FDR = false discovery rate, GO = gene ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes.

Table 4

Summaries for the function of 10 hub genes.

No.	Gene symbol	Full name	Function
1	SNAP25	Synaptosome Associated Protein 25	Regulating neurotransmitter release. May play an important role in the synaptic function of specific neuronal systems
2	GRIA2	Glutamate Ionotropic Receptor AMPA Type Subunit 2	Receptor for glutamate that functions as ligand-gated ion channel in the central nervous system and plays an important role in excitatory synaptic transmission
3	KCNJ9	Potassium Voltage-Gated Channel Subfamily J Member 9	The encoded protein, which has a greater tendency to allow potassium to flow into a cell rather than out of a cell, is controlled by G-proteins
4	SYN1	Synapsin I	Neuronal phosphoprotein that coats synaptic vesicles, binds to the cytoskeleton, and is believed to function in the regulation of neurotransmitter release
5	SLC32A1	Solute Carrier Family 32 Member 1	Involved in the uptake of gamma-aminobutyric acid (GABA) and glycine into the synaptic vesicles
6	SNCB	Synuclein Beta	This gene encodes a member of a small family of proteins that inhibit phospholipase D2 and may function in neuronal plasticity
7	GRM5	Glutamate Metabotropic Receptor 5	Plays an important role in the regulation of synaptic plasticity and the modulation of the neural network activity
8	GABRG2	GABA Type A Receptor Gamma2 Subunit	Component of the heteropentameric receptor for GABA, the major inhibitory neurotransmitter in the vertebrate brain. Functions also as histamine receptor and mediates cellular responses to histamine
9	SYP	Synaptophysin	Possibly involved in structural functions as organizing other membrane components or in targeting the vesicles to the plasma membrane. Involved in the regulation of short-term and long-term synaptic plasticity
10	CDH1	Cadherin 1	Cadherins are calcium-dependent cell adhesion proteins They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types

Table 5

The correlation and linear regression analysis between ACP and relevant gene expression.

	ACP			
	Spearman correlation coefficient		Multiple linear regression	
Gene symbol	ρ†	P value	β‡	P value
SNAP25	−0.702	<.001*	−0.040	.138
GRIA2	−0.673	<.001*	0.044	.041*
KCNJ9	−0.706	<.001*	0.021	.386
SYN1	−0.747	<.001*	0.083	.017*
SLC32A1	−0.813	<.001*	−0.009	.692
SNCB	−0.848	<.001	0.054	.108
GRM5	−0.680	<.001*	−0.005	.823
GABRG2	−0.830	<.001*	0.036	.326
SYP	−0.852	<.001*	−0.137	<.001*
CDH1	0.865	<.001*	0.154	<.001*

ACP = adamantinomatous craniopharyngioma, β = parameter estimate, ρ = Spearman correlation coefficient.
*Significant variables: $P < .05$.
†Spearman rank correlation coefficient between ACP and relevant characteristics.
‡Multiple linear regression analysis.

Table 6

Receiver operator characteristic curve analysis of hub gene expression for ACP.

Gene symbol	ACP			
	AUC	*P* value	95% CI	ODT
SNAP25	0.906	<.001*	0.831–0.980	11.476
GRIA2	0.889	<.001*	0.810–0.968	10.564
KCNJ9	0.908	<.001*	0.841–0.975	8.828
SYN1	0.932	<.001*	0.868–0.996	6.027
SLC32A1	0.970	<.001*	0.940–1.000	2.090
SNCB	0.990	<.001	0.976–1.000	2.920
GRM5	0.893	<.001*	0.828–0.959	7.121
GABRG2	0.980	<.001*	0.958–1.000	9.471
SYP	0.992^{max}	<.001*	0.980–1.000	5.672
CDH1	0.000	<.001*	0.000–0.000	1.400

ACP = adamantinomatous craniopharyngioma, AUC = area under curve, CI = confidence interval, max = the maximum of AUC, ODT = optimal diagnostic threshold.
*Significant variables.

in ACP patients. Since tumor progression and poor prognosis are often associated with the synaptic function of tumor cells,[13] we speculated that mutations in this gene were involved in the development and progression of ACP by causing changes in synaptic plasticity. These data suggest that the SYN1 gene and its target proteins can serve as potential, genetic, and molecular targets for ACP prevention and treatment.

Binding to small synaptic vesicles found in the nerve terminals, SYN1 possibly has an exocytotic regulatory role in linking the vesicles to the cytoskeleton and each other.[14–16] Furthermore, SYN1 is likely involved in neuronal development and the formation of synaptic contacts between neurons.[17–19] The mutations create changes in the SYN1 protein, potentially causing defects in synaptic vesicle traffic and nerve terminal function. Following its native position, SYN1 is found to be brain- and neuron-specifically expressed mediated by the promoter region of the SYN1 gene.[20] The SYN1 protein serves as a substrate for several different protein kinases, and phosphorylation is likely functioning in regulating this protein in the nerve terminal.

SYP coding protein is involved in forming intracellular vesicles and other membrane components, and it can regulate the short-term and long-term plasticity of synapses.[21] Since the interruption of synaptic plasticity is the basis of learning and memory, some scholars believe that SYP is involved in the occurrence and development of Alzheimer disease.[22] At the same time, studies have shown that the synapse complex protein syp-1 formed by its encoding protein affects the formation of some critical protein domains through phosphorylation, which further affects cell mitosis and plays an essential role in maintaining the normal cell cycle.[23] SYP is a specific marker protein of synaptic vesicles, and its density and distribution indirectly reflect the number and distribution of synaptic vesicles.[24] Other scholars have found that syp-5 can inhibit PI3K/AKT- and MAPK/erk-dependent hif-1 pathways and inhibit the migration and invasion of tumor cells and tumor angiogenesis.[25] Through bioinformatics technology, we found that ACP patients had low SYP expression and then speculated that the abnormal expression of synaptic vesicles and other synaptic signals might be involved in the development of ACP and suggested its potential significance as the target gene for the diagnosis and treatment of ACP. However, the signaling pathway affected by synaptic vesicles needs to be further studied.

SYP is a transmembrane glycoprotein found in small presynaptic vesicles of the nerve cells and microvesicles of the neuroendocrine cells[26] and is a major integral membrane protein of secretory vesicles. SYP, the most commonly expressed neural marker, exists widely in various primary central nervous system neoplasms lesions. The higher the tumor's degree of dedifferentiation, the higher the malignant degree. Therefore, its expression

level may be closely related to the malignant degree of ACP and the survival prognosis of patients.[27,28]

GRIA2 encoded glutamate receptors play an important role in central nervous system gated ion channels and excitatory synaptic transmission. Earlier studies found that the knockout of the GRIA2 gene in mice can affect learning and food-reward stimulation.[29] Recently, it has been found that the GRIA2 gene is differentially expressed in patients with a good prognosis of ovarian serous papillary adenocarcinoma after chemotherapy, suggesting the role of GRIA2 in determining the prognosis of patients with chemotherapy.[30] It has also been found that compared with normal individuals, the abnormal expression of GRIA2 in isolated fibrous tumors was statistically different.[31] However, some scholars believe this gene increases the invasion and migration of pancreatic cancer cells by activating the AMPA receptor and the classical MAPK pathway. GRIA2 is involved in the degeneration of the brain and spinal motor neurons caused by amyotrophic lateral sclerosis. Its mechanism may be the abnormal signal pathway caused by its transcriptional mRNA and the interference of Ca^{2+} homeostasis. Other studies have suggested that the glutamate system may be involved in the development of migraine.[32] However, through bioinformatics technology, we found that the low expression of GRIA2 in ACP patients may be the abnormal expression of the signal pathway related to the glutamate energy system, leading to the occurrence and development of ACP and suggesting that GRIA2 can be used as a potential target for ACP diagnosis and treatment.

Hub proteins are an essential part of interactors in the organism. They bind to different interacting partners, and most of which are transcription factors or co-regulators involved in signaling pathways. It shows remarkable pleiotropy and connects many cellular systems. Static centers interact with their partners simultaneously, while dynamic centers bind to different partners at different places and times.[33] Hub proteins transfer from the cytoplasm to the nucleus, triggered by phosphorylation and ubiquitination in regions of internal disorder. Hub proteins transmit external signals to the nucleus via the cell membrane and cytoplasm.[34] Hub proteins, which function at the center of multiple DNA-processing machines, are the basis of multiprotein complex remodeling and the integration of pathways such as DNA replication, damage response, and repair. These proteins often act as platforms or scaffolding around which dynamic DNA-processing mechanisms assemble and disassemble. A key characteristic of most Hub proteins in DNA-processing machines is that they bind DNA substrates and protein chaperones in specific directions, thus providing the necessary directionality to ensure proper functioning.[35]

However, our study still has many shortcomings, such as the lack of animal experiments to verify whether abnormal expression of these genes can trigger ACP. The experiment's sample
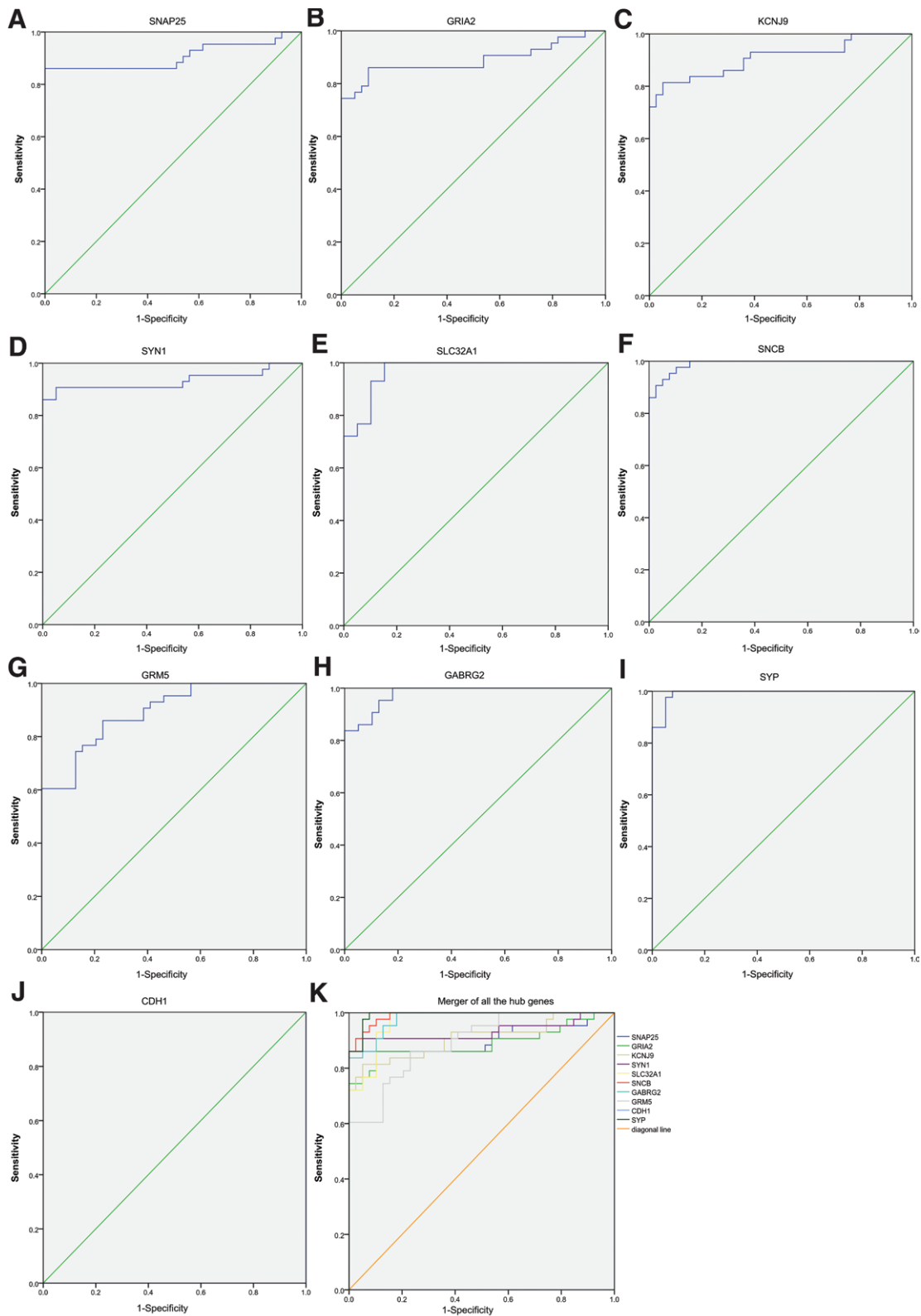
Figure 5. The receiver operator characteristic curves indicate that the hub genes could predict ACP sensitively and especially. (A) SNAP25, (B) GRIA2, (C) KCNJ9, (D) SYN1, (E) SLC32A1, (F) SNCB, (G) GRM5, (H) GABRG2, (I) SYP, (J) CDH1, (K) The merger of all hub genes. ACP = adamantinomatous craniopharyngioma.

size is small, which may affect the results to some extent. In future studies, larger sample sizes will be used for analysis, and more reliable statistical values will be obtained through higher sample sizes.

In a word, by screening the gene database, we find out that ACP patients differentially expressed genes are likely to play an essential role in the development of ACP. At the same time, these differentially expressed genes are of great value in the diagnosis
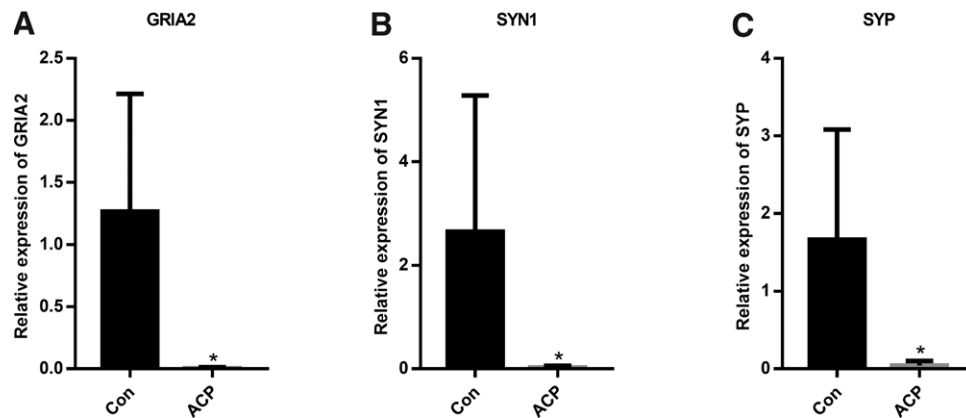
Figure 6. Relative expression of GRIA2, SYN1, and SYP by RT-qPCR analysis. *$P < .05$, compared with controls. RT-qPCR = quantitative real-time polymerase chain reaction.

and targeted therapy of tumors and can even be mined as a biological molecular target for the prognosis and prognosis of ACP patients, which has broad application prospects.

Author contributions

Y-FZ and S-YZ experimented and were significant contributors to writing and submitting the manuscript. BW and C-XS made substantial contributions to research conception. They also designed the draft of the research process. Y-FZ and LX were involved in critically revising the manuscript for important intellectual content. BW, L-WL and KJ analyzed the genic data regarding Glioblastoma. All authors read and approved the final manuscript.

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