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Article

Identification of Potential Biomarkers for Hypertensive Nephropathy by Bioinformatics Analysis

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Abstract. Hypertensive nephropathy (HN) is a common complication of chronic hypertension that leads to kidney damage. This study aimed to identify potential biomarkers and key pathways associated with HN using bioinformatics tools. Gene data related to HN were retrieved from GeneCards and the Comparative Toxicogenomics Database (CTD), resulting in 89 genes from GeneCards and 10,898 genes from CTD. A Venn diagram revealed 58 overlapping genes, which were then analyzed using Protein-Protein Interaction (PPI) networks and the CytoHubba plugin in Cytoscape. The Maximal Clique Centrality (MCC) algorithm identified 10 hub genes, including ACE, AGT, ACE2, AGTR1, and AGTR2, integral to the renin- angiotensin-aldosterone system (RAAS). Functional enrichment analysis using Gene Ontology (GO) and KEGG pathways revealed that the most significant biological process was regulating systemic arterial blood pressure by the Renin-Angiotensin system, with the renin-angiotensin system pathway being the most highly enriched. Further visualization using ShinyGo highlighted the involvement of key genes in the RAAS pathway. These findings provide valuable insights into the molecular mechanisms underlying HN and suggest that bioinformatics approaches can aid in the identification of specific biomarkers for early diagnosis, non-invasive monitoring, and targeted treatments for HN in the future.

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1. Introduction

Hypertension, shaped by genetic and environmental factors, is a significant risk factor for cardiovascular and cerebrovascular diseases. The kidney plays a dual role in hypertension, not only as a key contributor to its development but also as a primary target for damage caused by elevated blood pressure [1]. Hypertensive nephropathy (HN) develops through a complex interaction of pathophysiological processes that result in kidney damage. Central factors include alterations in hemodynamics, molecular signaling pathways, and epigenetic changes, all contributing to worsening renal injury and fibrosis. Hypertensive nephropathy (HT) is also a condition characterized by kidney damage caused by long-term high blood pressure, with a variable progression and the potential to lead to renal failure. Therefore, improving approaches for early diagnosis and identifying disease biomarkers in HTN remains a top priority [2-3].

Early hypertensive nephropathy (HN) detection is essential to prevent its progression to chronic kidney disease. Recent studies have highlighted various promising biomarkers, including urinary proteins, inflammatory markers, and microRNAs, potentially facilitating early diagnosis [4-5]. Recent studies have highlighted urinary podocalyxin and nephrin as potential biomarkers for hypertensive nephropathy (HN). Increased levels of urinary podocalyxin have been linked to early HN, showing significant elevation in patients with chronic hypertension compared to healthy individuals. This marker offers greater sensitivity for early detection than the urinary microalbumin/creatinine ratio (UM/CR) [5]. Similarly, urinary nephrin levels were found to be higher in 78.3% of normoalbuminuric patients with chronic hypertension, demonstrating high sensitivity (89.7%) and specificity (88.8%) for detecting early HN [4]. In other research, various biomarkers have been identified to uncover the potential mechanisms of hypertensive nephropathy (HN), including β2- microglobulin (β2-MG), transforming growth factor-β (TGF-β), and periostin [1]. Although some studies have identified potential biomarkers associated with hypertensive nephropathy (HN), the use of a single biomarker has limitations in uncovering the mechanisms of hypertension in the kidneys, and most of these mechanisms are still not fully understood.

In this study, genes associated with hypertensive nephropathy were constructed using GeneCards and the Comparative Toxicogenomics Database (CTD). Gene intersections were then filtered using a Venn diagram. A protein-protein interaction (PPI) network was built from the intersecting genes with strong interactions. Functional enrichment analysis was subsequently performed to identify biological processes, cellular components, and molecular functions. Additionally, the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was conducted on the genes present in the PPI network. Finally, based on the KEGG results, a gene network directly related to hypertensive nephropathy (HN) was proposed. These findings are expected to enhance our understanding of the occurrence and progression of HN.

2. Experimental Section

The research design used is a bioinformatics approach through in silico testing. In the first stage, gene data was constructed by obtaining genes related to hypertensive nephropathy from GeneCards (GC) and the Comparative Toxicogenomics Database (CTD). Next, the genes obtained from both databases were screened for intersecting genes using a Venn diagram to identify which genes are highly relevant and likely to be intensely involved in specific biological processes. The intersecting gene data were then used to build a protein-protein interaction (PPI) network using STRING v.10. The purpose of the protein-protein interaction is to predict significant interaction relationships [6]. The PPI parameters used are the full string type, an interaction cutoff of 0.900 (high confidence), and an FDR

stringency of 5%. Subsequently, k-means clustering was performed to group proteins based on their interaction patterns [7].

The PPI results were then imported and visualized in the Cytoscape v3.10.2 application. In Cytoscape, the cytoHubba plugin was used. CytoHubba was employed to identify essential nodes or hub genes within the PPI network. The parameters in cytoHubba were set to rank the top 10 nodes using the Maximal Clique Centrality (MCC) model. MCC was chosen because it is more effective than other methods [8]. After identifying the top 10 genes, functional enrichment analysis was performed using Gene Ontology and KEGG pathways. The functional enrichment analysis results identified genes that could serve as potential biomarkers related to hypertensive nephropathy (HN). An overview of the research flow can be seen in Figure 1.

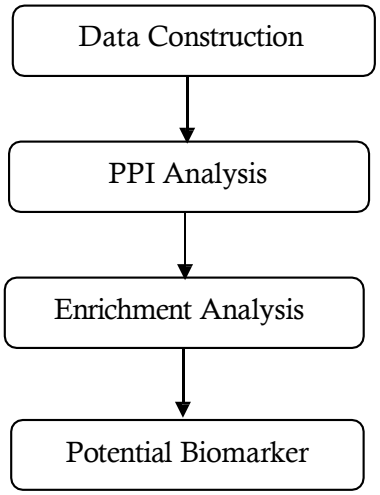

Figure 1. Schematic/Flowchart of research

2.1. Data Construction

The list of genes related to hypertensive nephropathy was obtained from the GeneCards database at https://www.genecards.org/ and the Comparative Toxicogenomics (CTD) database at https://ctdbase.org/ using the keyword "hypertensive nephropathy." The resulting gene lists were then exported in Excel format. Selection was performed on the genes obtained from both databases to identify the intersection of genes related to hypertensive nephropathy. The intersection results from both databases were then visualized using a Venn diagram created by inputting the gene lists from each database into the website https://bioinformatics.psb.ugent.be/webtools/Venn/. The output generated was a graphic in PNG format. Only the intersecting genes from both databases were further analyzed in the subsequent steps.

2.2. Protein-Protein Interaction (PPI)

The STRING application (Search Tool for the Retrieval of Interacting Genes), available at http://string-db.org, was utilized to construct the protein-protein interaction (PPI) network to explore further the interactions among the genes obtained from the Venn diagram selection. An interaction score of 0.900 represented high confidence and FDR stringency of 5%. Subsequently, k-means clustering was performed to group proteins based on their interaction patterns. The PPI results were then imported and visualized in the Cytoscape v3.10.2 application. Subsequently, the cytoHubba plugin, with the Maximal Clique Centrality (MCC) algorithm, was applied to identify hub genes with high connectivity within the PPI network [6-9].

2.3 Functional Enrichment Analysis

The Enrichr tool from Ma'ayan Laboratory was used for enrichment analysis to analyze the resulting genes' molecular function, biological function, and cellular components. The Enrichr tool takes a set of genes. It provides ontologies, pathways, transcription, and biological processes of cell types, molecular functions, cellular components, and signaling pathways related to HN. The adjusted P-value threshold for selecting significant GO terms or pathways is 0.05 [10]. In addition, enrichment analysis was also performed using KEGG pathways. The results of the selected KEGG pathways were then visualized using ShinyGO 0.80 (http://bioinformatics.sdstate.edu/go).

Utilizing the Shiny framework, which offers access to various powerful R packages for visualization and statistical analysis, we created a new tool based on the Ensembl annotation database and pathway databases from multiple sources. ShinyGO's distinctive features include showcasing query genes on pathway diagrams and PPI networks through API connections to KEGG and STRING and visualizing overlaps among enriched pathways via hierarchical clustering and interactive networks [11]. Based on the final analysis, several biomarker genes were linked to hypertensive nephropathy.

3. Results and Discussion

Data on genes associated with hypertensive nephropathy were downloaded from GeneCards and the Comparative Toxicogenomics Database (CTD) using the search keyword "hypertensive nephropathy". GeneCards is a comprehensive web-based database designed to facilitate human gene research by providing annotations divided into 18 categories, one of which is the "disorders" category that highlights diseases associated with specific genes [12]. The Comparative Toxicogenomics Database (CTD) is a comprehensive platform that integrates and manages toxicology data related to environmental chemicals and their effects on human health [13].

From the data construction results by collecting genes from two databases, 89 genes were obtained from GeneCards and 10,898 genes from CTD, all related to hypertensive nephropathy. From these thousands of genes, filtering was conducted to identify overlapping genes that are more significant and could serve as biomarkers. The filtering process resulted in 58 overlapping genes selected for further PPI analysis. The gene data filtering diagram is shown in Figure 2.

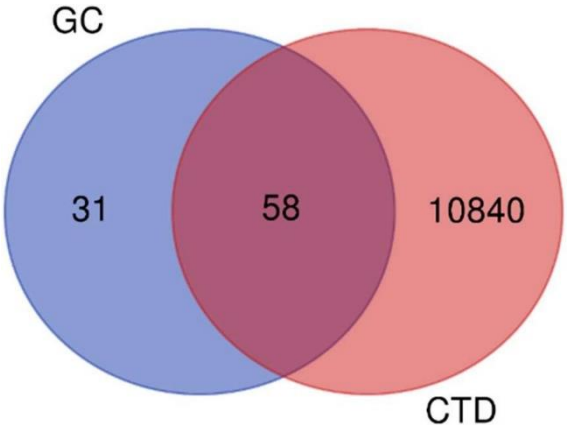

Figure 2. Potential gene intersection for hypertensive nephropathy (HN)

The analysis of 58 overlapping genes proceeded with PPI evaluation. Protein-protein interactions (PPI) are fundamental to numerous biological processes, including signal transduction, transcriptional regulation, and metabolic pathways. These interactions create functional networks that control cellular activities, making them vital for understanding disease mechanisms and aiding drug

development. Recent progress in experimental and computational techniques has enhanced our comprehension of PPI, uncovering their intricate roles and importance within biological systems. Additionally, PPI significantly influences cellular functions, impacting processes like metabolism and gene regulation[14-15].

PPI analysis was performed using the STRING v.12.0 webserver. The network was constructed with parameters set to a required score of 0.900 (high confidence) and an FDR stringency of 5%, followed by k-means clustering. From the PPI analysis of 58 genes, only 30 genes interacted with HN, forming 7 clusters with 7 different node colors. The different node colors indicate involvement in different biological pathways. The PPI network results are shown in Figure 3.

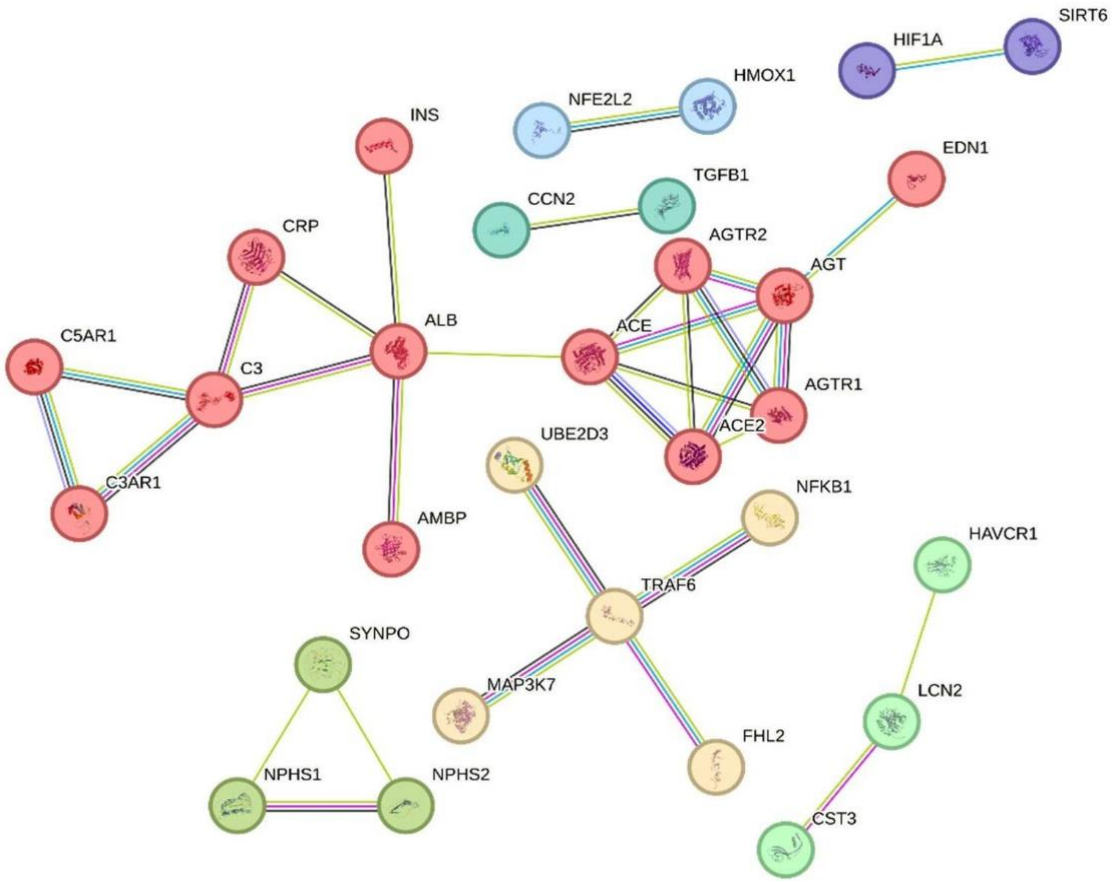

Figure 3. The PPI network results

In the PPI network depicted in Figure 3, the red nodes form the most significant cluster, consisting of 13 genes that exhibit extensive interactions compared to other clusters. This prominence is attributed to their association with a specific hypertension-related pathway, regulating blood volume by the renin-angiotensin system.

The findings from the PPI analysis emphasize the critical role of the red node cluster, which is intricately linked to the regulation of blood volume via the renin-angiotensin system. This connection aligns with the pathophysiology of hypertensive nephropathy, where the renin-angiotensin system plays a central role in regulating blood pressure. While the renin-angiotensin-aldosterone system (RAAS) may initially function as a compensatory mechanism in early kidney disease, prolonged activation can lead to worsening kidney damage. Elevated levels of renin and aldosterone in patients with chronic kidney disease disrupt kidney hemodynamics and anatomy, contributing to disease

progression. Persistent exposure to angiotensin II and aldosterone exacerbates kidney injury, highlighting the importance of RAAS inhibition as a key therapeutic approach in managing chronic kidney disease. This system, essential for maintaining vascular tone and fluid homeostasis, becomes dysregulated in hypertension, leading to increased susceptibility to kidney damage. Consequently, targeting the RAAS pathway has become a cornerstone in treatments to mitigate the effects of hypertensive nephropathy [16-18].

Identifying 13 genes within this significant cluster provides crucial insights into their role in the biological processes underlying hypertensive nephropathy. Their intense interaction within the network suggests their involvement as key contributors to disease mechanisms. This discovery enhances the understanding of hypertension-related kidney damage and paves the way for future research on developing targeted therapies and identifying biomarkers for early detection and treatment. In addition to the blood volume regulation pathway by renin-angiotensin, the PPI results also identified six other pathways involved in processes related to hypertensive nephropathy (HN). A description of the PPI clustering results involving various pathways associated with HN can be seen in Figure 4.

color	cluster Id	gene count	description
	Cluster 1	13	+ Regulation of blood volume by renin-angiotensin
	Cluster 2	5	TAK1-dependent IKK and NF-kappa-B activation
	Cluster 3	3	Mixed, incl. Glomerulosclerosis, and Inter-male aggressive behavior
	Cluster 4	3	CST3, HAVCR1, LCN2
	Cluster 5	2	Positive regulation of collagen biosynthesis
	Cluster 6	2	Regulation of HMOX1 expression and activity
	Cluster 7	2	HIF1A, SIRT6

Figure 4. The description of the PPI clustering results

The next step involves importing the PPI network data into the Cytoscape application, where the cytoHubba plugin is utilized. CytoHubba is vital for identifying crucial biological pathways involved in disease progression by analyzing complex biological networks to pinpoint hub genes and pathways central to disease mechanisms. Integrated into Cytoscape, this tool employs various topological analysis methods to rank network nodes, identifying essential proteins and pathways that could serve as potential therapeutic targets[19], [20]. An advanced analysis of the PPI network in cytoHubba, utilizing the Maximal Clique Centrality (MCC) method, was conducted to identify key genes with high connectivity within the network. This analysis identified the top 10 genes strongly linked to hypertensive nephropathy (HN) pathophysiology. The top 10 genes are presented in Figure 5.

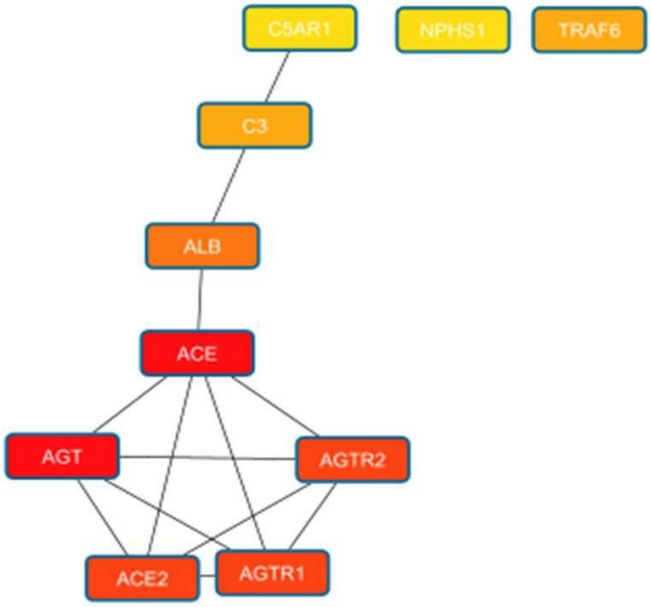

Figure 5. Top 10 hub genes related to hypertensive nephropathy

The analysis using Cytoscape identified 10 hub genes related to Hypertensive Nephropathy (HN), namely ACE, AGT, ACE2, AGTR1, AGTR2, ALB, C3, C5AR1, NPHS1, and TRAF6. The darker the color, the stronger the association with HN. It is known that ACE, AGT, ACE2, AGTR1, and AGTR2 are related to the renin-angiotensin system. The renin-angiotensin-aldosterone system (RAAS) is crucial in regulating blood pressure and fluid balance, involving complex interactions between several key proteins: ACE, ACE2, AGT, AGTR1, and AGTR2. These interactions are critical for cardiovascular health and disease, including hypertensive nephropathy. The molecular mechanisms underlying these interactions involve enzymatic conversions and receptor-mediated signaling pathways [18], [21-23].

Angiotensinogen (AGT) is the precursor to Ang I, which is cleaved by renin. The conversion of Ang I to Ang II by ACE is essential for activating AGTR1, triggering vasoconstriction, and raising blood pressure [23]. Although less well understood, AGTR2 is believed to play a role in mediating vasodilatory and anti-inflammatory effects, counteracting the actions of AGTR1 [22]. ACE converts angiotensin I (Ang I) into angiotensin II (Ang II), a potent vasoconstrictor that binds to AGTR1, leading to increased blood pressure and pro-inflammatory responses. ACE2, which is similar to ACE, counteracts these effects by converting Ang II into Ang-(1-7), which binds to the Mas receptor, promoting vasodilation and anti-inflammatory effects [21], [23].

The genes ALB, C3, and C5AR1 also play a role in hypertensive nephropathy (HN) mechanisms. The combined effect of ALB, C3, and C5AR1 in hypertensive nephropathy entails intricate interactions within the immune and complement systems, influencing kidney function and blood pressure control. ALB (albumin) indicates kidney damage, while C3 and C5AR1 are part of the complement system, involved in immune responses and inflammation. The interaction among these molecules plays a crucial role in developing hypertensive nephropathy by regulating immune cell activity and inflammatory pathways [24].

NPHS1 and TRAF6 also play a role in hypertensive nephropathy (HN). The interaction between NPHS1 and TRAF6 can affect the inflammatory and immune responses in the kidney, which may contribute to the progression of hypertensive nephropathy. Damage to the glomeruli and podocytes is a hallmark of HN, and mutations in NPHS1 can worsen this damage by disrupting the filtration barrier. TRAF6's involvement in immune signaling may further promote inflammation and fibrosis,

key features of hypertensive nephropathy [25]-26]. It has been shown that the 10 genes identified through PPI analysis have a strong association with hypertensive nephropathy (HN). Next, functional enrichment analysis was conducted to select the most potential genes and pathways closely related to the condition.

Enrichment analysis is a crucial computational method in genomics, helping researchers interpret complex omics data by identifying significant gene sets and their biological meanings. Enrichr, a popular web-based tool, facilitates this process by providing access to annotated gene sets from various libraries, allowing users to visualize and analyze enrichment results from different datasets to reveal hidden gene relationships [27]. In the enrichment analysis using Enrichr, the parameters include biological process, cellular component, and molecular function from Gene Ontology (GO) and enrichment analysis for KEGG pathways.

Gene Ontology (GO) is a standardized system used to describe the functions of genes across different organisms. It provides a structured vocabulary to represent gene functions in three main categories: Biological Process (BP), Molecular Function (MF), and Cellular Component (CC). Biological Process refers to the biological activities that a gene is involved in, such as metabolism or cell division. Molecular Function describes the specific biochemical activity of a gene product, such as binding or catalysis. Cellular Component refers to the location within the cell where the gene product is active, such as the nucleus or mitochondria. GO helps researchers understand gene function in a unified way, facilitating data integration and comparison across different species and studies [27]. The results of the top ten ranked GO enrichment analysis can be seen in Figure 6,7,8.

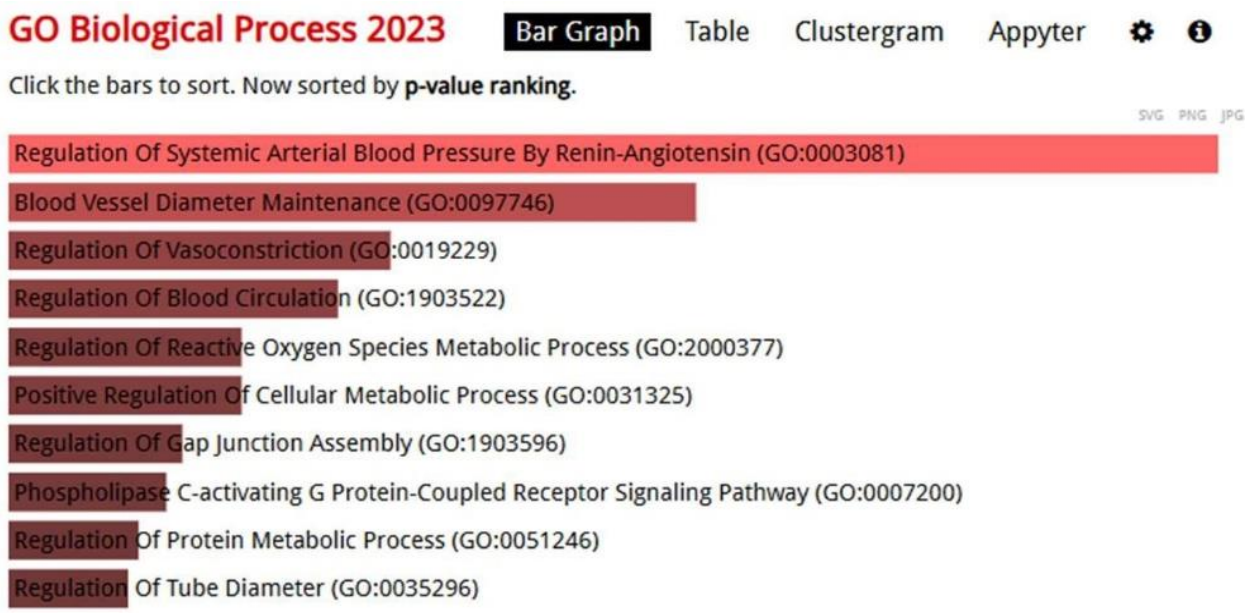

Figure 6. Gene ontology enrichment results for biological process

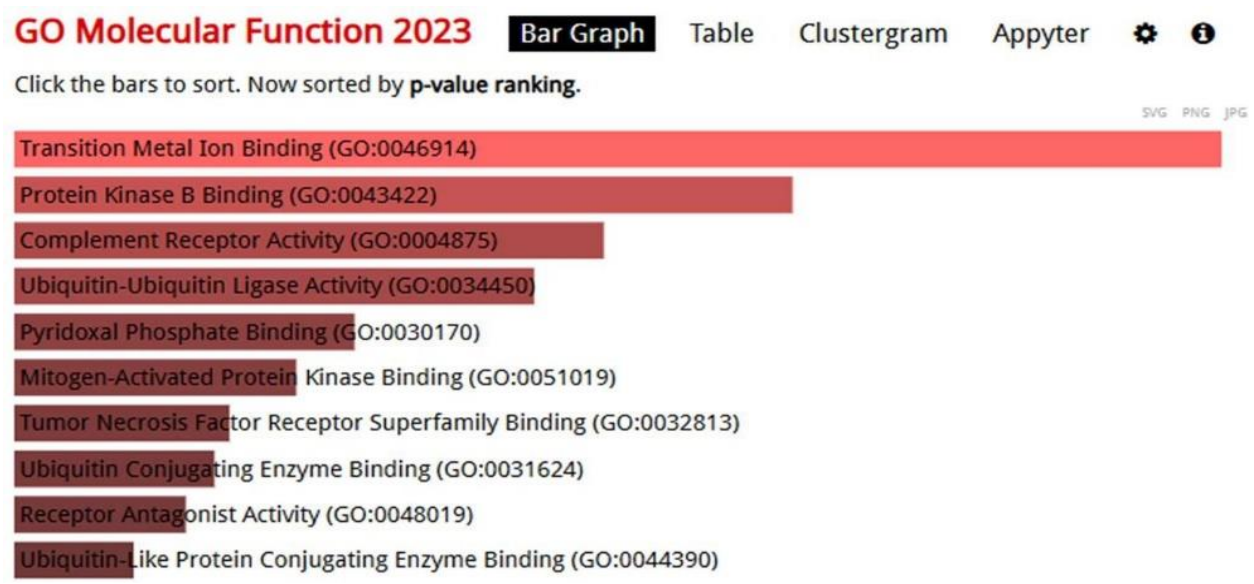

Figure 7. Gene ontology enrichment results for molecular function

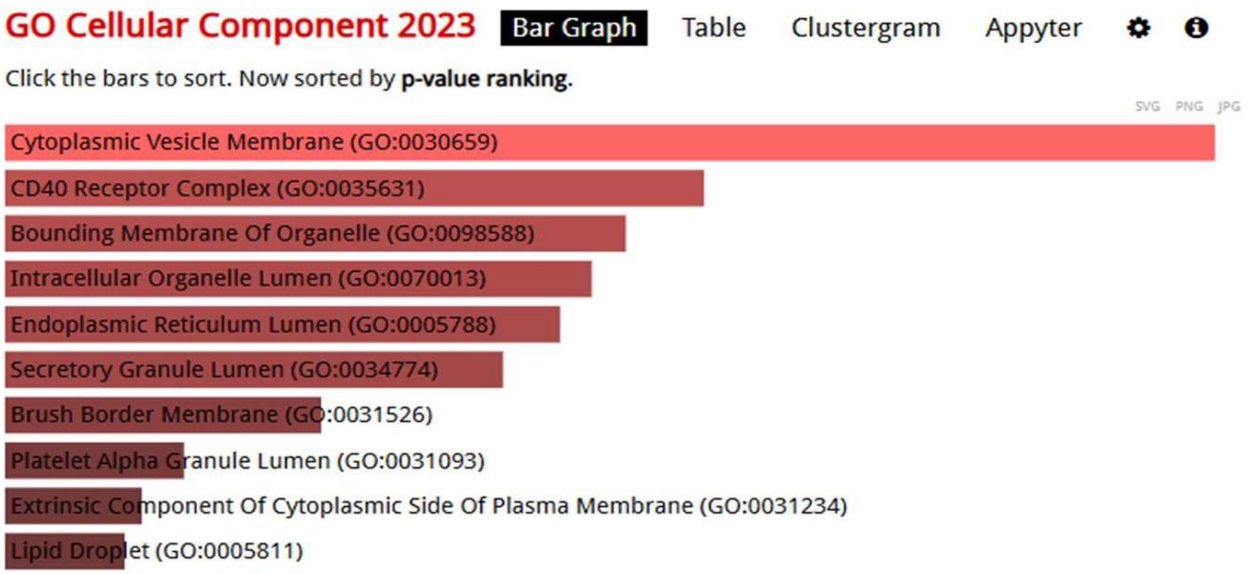

Figure 8. Gene ontology enrichment results for cellular component

The functional enrichment analysis results show that in the biological process mechanism, the regulation of systemic arterial blood pressure by Renin-Angiotensin is the most significant process with a P-value of 4.722 x 10-13. The significant molecular function is transition metal ion binding with a P-value of 1.254 x 10-3. The significant cellular component is the Cytoplasmic Vesicle Membrane, with a P-value of 7.915 x 10-4. In addition to GO, enrichment was also performed on the KEGG pathway, and the results are shown in Figure 9.

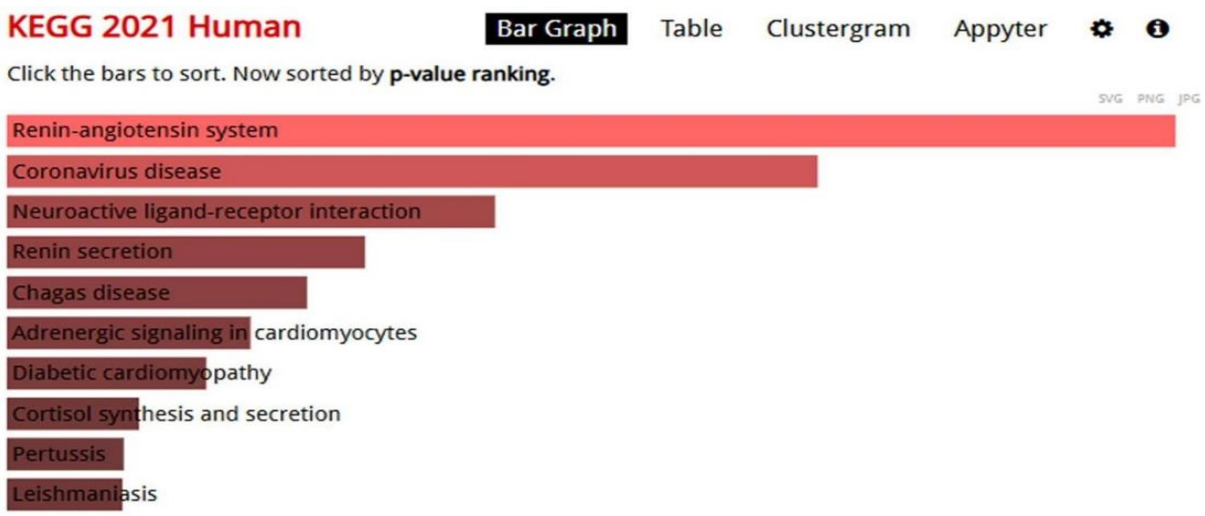

Figure 9. KEGG pathways enrichment results

The top ten pathways above are associated with hypertensive nephropathy. The P-value is representative, where a P-value < 0.01 indicates significant enrichment. It was found that the renin-angiotensin system pathway is the most significant, with a P-value of 3.168 x 10^-13. Subsequently, the results of the KEGG pathways were visualized using the ShinyGo 0.81 webserver. In the KEGG pathway section, selecting the renin-angiotensin signaling pathway displayed genes that were highlighted, indicating the expression of these genes. ShinyGo also allows the visualization of 20 pathways related to hypertensive nephropathy, as shown in Figure 10.

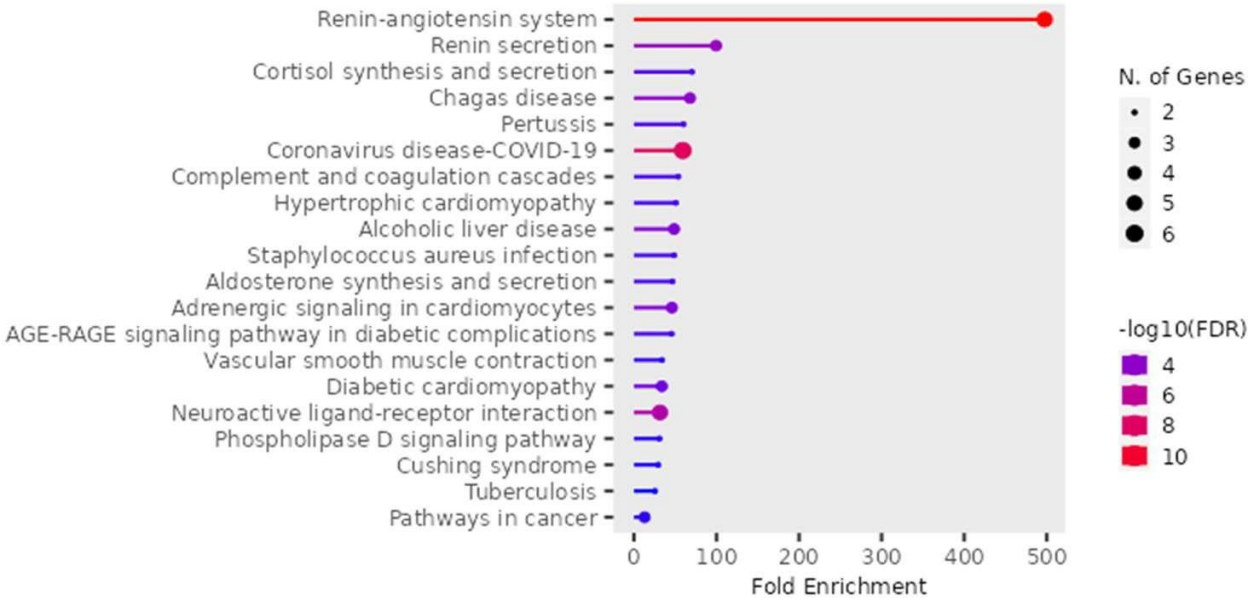

Figure 10. Enrichment analysis with ShinyGO

The enrichment visualization results using ShinyGo revealed that the renin-angiotensin system pathway is strongly associated with hypertensive nephropathy. Subsequently, in the RAS pathway,

the involvement of the 10 identified genes was visualized to highlight those most relevant to hypertensive nephropathy, as shown in Figure 11.

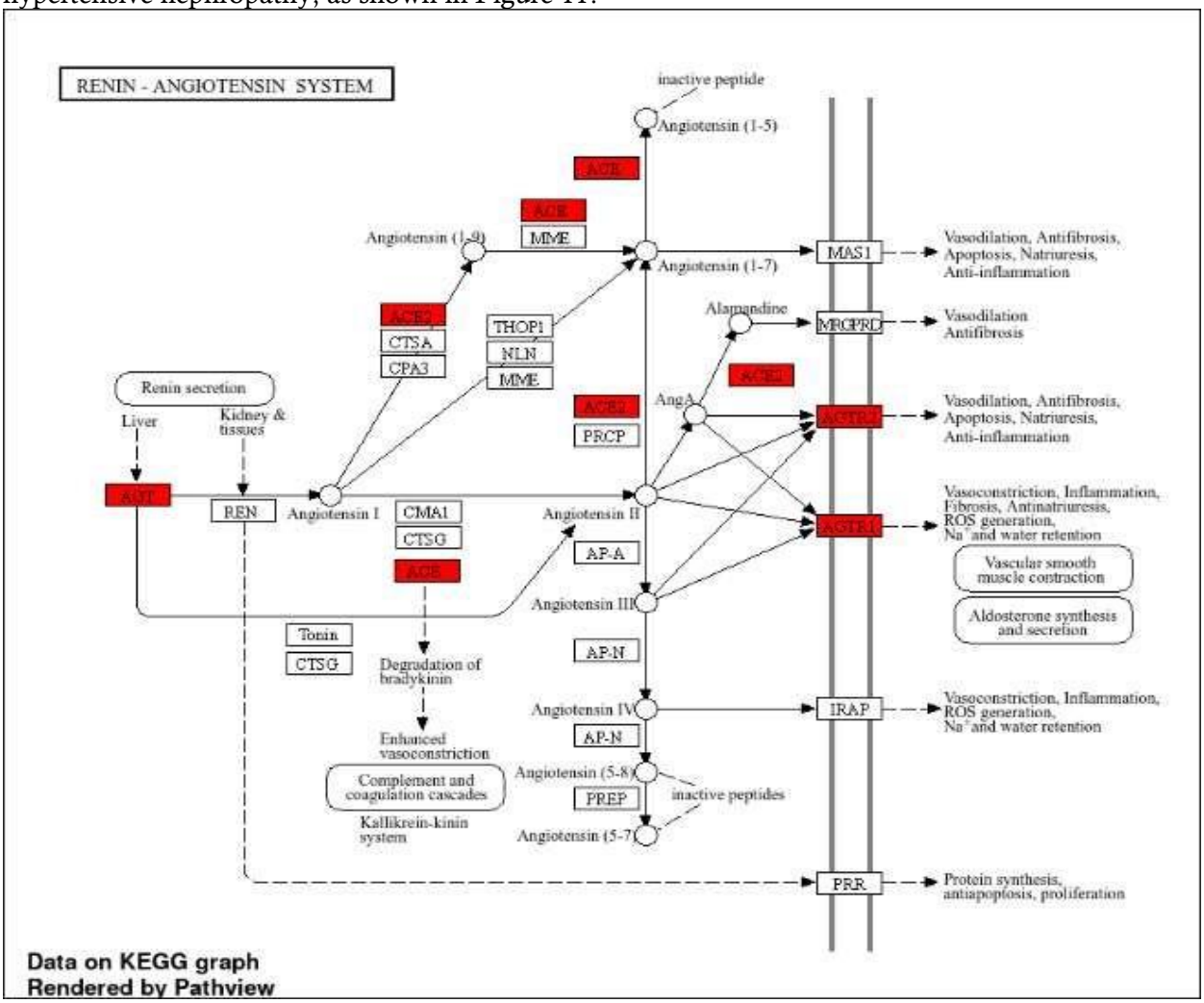

Figure 11. Visualization of RAS pathway

From the visualization results in Figure 9, it was observed that AGT, ACE, ACE2, AGTR1, and AGTR2 are critical genes that play a significant role in the renin-angiotensin system, which is central to the pathogenesis of hypertensive nephropathy (HN). AGT (angiotensinogen) is the precursor for synthesizing angiotensin I, which is converted into angiotensin II by ACE (angiotensin-converting enzyme). This enzyme, in turn, activates AGTR1 (angiotensin II type 1 receptor) and AGTR2 (angiotensin II type 2 receptor), which mediate vasoconstriction and influence blood pressure regulation. ACE2, a homolog of ACE, counteracts the effects of angiotensin II by converting it into angiotensin-(1-7), a molecule that has vasodilatory and anti-inflammatory properties. The interaction among these genes underscores their potential as biomarkers for diagnosing and understanding the mechanisms underlying HN [28].

The presence and expression of these genes can offer valuable insights into the progression of hypertensive nephropathy, especially in how dysregulation within the renin-angiotensin system contributes to kidney damage. Elevated levels of ACE, AGT, and angiotensin II are often observed in individuals with HN, suggesting that these molecules could serve as early indicators of the disease.

Furthermore, ACE2's role in mitigating the harmful effects of angiotensin II emphasizes its therapeutic potential in treating or preventing HN. Therefore, these genes not only play a fundamental role in the disease's pathophysiology but also hold promise as biomarkers for early detection and targeted treatment of hypertensive nephropathy[23], [29-30].

The importance of bioinformatics in developing specific biomarkers for hypertensive nephropathy (HN) cannot be overstated. By leveraging computational tools and vast biological data, bioinformatics allows for identifying key genes, pathways, and molecular interactions that contribute to the disease's progression. This non-invasive approach provides a powerful means to discover reliable biomarkers, which can be used for early diagnosis, monitoring, and personalized treatment of HN without invasive procedures. The analysis from this study, mainly through PPI network and pathway enrichment, has highlighted potential biomarkers such as AGT, ACE, ACE2, AGTR1, and AGTR2, which are crucial in understanding HN mechanisms. As such, bioinformatics can potentially revolutionize how we prevent and treat hypertensive nephropathy in the future, offering a more efficient and less invasive method for identifying at-risk individuals and tailoring therapeutic interventions, ultimately improving patient outcomes and quality of life.

4. Conclusion

In conclusion, this study highlights the significant role of bioinformatics in uncovering potential biomarkers and pathways associated with hypertensive nephropathy (HN). Through PPI network analysis and functional enrichment techniques, key genes such as AGT, ACE, ACE2, AGTR1, and AGTR2 were identified as crucial components in the pathogenesis of HN. These findings emphasize the importance of non-invasive methods, such as computational tools, in identifying biomarkers that could facilitate early diagnosis and personalized treatment for HN. Furthermore, integrating bioinformatics approaches opens up new possibilities for better prevention and management of hypertensive nephropathy, ultimately contributing to improved patient outcomes and reducing the need for invasive procedures.

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