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Original Research Article

Network pharmacology-based elucidation of the molecular mechanism underlying the anti-migraine effect of Asari Radix et Rhizoma

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Abstract

Purpose: *To determine the molecular mechanism involved in the anti-migraine effect of Asari Radix et Rhizoma (ARR) using network pharmacology.*

Methods: *The compounds present in ARR were identified through information retrieval from literature and public databases, and were screened based on absorption, distribution, metabolism, excretion and toxicity. Target genes related to the selected compounds and migraine were identified or predicted from public databases. Hub genes in ARR against migraine were identified through analysis of interactions in overlapping genes between compounds and migraine target genes, based on STRING database. Gene enrichment analysis of overlapping genes was performed using Database for Annotation, Visualization and Integrated Discovery.*

Results: *A total of 138 compounds were selected as potential bioactive compounds in ARR. Target genes related to the selected compounds (611 genes) and migraine (278 genes) were obtained, including 71 overlapping genes. The hub genes in the anti-migraine effect of ARR were BDNF, IL6, COMT, APP and TNF. Gene enrichment analysis showed the top 10 biological processes or pathways involved in the mechanism of anti-migraine action of ARR. The tissue source of the overlapping genes was not limited to the brain. The results from gene enrichment analysis revealed that the effect of ARR on migraine was holistic, which is characteristic of traditional Chinese medicines.*

Conclusion: *Network pharmacology has been used to decipher the molecular mechanism involved in the action of ARR against migraine. The results provide a scientific basis for the clinical effect of ARR on migraine.*

Keywords: *Asari Radix et Rhizoma, Migraine, Network pharmacology, Molecular mechanism, Hub genes*

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INTRODUCTION

Migraine is a chronic and sometimes progressive disease characterized by neurological disorders resulting in recurrent episodes of moderate or severe head pain and a myriad of neurological symptoms. Patients with migraine often have some characteristic symptoms such as

gastrointestinal symptoms e.g. emesis and nausea, photophobia, cutaneous allodynia, and phonophobia [1]. Moreover, migraine patients bear other neurological symptoms such as tinnitus, vertigo, and cognitive impairment [2]. Migraine exerts enormous economic burden and mental stress on the patient and the society at large [3]. Thus, there is need to develop safe and effective anti-migraine drugs.

Although anti-migraine western drugs have been developed all over the world [4], traditional Chinese medicines (TCMs) have also been widely used to treat migraine in China [5]. Asari Radix et Rhizoma (ARR), composed from the roots of *Asarum heterotropoides* Fr. Schmidt var. *mandshuricum* (Maxim.) Kitagawa, *A. sieboldii* Miq. var. *seoulense*, and *A. sieboldii* Miq., exhibits a wide range of pharmacological effects such as analgesic, sedative, anticonvulsant, hypotensive, anti-aging, and anti-inflammatory properties [6]. It is usually used as an herbal medicine to treat migraine in China [7,8]. However, not much is known about the molecular mechanisms underlying the anti-migraine effect of ARR. It is necessary to elucidate the mechanisms of anti-migraine action of ARR so as to rationally guide its clinical use.

With the rapid development of systems, network, and chemical biology, network pharmacology has emerged as a promising strategy in current drug discovery and development. It is used to study diseases from "network targets, multicomponent therapeutics" instead of "one target, one drug" [9]. This characteristic is consistent with the concept of holism. Coincidentally, the perspective of holism has long been central to TCMs treatments of various diseases [10]. Therefore, network pharmacology is a valuable tool for achieving holistic view of TCMs. The current TCMs issues based on how to study TCMs from point to face could be tackled with network pharmacology. Nowadays, network pharmacology is used widely to achieve comprehensive and systematic insight into the bioactive compounds and mechanisms of action of TCMs [11]. The aim of this study was to use network pharmacology to determine the molecular mechanism involved in the anti-migraine effect of ARR.

EXPERIMENTAL

Compounds collection and ADMET evaluation

The compounds present in ARR were identified from existing literature and three public databases: Symptom Mapping (SymMap, http://www.symmap.org/), Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://lsp.nwu.edu.cn/tcmsp.php), Encyclopedia of Traditional Chinese Medicine (ETCM, http://www.nrc.ac.cn:9090/ETCM/). PubChem CID, chemical structures, and SMILES of each compound were identified with PubChem (https://pubchem.ncbi.nlm.nih.gov/). All compounds were screened using ADMET evaluation with the aid of FAFDrugs4 (http://fafdrugs4.mti.univ-paris-diderot.fr/). The "PhysChem Filters" parameter of FAFDrugs4 was set as "*Drug-Like Soft*". Compounds were retained only if the result of ADMET evaluation was "Accepted".

Target genes related to the selected compounds

Target genes linked to the identified compounds were collected from the databases SymMap, TCMSP, ETCM and Therapeutic Target Database (TTD, http://bidd.nus.edu.sg/group/cjttd/), and were predicted by STITCH (http://stitch.embl.de/) and SwissTargetPrediction (http://swisstargetprediction.ch/) with "*Homo sapiens*" setting. When the information obtained was a protein name, UniProt (https://www.uniprot.org/) was used to transfer the protein name to gene symbol with "*Homo sapiens*" setting.

Target genes related to migraine

Migraine-related target genes were identified by retrieving Online Mendelian Inheritance in Man (OMIM, https://omim.org/), DisGeNET (www.disgenet.org/), TTD and TCMSP databases. The overlapping genes were identified by matching compounds-related target genes with migraine-related target genes with the aid of a Venn diagram plotted using the OmicShare tools, a free online platform for data analysis (www.omicshare.com/tools).

Protein-protein interaction (PPI) network analysis

The PPI network analysis of the overlapping genes was carried out on STRING (https://string-db.org/) with "*Homo sapiens*" setting. The constructed PPI network of overlapping genes was visualized using Cytoscape ver. 3.7.1 (https://cytoscape.org/). Nodes in networks represent genes, while edges suggest interactions among genes. The hub genes involved in the action of ARR against migraine were selected by setting threshold value of Degree in network. In this analysis, the bigger

the Degree values of genes, the more important the genes.

Gene enrichment analysis

Gene enrichment analysis of the overlapping genes was performed using Database for Annotation, Visualization and Integrated Discovery ver. 6.8 (https://david.ncifcrf.gov/) with "*Homo sapiens*" setting. The results of gene ontology (GO)-biological process, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, and tissue expression enrichment analyses were used to unravel the molecular mechanism involved in the anti-migraine effect of ARR. Bubble charts of GO-biological process and KEGG pathways of the overlapping genes were plotted using the OmicShare tools.

RESULTS

Compounds present in ARR and ADMET evaluation

A total of 259 compounds from ARR were identified through retrieval from extant literature, SymMap, TCMSP, and ETCM. Moreover, PubChem CID, chemical structures, and SMILES of each compound were confirmed through PubChem. Then, the SMILES of all compounds were imported into FAFDrugs4 for ADMET evaluation. The results suggested that 138 compounds exhibited good potential as active ingredients.

Target genes related to the 138 compounds and migraine

A total of 611 genes linked to the 138 compounds were identified through information retrieval from SymMap, TCMSP, ETCM, TTD, STITCH and SwissTargetPrediction databases. Genes related to migraine (278 genes) were collected from OMIM, DisGeNET, TTD and TCMSP databases. The Venn diagram shown in Figure 1 indicates that 71 overlapping genes were identified by matching the 611 genes related to the 138 compounds with the 278 genes related to migraine. The gene symbol and UniProt ID of 71 overlapping genes are listed in Table 1.

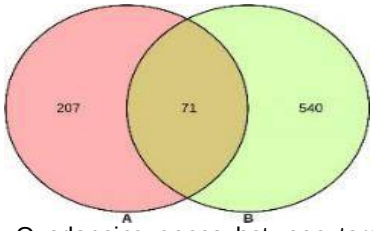

Figure 1: Overlapping genes between target genes related to migraine (A) and compounds in ARR (B)

Table 1: Gene symbol and UniProt ID of 71 overlapping genes

No.	Gene symbol	UniProt ID
1	SLC6A2	P23975
2	NOS2	P35228
3	SLC6A4	P31645
4	MMP3	P08254
5	HTR4	Q13639
6	GRM2	Q14416
7	HTR2B	P41595
8	CCR1	P32246
9	MAOB	P27338
10	CYP1A2	P05177
11	TRPM8	Q7Z2W7
12	BACE1	P56817
13	CYP2D6	P10635
14	GSTM1	P09488
15	NOS1	P29475
16	IL10	P22301
17	CA14	Q9ULX7
18	GNAS	O95467
19	HTR2C	P28335
20	MTRR	Q9UBK8
21	DRD5	P21918
22	MAOA	P21397
23	BDNF	P23560
24	KCNA3	P22001
25	DRD3	P35462
26	DAO	P14920
27	F7	P08709
28	EDNRA	P25101
29	DRD4	P21917
30	NOS3	P29474
31	DDC	P20711
32	GSTP1	P09211
33	GABRQ	Q9UN88
34	CNR1	P21554
35	PGR	P06401
36	SCN1A	P35498
37	TLR4	O00206
38	CYP19A1	P11511
39	GC	P02774
40	ADRB1	P08588
41	TNF	P01375
42	CCR2	P41597
43	CACNA1H	O95180
44	ADH1B	P00325
45	LDLR	P01130
46	DRD2	P14416
47	TYRP1	P17643
48	F2	P00734
49	TRPV3	Q8NET8
50	TRPA1	O75762
51	PTGS2	P35354
52	IL1B	P01584
53	REN	P00797
54	ESR1	P03372
55	HTR2A	P28223
56	CSNK1D	P48730
57	ADRB2	P07550
58	AR	P10275
59	ESR2	Q92731
60	GABRA3	P34903
61	CCL2	P13500
62	CYP3A4	P08684
63	IL6	P05231

Table 1: Gene symbol and UniProt ID of 71 overlapping genes *(continued)*

No.	Gene symbol	UniProt ID
64	SCN5A	Q14524
65	GRIA2	P42262
66	APP	P05067
67	COMT	P21964
68	SLC6A3	Q01959
69	TRPV1	Q8NER1
70	MMP9	P14780
71	DRD1	P21728

Hub genes involved in the action of ARR against migraine

As shown in Figure 2, the PPI network of overlapping genes consisted of 69 nodes and 487 edges. Based on Degrees of genes, the top 5 genes (BDNF, IL6, COMT, APP and TNF) were considered as the hub genes involved in the action of ARR against migraine. This indicated that these genes play important roles in the molecular mechanisms involved in the anti-migraine effect of ARR.

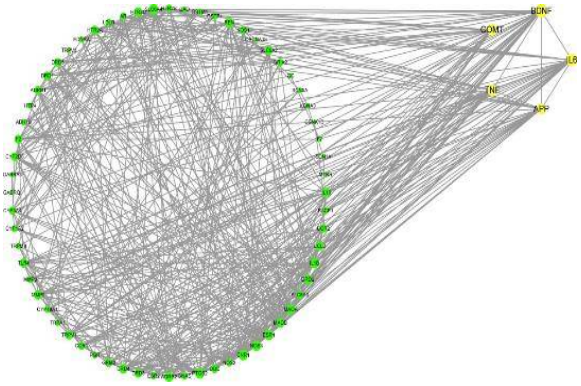

Figure 2: PPI network of overlapping genes including 69 nodes and 487 edges. Yellow nodes: hub genes involved in the anti-migraine effect of ARR

Gene enrichment analysis of 71 overlapping genes

The results of biological process enrichment analysis indicated that 265 biological processes were related to the anti-migraine effect of ARR, and the top 10 biological processes were response to drug, cellular calcium ion homeostasis, negative regulation of blood pressure, positive regulation of nitric oxide biosynthetic process, synaptic transmission, dopaminergic, behavioral response to cocaine, activation of adenylate cyclase activity, positive regulation of ERK1 and ERK2 cascade, dopamine catabolic process, and positive regulation of fever generation. The results are shown in Figure 3 and listed in Table 2.

The results of KEGG pathway enrichment analysis suggested that 52 pathways were related to the anti-migraine effect of ARR, and the top 10 pathways were neuroactive ligand-receptor interaction, cocaine addiction, dopaminergic synapse, serotonergic synapse, calcium signaling pathway, drug metabolism-cytochrome P450, inflammatory mediator regulation of TRP channels, tyrosine metabolism, gap junction, and amphetamine addiction. The results are shown in Figure 4 and listed in Table 3.

The results of tissue expression analysis suggested that the main tissue source of 71 overlapping genes was the brain (38 genes), and some other tissues were involved, including liver, blood, heart, breast, leukocyte, fibroblast, brain stem, G-protein coupled receptors, and breast cancer. The results are presented in Table 4.

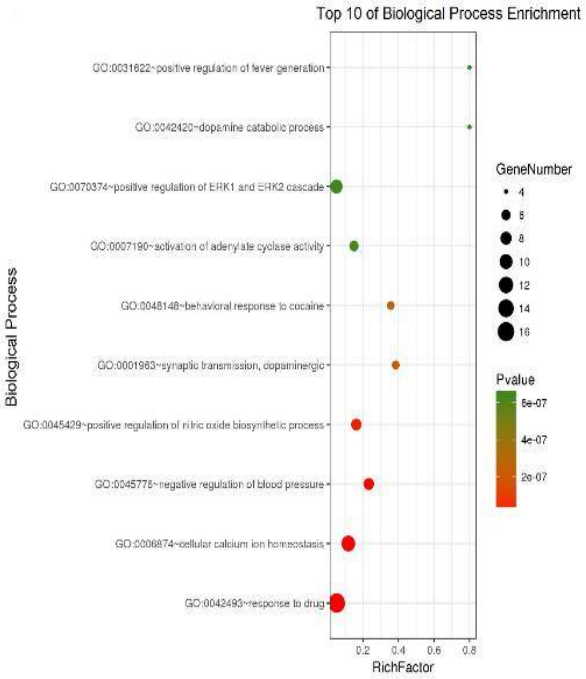

Figure 3: Bubble chart of the top 10 of GO-biological processes of 71 overlapping genes

DISCUSSION

In the present study, the molecular mechanism involved in the anti-migraine effect of ARR was systematically unraveled using network pharmacology. The results showed the hub genes, biological processes, and molecular pathways through which ARR acts against migraine. The tissue expressions of genes related to ARR action were also analyzed.

Table 2: Target genes in the top 10 of GO-biological processes

GO ID	Target genes
GO:0042493	IL6, DRD1, DRD3, PTGS2, DRD2, SLC6A2, SLC6A3, SLC6A4, TRPA1, MAOB, GNAS, COMT, HTR2B, HTR2C, IL10, HTR2A
GO:0006874	CCL2, DRD3, TRPM8, DRD2, CCR1, DRD5, CCR2, DRD4, HTR2B, HTR2C, HTR2A
GO:0045776	NOS1, DRD3, DRD2, DRD5, CNR1, NOS3, NOS2
GO:0045429	IL6, TNF, PTGS2, TRPV1, ESR1, IL1B, TLR4
GO:0001963	DRD1, DRD3, DRD2, DRD5, DRD4
GO:0048148	DRD1, DRD3, DRD2, DRD4, HTR2A
GO:0007190	EDNRA, DRD1, ADRB2, ADRB1, DRD5, GNAS
GO:0070374	IL6, TNF, CCL2, DRD2, CCR1, TLR4, HTR2B, HTR2C, HTR2A
GO:0042420	SLC6A3, MAOA, MAOB, COMT
GO:0031622	TNF, PTGS2, CNR1, IL1B

Table 3: Target genes in the top 10 of KEGG pathways

Pathway ID	Target genes
hsa04080	DRD1, DRD3, TRPV1, DRD2, GABRA3, DRD5, DRD4, HTR4, EDNRA, ADRB2, GRM2, ADRB1, GRIA2, CNR1, F2, HTR2B, HTR2C, GABRQ, HTR2A
hsa05030	DDC, DRD1, BDNF, GRM2, GRIA2, DRD2, SLC6A3, MAOA, MAOB, GNAS
hsa04728	DDC, DRD1, SCN1A, DRD3, GRIA2, DRD2, SLC6A3, DRD5, MAOA, DRD4, MAOB, GNAS, COMT
hsa04726	DDC, APP, PTGS2, MAOA, SLC6A4, MAOB, CYP2D6, HTR4, GNAS, HTR2B, HTR2C, HTR2A
hsa04020	DRD1, NOS1, DRD5, HTR4, EDNRA, ADRB2, ADRB1, CACNA1H, GNAS, NOS3, NOS2, HTR2B, HTR2C, HTR2A
hsa00982	GSTM1, CYP3A4, MAOA, MAOB, CYP2D6, ADH1B, CYP1A2, GSTP1
hsa04750	TRPM8, TRPV1, TRPV3, TRPA1, IL1B, GNAS, HTR2B, HTR2C, HTR2A
hsa00350	DDC, TYRP1, MAOA, MAOB, ADH1B, COMT
hsa04540	DRD1, ADRB1, CSNK1D, DRD2, GNAS, HTR2B, HTR2C, HTR2A
hsa05031	DDC, DRD1, GRIA2, SLC6A3, MAOA, MAOB, GNAS

Table 4: Tissue expression analysis of 71 overlapping genes

Tissues	Target genes
Breast	CYP3A4, ESR1, GNAS, SCN5A
Leukocyte	APP, ADRB2, BDNF, IL1B
Blood	AR, TYRP1, ADRB2, TNF, CCR1, MMP9, F2, KCNA3
Brain	DRD1, SCN1A, LDLR, CA14, DRD3, TRPV1, DRD2, SLC6A2, DRD5, SLC6A3, CYP2D6, SLC6A4, DRD4, ADH1B, KCNA3, COMT, EDNRA, BDNF, APP, CNR1, DAO, SCN5A, AR, NOS1, GABRA3, MAOB, ESR1, HTR4, ADRB2, GRM2, CSNK1D, GRIA2, BACE1, GNAS, HTR2B, HTR2C, GSTP1, HTR2A
Brain stem	CNR1, HTR2A
G-protein coupled receptors	CCR2, HTR4
Heart	GSTM1, ADRB2, CA14, HTR4, CACNA1H, SCN5A
Breast cancer	PGR, ESR1
Liver	CYP3A4, TYRP1, LDLR, MAOA, CYP2D6, ADH1B, CYP1A2, F7, GSTM1, APP, F2, GNAS, NOS2
Fibroblast	APP, IL6, MMP3

The ADMET evaluation serves to predict the bioactive potential of target compounds [12]. In this study, ADMET evaluation of 259 compounds present in ARR was carried out on FAFDrugs4 platform prior to searching target genes of the compounds. The results revealed that 138 compounds exhibited good potential as active ingredients.

STRING is an accepted database used to construct PPI networks of multiple genes [13]. In this study, PPI network of the 71 overlapping genes showed the degree of contribution of each gene to the mechanism involved in the anti-migraine action of ARR, while BDNF, IL6, COMT, APP and TNF were identified as the hub genes. The BDNF is an important regulatory factor of pain, and many investigations have indicated that it exerts an important influence on migraine pathophysiology [14]. It has been reported that migraine is positively associated with TNF-α, and negatively associated with IL-6 [15]. Some studies investigated the relationship between genetic polymorphisms of COMT and susceptibility to migraine, but the results were

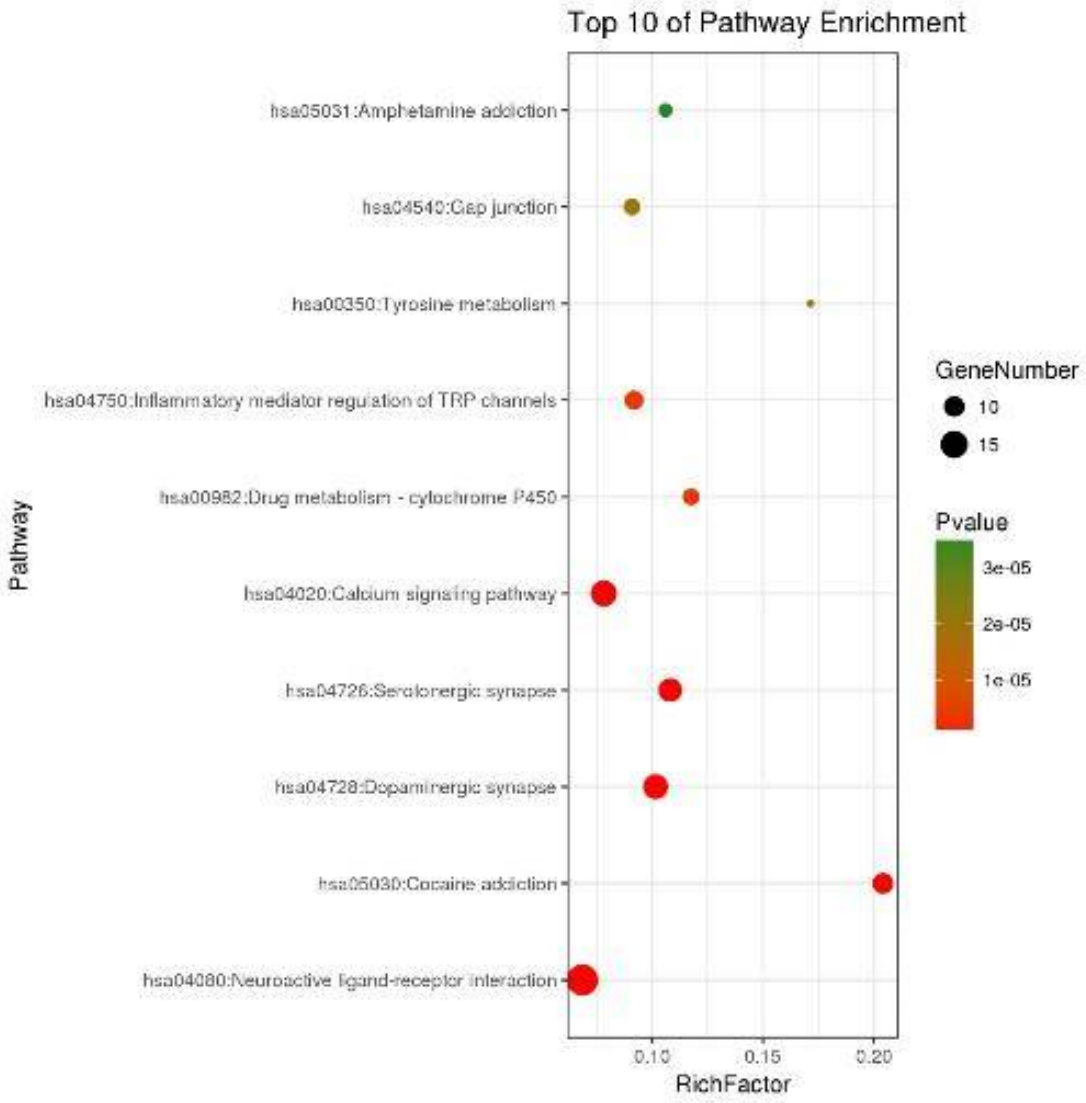

Figure 4: Bubble chart of the top 10 of KEGG pathways of 71 overlapping genes

inconsistent [16,17]. However, meta-analysis has indicated that COMT polymorphisms might decrease the risk of migraine [18]. Increases in levels of amyloid β-protein (the hydrolysate of amyloid-β precursor protein) suppresses the occurrence of migraine by inhibiting the activity of Na+-K+-ATPase in capillary endothelial cells, resulting in suppression of cortical spreading depression [19]. These reports indicate that the 5 hub genes (BDNF, IL6, COMT, APP and TNF) are directly related to the occurrence of migraine.

Gene enrichment analysis is aimed at functional classification of target genes and determination of the biological significance of phenotypic difference. In biological process enrichment analysis, 265 biological processes were related to the anti-migraine action of ARR, and the top 10 biological processes were considered as the main processes. In order to understand the biological processes associated with the anti-migraine effect of ARR more systematically and comprehensively, KEGG pathway enrichment analysis was carried out. Fifty-two pathways were associated with the anti-migraine potential of ARR, and the top 10 pathways were considered as the main pathways involved in the process. Some studies have reports the relationship between some biological processes or pathways and migraine. With respect to positive regulation of nitric oxide biosynthetic process, ample evidence has indicated that nitric oxide is a causative agent in migraine [20]. A study has revealed that dopamine induces migraine occurrence [21]. In addition, stimulation of dopaminergic neurons has been associated with induction of most symptoms of migraine, with patients showing high sensitivity to dopamine receptor [22]. In the inflammatory mediator regulation of TRP channels pathway, TRPV1 and TRPA1 interfere with the occurrence of migraine by regulating the release of calcitonin

gene-related peptide [23,24]. In the present study, the results obtained from biological process and KEGG pathway enrichment analyses have revealed the molecular mechanisms that underlie the anti-migraine action of ARR. The interactions among biological processes or pathways were obvious, based on the enrichment genes in these biological processes or pathways. Meanwhile, the brain was not the only tissue source of the 71 overlapping genes: some other tissues, such as liver, blood, heart, breast, leukocyte, fibroblast, brain stem, G-protein coupled receptors, and breast cancer, were also involved. In this respect, ARR showed holistic characteristic, which is similar to the characteristics of TCMs used for the treatment of various diseases. This suggests that the molecular mechanism involved in the anti-migraine effect of ARR may not be a single biological process or pathway but an interplay of several pathways/processes.

CONCLUSION

The molecular mechanism of action involved in the anti-migraine effect of ARR has been systematically and comprehensively investigated using network pharmacology. The results have identified the hub genes, major biological processes, and major pathways involved in the anti-migraine action of ARR. However, the tissue expression of genes related to the process is not limited to the brain. The interactions among biological processes, pathways and multi-tissue expressions of genes show that the action of ARR is holistic, which is consistent with the characteristics of TCMs. These results provide a scientific basis for the clinical application of ARR in migraine therapy.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Yun-Bin Jiang and Mei Zhong conceived and designed the study. Mei Zhong, Ting Huang and Zhong-Hua Dai collected the data. Yun-Bin Jiang, Xing-Bao Tao and Rong-Ping Yang analyzed the data, while Yun-Bin Jiang wrote the manuscript. All authors read and approved the manuscript for publication.

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