Mechanism of action of *Tripterygium wilfordii* for treatment of idiopathic membranous nephropathy based on network pharmacology

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**ABSTRACT**

**Background:** Although thunder god vine (*Tripterygium wilfordii*) has been widely used for treatment of idiopathic membranous nephropathy (IMN), the pharmacological mechanisms underlying its effects are still unclear. This study investigated potential therapeutic targets and the pharmacological mechanism of *T. wilfordii* for the treatment of IMN based on network pharmacology.

**Methods:** Active components of *T. wilfordii* were obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform. IMN-associated target genes were collected from the GeneCards, DisGeNET, and OMIM databases. VENNY 2.1 was used to identify the overlapping genes between active compounds of *T. wilfordii* and IMN target genes. The STRING database and Cytoscape 3.7.2 software were used to analyze interactions among overlapping genes. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses of the targets were performed using Rx64 4.0.2 software, colorspace, stringi, DOSE, clusterProfiler, and enrichplot packages.

**Results:** A total of 153 compound-related genes and 1485 IMN-related genes were obtained, and 45 core genes that overlapped between both categories were identified. The protein–protein interaction network and MCODE results indicated that the targets TP53, MAPK8, MAPK14, STAT3, IFNG, ICAM1, IL4, TGFB1, PPAR, and MMP1 play important roles in the treatment of *T. wilfordii* on IMN. Enrichment analysis showed that the main pathways of targets were the AGE signaling pathway, IL-17 signaling pathway, TNF signaling pathway, and Toll-like receptor signaling pathway.

**Conclusion:** This study revealed potential multi-component and multi-target mechanisms of *T. wilfordii* for the treatment of IMN based on network pharmacological, and provided a scientific basis for further experimental studies.

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**Introduction**

Idiopathic membranous glomerulonephritis (IMN) is an immune-mediated primary glomerular disease that usually manifests as nephrotic syndrome [1]. Although 30% of patients show spontaneous remission, persistent proteinuria occurs in 30–40% of cases, and may even develop to end-stage renal disease [2,3]. Although the treatment options for IMN include rituximab, glucocorticoids plus cyclophosphamide, and glucocorticoids plus calcineurin inhibitors, adverse events have been reported in association with these treatments. Rituximab is associated with adverse events, including infusion reaction, opportunistic infections, allergic reaction, and hypogammaglobulinemia, in addition to its slow effect and high cost [4]. Cyclophosphamide is associated with a variety of side effects, including gonad injury, malignancy, bone marrow suppression, and hemorrhagic cystitis [5]. Patients treated with calcineurin inhibitors are at risk for renal insufficiency and a high rate of relapse after drug withdrawal [6].

Thunder god vine (*Tripterygium wilfordii*) is a well-known traditional Chinese medicine that has been widely used for the treatment of various autoimmune diseases, such as nephritic syndrome, inflammatory bowel disease, systemic lupus erythematosus, and rheumatoid arthritis [7]. Although *T. wilfordii* has some adverse events, such as hepatotoxicity, nephrotoxicity,
and reproductive toxicity [8], clinical trials have demonstrated that *T. wilfordii* is effective for the treatment of IMN [9,10]. Guo et al. reported that *T. wilfordii* was effective for IMN patients with sub-nephrotic proteinuria, whether anti-PLA2R antibody is positive or negative [11]. And *T. wilfordii* was also more effective in preventing T cell proliferation and interferon-gamma production than FK506 [12]. Therefore, *T. wilfordii* can induce remission in patients who have no response to prednisone and calcineurin inhibitor. Compared with cyclophosphamide, the reproductive toxicity of *T. wilfordii* is reversible. In addition, *T. wilfordii* is inexpensive and available as a tablet. However, little is known about the molecular mechanisms underlying the effects of *T. wilfordii* on IMN.

The incorporation of traditional Chinese medicine into clinical therapy via network pharmacology can provide insights into the possible mechanisms of action and enhance the specificity and effectiveness of the treatment regimen [13]. Here, we explored the possible molecular mechanism underlying the effects of *T. wilfordii* on IMN using a network pharmacology approach.

**Materials and methods**

**Identification and screening of active compounds and targets for *T. wilfordii* and therapeutic targets for IMN**

The active compounds and potential target proteins of *T. wilfordii* were obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (http://tcmspw.com) [14] according to the parameters of absorption, distribution, metabolism, excretion (ADME) [15,16]. The criteria included drug likeness (DL) $\geq 0.18$, oral bioavailability (OB) $\geq 30\%$, hydrogen bond donors (Hdon) $\leq 5$, and hydrogen bond acceptors (Hacc) $\leq 10$. Using a systematic model established based on Random Forest and Support Vector Machine approaches, the proteins with the scores predicted by both methods of RF and SVM larger than 0.8 and 0.7 respectively were chosen as targets [17]. The gene names of targets were further obtained from the UniProt Knowledgebase (UniProtKB) (http://www.uniprot.org).

IMN-associated target genes were gathered from the GeneCards (https://www.genecards.org/), DisGeNET (https://www.disgenet.org/home/), and OMIM (https://omim.org/) databases. In addition, the genes that overlapped between compounds of *T. wilfordii* and IMN target genes were identified and visualized by VENN 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/).

**Construction of drug–disease target protein–protein interaction network**

The overlapping genes between compounds of *T. wilfordii* and IMN target genes were imported into STRING (https://string-db.org/cgi/input.pl) [18] to construct and visualize the protein–protein interaction (PPI) network. In addition, CytoV 3.7.2 software [19] was used to further analyze and visualize the PPI network. We identified the most important clusters within the PPI network with the criteria of Molecular Complex Detection (MCODE) score $>3$, degree cutoff $= 2$, node score cutoff $= 0.2$, k-score $= 2$, max depth $= 100$, and the number of nodes $\geq 10$ [20]. The node size reflected the MCODE scores, with larger nodes indicating higher MCODE scores.

**Pathway analysis of *T. wilfordii* for IMN and the drug–compound–target–signaling pathway network**

Then Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Gene and Genomes (KEGG) pathway enrichment of the significant clusters were analyzed using the Rx64 4.0.2 software, colorspace, stringi, DOSE, clusterProfiler, and enrichplot packages [21,22]. The screening conditions were adjusted for $p < 0.05$ and $q < 0.05$, and the results of GO enrichment analysis included three different levels: biological process (BP), molecular function (MF), and cellular component (CC) [23]. We used Cytoscape 3.7.2 to construct and visualize the drug–compound–target–signaling pathway network.

**Results**

**Identification and screening of active compounds and targets for *T. wilfordii* and therapeutic targets for IMN**

A total of 42 active compounds of *T. wilfordii* were obtained by searching the TCMSP database, and using ADME parameters (Table 1). After obtaining the target data from the TCMSP database and deleting duplicated items, 153 targets were identified. Then we used the UniProtKB database to normalize the protein targets collected from the TCMSP database. There were 1007 therapeutic targets of IMN in GeneCards database, 479 therapeutic targets of IMN in OMIM database, and 80 therapeutic targets of IMN in DisGeNET database were found. A total of 1485 therapeutic targets of IMN were acquired after removing duplicates.
Drug–disease target PPI network

A total of 77 genes overlapped between the 153 genes related to *T. wilfordii* and the 1485 genes related to IMN (Figure 1, Table 2). To establish the relationships between the overlapping genes, we uploaded the overlapping genes to the STRING database. A PPI network was built with 77 nodes and 1009 edges (Figure 2). The average node degree was 26.20, and the average local clustering coefficient was 0.69. The PPI network indicated complex relationships between these genes. The results were used for further analysis using Cytoscape software. MCODE was used to analyze the most significant module and obtained 45 core target nodes. Details of the clusters are presented in Table 3. The network was constructed as shown in Figure 3. The top 10 targets—TP53, MAPK8, MAPK14, STAT3, IFNG, ICAM1, IL4, TGFβ1, PPARG, and MMP1 had higher MCODE score in this process, which explained their significance in the network.

**GO enrichment analysis of *T. wilfordii* for the treatment of IMN**

A total of 1601 BP terms, 100 MF terms, and 19 CC terms were enriched for the 45 target genes. We selected the top 20 according to the p-value as shown in Figure 4. The results showed that *T. wilfordii* impacts IMN through various BPs, including response to lipopolysaccharide, response to molecule of bacterial origin, positive regulation of hemopoiesis, positive regulation of cytokine production, myeloid cell differentiation, cellular response to biotic stimulus, aging,
regulation of cell-cell adhesion, leukocyte cell-cell adhesion, and myeloid leukocyte differentiation. In the MF classification, the effects of *T. wilfordii* on the treatment of IMN were mainly manifested through cytokine receptor binding, RNA polymerase II-specific DNA-binding transcription factor binding, ubiquitin-like protein ligase binding, cytokine activity, DNA-binding transcription activator activity and RNA polymerase II-specific, DNA-binding transcription activator activity, nuclear receptor, ligand-activated transcription factor activity, DNA-binding transcription activator activity, nuclear receptor, ligand-activated transcription factor activity, DNA-binding transcription activator activity, ubiquitin protein ligase binding. According to the enrichment results of CC, membrane was the main classification of the target proteins. These may be the basis of the effects of *T. wilfordii* in the treatment of IMN.

**KEGG pathway enrichment analysis of *T. wilfordii* for the treatment of IMN**

The results of KEGG pathway enrichment analysis indicated 45 genes were markedly enriched within 32 items. We selected the top 20 items according to the *p*-value as shown in Table S1, Figure 5 and Figure S1–S4, which indicated that main processes of *T. wilfordii* in treating IMN included AGE-RAGE signaling pathway in diabetic complications, IL-17 signaling pathway, TNF signaling pathway, and Toll-like receptor signaling pathway.

**Drug–compound–target–signaling pathway network**

A drug–compound–target–pathway network of *T. wilfordii* for IMN treatment was constructed (Figure 6). The
integrative network suggested that the therapeutic effects of *T. wilfordii* on IMN may be attributable to the active components kaempferol acting on targets (MAPK8, ICAM1, VCAM1, MMP1, JUN, TNF, AKT1, CASP3, STAT1, RELA, SELE), triptolide acting on targets (STAT3, IFNG, IL4, TGFβ1, CD40, VEGFA, CXCL8, FOS, CD86, CD80) that regulate key pathways (AGE-RAGE signaling pathway, IL-17 signaling pathway, TNF signaling pathway, and Toll-like receptor signaling pathway), and nobiletin acting on targets (MMP9, CREB1), that regulate IL-17 signaling pathway and TNF signaling pathway.

**Discussion**

Clinical studies have shown that *T. wilfordii* appears to be promising and safe for the treatment of IMN patients [10,11]. However, the mechanism remains unclear. In the present study, we used a network pharmacological approach to identify the major constituents, potential therapeutic targets and significant pathways of *T. wilfordii* for IMN. We found that the main active components of *T. wilfordii* (tripolide, kaempferol, and nobiletin) play important roles in the treatment of IMN. Kaempferol, the main component of *T. wilfordii*, has been suggested to inhibit hyperglycemia-induced activation of inflammatory cytokines, TGF-β1 expression, and oxidative stress in NRK-52E and human renal tubular epithelial cells [24]. Triptolide, is extracts of *T. wilfordii* and frequently used to treat inflammatory and autoimmune disease, e.g., systemic lupus erythematosus, nephritis, psoriasis, and rheumatoid arthritis [25]. And, triptolide can inhibits the secretion of many cytokines, adhesion molecules, and chemokines and affects the functions of renal tubular epithelial cells [26], and reduces established proteinuria and podocyte injury in IMN [27]. In addition, nobiletin has anti-oxidant, anti-inflammatory and anti-apoptotic effects, and can attenuated tubular injury by inhibiting activation of apoptotic pathways and DNA damage [28].

Using STRING, MCODE, and combined the results of KEGG, we found the MAPK8, STAT3, IFNG, ICAM1, IL4, TGFβ1, MMP1 may be important targets of *T. wilfordii* in the treatment of IMN. MAPK8, also known as JNK1, belong to the family of MAPKs. Activation of MAPK8 correlates with a variety of cellular responses, including inflammation, oxidative stress and apoptosis [29]. Moreover, complement membranous attack complex could activate nicotinamide adenine dinucleotide phosphate hydrogen oxidase and JNK in podocytes, which

**Table 3.** Details of 2 significant modules in the PPI network.

| Cluster | Score (Density/#Nodes) | Nodes | Edges | Node IDs |
|---------|------------------------|-------|-------|----------|
| 1       | 28.625                 | 33    | 458   | STAT3, VCAM1, MMP1, CREB1, TGFβ1, IL4, CXCL8, STAT1, IFNG, CD40, AR, NOS2, PPARG, CXCR4, PTGS2, IL2, HMox1, ICAM1, CDK11A, TPS3, MAPK8, JUN, TNF, VEGFA, AKT1, NOS3, CASP3, CASP9, FOS, RELA, MMP9, TIMP1, MAPK14, CCR7, PLA2U, SELE, KDR, XIAP, CASP8, CD86, CD80, CD274, ESR1, HSP90AA1, NR3C1 |
| 2       | 4.364                  | 12    | 24    | CCR7, PLAU, SELE, KDR, XIAP, CASP8, CD86, CD80, CD274, ESR1, HSP90AA1, NR3C1 |

**Figure 3.** MCODE module of targets’ intersection of IMN and *T. wilfordii*. The nodes represent important targets in the most significant MCODE module from PPI network, and the size of the nodes represent the MECOD score (larger nodes indicate higher score).
could induce production of ROS in these cells, and a high amount of ROS has been documented to be associated with proteinuria in patients with MN [30]. STAT3, a member of the STAT family, is known to be activated by some upstream cytokines, such as TNF-α, IL-6 [31]. The biological functions of the STAT3 included cell proliferation, differentiation, survival and angiogenesis. Activation of STAT3 contributes to proliferation of extra-capillary glomerular epithelial cells and extent of injury in glomerulonephritis [31,32]. And STAT3 signaling was activated in the kidneys of mice [33] and human [33] with glomerulonephritis. Sublytic C5b-9, also called membrane attack complex, can attack podocytes to cause injuries which promoted the occurrence and development of MN and finally led to ESKD [34]. In C5b-9-induced podocyte injury model, STAT3 inhibitor had a protective effect on CXCL12-treated podocytes [34]. IFNG, one of the most important regulatory cytokines produced in response to viruses and microbe, stimulates Th-1 clonal expansion and inhibits Th-2 expansion, and the balance between Th-1 and Th-2 CD4 cells is relevant to autoimmunity [35]. The data from single-cell RNA sequencing showed that IFNG is up-regulated in MN patients [36]. MN is generally considered as an immune complex-mediated disease with predominant Th-2 nephritogenic immune, and

Figure 4. GO (BP, MF, CC) analyses of the therapeutic target genes of T. wilfordii for treatment of IMN. Each bar represents a GO term on the vertical axis. The number of genes enriched in each term is shown on the horizontal axis. The color of each bar represents the adjusted p-value of each GO term. Redder color indicates smaller adjusted p-value.
correlated significantly with urinary protein excretion [37]. In addition, in patients with MN, Th2 subclass IgG production is stimulated by IL-4 at the microenvironment level, and other mechanisms of proteinuria in MN could be the direct effect of IL-4 on podocytes [38]. These proteins are related to inflammation, immunity, oxidative stress and apoptosis. Based on STRING, MCODE results, and combined with the GO analysis results, the potential mechanism of treating IMN of \textit{T. wilfordii} is probably associated with its participance in BPs of response to lipopolysaccharide, response to molecule of bacterial origin, and positive regulation of cytokine production. These BPs are related to immune response, and inflammation [39]. IMN is a pathological pattern of glomerular damage caused by an autoimmune response. Immune complex deposition, thickness of glomerular basement membrane, and changes in the podocyte morphology are responsible for the development of proteinuria, which is caused by the

targeted binding of auto-antibodies to podocytes [40]. In addition, there are close associations between IMN and inflammation, and chronic inflammation is one of important characteristic of MN [41]. Through regulation of immune-related cell and inflammatory mediators, \textit{T. wilfordii} show potent immunosuppressive activity, thus it has the great potential for the treatment of immune diseases [42].

KEGG enrichment analysis showed that the pharmacological effects of \textit{T. wilfordii} on IMN were closely related to well-known IMN-associated pathways, such as the AGE-RAGE signaling pathway, IL-17 signaling pathway, TNF signaling pathway, and Toll-like receptor signaling pathway. This indicates that \textit{T. wilfordii} may act through multiple pathways. The AGE-RAGE signaling pathway is related to the autoimmune disorder, inflammation, and tissue damage in MN rats, and AGE inhibition could reduce the inflammatory reactions and oxidative lesions in MN [43]. IL-17 plays crucial roles in
the development of inflammatory autoimmune diseases. In a previous study, IL-17 mRNA was overexpressed in kidney biopsy specimens from IMN patients [44]. The inhibitory effect of *T. wilfordii* on IL-17 has been demonstrated in autoimmune diseases including psoriasis, ankylosing spondylitis, and rheumatoid arthritis [45–47], but not in IMN yet. In another study, TNF-α was directly cytotoxic to many glomerular cell types and promoted procoagulant activity with the formation of microthrombi that could contribute to renal vein thrombosis associated with MN [48]. Anti-TNF-α therapy attenuates renal immune cell infiltration in experimental MN [49]. Of note, it has been identified that multiglycoside of *T. wilfordii* could inhibit the activation of NF-κB and suppress overexpression of inflammatory cytokines TNF-α, IL-1β, and MCP-1 in kidney of MN rats, which may contribute to the improvement in podocyte injury in IMN [50]. Toll-like receptor, as a classic example of a pattern recognition receptor, participates in autoimmune disorders. Signals generated by Toll-like receptor are transduced through NF-κB signaling and MAP kinase pathways to recruit proinflammatory cytokines and costimulatory molecules, which promote inflammatory responses [51]. TLR4, a member of Toll-like receptor family, is related to in podocyte apoptosis, and progress of disease in MN patients [52,53]. It has been reported that *T. wilfordii* can ameliorate renal tubulointerstitial fibrosis through suppression of the TLR4/NF-κB pathway in diabetic rats [54], but not in IMN yet.

We explored the potential molecular mechanisms of action of *T. wilfordii* in the treatment of IMN from an integrated and systematic perspective, and our results provide a theoretical basis for further experimental studies. The limitations of present study is that our findings are only based on network pharmacology approaches. Therefore, it is worth noting that our findings provide only hints, and they must be verified by actual experimental data.

**Conclusion**

*T. wilfordii* has a potential multi-component, multi-target, and multi-pathway molecular mechanism of action for the treatment of IMN. MAPK8, STAT3, IFNG, ICAM1, IL4, TGFβ1, MMP1 may be the most important direct targets of *T. wilfordii*. The mechanism of action may be related to the AGE-RAGE signaling pathway, IL-17 signaling pathway, TNF signaling pathway, and Toll-like receptor signaling pathway. This study provides a basis for further studies of *T. wilfordii* in the treatment of IMN.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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