# Network Pharmacology-Based Analysis of the Role of Traditional Chinese Herbal Medicines in the Treatment of Migraine

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# Classical Pharmacodynamics


A ‘single-drug’, ‘single-target’ paradigm has dominated drug discovery for decades

Some have argued this paradigm has limited the drug discovery space

# Migraine Headache

A recurring, disabling neurological condition (affects 10-20% of population)

*Pathophysiology:* Several distinct stages with complex symptomology

- Hypothalamic activation
- Cortical spreading depression (CSD)
- Trigeminal-vascular activation (peripheral & central)
- Neuropeptide imbalances: 5-HT, DA, GABA-A, CGRP, substance P, Glu, etc.

*Biomedical treatment*: **abortive** & **prophylactic**

**Sumatriptan**
Imitrex®

**$5\text{-HT}_{1D}$, $5\text{-HT}_{1B}$**
Serotonin receptor agonist

**Mechanism of Action**
Blocks CGRP release
Inhibits vasodilation
Decreases trigeminal activity

**Migraine Therapies**

**Abortive**
- NSAIDs
- Triptans
- DA agonists

**Prophylaxis**
- Beta blockers
- TCAs
- Topiramate
- MAOIs
- CGRP inhibitors
- Botox
- Gabapentin

*Outcomes:* mixed results (triptans give ~68% pain-free response in 2 hours, ~54% in 24 hours

Thorlund K, Mills EJ, Wu P, et al. Comparative efficacy of triptans for the abortive treatment of migraine: a multiple treatment compa rison meta-analysis. *Cephalalgia.* 2014;34(4):258-267

# Migraine Headache & Genes

Migraine is a complex genetic/polygenetic disorder

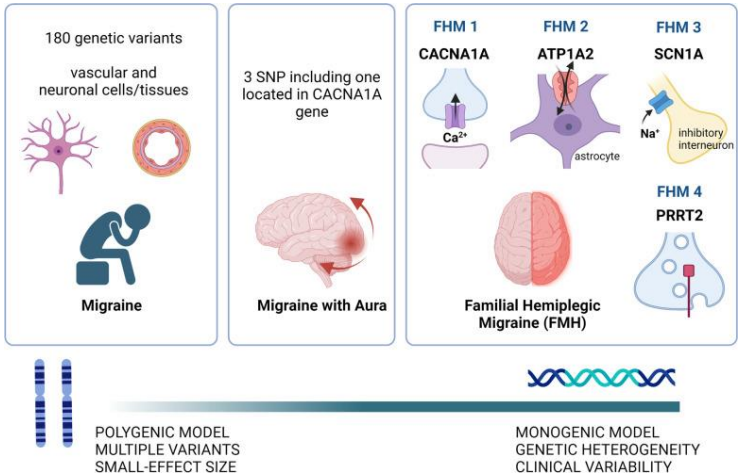
GWAS have identified over **180 genetic variants** in common migraine

Most involve complex networks of **neuronal** or **vascular** molecular abnormalities

Shared genetic factors between migraine and co-morbidities (depression, high blood pressure, etc.)

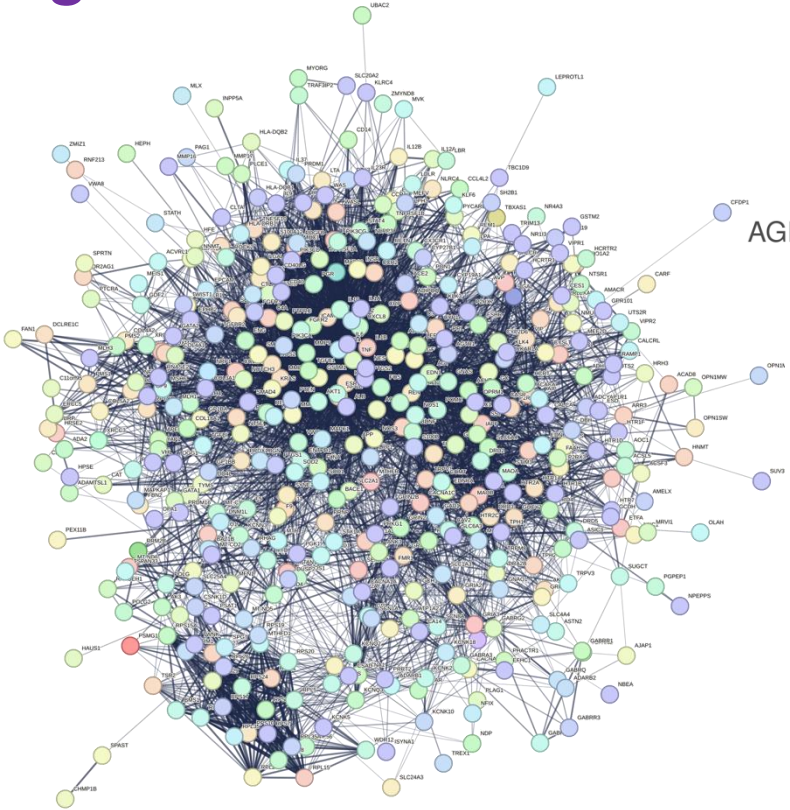
**GeneCards** (www.genecards.org)
- 1500 total genes (accessed 3/12/24)

**Disgenet** (www.disgenet.org)
- 512 total genes (accessed 3/12/24)

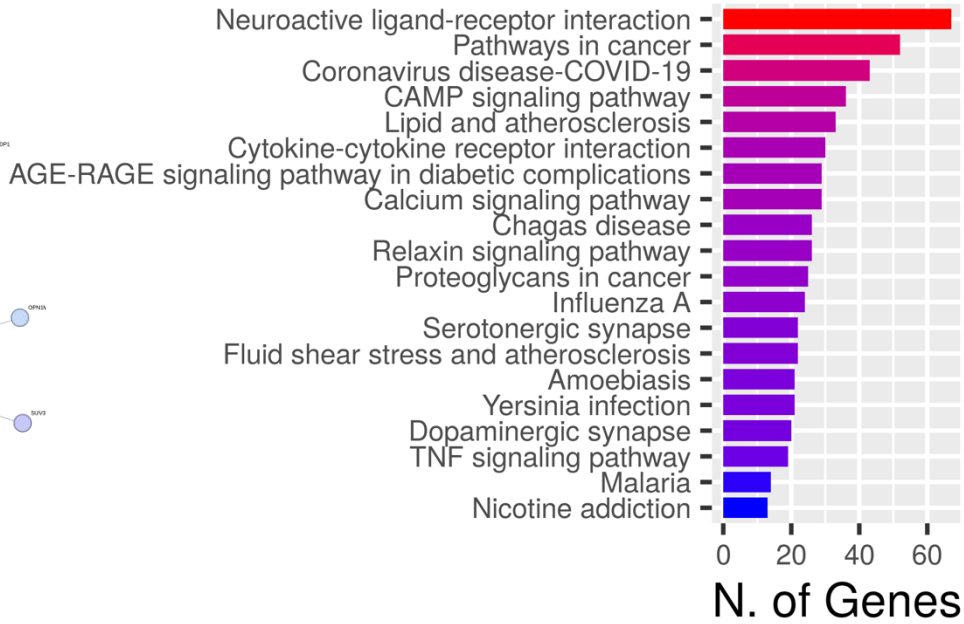



Grangeon L, Lange KS, Waliszewska-Prosół M, Onan D, Marschollek K, Wiels W, Mikulenka P, Farham F, Gollion C, Ducros A; European Headache Federation School of Advanced Studies (EHF-SAS). Genetics of migraine: where are we now? J Headache Pain. 2023 Feb 20;24(1):12.

# Migraine PPI Network & Major Pathways



**Disgenet & STRING (v2.0)** (www.string-db.org)
481 nodes | 6073 edges | av. Node degree: 25.3

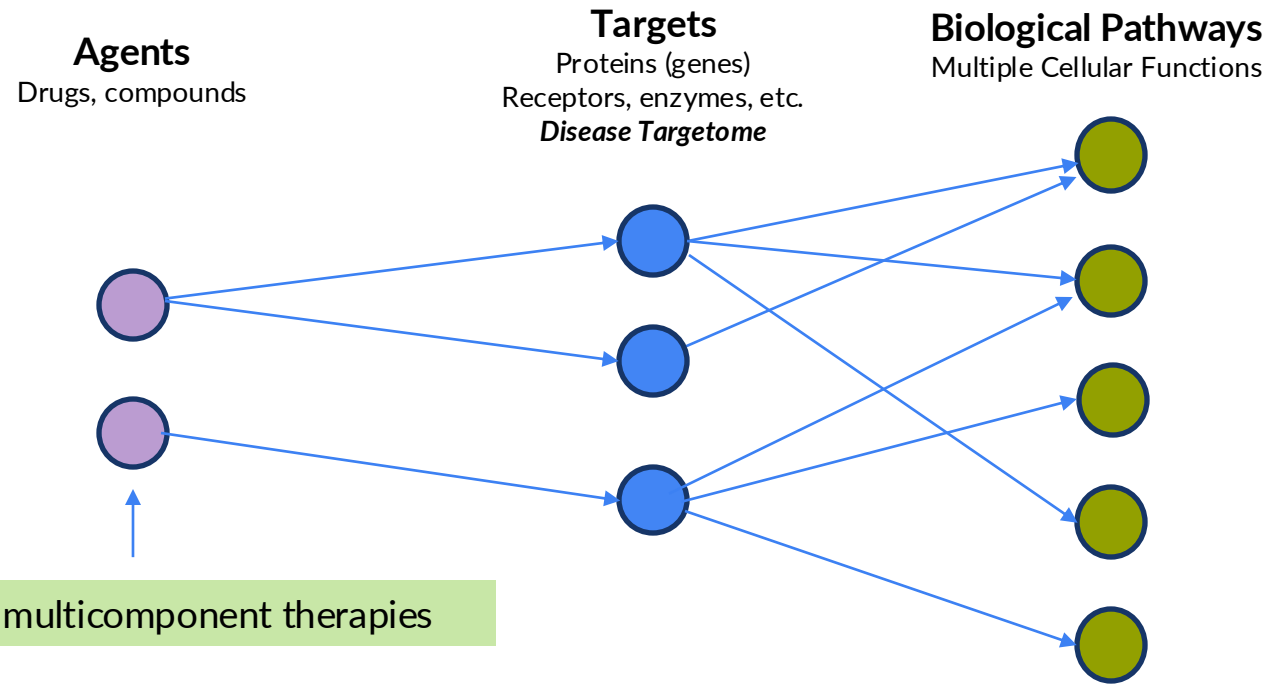



Immune-inflammation, 5-HT/DA, Ca-signaling pathways

**ShinyGO 0.80** (www.bioinformatics.sdstate.edu)

# Pharmacodynamics 2.0

'Omics' (genomics, protein-protein interactions, etc.) studies reveal that disease typically involves multiple targets and pathways




Role of multicomponent therapies

# Network Pharmacology

Integrative approach using computational methods

- Predicts how **multicomponent therapies** interact with disease networks
- Emphases a “network-target, multicomponent-therapy” paradigm
- Uses **network topology & centrality measures** to identify most important nodes in the ‘targetome’
- **Betweenness centrality** (number of nonredundant shortest paths traveling through a node) appears to be more important than degree centrality
- Idea is that you may not want to target hubs (based on degree centrality), but rather non-hub bottlenecks in the network (less lethality)

Review Article | Published: 20 October 2008

## Network pharmacology: the next paradigm in drug discovery

Andrew L Hopkins

*Nature Chemical Biology* **4**, 682–690 (2008) | Cite this article

**18k** Accesses | **2523** Citations | **59** Altmetric | Metrics

### Abstract

The dominant paradigm in drug discovery is the concept of designing maximally selective ligands to act on individual drug targets. However, many effective drugs act via modulation multiple proteins rather than single targets. Advances in systems biology are revealing a phenotypic robustness and a network structure that strongly suggests that exquisitely selective compounds, compared with multitarget drugs, may exhibit lower than desired clinical efficacy. This new appreciation of the role of polypharmacology has significant

Hopkins AL. Network pharmacology: the next paradigm in drug discovery. *Nat Chem Biol.* 2008;4(11):682-690.

# Traditional Chinese Medicine (TCM)

## An Ancient Medical System

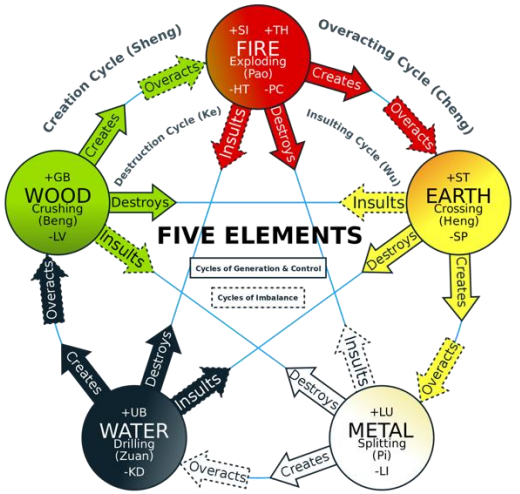
- *Modalities:* acu-moxa therapy, herbal medicine, qi gong, diet, etc.
- Historical records to ~200 BCE
- Many different systems and “schools” within Chinese medicine
- Practiced alongside biomedical approaches in China
- Emphasis on qualitative, systems-based approaches to disease
- Pattern-differentiation: organ + organ state

## Chinese Herbal Medicine

- Complex, multi-component herbal preparations used to treat disease
- Increasing number of high quality RCTs show efficacy
- Mechanistic understanding of herbal action is often lacking
- A single formula may have 10 herbs, with thousands of constituents
- Current research: formulas target multiple biological pathways

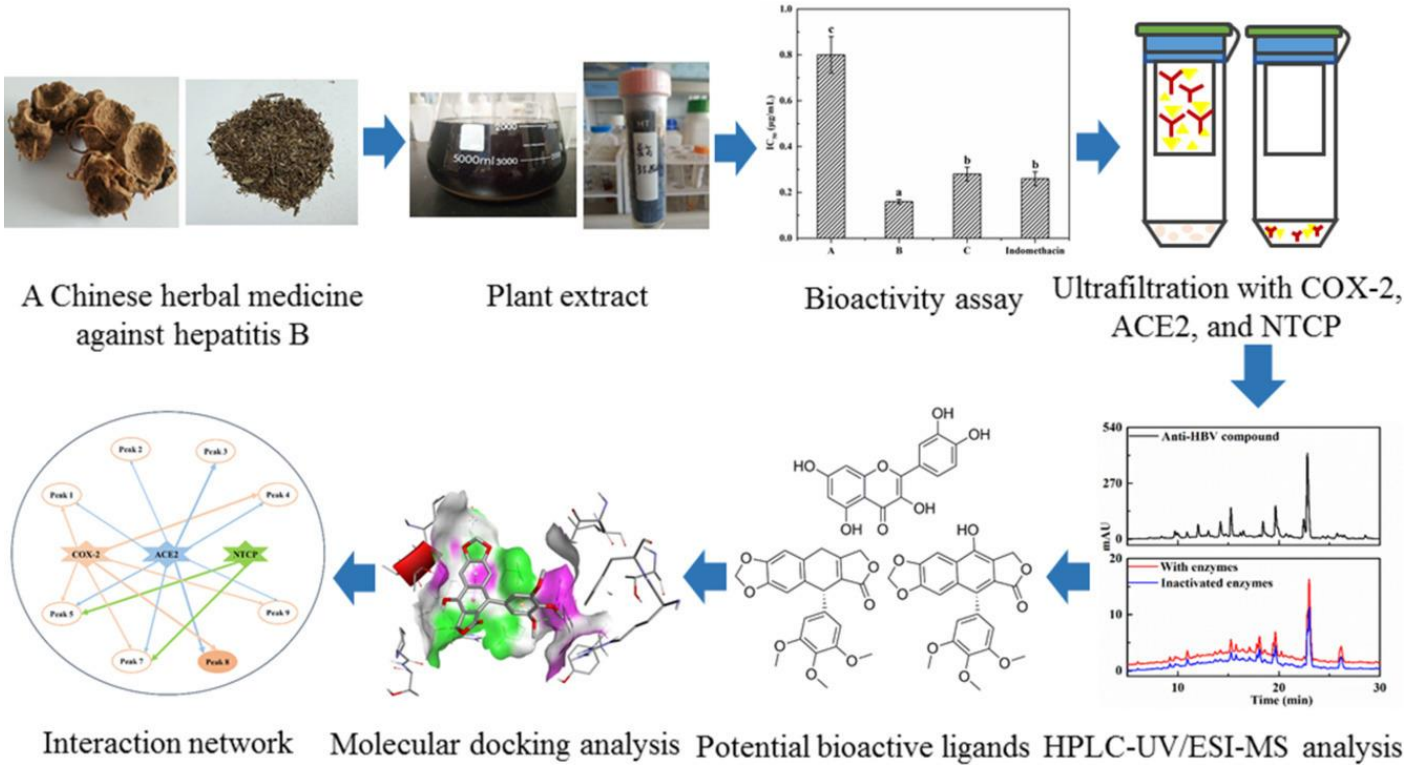
## Bioinformatics and Chinese Herbal Medicine

- Databases containing herb-compound-target information now exist
- Network pharmacology can now be applied to elucidate herbal formula actions and potentially expand the drug discovery space




Noor F, Tahir Ul Qamar M, Ashfaq UA, Albutti A, Alwashmi ASS, Aljasir MA. Network Pharmacology Approach for Medicinal Plants: Review and Assessment. *Pharmaceuticals (Basel).* 2022;15(5):572. Published 2022 May 4. doi:10.3390/ph15050572

# Gathering Constituent Data from TCM Herbs




Huixia Feng, Guilin Chen, Mingquan Guo. Potential multifunctional components explored in Chinese herbal medicine against hepatitis B combining multi-target affinity ultrafiltration HPLC/MS. Phytomedicine Plus, Volume 3, Issue 1, 2023.

# *Project:* Apply Network Approaches to Help Understand Chinese Herbal Action in Migraine

## TCM Herb Targets

Gather ***migraine herbs*** based on available studies

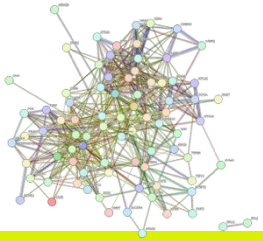
Gather constituents & known ***herb gene-protein targets***

*Batman2.0*
*ETCM*

## Migraine Targets

Gather ***migraine gene-protein targets***

*Disgenet*



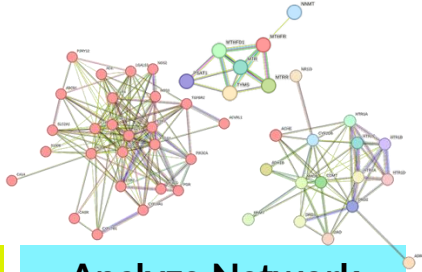
## PPI Network

Find ***common targets*** between both lists

Obtain ***PPI Interaction Network***

Graph the network

*Python*
*STRING database*



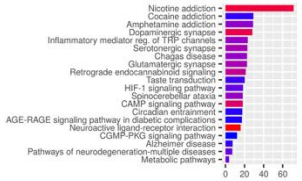
## Analyze Network

***Components***
Use largest component

***Betweenness Centrality***
Pick top 20 based on BC

***Clusters***
Use clustering algorithm to find major clusters

*Networkx*




## Find Pathways

Gene ontologies
KEGG pathways
Reactome

*ShinyGO 0.80*
*Reactome*

## Conclusions

# Find TCM Herb Targets

**Commonly Used TCM Herbs in Migraine[1]**

| Herb (Pin yin) | Genus/Species | Studies |
|---|---|---|
| **Chuan xiong** | *Ligusticum striatum (rhizome)* | 31 |
| **Bai zhi** | *Angelica dahaurica (root)* | 18 |
| **Bai shao** | *Peonia lactiflora (root)* | 16 |
| **Dang gui** | *Angelica sinensis (root)* | 12 |
| **Chai hu** | *Bupleurum chinense (root)* | 11 |
| **Tian ma** | *Gastrodia elata (rhizome)* | 13 |
| **Gou teng** | *Uncaria rhynachophylla (rhizome)* | 8 |
| **Xi xin** | *Asarum sieboldii (herb)* | 9 |
| **Jing jie** | *Schizonepeta tenuifolia (herb)* | ? |

## Compounds

Using full herb list

### *Batman2.0 Database*

- Adjusted p-value: 0.05
- Druggable score: 0.9
- **Unique constituents: 2332 (!)**
- **Gene targets: 6587 (!)**

### *ETCM*

Encyclopedia of Traditional Chinese Medicine

- Can't adjust druggable scores, etc.
- **Unique constituents: 506**
- **Gene targets: 751**

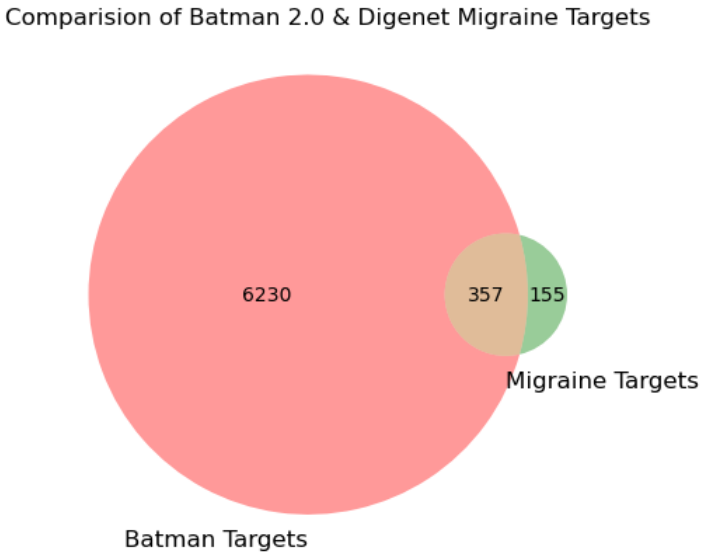
Other databases mentioned in literature

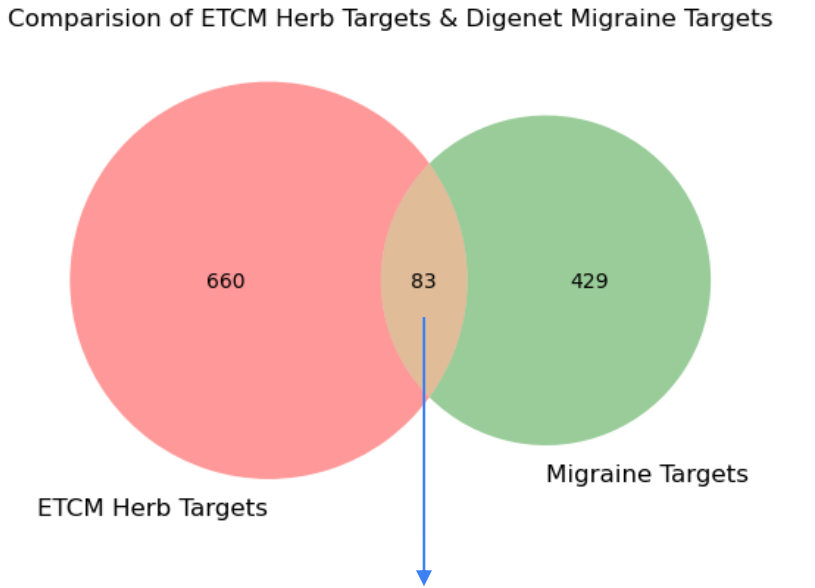
- No longer are active
- Can't be loaded

1) Lyu S, Zhang CS, Guo X, Zhang AL, Sun J, Lu C, Xue CC, Luo X. Oral Chinese Herbal Medicine as Prophylactic Treatment for Episodic Migraine in Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Evid Based Complement Alternat Med. 2020 Dec 28;2020:5181587

# TCM Herb & Migraine Target Overlap

**Using Disgenet database to obtain migraine targets ('Common migraine')** → 512 migraine-associated genes

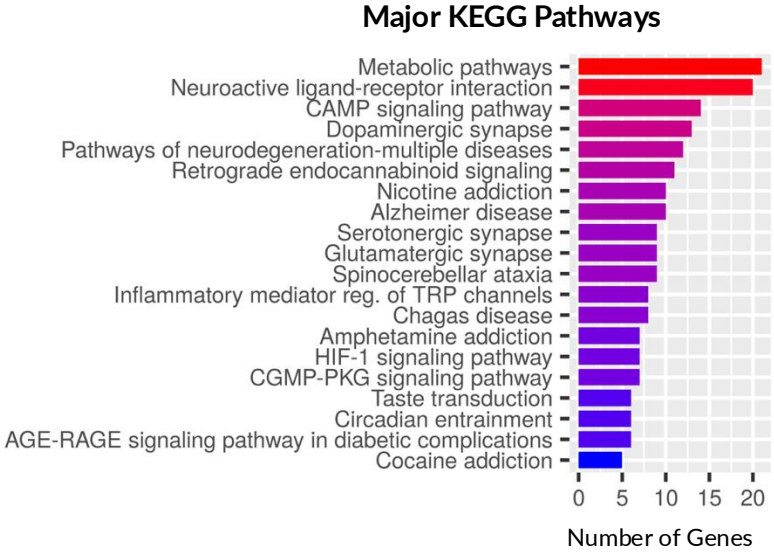


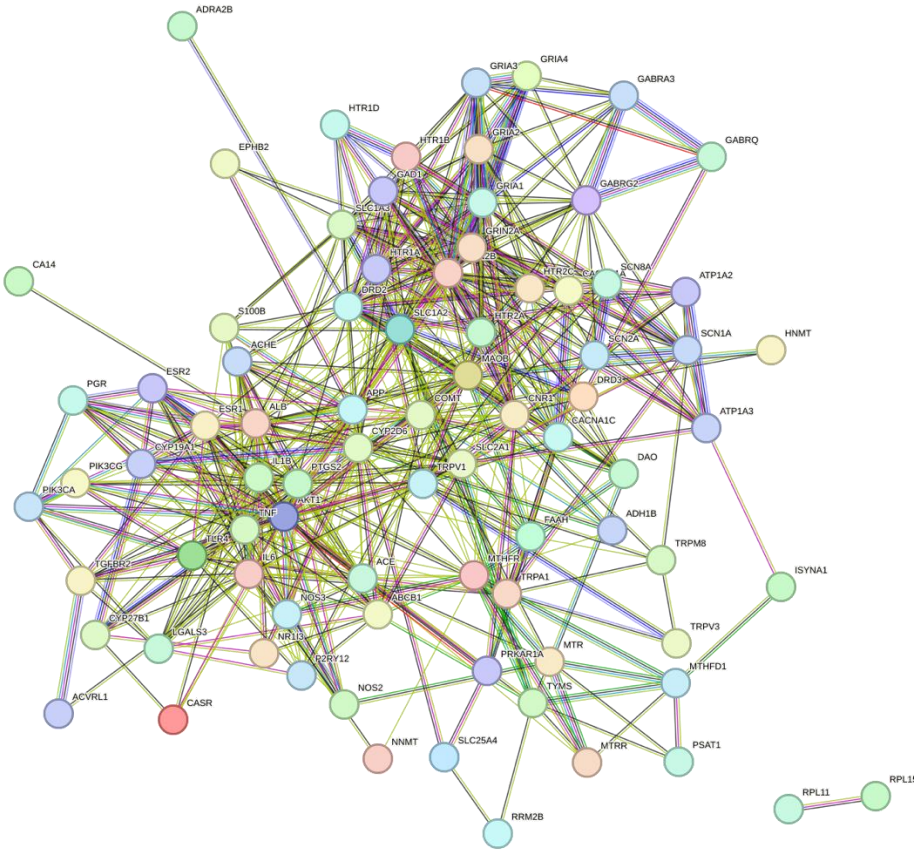

Used gene list for further analysis
**Overlap Targets (OT)**

# Overlap Targets (OT)-PPI Network

- Nodes: 82
- Edges: 543
- Components: 2
- Avg. node degree: 13.2
- Avg. local clustering coefficient: 0.579

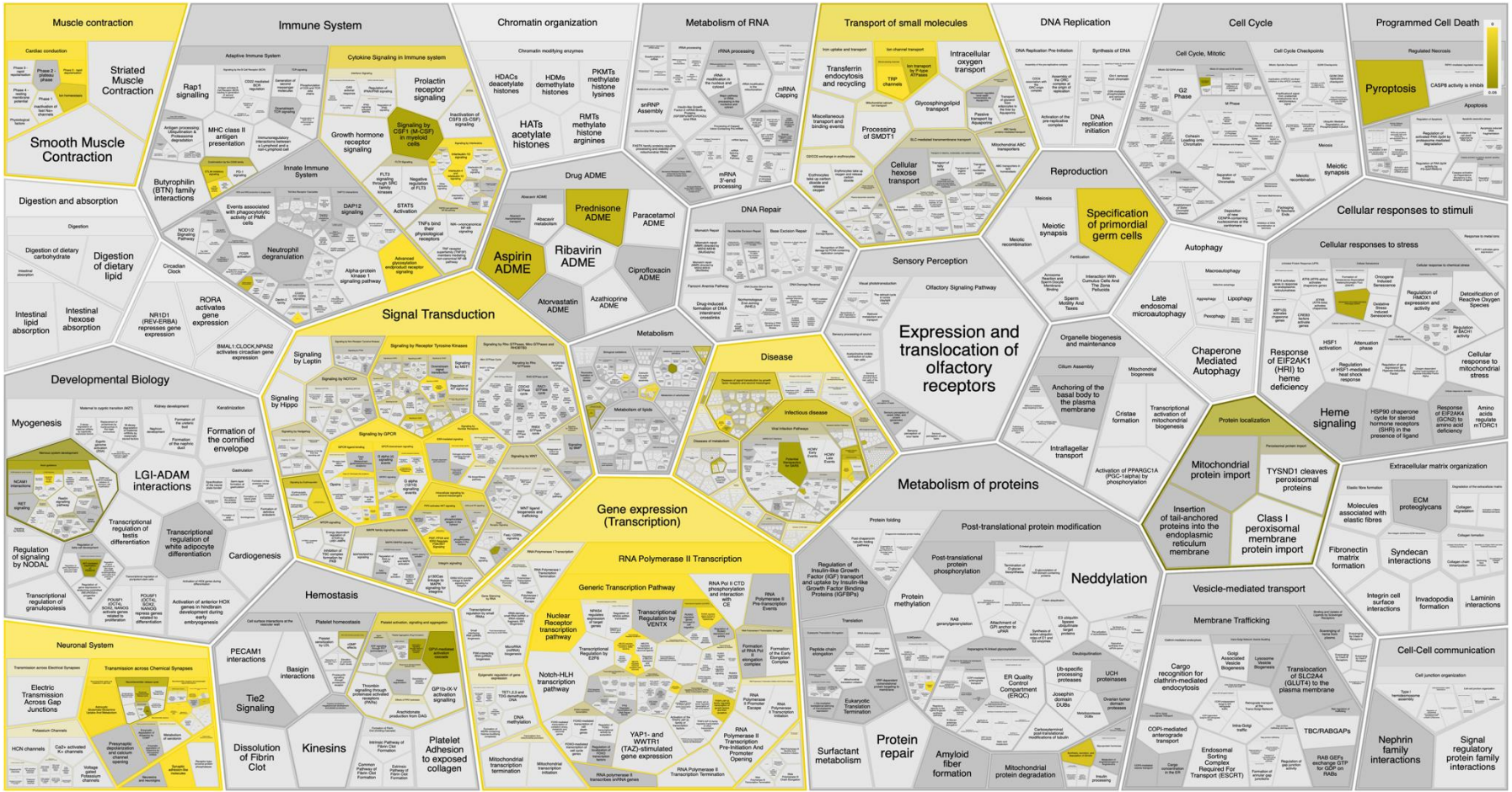



**ShinyGO 0.80**
http://bioinformatics.sdstate.edu/go/




**STRING**
https://string-db.org

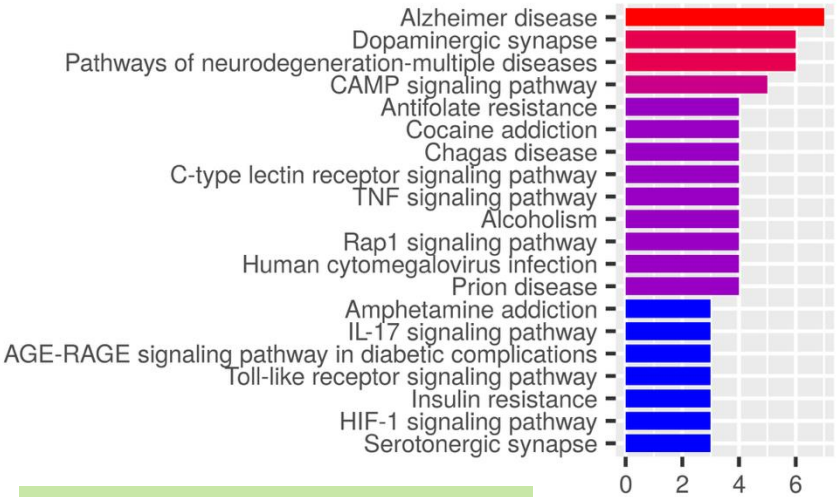
# OT Reacfoam (https://reactome.org/)



# OT-PPI Betweenness Centrality

- Nodes: 20 (Used top 20 genes)
- Edges: 95
- Avg. node degree: 9.5
- Avg. local clustering coefficient: 0.662

**Major KEGG Pathways**

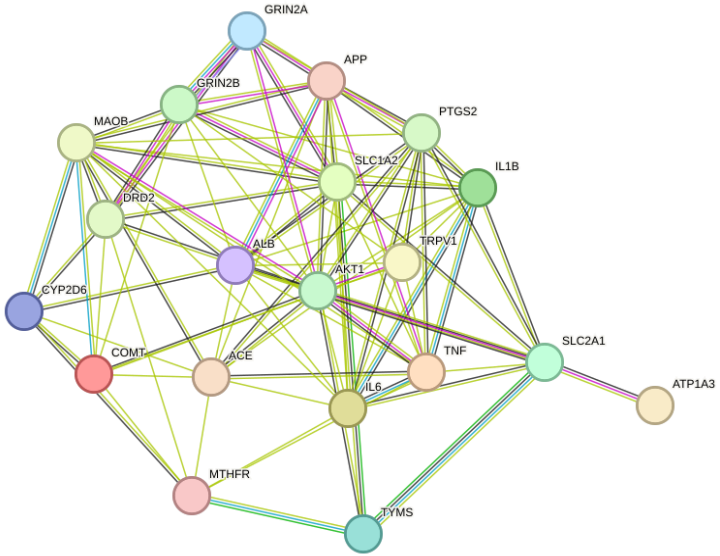



*DA, 5HT, inflammation pathways*

**ShinyGO 0.80**
http://bioinformatics.sdstate.edu/go/

**Top 20 Genes**

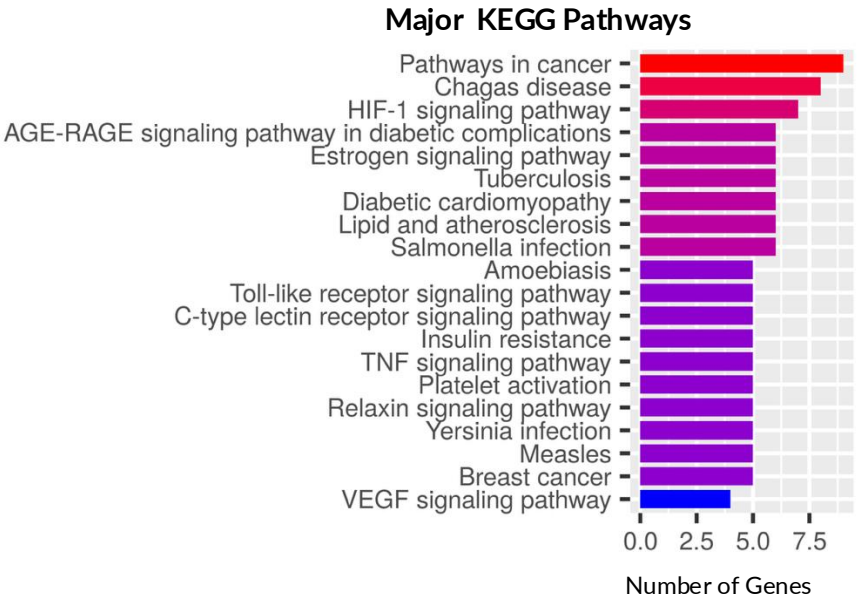
AKT1
ALB
IL6
APP
COMT
GRIN2B
DRD2
MTHFR
TYMS
MAOB
TRPV1
SLC1A2
SLC2A1
TNF
GRIN2A
IL1B
PTGS2
ACE
ATP1A3
CYP2D6




**STRING**
https://string-db.org

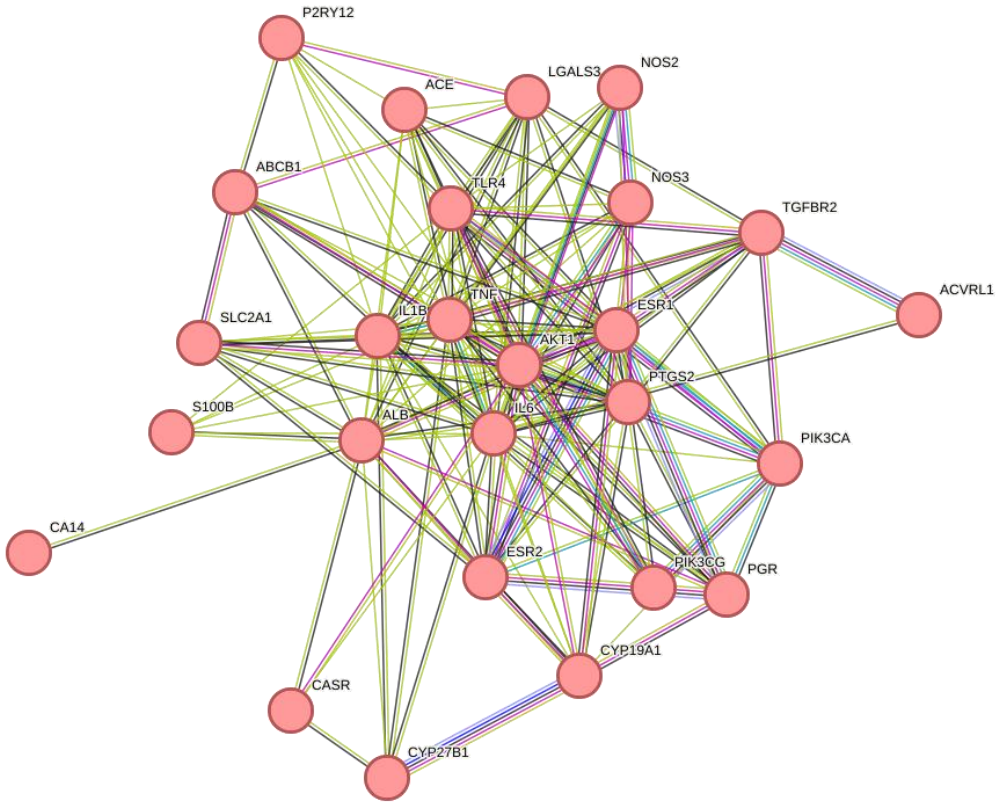
# OT-PPI Cluster Analysis: Cluster 1

**MCL (Markov) Clustering:** Detected 10 Clusters




*Inflammation, Vascular, Estrogen Signaling*

**ShinyGO 0.80**
http://bioinformatics.sdstate.edu/go/

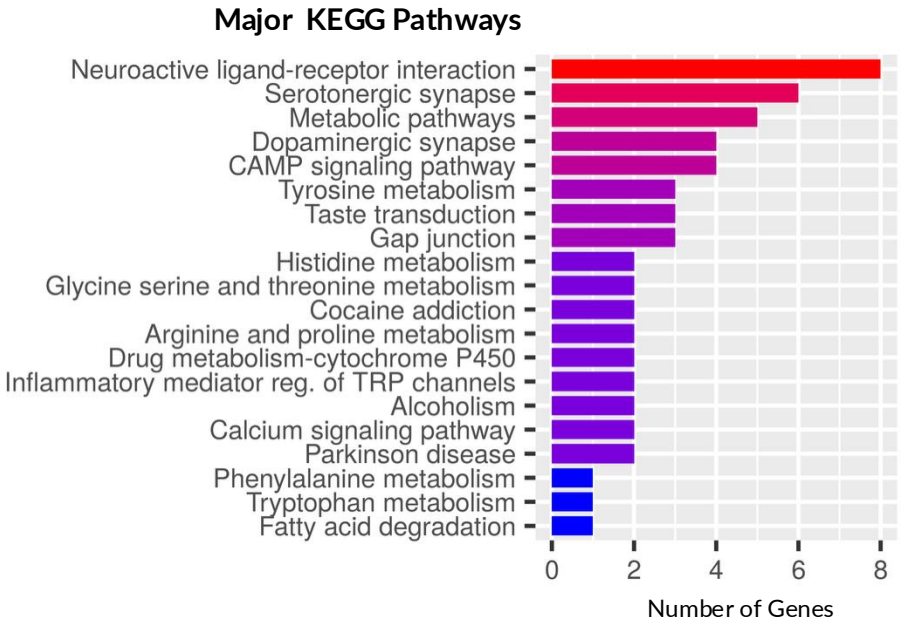


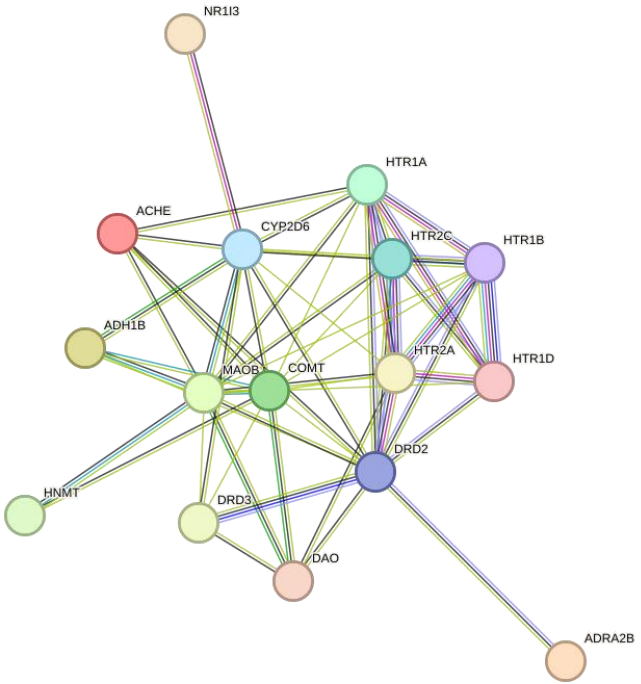

26 nodes | 153 edges

**STRING** https://string-db.org

# OT-PPI Cluster 2

## Based on MCL (Markov) Clustering




*5HT, DA Signaling*

16 nodes | 56 edges

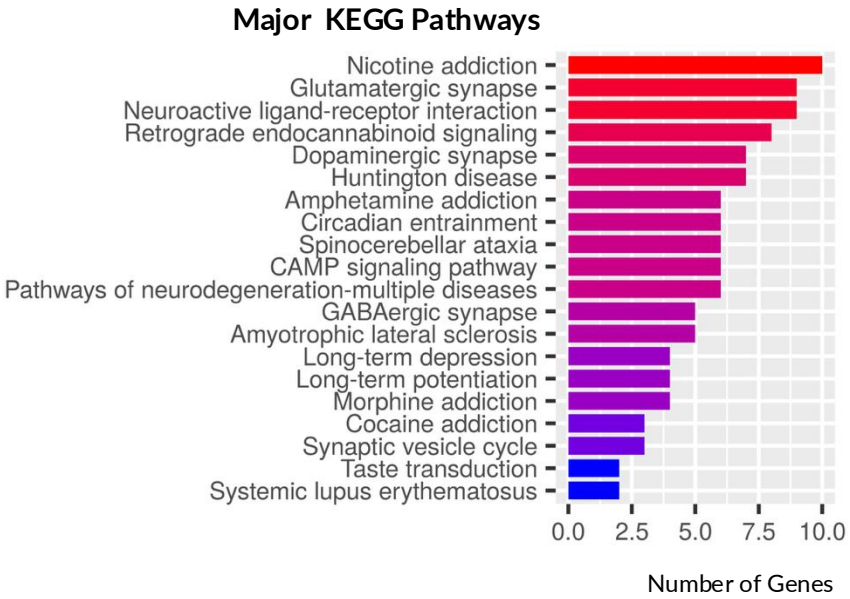
**STRING** https://string-db.org

**ShinyGO 0.80**

http://bioinformatics.sdstate.edu/go/

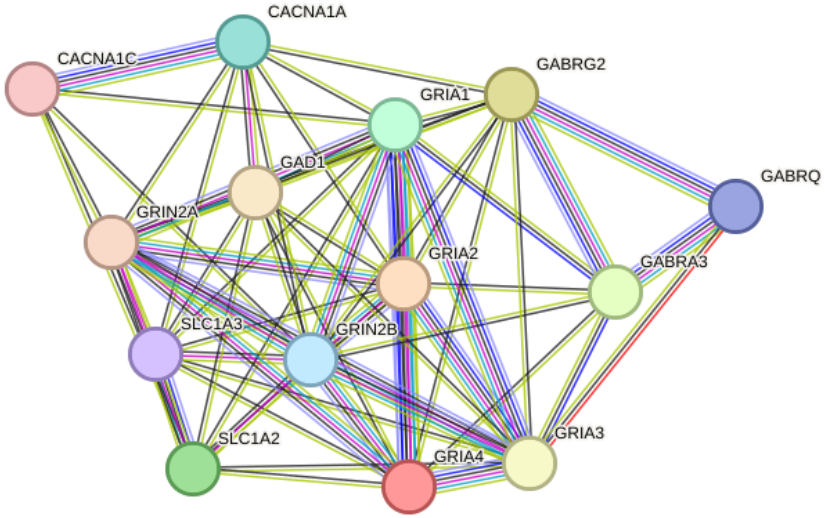
# OT-PPI Cluster 3

## Based on MCL (Markov) Clustering




*GABA, Glu, DA Pathways*

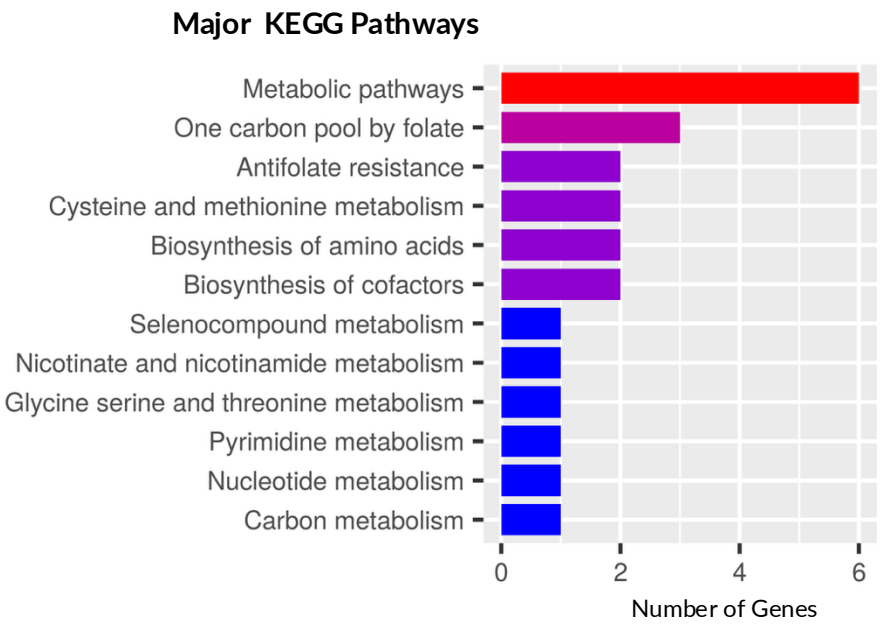
**ShinyGO 0.80**
http://bioinformatics.sdstate.edu/go/

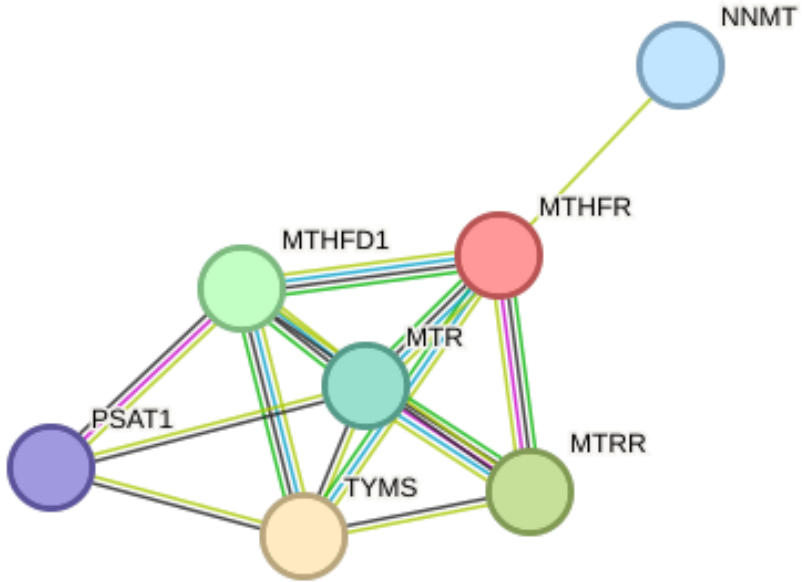



14 nodes | 64 edges
**STRING** https://string-db.org

# OT-PPI Cluster 4

## Based on MCL (Markov) Clustering




*Amino acid, nucleotide, methylation pathways*

**ShinyGO 0.80**
http://bioinformatics.sdstate.edu/go/

7 nodes | 14 edges
**STRING** https://string-db.org

# Conclusions

## Summary

- The 9 most used Chinese herbs for migraine prophylaxis were obtained through a literature review
- Targets for these herbs were obtained using the 'Encyclopedia of Traditional Chinese Medicine (ETCM)' database
- Migraine targets were obtained using the Digenet.org database
- The intersection between these lists were compiled into a PPI network using the STRING database
- The resulting PPI network was subject to network analysis (betweenness centrality, cluster analysis)
- The top 20 KEGG pathways were obtained for each method

## Findings

- A 'Chinese Herbal Network Pharmacology' method can reveal mechanistic insights into biological pathways activated
- Analysis of an herb-disease-target network reveals that commonly used Chinese herbs target inflammatory, neurotransmitter (serotonin, dopamine, GABA, glutamate), endothelial, and estrogen signaling pathways
- Further understanding these pathways could inspire novel research into the effects of Chinese herbal medicines

## Limitations

- Quality of constituent-target information in known databases is suspect
- Lack of oral bioavailability and specific target binding information (up-regulates or inhibits target?)
- Need further research to determine if betweenness centrality and Markov clustering are most useful measures