A retrospective study on professor Gui-Qi Xuan’s experience in traditional Chinese medicine treatment on attention-deficit hyperactivity disorder: based on data mining and network pharmacology

Jue Hu1, Dan-Fei Chen1, Fang-Fang Li1, Nuo Chen1, Chun-Lu Ye1, Ke-Pin Yu1, Xiao-Bo Xuan2, Gui-Qi Xuan3, Jian Chen1*

1First Clinical School, Zhejiang Chinese Medical University, Hangzhou 310053, China. 2Pediatrics Department of Traditional Chinese Medicine, Zhejiang Provincial Hospital of Traditional Chinese Medicine, Hangzhou 310006, China.

E-mail: chenj670@163.com

Abstract
Background: To investigate the clinical medication approach of Professor Guiqi Xuan (Prof. Xuan) in treating pediatric patients with attention-deficit hyperactivity disorder (ADHD) and the potential mechanism of the core herbal prescription. Methods: Following medical record information pretreatment, the Traditional Chinese Medicine (TCM) inheritance computing platform system V3.0 was utilized to analyze the standardized data. The associate rules were summarized to identify the core prescription for treating ADHD. The extracted core herbal prescription’s active compounds and potential targets were used to establish a protein-protein interaction network of active ingredient-disease targets. Cytoscape 3.9.1 software was used to analyze the network’s topological parameters to obtain the key active ingredients and their targets. The Bioconductor data package of R4.0.2 was used to analyze the gene ontology biological functions and Kyoto Encyclopedia of Genes and Genomes pathways of key targets. Results: Two hundred and twenty-seven entries derived from TCM record information were selected. Through data mining, it was found that 62.5% of pediatric patients had short-tempered behavior, nearly half had sleep problems, and 30%–40% had picky eating and polyphagia issues. The highest-frequency syndrome type was kidney deficiency and liver hyperactivity. Deficiency, fire, phlegm, and dyspeptic food were the main pathological factors for ADHD. Prof. Xuan’s treatment of ADHD mainly focused on replenishing kidney essence and subduing Yang (active, external, ascending, warm, bright, functional and excited pertain to Yang). The core herbal prescription for ADHD included Yuan-zhi, Yi-zhi, Gui-jia, Bai-shao, Long-chi, Ci-shi, Shi-chang-pu, Yu-jin, Fu-shen, and Huang-jing. The protein-protein interaction network showed that MAOA, ADRB2, FOS, MAOB, and SLC6A3 were the five key targets essential in treating ADHD with core herbal prescriptions. The gene ontology biological function of crucial targets mainly involved G protein-coupled amine receptor activity, catecholamine binding, and neurotransmitter transmembrane transporter activity. Analysis of Kyoto Encyclopedia of Genes and Genomes pathways showed that the dopaminergic synapse signaling and neuroactive ligand-receptor interaction pathways were significantly enriched and may be the primary routes for the main treatment of ADHD. Conclusion: Prof. Xuan’s treatment of ADHD has achieved satisfactory clinical effects by supplementing the kidney, replenishing the essence, opening the orifices, nourishing the Yin (static, internal, descending, cold, dim, organic, depressed and pertain to Yin), and subduing the Yang. The major prescription predominantly affects catecholamine binding, neuroactive ligand-receptor interaction, G protein-coupled amine receptor function, and signaling pathways for dopaminergic synapses. Our findings showed that the methodology and software used in this research could explore and analyze the mechanism behind Prof. Xuan’s clinical medication rule for treating ADHD in children.

Keywords: Chinese traditional medicine; Data mining; Retrospective clinical studies; Network pharmacology; Attention-deficit hyperactivity disorder; Mechanism of action
Background

According to the DSM-5, attention-deficit hyperactivity disorder (ADHD) is a typical, early-onset, chronic developmental disease affecting children and adolescents. Three main symptoms of ADHD are inattentiveness, impulsivity, and/or motor restlessness, which appear in many contexts and last for at least six months. Beyond what would be anticipated, given the patient’s age, developmental stage, and IQ, these basic symptoms are present [1]. The global epidemiological Prevalence of the DSM-5 is 5.3%, and it is more prevalent in men than in women [1]. According to the research of a national-scale psychiatric epidemiological survey for children and adolescents in China, ADHD was shown to be the most common mental condition, with a prevalence rate as high as 6.4% [2]. At least 75% of people with ADHD also have another mental disorder, and 60% of those people have multiple comorbid mental disorders, including conduct disorder, major depressive disorder, tic disorder, and oppositional defiant disorder, which can make it more difficult to diagnose and treat patients and worsen their prognosis [3, 4]. Inattentiveness has been more pronounced in kids of elementary school age and has worsened as external pressures have grown. A college degree and a lower socioeconomic position are about four times less likely to occur in ADHD youngsters than in their peers [5]. Their likelihood of breaching the law has grown by two to three times [6, 7]. Preschoolers to 13-year-olds had a suicide risk of about six times greater than their peers [8]. Additionally, there is a link between the frequency of suicidality and the severity of ADHD [9]. The primary factor contributing to the 50% increase in Mortality reported among people with ADHD across all age categories is accident propensity, especially with respect to driving accidents [10, 11]. With the increasing incidence of the disease, it has recently become a hot issue that parents and teachers pay attention to [2].

The causes of ADHD are complex and diverse. The exact pathogenesis and mechanism remain unknown, but they may involve significant genetic components and various neurobiological changes [12]. Psychological education, behavioral therapy, and psychoactive medications are the three primary therapeutic modalities [13]. Western drugs, particularly Methylphenidate (MPH) and atomoxetine, are still widely used to treat ADHD and have been shown to be effective in promoting positive long-term outcomes and behavior [14, 15]. However, numerous studies have linked MPH, the preferred medication for ADHD, to a wide range of adverse effects [16]. Despite this, some data suggest that it may reduce appetite, headaches, stomach discomfort, and insomnia, while also causing obsessive-compulsive behavior, melancholy, and anxiety [17–20]. These adverse effects have limited the use of MPH in clinical settings. Therefore, it is not surprising that physicians and parents of children with ADHD have sought alternative, effective, and well-tolerated treatments [21].

Holistic, integrative medicine, which offers an alternative or supplement to traditional pharmaceutical and cognitive-behavioral therapies for ADHD, includes complementary and alternative medicine [22–24]. Complementary and alternative medicine aims to enhance psychological, emotional, and neurobiological functioning and overall treatment outcomes by working in conjunction with other mind-body treatments (such as mindfulness, biofeedback, and yoga). In many countries, Traditional Chinese Medicine (TCM) has been used as an alternative form of medicine for the prevention and treatment of psychiatric disorders, such as schizophrenia, ADHD, Alzheimer’s disease, and Tourette syndrome [25–28].

TCM treatment for ADHD has its unique advantages and characteristics, as evidenced by its clinical total effective rate and hyperactivity index score [29]. XUAN’s Pediatrics, founded in the late Qing Dynasty, is a renowned TCM pediatrics institution in Zhejiang Province with distinctive features. Professor Gui Xu (Prof. Xu), a representative descendant of the third generation of Xuan’s Pediatrics, is a highly respected TCM practitioner and professor at Zhejiang University of TCM. With nearly 60 years of clinical experience, teaching, and scientific research, Prof. Xu has extensive practical expertise and outstanding clinical outcomes, particularly in treating complex nervous system diseases in children, such as Tourette syndrome, ADHD, febrile seizures, epilepsy, and autism. His treatment of ADHD, which involves supplementing the kidney, replenishing the essence, opening the orifices, nourishing the Yin (static, internal, descending, cold, dim, organic, depressed and pertain to Yin), and subduing the Yang (active, external, ascending, warm, bright, functional and excited pertain to Yang), has achieved satisfactory clinical results.

TCM network pharmacology takes a holistic view and considers the complex relationships within the organism, establishing a formula research model based on biological network regulation that goes beyond simple biological indices. TCM’s main features include a holistic view, TCM syndrome differentiation and treatment, and prescription drugs. This study aims to summarize Professor Xuan’s prescription and medication rules in ADHD treatment and analyze their action pathways.

Methods

General data

All medical cases included in this study were from January 2018 to January 2022, and all patients received outpatient care from Prof. Xuan. His pupils meticulously recorded the patient’s name, age, sex, visit date, clinical symptoms, and prior medical history. The necessary examinations, diagnoses, etiology, treatment philosophies, prescriptions, and dosages were also collected. The diagnosis of ADHD was consistent with both Chinese and Western diagnostic criteria, and the information was complete [1, 30]. Exclusion criteria for this study were: (1) patients with comorbid tic disorder, childhood autism, oppositional defiant disorder, and other mental and psychological diseases, or those with apparent organic lesions based on head CT, MRI, and other examinations; (2) children with acute asthma attack, acute respiratory tract infection, acute otitis media, acute gastroenteritis, and other acute diseases requiring treatment; (3) patients who did not meet the standards of traditional Chinese and Western medicine; (4) patients who did not accept standard treatment.

Information gathering

For Professor Xuan’s diagnosis, all data were inputted into the “Microsoft Excel 2019” management system program (Microsoft Corporation, Washington, USA). Excel patterns were generated using treatment-related information, and a medical case database was created. Instances that matched the research requirements were then selected.

During the preprocessing of medical record data, several batches of students who worked with Professor Xuan encountered issues with the regular expression of four diagnostic information and nonstandard expression of pathogenesis due to their varied and comprehensive talents. Therefore, it was necessary to pretreat medical records by: (1) correcting incorrect words and (2) standardizing and unifying vocabulary based on criteria from normative textbooks such as Chinese Medicine Diagnostics, Traditional Chinese Pharmacology, Formulas of Chinese Medicine, and Chinese Internal Medicine [31–34]. For example, “absent-minded in class”, “be in a daze”, and “be careless” were unified as “attention deficiency”, while “kidney essence deficiency”, “kidney essence consumption”, and “kidney essence damage” were unified as “kidney essence deficiency”.

Data mining

The standardized data was processed using the TCM Inheritance Computing Platform System V3.0 (Alliance of Large Varieties of TCM, Beijing, CHN). The “Statistical Analysis - Data Analysis” function was used to calculate the frequency of drug usage, the frequency of the Four Qi (it reflects the body’s effect of inclination of the ups and downs of Yin and Yang and the Semi-interior phase change after the drugs acting on the body), the frequency of the Five Tastes, the frequency of the channel tropism, and the frequency of drug efficacy
categories. The results were then exported. The “Statistical Analysis - Formula Analysis” function was used, with the “support frequency” set to 120 (indicating that the drug combination appears in at least 120 prescriptions, with a minimum support rate of 52.9%, meaning that the probability of both the antecedent and consequent drugs appearing in the drug combination is at least 52.9%). The minimum “confidence degree” was set to 0.95 (indicating that when the antecedent appears in the drug combination, the probability of the consequent also appearing is at least 95%). Setting a lower support count helps to grasp the overall situation of the Chinese medicine prescription, while a higher support count can show the combination of high-frequency drugs. The support count and confidence degree represent the probability of two events occurring. The higher the values, the more significant the positive significance. Pattern analysis was performed using a clustering model with three clusters, including cluster analysis, extracting combinations, and displaying them in a network format.

Pharmacological mechanism analysis
The main components of any Chinese medicine are available on the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), a digital information portal. Through the TCMSP (https://old.tcmsp-e.com/tcmsp.php) and literature search, the active ingredients of each drug in the core prescription were integrated. The relevant target information of the active components was analyzed using the drug-likeliness (DL) ≥ 0.18 and oral bioavailability (OB) ≥ 30% criteria.

The protein targets of ADHD were found using the term “attention-deficit hyperactivity disorder” in the Gene database. After selecting these targets as disease candidates, we looked for the gene names of the protein targets in the UniProt database. Wayne’s analysis was performed using Venny 2.1 software. The screened active ingredient targets were then cross-linked with disease-related targets, and the information of potential targets worth duplicating to ADHD was deleted. Cytoscape 3.9.1 software was used to construct “Chinese medicine - ingredient - common target” and “common target interaction” network diagrams. The crossover genes of drugs and diseases were uploaded to the String protein interaction database (https://cn.string-db.org/) to obtain protein-protein interaction (PPI) network TSV files. After exporting the complete node relationship information, Cytoscape 3.9.1 was used to build a target-target PPI network, and the primary objectives were eliminated in order of degree. To identify key active ingredients and their targets, the network’s topological parameters were analyzed with Cytoscape 3.9.1. The Bioconductor data package of R4.0.2 was used to analyze key targets’ gene ontology (GO) biological function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. The P-value was set as 0.05 according to statistical significance, and the results were outputted by drawing bar plot histograms. Finally, the results were analyzed to discuss the therapeutic mechanism of core prescriptions for ADHD.

Results
General information statistics of medical records
The 88 patients in this study were classified by gender, with 79 boys (89.77%) and 9 girls (10.23%). The male to female ratio was approximately 8.76:1. The age distribution was wide but mostly concentrated in the 7-10-year-old range, accounting for approximately 77.27% of the total (Figure 1). The frequency of children under the age of 6 and those over 12 years old was lower, suggesting that the main patient group was school-age children.

Frequency distribution of four diagnostic information
In the TCMS system data, a total of 154 symptoms were observed in 88 ADHD patients. More than 33.7% of the patients exhibited symptoms such as hyperactivity, attention deficiency, short temper, restless sleep, underachievement, picky eating, indigestion, and overeating. During 227 subsequent visits, tongue and pulse data analysis revealed that the most frequently occurring symptom was a reddened tongue (214), followed by a fine rapid and string-like pulse (181) and a thin white coating (163). The high-frequency symptoms, including thin white coating, fine rapid pulse, thin white greasy coating, and thin yellow tongue coating, were all at the forefront of the symptom frequency chart (refer to Figure 2 and Figure 3 for details).

Frequency distribution of syndrome type
According to the frequency distribution of syndrome types (refer to Figure 4), the most common symptom type, accounting for 58.15% of all symptom types, was kidney deficiency and liver hyperactivity.

Figure 1 Statistical chart of age distribution

Figure 2 Statistical chart of tongue and pulse information

Figure 3 Statistical chart of main symptoms

Figure 4 Statistical chart of pathological factors’ frequency distribution
Spleen deficiency and liver hyperactivity, deficiency of liver-kidney Yin, insufficiency of the liver and kidney, and yin deficiency with efflulent fire accounted for 14.54%, 7.93%, 6.17%, and 4.47%, respectively. More than 90% of the patients exhibited “deficiency” symptoms on a regular basis. This result indicated that the primary pathological causes of ADHD were deficiencies, fire, phlegm, and a dyspeptic diet, which was consistent with the frequency distribution of syndromes.

**Herbs’ usage frequency and effect**
Among the 115 herbs, the top ten high-frequency herbs were Yi-zhi, Gui-jia, Bai-shao, Long-chi, Duan-ci-shi, Shi-chang-pu, Yuan-zhi, Yu-jin, Fu-shen, and Huang-jing. Table 1 displays the top 46 high-frequency herbs. The focus was mainly on nourishing the kidney and opening the orifices, as well as nourishing the Yin and submerging the Yang. Gui-jia, Long-chi, Shi-chang-pu, and Yuan-zhi were part of the “Sagely Confucius Pillow Elixir” Simiao Essential Prescriptions Worth a Thousand Gold for Emergencies (Si-Mao Sun, 541–682 C.E.), which can regulate the DA neural signal transduction of the prefrontal cortex- striatal circuit in the brain [35]. Zou Wengjing’s clinical randomized controlled study suggested that the efficacy of supplemented “Sagely Confucius Pillow Elixir” in treating children with ADHD was better than that of Western medicine (P < 0.01) [36].

**The effect, property, taste and meridian distribution of herbs**
The statistical data indicated that tonifying medicine was the most commonly used, which was consistent with the pathological factors of kidney deficiency (Figure 5). Among the four property attributes, cold herbs were the most frequently used, accounting for 42.20%. Cold herbs can clear away heat and nourish Yin. Previous research has suggested that cold herbs can increase the 5-HT content, inhibit tyrosine hydroxylase, and reduce the synthesis of NA and DA in the brains of rats, showing a state of central inhibition [37, 38]. The kidney and heart meridians of herbs accounted for 22.70% and 20.16%, respectively. Additionally, bitter and sweet were the most commonly used among the five flavor attributes, accounting for 28.62% and 27.67%, respectively (Figure 6). According to the theory of five flavors, sweet herbs can invigorate the kidney and spleen, nourish the heart, and soothe the nerves to relieve the symptoms of hyperactivity, restlessness, and inattentiveness in children. Bitter herbs can purify fire and strengthen Yin, dry dampness and strengthen the spleen, clear the fire of the heart and liver to relieve the symptoms of restlessness and impulsiveness in children.

<table>
<thead>
<tr>
<th>No.</th>
<th>Herbs</th>
<th>Frequency</th>
<th>Percentage</th>
<th>No.</th>
<th>Herbs</th>
<th>Frequency</th>
<th>Percentage</th>
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<td>1</td>
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<td>Da-zao</td>
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<td>25</td>
<td>Gan-cao</td>
<td>24</td>
<td>10.57%</td>
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<td>3</td>
<td>Gui-jia</td>
<td>219</td>
<td>96.48%</td>
<td>26</td>
<td>Huai-xiao-mai</td>
<td>23</td>
<td>10.13%</td>
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<td>4</td>
<td>Bai-shao</td>
<td>219</td>
<td>96.48%</td>
<td>27</td>
<td>Bin-lang</td>
<td>21</td>
<td>9.25%</td>
</tr>
<tr>
<td>5</td>
<td>Long-chi</td>
<td>216</td>
<td>95.15%</td>
<td>28</td>
<td>Mai-dong</td>
<td>20</td>
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</tr>
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<td>29</td>
<td>Sheng-shan-zha</td>
<td>20</td>
<td>8.81%</td>
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<tr>
<td>7</td>
<td>Shi-chang-pu</td>
<td>213</td>
<td>93.83%</td>
<td>30</td>
<td>Di-gu-pi</td>
<td>19</td>
<td>8.37%</td>
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<td>8</td>
<td>Yu-jin</td>
<td>180</td>
<td>79.30%</td>
<td>31</td>
<td>Shu-di-huang</td>
<td>19</td>
<td>8.37%</td>
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<td>9</td>
<td>Fu-shen</td>
<td>154</td>
<td>67.84%</td>
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<td>Sheng-di-huang</td>
<td>18</td>
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<td>10</td>
<td>Huang-jing</td>
<td>123</td>
<td>54.19%</td>
<td>33</td>
<td>Bai-zhu</td>
<td>18</td>
<td>7.93%</td>
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<td>11</td>
<td>Bai-zi-ren</td>
<td>117</td>
<td>51.54%</td>
<td>34</td>
<td>Mu-li</td>
<td>18</td>
<td>7.93%</td>
</tr>
<tr>
<td>12</td>
<td>Dan-shen</td>
<td>111</td>
<td>48.90%</td>
<td>35</td>
<td>Bai-he</td>
<td>17</td>
<td>7.49%</td>
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<td>13</td>
<td>Gou-qi-zhi</td>
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<td>14</td>
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<td>87</td>
<td>38.33%</td>
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<td>Ban-xia</td>
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<td>Zhi-ke</td>
<td>65</td>
<td>28.63%</td>
<td>38</td>
<td>Tai-zhi-shen</td>
<td>16</td>
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<td>16</td>
<td>Sang-shen</td>
<td>63</td>
<td>27.75%</td>
<td>39</td>
<td>Xia-ku-cao</td>
<td>16</td>
<td>7.05%</td>
</tr>
<tr>
<td>17</td>
<td>Ji-nei-jin</td>
<td>62</td>
<td>27.31%</td>
<td>40</td>
<td>Gua-lou-pi</td>
<td>16</td>
<td>7.05%</td>
</tr>
<tr>
<td>18</td>
<td>He-shou-wu</td>
<td>57</td>
<td>25.11%</td>
<td>41</td>
<td>Dan-dan-xing</td>
<td>14</td>
<td>6.17%</td>
</tr>
<tr>
<td>19</td>
<td>Suan-zao-ren</td>
<td>50</td>
<td>22.03%</td>
<td>42</td>
<td>Lu-dou-yi</td>
<td>13</td>
<td>5.73%</td>
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<tr>
<td>20</td>
<td>Dang-gui</td>
<td>48</td>
<td>21.15%</td>
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<td>Sang-Piao-xiao</td>
<td>13</td>
<td>5.73%</td>
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<tr>
<td>21</td>
<td>Wu-wei-zi</td>
<td>36</td>
<td>15.86%</td>
<td>44</td>
<td>Shan-yao</td>
<td>11</td>
<td>4.85%</td>
</tr>
<tr>
<td>22</td>
<td>Fu-ling</td>
<td>33</td>
<td>14.54%</td>
<td>45</td>
<td>Ba-ji-tian</td>
<td>11</td>
<td>4.85%</td>
</tr>
<tr>
<td>23</td>
<td>Chai-hu</td>
<td>28</td>
<td>12.33%</td>
<td>46</td>
<td>Mai-ya</td>
<td>11</td>
<td>4.85%</td>
</tr>
</tbody>
</table>
Cluster analysis and association rules analysis

The K-means algorithm and regression model cluster analysis were used to set the number of clusters to three, resulting in the extraction of three core combinations (Table 2 and Figure 7). For association rule analysis, the supportive degree number was set to 120 and the confidence degree to 0.95, resulting in the extraction of 481 pieces of data, with the top 10 herb combinations with frequencies ≥ 210 displayed in Table 3. Table 4 displays the top 10 pieces of data with a confidence degree of 1. Finally, the core prescriptions obtained from the network topology of TCM were listed in Figure 8.

Based on Prof. Xuan's clinical medication experience and the above analysis results, we referred to the core herbal prescription, which includes Yuan-zhi, Yi-zhi, Gui-jia, Bai-shao, Long-chi, Ci-shi, Shi-chang-pu, Yu-jin, Fu-shen, and Huang-jing, as the intersection of the top ten high-frequency herbs and the core combinations of the cluster and association rules analysis (Figure 9).

Pharmacological mechanisms of core herbal prescription

By filtering the pharmacokinetic characteristics of OB ≥ 30% and DL ≥ 0.18 from TCMSp, we further retrieved the constituents of the ten herbs utilised in the core herbal prescription to examine the pharmacological mechanisms of these herbs. After removing duplicate values, we obtained 21 active ingredients. The TCMSp database provided us with 538 pharmacological targets for these ten herbs, which includes a targeted gene list of 21 for ADHD, such as GABRA1, NR3C2, CHRMI, ADRA1A, ADRA1B, ADRB2, SLC6A4, OPRM1, ADH1C, ADRB1, MAOB, AR, FOS, MMP9, IGF2, GSK3B, MTOR, SLC6A2, ADRA2A, SLC6A3, and MAOA (Figure 10).

Using Cytoscape 3.9.1 software, we created a network diagram based on the active ingredient information and corresponding target information to establish the core action network for herbal ingredients, targets, and diseases. According to the network topology study, the network had 169 network edges and 41 network nodes.

Figure 5 Effect of high-frequency herbs

Figure 6 Property, taste, and meridian distribution of herbs. (A) Radar map of nature of herbs of 227 clinical case prescriptions; (B) Radar map of five flavors of 227 clinical case prescriptions; (C) Radar map of meridien entry of 227 clinical case prescriptions.

<table>
<thead>
<tr>
<th>No.</th>
<th>Core Combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yuan-zhi, Gui-jia</td>
</tr>
<tr>
<td>2</td>
<td>Shi-chang-pu, Bai-shao, Yuan-zhi, Yi-zhi, Long-chi</td>
</tr>
<tr>
<td>3</td>
<td>Yi-zhi, Yuan-zhi, Ci-shi, Bai-shao, Gui-jia</td>
</tr>
</tbody>
</table>

Table 2 Core combinations of cluster analysis

<table>
<thead>
<tr>
<th>No.</th>
<th>Core Combinations</th>
<th>Frequency</th>
<th>Support degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yuan-zhi, Yi-zhi</td>
<td>220</td>
<td>96.92%</td>
</tr>
<tr>
<td>2</td>
<td>Yuan-zhi, Gui-jia</td>
<td>217</td>
<td>95.59%</td>
</tr>
<tr>
<td>3</td>
<td>Yuan-zhi, Ci-shi</td>
<td>215</td>
<td>94.71%</td>
</tr>
<tr>
<td>4</td>
<td>Yuan-zhi, Bai-shao</td>
<td>215</td>
<td>94.71%</td>
</tr>
<tr>
<td>5</td>
<td>Yi-zhi, Gui-jia</td>
<td>214</td>
<td>94.27%</td>
</tr>
<tr>
<td>6</td>
<td>Yuan-zhi, Yi-zhi, Gui-jia</td>
<td>214</td>
<td>94.27%</td>
</tr>
<tr>
<td>7</td>
<td>Yuan-zhi, Long-chi</td>
<td>214</td>
<td>94.27%</td>
</tr>
<tr>
<td>8</td>
<td>Yi-zhi, Bai-shao</td>
<td>213</td>
<td>93.83%</td>
</tr>
<tr>
<td>9</td>
<td>Yi-zhi, Ci-shi</td>
<td>213</td>
<td>93.83%</td>
</tr>
<tr>
<td>10</td>
<td>Gui-jia, Bai-shao</td>
<td>213</td>
<td>93.83%</td>
</tr>
</tbody>
</table>

(Note: support frequency ≥ 213, confidence degree = 0.95)
Table 4 Top ten association rule analyses

<table>
<thead>
<tr>
<th>No.</th>
<th>Association rules</th>
<th>Confidence degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gui-jia, Yu-jin, Fu-shen → Shi-chang-pu</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Ci-shi, Shi-chang-pu, Fu-shen → Yuan-zhi</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Long-chi, Ci-shi, Fu-shen → Yuan-zhi</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Yi-zhi, Ci-shi, Yu-jin, Fu-shen → Shi-chang-pu</td>
<td>1</td>
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<td>5</td>
<td>Long-chi, Ci-shi, Shi-chang-pu, Yu-jin, Fu-shen → Yuan-zhi</td>
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<td>6</td>
<td>Yuan-zhi, Long-chi, Ci-shi, Yu-jin, Fu-shen → Shi-chang-pu</td>
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<td>7</td>
<td>Yi-zhi, Huang-jing → Yuan-zhi</td>
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<td>8</td>
<td>Bai-shao, Long-chi, Shi-chang-pu, Yu-jin, Fu-shen → Yuan-zhi</td>
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<td>9</td>
<td>Yuan-zhi, Bai-shao, Long-chi, Yu-jin, Fu-shen → Shi-chang-pu</td>
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<tr>
<td>10</td>
<td>Ci-shi, Yu-jin, Fu-shen → Yuan-zhi</td>
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Figure 7 Prescription cluster analysis diagram (K-means algorithm and regression model cluster analysis). (A) Cluster analysis chart of 227 clinical case prescriptions (K-means algorithm + clustering); (B) Cluster analysis chart of 227 clinical case prescriptions (K-means algorithm + regression simulation).

Figure 8 Network topology of core prescriptions (support frequency = 120, confidence degree = 0.95)
with a network density of 0.117 and a network concentration of 0.429. The typical route length was 2.276, and the network heterogeneity was 0.882. The disorders, core prescription, targets, and substances were changed in size, shape, and color, and the target-interaction connection was shown (Figure 11). The top 10 active ingredients of the drug against the disease are beta-sitosterol, Stigmasterol, kaempferol, (2R)-7-hydroxy-2-(4-hydroxyphenyl) chroman-4-one, baicalein, sitosterol, Frutinone A, 8-Isopentenyl-kaempferol, DFV, and 4',5-Dihydroxyflavone. The top 5 targets are GABRA1, ADRB2, NR3C2, AR, and ADRA1B.

It was decided to incorporate the common targets of ADHD medications and the String database. To gather information about protein interactions, the multiple protein tool was deployed, with Homo sapiens as the limiting condition. The final model contains a total of 31 nodes, 244 edges, and a degree of 15.7 for each node in the network. The local clustering coefficient was found to be 0.754 on average. Figure 12 demonstrates that the whole collection of data on the relationships between nodes was first exported and then imported into Cytoscape. Based on the visualization processing degree value, the primary genes that comprised the PPI network were disregarded. Figure 13 depicts the top five nodes in terms of degree values, which are labelled as follows: MAOA, ADRB2, FOS, MAOB, and SLC6A3. These targets may be highly significant in the treatment of ADHD with conventional medications.

We subjected the 21 common targets of core prescription and ADHD to enrichment analysis using the Bioconductor data package of R4.0.2. The GO functions of the core target genes of core prescriptions in the treatment of OP mainly involve G protein-coupled amine receptor activity, catecholamine binding, neurotransmitter transmembrane transporter activity, and neurotransmitter receptor activity involved in the regulation of postsynaptic membrane potential. Excel was used to select the top 20 of these for visualization processing (Figure 14).

The KEGG pathway study produced 28 pathways, including Neuroactive ligand-receptor interaction, Dopaminergic synapse, Amphetamine addiction, cGMP-PKG signaling pathway, IL-17 signaling pathway, cAMP signaling pathway, and Serotonergic synapse. Figure 15 displays the top 20 KEGG signal enrichment analysis results. Figure 16 shows a detailed diagram of the core prescription for treating ADHD through the top two key signaling pathways.

Discussion

It has been suggested that the primary approach to developing TCM theory is through researching the academic philosophy and clinical experience of Chinese doctors, which is reflected in the treatment of numerous medical cases. Conducting multi-level and in-depth research is an effective measure to inherit and innovate TCM’s academic thought and clinical experience. This research outlines the clinical experience of Prof. Xuan in treating ADHD from the perspectives of syndrome, pathophysiology, medication, and likely mechanism of the core prescription. Data mining revealed that
Figure 11 Network of herbal ingredient-target-illness. (A) Disease in red, drugs in yellow, pink represents the active ingredients of drugs and green represents the common targets for drugs and diseases; (B) The larger the circular area and the darker the color of the node, the higher its degree value and the more critical it is in the network. ADHD, attention-deficit hyperactivity disorder.

Figure 12 Interaction diagram between herbs and potential targets of ADHD. ADHD, attention-deficit hyperactivity disorder.

Figure 13 Core protein histogram
Figure 14 Barplot histogram of the signaling pathway of key target enrichment of core prescription in the treatment of ADHD. ADHD, attention-deficit hyperactivity disorder.

Figure 15 Barplot histogram of the signaling pathway of key target enrichment in the treatment of ADHD. ADHD, attention-deficit hyperactivity disorder.
Figure 16 Neuroactive ligand-receptor interaction and Dopaminergic synapse signaling pathways of core prescription in the treatment of ADHD (Red represents related genes or proteins). (A) Schematic diagram of the Neuroactive ligand-receptor interaction pathway of core prescription for ADHD; (B) Schematic diagram of the Dopaminergic synapse pathway of core prescription for ADHD. ADHD, attention-deficit hyperactivity disorder.
deficiency, fire, phlegm, and dyspeptic food were the main pathological factors for ADHD. The syndrome of ADHD is complex and variable, with kidney essence deficiency being the fundamental internal etiology of the disease, which belongs to root vacuity and tip repletion with an imbalance of Yin and Yang. The main pathogenesis is kidney-essence deficiency and water failing to nourish wood, leading to hyperactivity of liver yang. Impulsiveness, hyperkinesia, inattention, lack of self-control, reddened eyes, thin white coating, and fine rapid string-like pulse are all manifestations of liver-yang hyperactivity and kidney-yin deficiency. According to TCM philosophy, the kidney produces marrow, which comes from essence, is collected up, and finally fed to the brain. The brain is associated with thinking, memory, and consciousness and is referred to as “the sea of marrow” and “the abode of the spirit”. Therefore, a lack of renal essence might result in lessened brain marrow debilitation, which would then cause attention impairments. Li and colleagues examined the TCM syndrome features in 170 ADHD youngsters and discovered that the most prevalent symptom was “deficiency in renal essence and marrow in the brain” [39]. Ma and colleagues initially proposed the theory that a maturational delay in the formation of brain marrow may be associated with ADHD [40]. They suggested that the pathogenic processes of ADHD were renal essence deficit, marrow maturational delay, and Yin and Yang imbalance. This idea is in line with the concept that the pathophysiology of ADHD involves the maturation lag of cortical regions [41]. Furthermore, congenital conditions such as dystonia, low birth weight, injuries, and premature labor are significant risk factors for kidney essence deficiency. These conditions can also cause blood flow to decrease and blood stasis, which may contribute to insufficient cerebral blood flow associated with ADHD. Nearly half of the 88 children who visited the clinic had sleep problems, and 30%–40% had issues with picky eating and polyphagia. Research by Denise Bijlenga found that ADHD was highly associated with delayed sleep phase disorder, indicating a delayed circadian rhythm as an underlying mechanism [42]. Dyspeptic food cannot be neglected as a factor for ADHD. The Toddler Description (Youkefahui 1549 AD) states, “Children are often deficient in the spleen, not comparable to adults, young and ignorant and greedy in their appetites”. An improper diet may harm the spleen and stomach, impair their ability to transport and change substances, and cause a buildup of moisture, phlegm, and heat that can exacerbate clinical symptoms. An increasing number of studies have linked eating disorders, particularly bulimia nervosa, to ADHD [43]. Reinblatt et al. (adjusted OR = 12.68) found that loss of control eating was much more common in children with ADHD [44]. Dopamine and noradrenergic circuits in the brain, which are involved in processing rewards, are thought to be the cause of ADHD. This explains why drug addiction problems are so prevalent in individuals with ADHD [45]. It is probable that loss of control sufferers have impulse control issues similar to those observed in ADHD.

This study gathered 227 prescriptions, which included 115 Chinese herbs. Prof. Xuan’s approach to treating ADHD primarily focuses on supplementing the kidney and replenishing the essence, opening the orifices, nourishing the Yin, and subduing the Yang. Additionally, it involves methods such as resolving phlegm, clearing heat, and removing blood stasis, while also soothing the nerves and nurturing the mind. Kidney tonifying is categorized into tonifying kidney Yin, kidney Qi, and kidney Yang. Children with kidney Yin deficiency are more prone to suffer as they tend to have excess Yang and lack Yin. Huang-jing, Sheng-di, Zhi-shou-wu, Yu-rou, Gui-ban, and Sang-shen are the typical medicines used for tonifying kidney Yin. On the other hand, Ba-jì-tian, Bu-gu-zhi, Du-zhong, and Rou-cou-rong are the common medicines used for tonifying kidney Yang. Clinically, Huang-jing and Gou-qì are usually applied to invigorate kidney Qi.

In the core prescription, Huang-jing is abundant in nonstarch polysaccharides, oligosaccharides, steroids, triterpenoid saponins, flavonoids, and other nutritional and functional components [46]. Prof. Xuan has suggested that Huang-jing not only nourishes the kidney and replenishes the essence but also has a slight effect of invigorating the spleen Qi. Children with excess Yang should not blindly use Qi tonifying herbs such as Dang-shen. Yi-zi is acrid and pungent, enters the kidney meridian, invigorates the kidney, solidifies the essence, opens the orifices, and calms the mind. It has been demonstrated that chemical components such as protocatechuic acid, nootropic ketone B, and chrysin in Yizhihun have good neuroprotective functions, which can enhance cognitive function and memory [47]. Gui-jia and Long-chi are both spiritual objects. One can enter the kidneys and calm the mind, while the other enters the liver and soothes the soul. When combined with magnets, they can benefit the kidneys, calm the liver, and subdue Yang to soothe the mind and treat hyperactivity. Li Caixia et al. discovered the molecular mechanism of Gui-jia extracts inducing neural stem cells to differentiate into neuronal cells, providing a theoretical basis for modern medicine for TCM to strengthen the kidney and strengthen the brain. Yuan-zhi returns to the heart and kidney meridian, soothes the nerves, and nourishes the mind [48]. It is used for its antipsychotic, sedative, cognitive-improving, neuroprotective, and antidepressant effects [49]. When combined with Yu-jin and Shi-chang-pu to regulate Qi, dispel phlegm, and open the orifices, it can use movement to control movement and combine movement and stillness. Ni Xiqiang and others found that the Yuanzhi-Shichangpu drug pair could reduce the spontaneous activity and impulsive behavior of ADHD model rats [50]. The mechanism may be related to Yuanzhi-Shichangpu increasing the dopamine content in the prefrontal cortex of ADHD mice, decreasing dopamine metabolism, increasing blood, and astringing Yin, calming liver Yang. Fu-shen is sweet and light in taste, which is good at infiltrating and draining water and dampness. By combining various herbs, kidney essence is nourished, Yin is replenished, Yang is subdued, the mind is refreshed, and the orifices are opened.

This study investigated the active ingredients and targets of TCM’s primary herbal treatments for ADHD using network pharmacology. The study thoroughly examined the intervention’s impact and the core medication’s mechanism on ADHD. In the “component-target-disease” network analysis, the top 5 targets were identified as GABRA1, ADRB2, NR3C2, AR, and ADRAlB. GABRA1 encodes the gamma-aminobutyric acid (GABA) receptor, which plays a role in inhibiting the reception of neuronal signals. Disruption of GABAergic pathways can lead to various central nervous system disorders. It was discovered that mice with GABA transporter 1 knocked out exhibited features such as excitability, hyperactivity, and impaired learning and memory abilities, similar to those of childhood ADHD [51]. Shuliu et al. Identified ADRB2 mRNA as a fat mass and obesity-associated gene (FTO) target [52]. According to one case report, FTO overexpression (16q11.2-16q13 duplication) may create an ADHD phenotype linked with obesity and mental retardation [53]. FTO in the brain has been shown to influence postnatal mouse growth, dopaminergic midbrain circuitry activity, memory processes in the prefrontal cortex and hippocampus, brain development, adult neurogenesis, and axonal regeneration [54-57]. The salt corticosteroid receptor gene NR3C2 is involved in triggering stress responses, and its genetic polymorphisms have a greater association with violent aggression, which may correlate with ADHD impulsivity and oppositional defiance [58]. The predominance of ADHD disruptive behavior disorders in males can be explained by the involvement of X-linked genes or genes related to androgen metabolism, while AR are both. AR acts as a transcription factor regulating euchromatic gene expression and plays an important role in neurological, reproductive, cardiovascular, immune, and axonal developmental and homeostatic maintenance. D E Comings et al. found that AR haploinsufficiency was significantly associated with ADHD (P < 0.0001) [59]. ADRA1B is one of the α-adrenergic receptors, which is closely related to neurotransmitter transmission, and Hawi et al. reported that ADRA1B gene polymorphism loci are associated with attention deficit hyperactivity disorder [60]. Based on PPI network analysis, the top five targets in terms of value were identified as MAOA, ADRB2, FOS, MAOB, and MLG3A3. These five key targets may be crucial when using a basic herbal prescription to treat ADHD. Monoamine oxidases A and B (MAO-A and MAO-B) regulate the levels of neurotransmitters in the central nervous system.
by decomposing biogenic amines such as dopamine and serotonin. MAO-A is an enzyme located on the outer mitochondrial membrane and encoded by the X-linked MAO-A gene. It regulates the homeostasis of key monoamine neurotransmitters (including serotonin, dopamine, epinephrine, and norepinephrine), thereby affecting human mood and behavior. According to Katharina Domschke, the greater activity variation of monoamine oxidase A (941G) may be a risk factor in the development of ADHD, which is consistent with the hypodopaminergic and/or hyposerotonergic etiology of the illness [61]. The dopaminergic, serotonergic, and noradrenergic systems have been implicated in the development of ADHD, according to pharmacological and genetic studies. Studies have shown that the pathological mechanisms of ADHD involve the DA, 5-HT, and NE systems. Functional imaging, genetic, and biochemical studies have shown that children with ADHD have a state of hypodopaminergic function, and MAOA knockout mice show increased aggressive behavior accompanied by increased levels of DA, 5-HT, and NE in the brain [62]. Compared to MAO-A, MAO-B may have a smaller impact on ADHD. Research results have shown no significant difference in mRNA expression levels of single MAO-B gene in the blood cells of children with ADHD and healthy children [63]. This suggests that the function of the MAO-B gene may not be directly related to attention deficit hyperactivity disorder, and more research and exploration are still needed in the future. ADRB2 has been described above. C-fos is a normal gene found in human or animal cells that belongs to the immediate-early response gene family. As a nuclear protein transcription factor, FOS is required for cell growth, division, proliferation, differentiation, and even programmed death [64]. The expression of c-fos and fosB regulates neuroplasticity in the developing brain by modulating downstream genes such as substance P, dynorphin, and cdk5 [65]. C-fos silencing resulted in behavioral changes such as learning impairment, hyperactivity, and aberrant sexual behavior [66]. SLC6A3/DAT is a soluble transporter that can reuptake Dopamine from the synaptic cleft back to pre- and postsynaptic neurons, thereby terminating the activity of dopaminergic neurotransmitters. Mice knocked out of the DAT gene are hyperactive in behavior, can adapt to a new environment for a longer time, and show memory deficits in an eight-arm maze model that detects spatial memory [67]. SLC6A3 is increasingly recognized as a risk factor with a growing number of familial mutants associated with neuropsychiatric and neurological disorders, including ADHD (low activity variant) [68]. The biological functions of the key targets in GO mainly involve G protein-coupled amine receptor activity, sodium-chloride symporter activity, catecholamine binding, neurotransmitter transmembrane transporter activity, and neurotransmitter receptor activity involved in the regulation of postsynaptic membrane potential, among others. The core prescription may treat ADHD by regulating neurotransmitter activity, participating in the transcription of amino acid sequences, activating the Wnt signaling pathway, regulating transporter activity, and lipidation modification. KEGG pathway analysis includes neuroactive ligand-receptor interaction, dopaminergic synapse, amphetamine addiction, cGMP-PKG signaling pathway, IL-17 signaling pathway, CAMP signaling pathway, drug metabolism-cytochrome P450, synaptic vesicle cycle, tyrosine metabolism, and serotonergic pathway synapse, which may be related to the treatment of ADHD by the core prescription. Among them, neuroactive ligand-receptor interaction and dopaminergic synapse signaling pathways are highly enriched, which might be the key pathways for treating ADHD. Neuroactive ligand-receptor interaction signaling pathways can be involved in the regulation of biological functions such as circadian rhythm, emotion, learning, and memory after binding to the corresponding receptor [69]. Noncoding Structural Variations that are highly relevant to the pathogenesis of ADHD and the control of gene expression in relation to particular ADHD phenotypes cluster around neuroactive ligand–receptor interaction pathways that are involved in neuronal brain function [70]. For example, when CHRM3 expression is upregulated, it can enhance cholinergic function and promote improved learning and memory abilities [71]. The core prescription may enhance learning and cognitive ability, improve mood, and relieve ADHD symptoms by regulating the expression of CMMR and GABR in dopaminergic synaptic signaling pathways [72]. The dopamine dysfunction hypothesis has become one of the main hypotheses to explain the pathogenesis of ADHD [73]. Investigations of humans in both experimental settings and clinical have yielded evidence that disturbances in dopaminergic signaling pathways are closely associated with numerous neurological and psychiatric conditions, including ADHD, which is primarily characterized by presynaptic deficits with increases in dopamine transporter binding [74]. In dopaminergic synaptic signaling pathways, the core formula may relieve ADHD symptoms by modulating MAO (inactivation of Dopamine in the synaptic cleft), DAT (dopamine reuptake back into presynaptic neurons and stop the activity of dopaminergic neurotransmitters), GSK3 (associated with increased neuronal progenitor proliferation, suppressed neural differentiation, and memory impairment at the synaptic level), and c-fos (involved in the regulation of neuropeptides and the formation of memory at the cellular level [75–77]). Additionally, D-amphetamine has been shown to increase circulating cortisol, an effect that appears to be related to the ability of these substances to trigger DA release in the central nervous system [78]. This research utilized data mining to summarize the primary herbs used by Prof. Xuan to treat ADHD. The network pharmacological study findings suggest that a core herbal prescription may have better anti-ADHD benefits by utilizing multicomponent, multtarget, and multilingual pathways. However, this study had certain limitations. Firstly, the data's magnitude may skew the research findings. Additionally, the candidate active substances OB ≥ 30% and DL ≥ 0.18 may overlook certain active ingredients and targets. Secondly, there are various data mining techniques for creating therapeutic recommendations, including complex network approaches, topic models for analyzing dialectical treatment rules, and Markov decision process models for maximizing dynamic schemes based on syndrome differentiation [79]. Despite this, our data mining technique is relatively straightforward. Lastly, the occurrence and development of diseases are dynamic evolution processes. Most clinical medical records of famous TCM veteran doctors have complex conditions and long treatment cycles. Therefore, exploring the dynamic treatment plan analysis strategy that conforms to Chinese medicine diagnosis and treatment characteristics is a problem that we need to consider. In future research, we will explore the dynamic treatment plan analysis strategy of ADHD and establish an effective method for individualized medicine and TCM experience inheritance. We will conduct trials to identify the most efficient targets and pathways, which will serve as a strong foundation for the development of novel medications.

**Conclusion**

The main pathological factors of children's ADHD are deficiency, fire, phlegm, and dyspeptic food. Prof. Xuan's treatment of ADHD has achieved satisfactory clinical effects by supplementing the kidney, replenishing the essence, opening the orifices, nourishing the Yin and subduing the Yang. The core prescription mainly intervenes in G protein-coupled amine receptor activity, catecholamine binding, Neuroactive Ligand-Receptor Interaction and Dopaminergic synapse signaling pathways. Our results suggested that the method and software used in this study could effectively explore the clinical medication rule of Prof. Xuan in treating children with ADHD and analyze the mechanism.

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