A New Prospect for the Treatment of Nephrotic Syndrome Based on Network Pharmacology Analysis

Rini Varghese , Anuradha Majumdar [*]

Bombay College of Pharmacy, Kalina, Santacruz (E), Mumbai, Maharashtra, 400098, India

A R T I C L E I N F O

Keywords:
Network pharmacology
Nephrotic syndrome
Chronic kidney diseases
All trans retinoic acid
Valproic acid
Synergism

A B S T R A C T

Network pharmacology is an emerging field which is currently capturing interest in drug discovery and development. Chronic kidney conditions have become a threat globally due to its associated lifelong therapies. Nephrotic syndrome (NS) is a common glomerular disease that is seen in paediatric and adult population with characteristic manifestation of proteinuria, oedema, hypoalbuminemia, and hyperlipidemia. It involves podocyte damage with tubulointerstitial fibrosis and glomerulosclerosis. Till date there has been no specific treatment available for this condition that provides complete remission. Repurposing of drugs can thus be a potential strategy for the treatment of NS. Recently, epigenetic mechanisms were identified that promote progression of many renal diseases. Therefore, in the present study, we investigated two epigenetic drugs valproic acid (VPA) and all-trans retinoic acid (ATRA). Epigenetic drugs act by binging about changes in gene expression without altering the DNA sequence. The changes include DNA methylation or histone modifications. The targets for the two drugs ATRA and VPA were collated from ChEMBL and Binding DB. All the genes associated with NS were collected from DisGeNET and KEGG database. Interacting proteins for the target genes were acquired from STRING database. The genes were then subjected to gene ontology and pathway enrichment analysis using a functional enrichment software tool. A drug-target and drug-potential target-protein interaction network was constructed using the Cytoscape software. Our results revealed that the two drugs VPA and ATRA had 65 common targets that contributed to kidney diseases. Out of which, 25 targets were specifically NS associated. Further, our work exhibited that ATRA and VPA were synergistically involved in pathways of inflammation, renal fibrosis, glomerulosclerosis and possibly mitochondrial biogenesis and endoplasmic reticulum stress. We thus propose a synergistic potential of the two drugs for treating chronic kidney diseases, specifically NS. The outcomes will undoubtedly invigorate further preclinical and clinical explorative studies. We identify network pharmacology as an initial inherent approach in identifying drug candidates for repurposing and synergism.

1. Introduction

Globally and in the developing countries, the prevalence of chronic kidney diseases (CKD) is considerably high with a significant number of patients in need of renal replacement therapy (Glassock et al., 2017) (Abrahamet al, 2016) (Avner et al., 2009) (Singhet al, 2013). One among the chronic renal conditions is nephrotic syndrome (NS) which is identified by its defining features of proteinuria, oedema, hypoalbuminemia, and hyperlipidemia involving podocyte loss, renal fibrosis and glomerulosclerosis (Avner et al., 2009) (Doucet et al., 2007). NS can be classified into several types viz. focal segmental glomerulosclerosis, minimal change disease, membranous nephropathy and IgA nephropathy which are categorized based on their underlying causes (Varghese

and Majumdar, 2021). NS necessitates chronic treatment with steroids or other immunosuppressants, angiotensin converting enzyme inhibitors and monoclonal antibodies as there are no specific targeted treatments available so far (Saleem and Bierzynska, 2017) (Korbet, 2012). In most of the cases, patients are found to be steroid resistant and they ultimately progresses to end stage renal disease requiring kidney transplantation or dialysis. It thus becomes crucial to find alternative treatments that can attenuate proteinuria, glomerulosclerosis and renal fibrosis associated with the condition of NS (Avner et al., 2009) (Geeet al, 2015) (Mekahliet al, 2009).

Valproic acid (VPA) is a popular anti-convulsant and an antineoplastic agent (Cinatlet al, 1997). It is a short chain fatty acid which was found to inhibit class I and class II histone deacetylases (HDACs) and regulate cell differentiation, apoptosis, proliferation and

---

Abbreviations

CKD	chronic kidney diseases
NS	nephrotic syndrome
VPA	valproic acid
ATRA	all trans retinoic acid
HDAC	histone deacetylase
HDACi	histone deacetylase inhibitor
MCD	minimal change disease
RARs	retinoic acid receptors
SRNS	steroid resistant nephrotic syndrome
SSNS	steroid sensitive nephrotic syndrome
INS	idiopathic nephrotic syndrome
FSGS	focal segmental glomerulosclerosis
KEGG	Kyoto Encyclopedia of Genes and Genomes
GO	gene ontology
BP	biological process
MF	molecular function
CC	cellular component

---

immunogenicity in cancerous cells, thus attenuating the process of metastasis. Histone deacetylase inhibitors (HDACi) are the compounds that increase acetylation of histone and non-histone proteins by inhibiting the enzymatic activity of HDAC. HDACi are known to manifest their anti-inflammatory and antifibrotic effects in some organs, but their effect on the progression of renal disease is studied to a lesser extent; however, several studies have shown beneficial effect of HDACi in attenuating proteinuria and kidney injury in murine models (Varghese and Majumdar, 2021) (Van Benedenet al, 2011). Studies have shown that VPA can attenuate kidney injury and proteinuria (Van Benedenet al, 2011) (Khan et al., 2015) in diabetic nephropathy model in rats and adriamycin murine models, nevertheless the mechanisms underlying the disease and the action of drug are not clearly understood. All-trans retinoic acid (ATRA) is a natural derivative of vitamin A, known to play a role as an antioxidant and regulator of cell differentiation, apoptosis and attenuation of inflammation. ATRA acts by binding to retinoic acid receptors (RARs). Binding of ATRA to RARs give rise to a relaxed chromatin structure via activation of RARs and thereby activating gene transcription. It has also been known to partake in the protective role in certain renal diseases leading to attenuation of proteinuria (Zhang et al., 2018) (Liu et al., 2008). Several studies have used VPA and ATRA in combination for the treatment of myelodysplastic syndrome and acutepromyelocytic leukemia (Ciminoet al, 2006) (Kuendgenet al, 2005) (Buget al, 2005) and showed good tolerability of this combination. This particular combination was observed to be safe and was associated with induction of hypomethylation of DNA and histone acetylation (Sorianoet al, 2007). Epigenetic mechanisms have been known to contribute in the progression of diseases such as cancer, inflammatory disorders, neurological diseases (Berdasco and Esteller, 2013). Additionally, in renal conditions involving sclerosis and fibrosis, epigenetic mechanisms have been recognized recently (Hadden and Advani, 2018). The therapy of combining VPA and ATRA can therefore be a novel therapeutic approach in treating NS.

Network pharmacology has been greatly utilized for exploring new drugs. It is also used to investigate already existing drugs for repurposing (dan Xionget al, 2018) (Hopkins, 2008). Another application of network pharmacology is to understand the mechanisms of drug formulations which have multiple drug components in it. Therefore it is widely used to explore traditional herbal formulations (dan Xionget al, 2018) (Chandran et al., 2015a) (LI and ZHANG, 2014). Lately, increasing attention has been paid to the application of the network pharmacology. It also finds it application in uncovering efficacious drugs for treating an array of diseases with common pathogenesis and to

unravel the mechanism of action of drugs (Chandran et al., 2015b) (Yuet al, 2016) (Qiet al, 2016). Thus, in the present study, we explored the synergistic potential of VPA and ATRA for the treatment of NS using network pharmacology approach.

2. Methods

2.1. Collection of drug targets and their associated diseases

The targets for the two drug compounds ATRA and VPA were obtained from the data base ChEMBL (https://www.ebi.ac.uk/chembl/) and Binding DB (https://www.bindingdb.org/bind/index_original.jsp) (Chandran et al., 2015b) (Liu et al., 2007). With the aim of retrieving the targets from Binding DB, the structures of the two drug compounds were first retrieved from ChEMBL in .sdf format. The .sdf files were then entered and queried in the Binding DB so as to obtain the targets. All targets were obtained specifically by filtering the organism as "Homo sapiens". Targets from both the databases having Uniprot IDs were combined and the targets that overlapped and were in duplicates were carefully screened and eliminated. The gene and the gene names for all the drug targets were obtained from UniProt using the UniProt IDs of the targets (Bairochet al, 2005). The disease in which each drug target participated, were obtained from DisGeNET (https://www.disgenet.org/search) which is a web server that provides the data regarding various genes and its associated diseases. Data regarding each drug target genes associated with different diseases were obtained and collated from this database (Deu-pons et al., 2015) (Yuet al, 2019).

2.2. Collection of genes of NS

The genes specifically associated with NS were obtained from two databases DisGeNET (https://www.disgenet.org/search) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.kegg.jp/kegg/pathway.html) (Deu-pons et al., 2015) (Kanehisa, 2002). The genes were identified by searching for terms specific to nephrotic syndrome in the databases viz. nephrotic syndrome, focal glomerulosclerosis, childhood nephrotic syndrome, idiopathic nephrotic syndrome, nephrotic syndrome-minimal change disease, steroid sensitive nephrotic syndrome, nephrotic range proteinuria, glomerulosclerosis (disorder), IgA glomerulonephritis, glomerulonephritis, membranous glomerulonephritis, mesangioproliferative glomerulonephritis, proteinuria, steroid resistant nephrotic syndrome, tubulointerstitial fibrosis, renal interstitial fibrosis, steroid dependent nephrotic syndrome, focal segmental glomerulosclerosis (primary and secondary), renal fibrosis, nephrotic syndrome-frequently relapsing, nephrotic syndrome with focal and segmental sclerosis, steroid resistant nephrotic syndrome of childhood, and renal sclerosis. All the overlapping genes were screened and then eliminated to avoid duplication.

2.3. Potential target identification, gene mapping and protein-protein interactions

The targets collected for ATRA and VPA were then mapped to the collected genes associated with nephrotic syndrome to identify and obtain shared genes. These shared genes were predicted to be the potential targets of ATRA and VPA for treating NS (Gao et al., 2018). Additionally, we also identified and obtained proteins that interacted with these potential targets from STRING database (https://string-db.org/) having a highest threshold confidence score of 0.9 (Jensenet al, 2009) (Gao et al., 2018). Intersecting gene targets between SRNS and SSNS, and the overlapping targets between ATRA, VPA, SRNS and SSNS were also identified.

2.4. Gene Ontology (GO) and pathway enrichment analysis

GO: biological process (BP), molecular function (MF) and cellular

component (CC) and pathway enrichment analysis were carried out with the help of Reactome (https://reactome.org/) and FunRich database using special software tool for functional enrichment analysis called FunRich, version 3.1.3. with p-value <0.05 (hypergeometric test) (Pathan et al, 2015) (Rouka et al., 2020).

2.5. Construction of networks

2.5.1. Network construction

A network consists of multiple nodes and edges. Nodes are the points of communication while the edges are the lines that communicate between the nodes. We constructed different networks from the retrieved data. Drug-Target and Drug-Target-Protein interaction networks were constructed using Cytoscape 3.8.2. application which is an open source software based on java (Shannon et al, 2003).

2.5.2. Analysis and evaluation of networks

A cytoscape plugin called network analyzer was used to analyse and evaluate the networks (de Jong et al., 2003). All the nodes of the network were analysed using an index called "degree" given by the analyser (Yu et al, 2019). Degree value corresponds to the number of edges between a node and another node in the network. This index play a key role in the network and the value is positively associated with the significance of a node in the network (Die et al, 2018) (Raman et al., 2014).

2.6. Three way analysis of dexamethasone, ATRA and VPA

Since corticosteroids are the first line of therapy for patients with NS, we carried out a three way analysis of the steroidal drug dexamethasone together with ATRA and VPA with an aim to identify the overlapping targets and involvement of the targets of dexamethasone in NS. The targets of dexamethasone were obtained in the same way as that of ATRA and VPA from ChEMBL and Binding DB, as described in section 2.1.

3. Results

3.1. Collection of targets for ATRA and VPA and their associated diseases

On performing the network analysis of drug compounds ATRA and VPA, we acquired a total of 160 targets for VPA and 138 targets for ATRA. Out of these, 103 targets were common to both ATRA and VPA. These 103 targets included kidney related as well as other disease related targets. Thus, in all 195 targets were identified with a total of 92 uncommon or non-overlapping targets and 103 common or overlapping targets for ATRA and VPA (Fig. 1 and Fig. 6). All 195 targets obtained for ATRA and VPA were entered in DisGeNET database. We observed that out of 195, 114 targets were associated with different kidney related diseases such as nephrotic syndrome, diabetic nephropathy, kidney failure, renal cell carcinoma, renal hypertension, lupus nephritis, chronic kidney disease etc. Thus, out of the 103 targets that were common for ATRA and VPA, 65 genes were involved in kidney associated diseases (Fig. 1). Subsequently, a drug-target network was constructed using cytoscape (Fig. 6).

3.2. Collection of genes of NS

The genes specifically involved in nephrotic syndrome were collected from KEGG and DisGeNET database. Each of the input terms and their corresponding number of genes obtained are-nephrotic syndrome: 384, focal glomerulosclerosis: 281, childhood nephrotic syndrome: 23, idiopathic nephrotic syndrome: 51, nephrotic syndrome-minimal change disease: 85, steroid sensitive nephrotic syndrome: 36, nephrotic range proteinuria: 7, glomerulosclerosis (disorder): 221, IgA glomerulonephritis: 456, glomerulonephritis: 391, membranous glomerulonephritis:197, mesangioproliferative glomerulonephritis: 21, proteinuria: 239, steroid resistant nephrotic syndrome: 73, tubulointerstitial fibrosis: 328, renal interstitial fibrosis: 138, steroid dependent nephrotic syndrome: 6, primary focal segmental glomerulosclerosis: 18, secondary focal segmental glomerulosclerosis: 4, focal
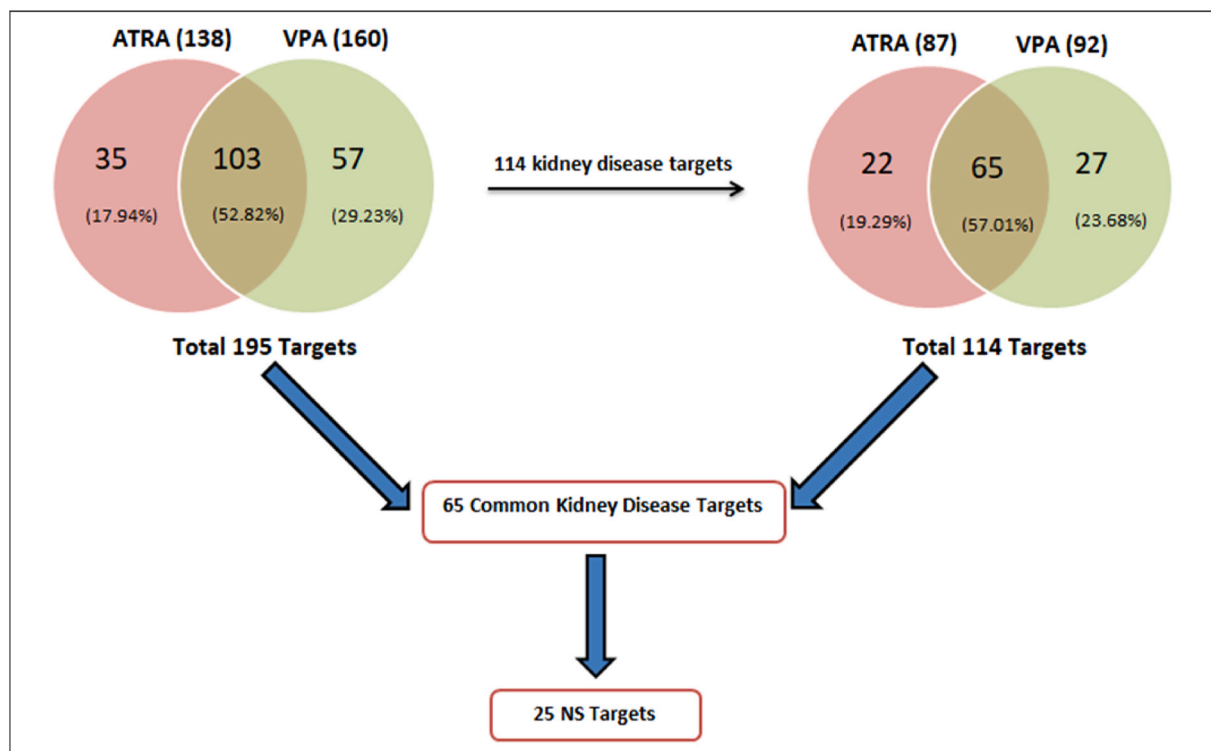

Fig. 1. Venn diagram for overlapping targets between ATRA and VPA and targets associated with kidney diseases. ATRA, all trans retinoic acid; VPA, valproic acid; NS, nephrotic syndrome.

segmental glomerulosclerosis: 42, renal fibrosis: 570, nephrotic syndrome-frequently relapsing: 5, nephrotic syndrome with focal and segmental sclerosis: 3, steroid resistant nephrotic syndrome of childhood: 73, and renal sclerosis: 41. Thus a total of 3693 NS genes were obtained from DisGeNET. Also, we observed that all the genes obtained from KEGG (33 genes) intersected with the genes obtained from DisGeNET. Additionally, genes of multiple input terms overlapped. Therefore, following elimination of duplicate genes and/or similar terms, a total of 1733 genes related to nephrotic syndrome were identified (Supplementary Table S1).

3.3. Potential target identification, gene mapping and protein-protein interactions

On mapping all the 195 collected targets of ATRA and VPA with 1733 genes associated with NS, we obtained 41 shared genes which were identified as potential targets for NS (Fig. 6). Out of the 41 genes, 25 genes were common to ATRA and VPA and the remaining 16 genes included 8 genes each of ATRA and VPA (Table 1 and Table 2). Additionally, 50 protein targets associated with these 25 common genes were acquired from STRING database (Fig. 5 and Supplementary Table S2). Subsequently, a drug-target-protein interaction network was constructed (Fig. 5).

3.3.1. Identifying overlapping genes between SRNS, SSNS, ATRA and VPA

With an aim to identify intersecting genes between SRNS and SSNS, and the overlapping targets between ATRA, VPA, SRNS and SSNS, we analysed all the targets that were obtained for NS. There were a total of 73 genes (after eliminating duplicates) specifically for the terms "steroid resistant nephrotic syndrome" and "steroid resistant nephrotic syndrome of childhood". A total of 36 genes for the term "steroid sensitive nephrotic syndrome" were obtained. 16 target genes were common between SRNS and SSNS. Out of which NR3C1 gene was common between SRNS, SSNS, ATRA and VPA. ABCB1, HDAC2, NFKB1 coincided between SRNS or SSNS, ATRA and/or VPA. All the four genes i.e., NR3C1, ABCB1, HDAC2, NFKB1 were the target genes involved in SRNS (Figs. 6 and 7).

Table 1

25 nephrotic syndrome (NS) related genes common to all trans retinoic acid (ATRA) and valproic acid (VPA).

UniProt IDs	Gene	Gene description
P50052	AGTR2	Type-2 angiotensin II receptor
P28482	MAPK1	Mitogen-activated protein kinase 1
P41597	CCR2	C-C chemokine receptor type 2
O76074	PDE5A	cGMP-specific 3',5'-cyclic phosphodiesterase
Q16539	MAPK14	Mitogen-activated protein kinase 14
P00533	EGFR	Epidermal growth factor receptor
P33261	CYP2C19	Cytochrome P450 2C19
P08246	ELANE	Neutrophil elastase
P17948	FLT1	Vascular endothelial growth factor receptor 1
P51681	CCR5	C-C chemokine receptor type 5
P25101	EDNRA	Endothelin-1 receptor
P03956	MMP1	Interstitial collagenase
P14780	MMP9	Matrix metalloproteinase-9
P03372	ESR1	Estrogen receptor
P37231	PPARG	Peroxisome proliferator-activated receptor gamma
P21554	CNR1	Cannabinoid receptor 1
P04150	NR3C1	Glucocorticoid receptor
P04626	ERBB2	Receptor tyrosine-protein kinase erbB-2
P17252	PRKCA	Protein kinase C alpha type
P29274	ADORA2A	Adenosine receptor A2a
P35354	PTGS2	Prostaglandin G/H synthase 2
P27361	MAPK3	Mitogen-activated protein kinase 3
Q16236	NFE2L2	Nuclear factor erythroid 2-related factor 2
P08575	PTPRC	Receptor-type tyrosine-protein phosphatase C
P08684	CYP3A4	Cytochrome P450 3A4

Table 2

41 (25 overlapping genes and 16 non-overlapping) genes involved in nephrotic syndrome (NS), renal sclerosis and fibrosis, glomerulonephritis and proteinuria. MCD: minimal change disease; SRNS: steroid resistant nephrotic syndrome; SSNS: steroid sensitive nephrotic syndrome; INS: idiopathic nephrotic syndrome; FSGS: focal segmental glomerulosclerosis.

Disease	Common NS Genes associated with the disease	Uncommon (non-overlapping) NS genes associated with the disease
MCD, SRNS, SSNS, INS	MAPK1	F3 (VPA)
	PDE5A	HDAC2 (VPA)
	MAPK14	LTA4H (VPA)
	CYP2C19	NFKB1 (VPA)
	FLT1	ABCB1 (ATRA)
	EDNRA	HIF1A (ATRA)
	MMP1	
	ESR1	
	NR3C1	
	PTGS2	
	CYP3A4	
Renal fibrosis	AGTR2	HDAC2 (VPA)
	MAPK1	HDAC1 (VPA)
	MAPK14	GSK3B (VPA)
	EGFR	HDAC4 (VPA)
	ELANE	NFKB1 (VPA)
	CCR5	VDR (ATRA)
	EDNRA	NR1H4 (ATRA)
	MMP1	MMP2 (ATRA)
	MMP9	ABCB1 (ATRA)
	PPARG	AR (ATRA)
	CNR1	HIF1A (ATRA)
	ERBB2	SMN1 (ATRA)
	PRKCA	
	ADORA2A	
	PTGS2	
	MAPK3	
	NFE2L2	
	PTPRC	
Glomerulosclerosis, FSGS	AGTR2	HDAC9 (VPA)
	MAPK1	HDAC2 (VPA)
	CCR2	HDAC1 (VPA)
	MAPK14	RARA (ATRA)
	MMP1	MMP2 (ATRA)
	MMP9	HIF1A (ATRA)
	PPARG	
	PRKCA	
	PTGS2	
	MAPK3	
	NFE2L2	
	PTPRC	
Glomerulonephritis, IgA glomerulonephritis, membranous glomerulonephritis	AGTR2	RARA (ATRA)
	MAPK1	VDR (ATRA)
	CCR2	MMP2 (ATRA)
	PDE5A	ABCB1 (ATRA)
	MAPK14	SMN1 (ATRA)
	EGFR	
	ELANE	
	CCR5	
	MMP1	
	PPARG	
	CNR1	
	NR3C1	
	PRKCA	
	MAPK3	
	NFE2L2	
	PTPRC	
Proteinuria, Nephrotic range proteinuria	FLT1	F3 (VPA)
	PTGS2	

3.4. Pathway enrichment and GO analysis

Pathway enrichment analysis of 25 overlapping genes using FunRich gave 241 enriched pathways with a p-value <0.05 and analysis in Reactome database gave 145 enriched pathways (Supplementary Table S3). No pathways were found for the gene NFE2L2 in Reactome

database. The top 20 significantly enriched pathways obtained from FunRich and Reactome database are shown in Fig. 2. A comparison of biological pathway of 25 common NS genes and 16 non-overlapping genes was performed using FunRich tool from reactome database. A doughnut chart of the overlaid pathways is represented in Fig. 3. The analysis showed that the genes were involved in pathways of activation of matrix metalloproteinases, collagen degradation, neutrophil degranulation, extra nuclear estrogen signalling, degradation of extracellular matrix, SUMOYlation of intracellular receptors, nuclear receptor transcription pathway, IL-4 and IL-13 signaling, regulation of PTEN gene transcription, and PPARA activates gene expression.

Gene ontology: Biological Process (BP), Molecular Function (MF) and Cellular component(CC) enrichment carried out (Supplementary Table S4) using FunRich tool ($p < 0.05$) gave 604 biological processes such as cell surface signaling pathway, cellular response to reactive oxygen species, positive regulation of MAP kinase activity, regulation of

blood pressure, regulation of inflammatory response, cellular response to TNF, cytokine mediated signaling pathway, renin-angiotensin regulation of aldosterone production, endoplasmic reticulum unfolded protein response, positive regulation of release of cytochrome *c* from mitochondria, regulation of mitochondrial membrane potential etc. The different molecular function (MF) that were enriched were enzyme binding, ligand binding, MAP kinase activity, nitric oxide synthase regulator, ATP binding, chromatin binding, transcription cofactor binding. The enriched cellular components (CC) were plasma membrane, cytoplasm, endoplasmic reticulum lumen, mitochondria, cell surface, chromatin etc. The top 5 enriched GO: BP, MF, CC are shown in Fig. 4.

3.5. Network analysis

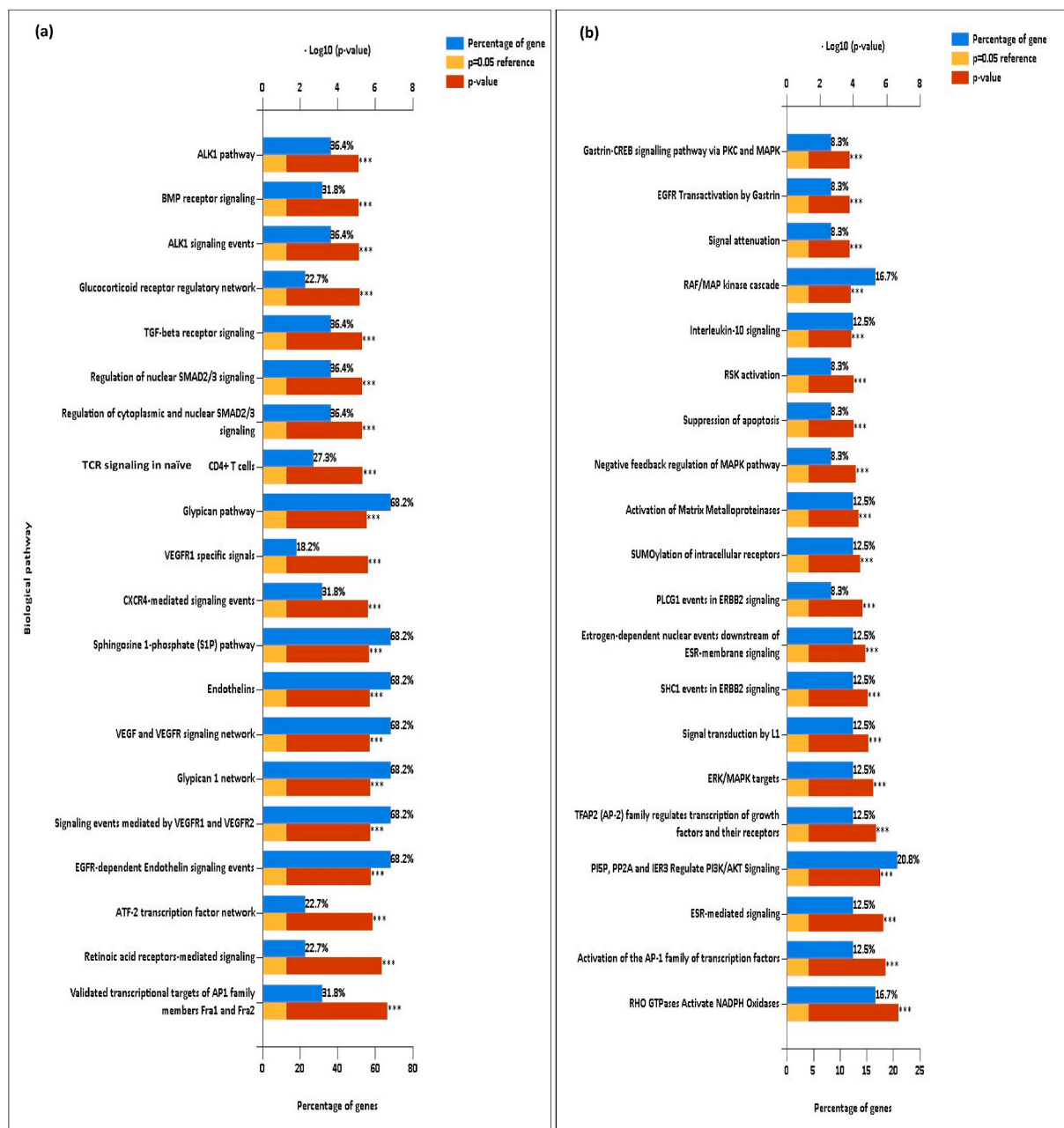A drug-target-protein interaction network of 25 overlapping targets

Fig. 2. Top 20 significantly enriched biological pathways of 25 nephrotic syndrome (NS) related genes obtained using FunRich tool from **(a)** FunRich database and **(b)** reactome database. ***$p < 0.001$.
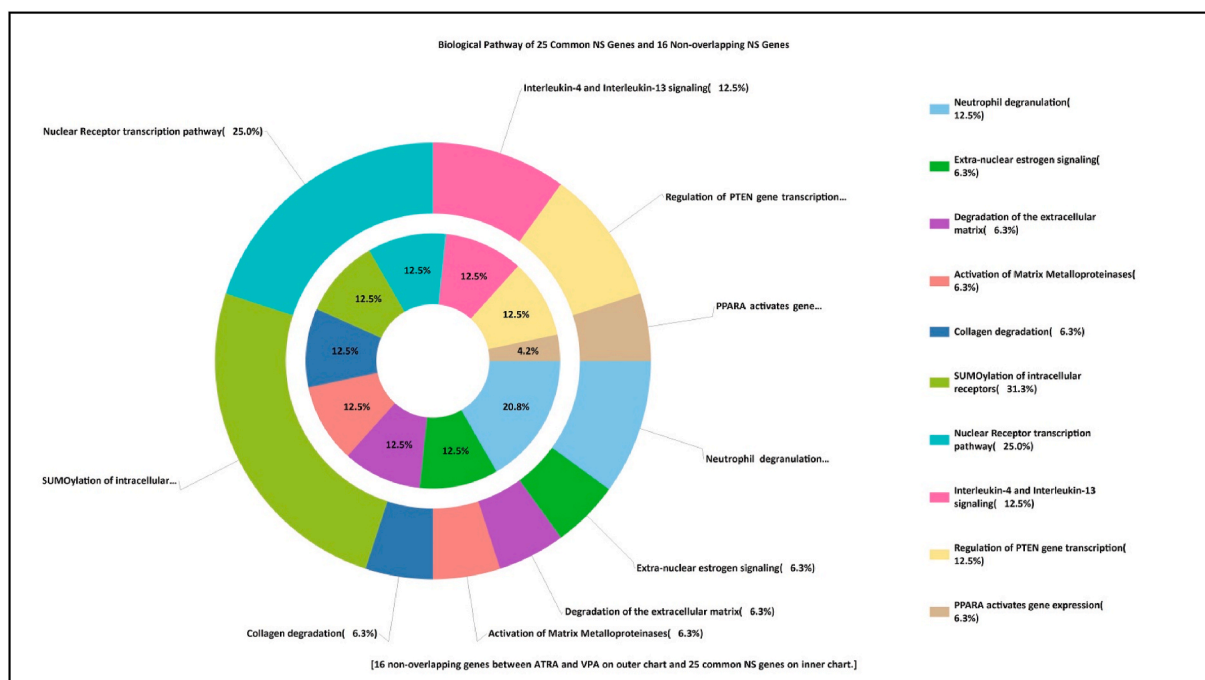
Fig. 3. Doughnut chart of biological pathways of 25 common NS genes and 16 non-overlapping genes created using FunRich tool.

of ATRA and VPA and the proteins which interacted with these targets was constructed using the Cytoscape 3.8.2 software (Fig. 5). In the network, there were in all 77 nodes and 418 edges. The average degree value that was calculated by Network Analyzer was 10.857. The 25 targets interacted with 50 proteins in 368 ways (Supplementary Table S5). There were a total of 33 nodes in the network having degree values greater than the average degree value (Supplementary Table S6). In the drug-target network, there were 197 nodes and 298 edges representing the targets of the two drugs ATRA and VPA (Fig. 6).

3.6. Three way analysis of dexamethasone, ATRA and VPA

126 genes targets were obtained for dexamethasone from ChEMBL and Binding DB after elimination of all the duplicate gene targets (Supplementary Table S7). From 126 genes, 106 genes intersected between ATRA and/or VPA. Out of 103 NS genes that were common between ATRA and VPA, 94 genes intersected with dexamethasone. Five genes of SRNS namely NR3C1, RELA, ABCB1, TNF, ALB overlapped with the target genes of dexamethasone.

4. Discussion

Network pharmacology is a growing field that is used widely in the area of drug discovery process. It involves computational biology with the amalgamation of systems biology and omic technology (Zhang et al., 2013) (Hopkins, 2008). Many of the drugs that are used to treat cancer, heart diseases, neurological disorders and other chronic illnesses can act on multiple targets acting via numerous biological pathways (Luo et al., 2014). Thus it becomes necessary to understand the complex pathways that a drug can act in different diseases. Moreover, it is implicated that drugs when used in combination can have an impact on multiple targets and can be effective in controlling complex diseases as compared to single drug that binds to a specific target (Zimmermann et al., 2007). Several studies were carried out that involved herbal medicines and traditional Chinese medicines that unravelled their therapeutic benefits in treating diabetic kidney disease, renal failure and renal fibrosis. Network pharmacology approach was used in these studies to identify the mechanisms of the drugs. A network pharmacology study carried out by Lifei Gu et al., identified the mechanisms and active components of *Abelmoschus manihot* (L.) Medik. It is a herbal plant and it's extract is used in the patented medicine for treating chronic nephritis in China (Zhang, 2020). A study carried out by Dong et al., identified the targets of astragalus membranaceous-angelica sinensis compound to understand the potential mechanism of the compound in diabetic nephropathy using network pharmacology (Dong et al., 2021). Qian et al., and Yan Li et al., used the network pharmacology approach to unravel the mechanism of Cordyceps Cicadae and Cordyceps sinensis respectively in diabetic nephropathy (Qianet al, 2021) (Liet al, 2021). Another study carried out by Inoue et al., analysed the beneficial effects of VPA using bioinformatics tool (Inoueet al, 2019a). Understanding the network of interactions between drugs and protein molecules using network pharmacology, contributes to identifying potential drugs and their targets in treating diverse complex diseases. This can be valuable for drug repurposing and identifying synergism between drugs (Luo et al., 2014) (Zimmermann et al., 2007) (Il Goh et al., 2007).

In the present study we used this network pharmacology method to explore the targets and synergistic potential of two drugs ATRA and VPA in treating NS. The drugs have been known for their anti-inflammatory, anti-neoplastic activity and have been much explored and used in treating cancer alone as well as in combination. However, not much has been investigated with respect to their synergistic potential in treating chronic kidney disease like NS. We first obtained all the targets of ATRA and VPA from ChEMBL and Binding DB. ChEMBL and Binding DB are chemical databases of molecules that have drug like properties and biological activities that integrate genomic data, contribute to understanding interaction of proteins and ligands and helps in identifying new drugs and obtaining the targets for the drug molecules. Once the targets were obtained, each target was then searched in DisGeNET database to screen for targets associated with kidney diseases. DisGeNET is a database that focuses on human genes and their association to diseases and KEGG is database specially designed to give data regarding genomes, pathways, drugs and diseases. We retrieved 114 targets in total out of 195 targets of ATRA and VPA that had a share in different kidney diseases such as nephrotic syndrome, diabetic nephropathy, kidney failure, renal cell carcinoma, renal hypertension, lupus nephritis, chronic kidney disease, glomerulosclerosis, and renal fibrosis. It was observed that the
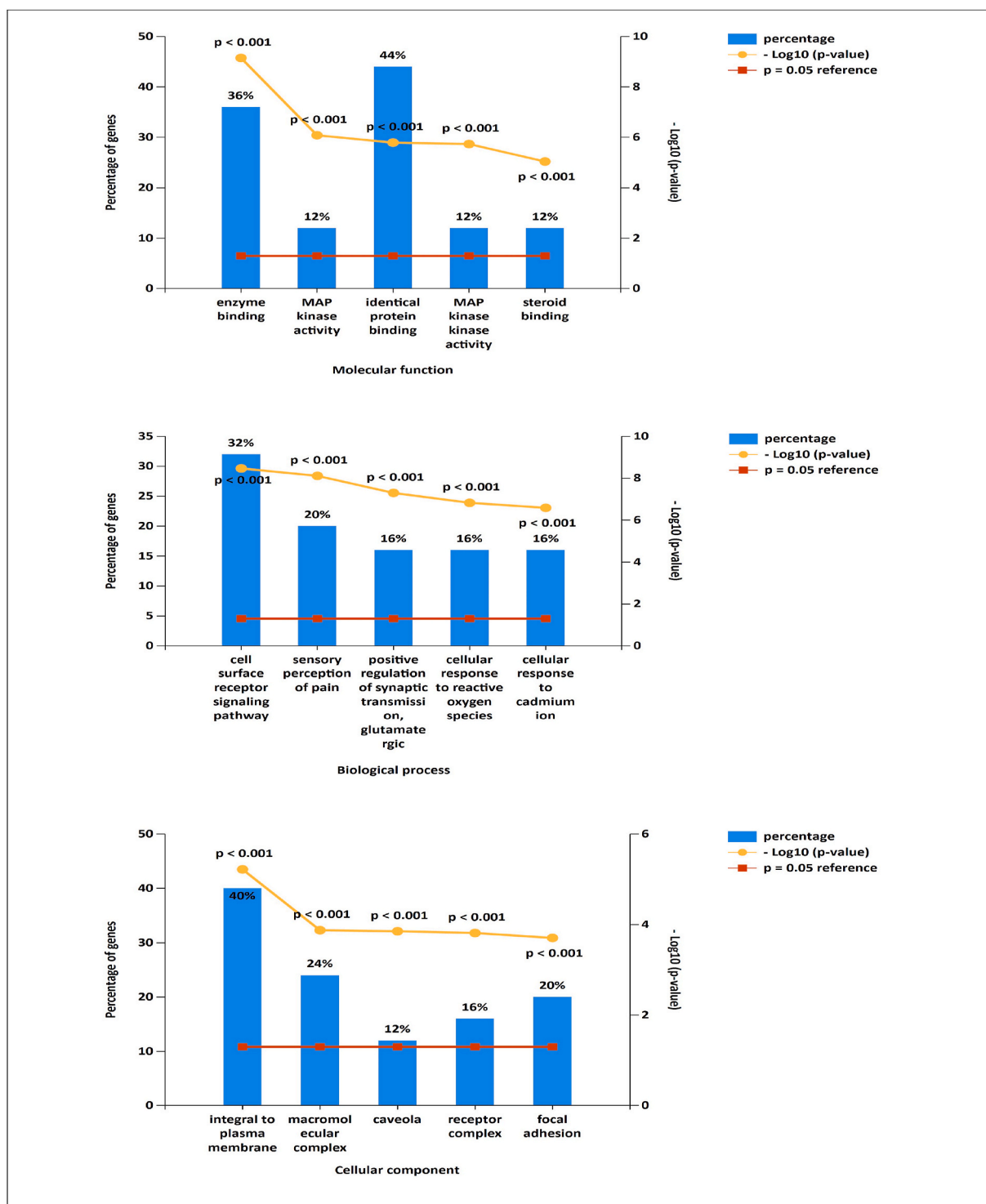
Fig. 4. Gene Ontology enrichment data of 25 common NS genes using FunRich tool (a.) Top 5 enriched molecular function (b.) Top 5 enriched biological process (c.) Top 5 enriched cellular component.

drugs had 65 targets in common for ATRA and VPA. Secondly, all the genes associated with NS were collected from DisGeNET and KEGG and were then mapped to the target genes of ATRA and VPA. The results revealed 41 targets that overlapped with NS genes out of which 25 genes were common for ATRA and VPA suggesting a potential role of the two drugs in treating NS. We constructed drug-target and drug-target-protein interaction networks in cytoscape and 50 interacting proteins of the targets were identified from STRING database. The STRING database gives information pertaining to known and predicted protein-

protein interactions. Some of the interacting proteins identified were PIK3R1, EGF, STAT3, VEGFA, VEGFB and KEAP1 (Fig. 5).

In the three way analysis of dexamethasone, ATRA and VPA, the four intersecting genes (excluding ALB) between SRNS and dexamethasone were either the targets of ATRA and/or VPA or were identified to play a role in inflammatory pathways in the pathway and GO analysis of intersecting NS genes between ATRA and VPA. Thus our analysis indicates that ATRA and VPA can act on several genes involved in SRNS. Additionally, ALB gene that encodes for the protein albumin was
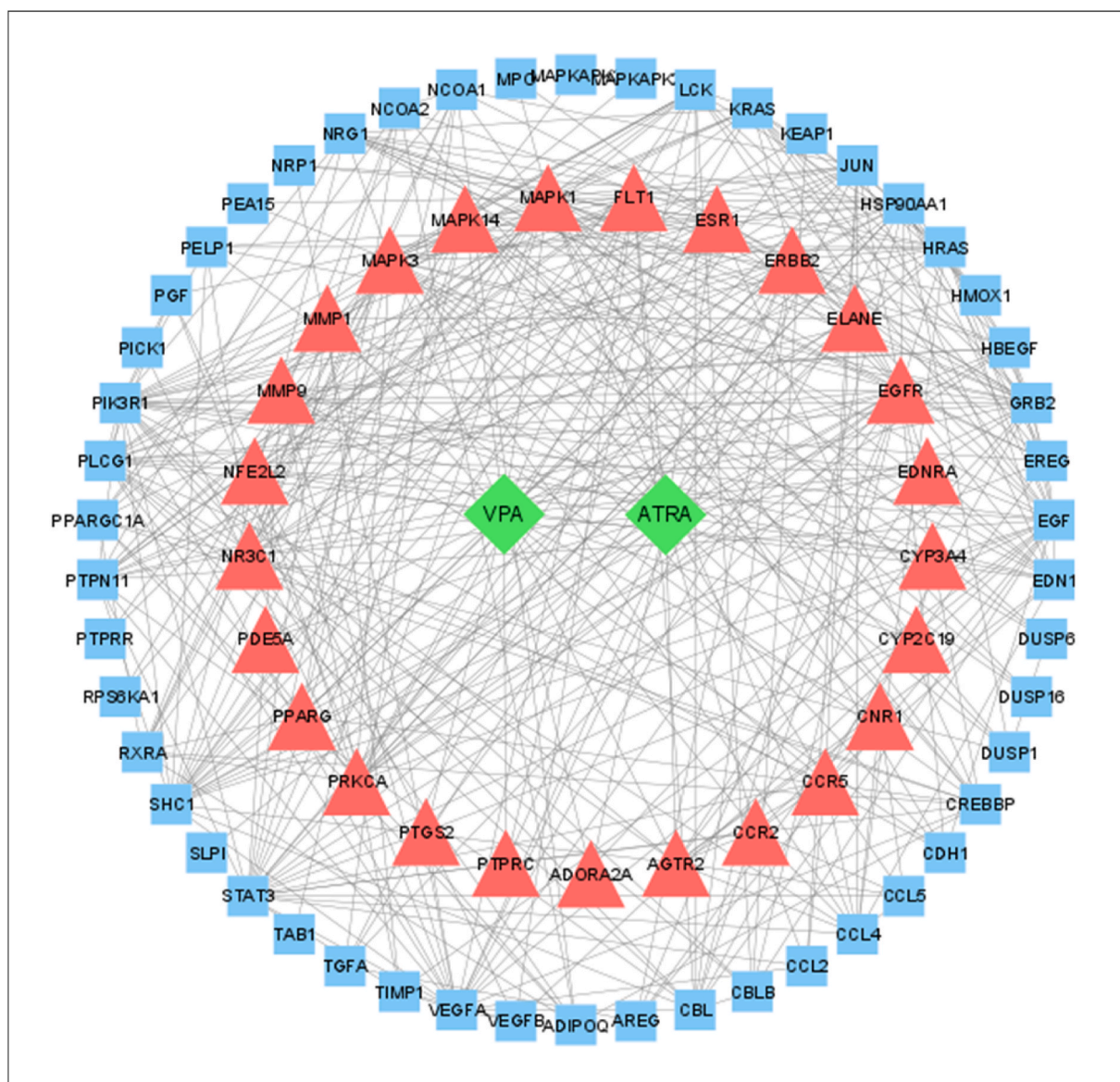
Fig. 5. Drug-NS specific target-protein interaction network. Triangle nodes represent the 25 targets specific to nephrotic syndrome (NS) that are common to all trans retinoic acid (ATRA) and valproic acid (VPA). Square nodes represent 50 different proteins that interact with NS specific targets. The edges represent interaction between the targets and proteins.

identified as one of the five targets of dexamethasone. 106 target genes of dexamethasone intersected with ATRA and VPA which shows that the two drugs can act on the targets of the steroidal drug dexamethasone. The remaining 20 genes of dexamethasone also intersected with the protein targets obtained for ATRA and VPA and were also involved in pathways identified in FUNRICH pathway analysis and GO analysis. HDAC2 was identified as one of the target gene of SRNS and an increase in HDAC2 enzyme has been recently identified to play a central role in causing podocyte damage, proteinuria and glomerulosclerosis (Liu et al., 2020). VPA being an HDAC inhibitor can act on HDAC2 and thus could play a vital role in steroid resistance by preventing podocyte damage and protein leakage.

Analysis of pathways is carried out to understand the mechanisms and pathways of target genes participating in various biological functions. Therefore, we carried out the pathway enrichment analysis, the results of which revealed that multiple common targets of ATRA and VPA took part in multiple pathways such as TGF-β signalling, PI3K/AKT signalling, RHO-GTPases, ERK/MAPK targets signalling, activation of MMPs, pathways of inflammation, suppression of apoptosis (Fig. 2

Supplementary Table S3). The TGF-β is a cytokine that is intently linked to many glomerular diseases and in a diverse podocyte diseases such as membraneous nephropathy, diabetic nephropathy, and FSGS, its receptors become upregulated (Zhou and Yang, 2008). TGF-β exerts its action through SMAD-independent pathways, including Rho GTPases, MAP kinases, ERK, Cdc42, ILK, β-catenin, and PI3K–Akt–mTOR cascades (Hallet al, 2018) (Zhou and Yang, 2008). The PI3K-Akt signalling pathways are associated with podocyte apoptosis and their activation regulates apoptosis (Ren and You Yu, 2016) (Manning and Cantley, 2007) (Song et al., 2005). The drugs VPA and ATRA regulate TGF-β signalling and partake in PI3K-Akt and MAPK pathways in several types of cancer (Liu et al., 2008) (Qiet al, 2019) (Duenas-Gonzalez et al., 2008) (Fenget al, 2012). Thus our results of functional and pathway analysis indicates the potential synergistic involvement of ATRA and VPA, in chronic renal diseases including NS by acting on biological pathways contributing to NS.

Renal fibrosis and glomerulosclerosis is a typical manifestation in several types of NS. Glomerulosclerosis involves tissue scaring and thickening of glomeruli of the kidneys. Renal fibrosis occurs due to long
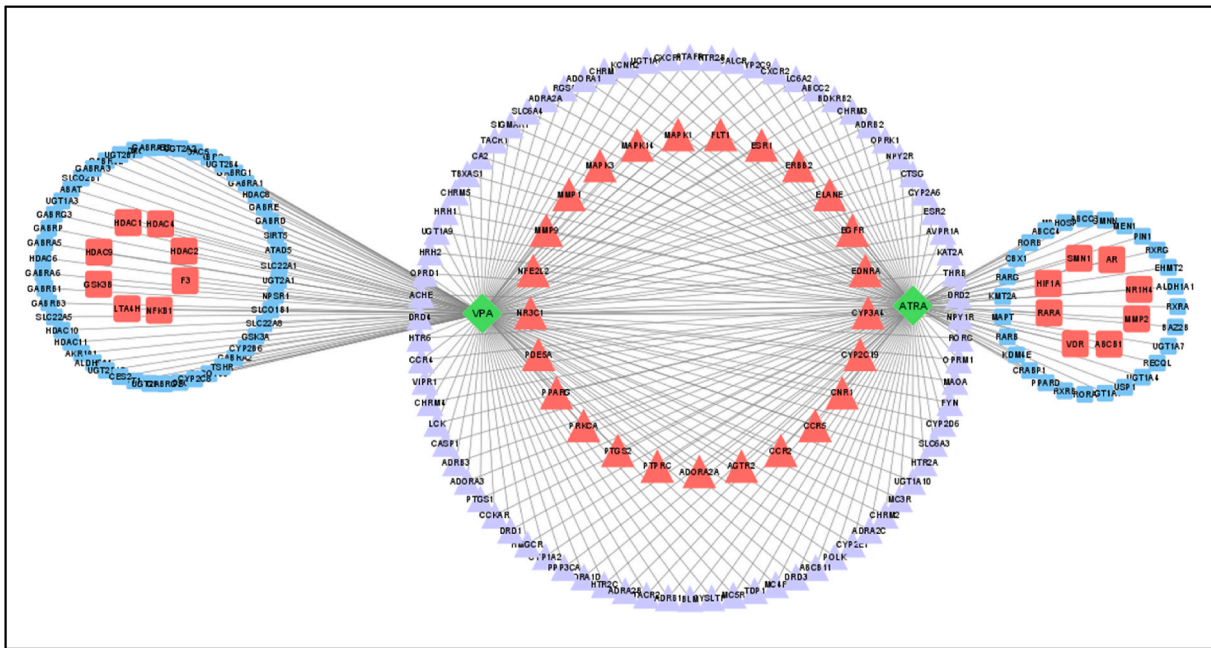
Fig. 6. Drug-Target network; Diamond nodes in green colour represent the two drug compounds all trans retinoic acid (ATRA) and valproic acid (VPA). The triangle nodes represent the targets which are common for ATRA and VPA and square nodes represents the non-overlapping targets of ATRA and VPA. Red coloured triangle nodes indicate the 25 targets related to nephrotic syndrome. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
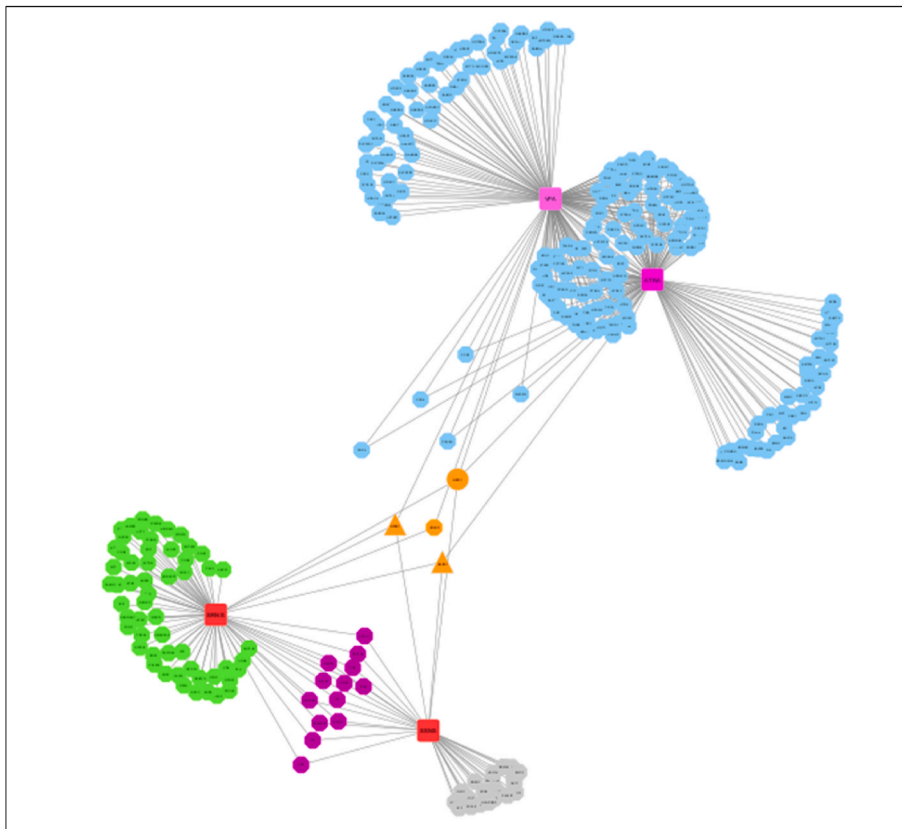

Fig. 7. Network of overlapping targets between ATRA, VPA, SRNS and SSNS. The green and the grey nodes represent the target genes of SRNS and SSNS. The purple nodes indicate the overlapping genes between SRNS and SSNS. Blue nodes represent the targets of the two drugs ATRA and VPA. The square nodes in pink and red represents the two drugs ATRA, VPA and SRNS, SSNS respectively. Four genes i.e., NR3C1, ABCB1, HDAC2, NFKB1 (represented as orange nodes) were the target genes involved in SRNS. SRNS: steroid resistant nephrotic syndrome; SSNS: steroid sensitive nephrotic syndrome; ATRA: all trans retinoic acid; VPA: valproic acid. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

term inflammation resulting in production of growth factors, angiogenic factors, cytokines and proteinase. All of these factors stimulate excessive accumulation of extracellular matrix components through epithelial to mesenchymal transition (EMT), which leads to production of myofibroblasts and induces accelerated kidney dysfunction and failure (Liu, 2010) (Iwano et al., 2002). Renal fibrosis is the chief process in the progression of NS along with glomerulosclerosis and changes in renal vasculature (Boor et al., 2007). It serves as a final common pathway

leading to almost all forms of chronic kidney diseases (Nangaku, 2004). Our findings revealed that the 25 targets genes common to ATRA and VPA participated in renal fibrosis in steroid resistant nephrotic syndrome, steroid sensitive nephrotic syndrome, idiopathic nephrotic syndrome, focal segmental glomerulosclerosis, glomerulonephritis, and nephrotic range proteinuria (Table 2). Out of the 25 gene targets, 18 targets were associated with renal and tubulointerstitial fibrosis, 12 genes were involved in glomerulosclerosis and 11 genes were involved in different types of NS such as idiopathic nephrotic syndrome, minimal change disease, steroid resistant and steroid sensitive nephrotic syndrome. MAPK1, MAPK14, MMP1, and PTGS2 were the key targets that were associated with renal fibrosis, glomerulosclerosis and NS. Pathway analysis in Reactome and FunRich revealed the involvement of genes in immune mediated pathways such as interleukins signalling, signalling events mediated by VEGFR1 and VEGFR2 signalling. These immune mediated receptors have been known to participate in nephrotic syndrome (Ereminaet al, 2003). Other enriched pathways identified include RAF/MAP kinase pathway, transcription pathways, MAPK pathways, and activation of MMPs. MMPs and VEGF are known to contribute in renal fibrosis and renal damage (Boor et al., 2007) (Catania et al., 2007). The analysis thus reveals the involvement of ATRA and VPA targets in these pathways.

PGC-1α plays a major role in mitochondrial biogenesis and is considered as a master regulator (Hasegawa and Inagi, 2020). PPAR-γ is another key transcription factor that is involved in a variety of cellular functions such as energy metabolism, regulation of mitochondrial function, oxidation of fatty acids and regulation of anti-oxidant defence mechanisms. Our analysis identified PGC-1α as the interacting protein in the network (Corona and Duchen, 2016). In NS, oxidative stress is generated in podocytes which contributes to tissue injury, inflammation, and podocyte detachment (Massyet al, 2005). Nuclear factor erythroid 2-related factor 2 (NFE2L2) also called as NRF2 is an important transcription factor involved in protection of oxidative stress, antioxidant machinery acting as well as mitochondrial biogenesis via NRF2-KEAP1 pathway (Ruiz et al., 2013) (de Zeeuwet al, 2013). NRF2 (NFE2L2) was noted to be a common target for ATRA and VPA and KEAP-1 was identified as the interacting protein thereby directing their synergistic role in anti-oxidant mechanisms and in regulating mitochondrial biogenesis in NS, possibly in podocytes. Further studies are however essential to confirm their action and efficacy in pathogenesis of NS. We also identified the gene JUN, which encodes for activator protein-1 (AP-1) transcription factor c-Jun. This transcription factor is known for its anti-apoptotic role in cancer and is an important target for Jun-N terminal kinase (JNK). c-Jun and JNK are also associated with endoplasmic reticulum (ER) stress and the transcription factor c-Jun is known to act against ER stress in neuronal and hepatic tissues (Senft and Ronai, 2015). Our network analysis revealed that 9 overlapping genes of ATRA and VPA that played a role in NS namely, MAPK1, MAPK14, MAPK3, STAT3, PPARG, NR3C1, NCOA1, NFE2L2 and VEGFA interacted with JUN, suggesting a possibility of targeting these genes in treating NS with respect to ER stress (Supplementary Table S3). Pathway analysis also revealed AP-1 mediated transcription signalling pathways involving ATF2 which is member of AP-1 complex further suggesting a role of ATRA and VPA synergistically in ER stress pathways. However, studies are further required to be carried out in renal tissues in order to validate and confirm the role of these drugs in ER stress. Further, we identified genes such as ADORA2A and ELANE that were common for ATRA and VPA. Data regarding their contributing roles in NS is meagre which necessitates further explorative studies in understanding the action of ATRA and VPA synergistically through these targets.

The analysis of GO (BP, MF and CC) revealed that the 25 genes participated in cell surface signaling pathway, cellular response to reactive oxygen species, regulation of mitochondrial membrane potential, positive regulation of MAP kinase activity, regulation of blood pressure, regulation of inflammatory response, cellular response to TNF, cytokine mediated signaling pathway, renin-angiotensin regulation of

aldosterone production, endoplasmic reticulum unfolded protein response, positive regulation of release of cytochrome c from mitochondria and were involved in various function viz. ligand binding, MAP kinase activity, nitric oxide synthase regulator, ATP binding, chromatin binding, transcription cofactor binding. The genes were localized in different cellular components such as plasma membrane, cytoplasm, endoplasmic reticulum lumen, mitochondria, cell surface, chromatin etc. Thus the GO enrichment data further strengthens the role of synergistic activity of ATRA and VPA in treating NS possibly through pathways of inflammation, gene transcription, oxidative stress, mitochondrial and ER pathways.

Apart from the overlapping 25 genes associated with NS, additional 16 genes identified for ATRA and VPA were NFKB, HDAC 1, 2, 4, and 9, F3, GSK3B, LTA4H, RARA, VDR, NR1H4, MMP2, ABCB1, AR, HIF1A, and SMN1 (Table 2). Our results revealed that these 16 targets have their involvement in renal sclerosis and fibrosis associated with different types of NS thus indicating that these targets can be aimed and explored so as to identify their roles in treating NS when ATRA and VPA is administered to obtain synergistic outcomes. The overlaid doughnut plot of biological pathways revealed that the 16 genes participated in pathways of collagen degradation, degradation of ECM, activation of MMP, and interleukin signalling further suggesting their potential role in NS pathogenesis.

MAPK3, MAPK1, MAPK14 were among the targets having major involvement in the drug-target-protein interaction network as well as they were involved in multiple biological pathways suggesting their significant role in NS. Thus, the activity of MAPK in NS may be explored when ATRA and VPA is given in combination.

HIFA was identified as one of the targets of ATRA and NF-κ B as one of the targets of VPA. HIFA is associated with changes in inducing podocyte foot process, thickening of glomerular basement membrane, and accumulation of collagen (Steenhardet al, 2010). Production of proinflammatory cytokines that are controlled by NF-κ B is responsible for progression of inflammation while inhibition of this pathway attenuates renal inflammation and fibrosis (Mezzanoet al, 2001). This therefore supports the potential anti-inflammatory role of HDAC inhibitors pointing towards the beneficial role of VPA in NS. Although the exact mechanism of action of ATRA and VPA that display protective action on podocytes, its structure and function remains elusive, several studies have demonstrated the benefits and probable mechanisms of these drugs in renal physiology. In our analysis, HDAC 1, 2, 4 and 9 were the targets of VPA that were identified to be associated with NS. A recent study carried out by Inoue et al., demonstrated that modification (inhibition) of HDAC 1 and 2 in podocytes diminished albuminuria and glomeruloscelrosis via regulation of early growth response 1 (EGR1) in podocytes (Inoueet al, 2019a). EGR1 is a transcription factor which is responsible for regulating actin cytoskeleton structure and podocyte death. An increase in EGR1 expression has been noted in the podocytes of patients with NS. Also, HDAC4 and HDAC 9 expression was increased following podocyte injury inhibiting autophagy, reducing nephrin and podocin expression. Therefore, silencing of HADC 4 and 9 expressions can attenuate injury to podocytes (Inoueet al, 2019a) (Liuet al, 2016) (Wanget al, 2014). Thus it was demonstrated from the study that HDACs plays a crucial role in podocyte injury and damage, and HDAC 1 and 2 is primarily involved in the mechanism of podocyte damage through an increased expression of EGR1 and treatment with HDAC inhibitor VPA led to a reduced EGR1 expression (Inoueet al, 2019b). Several other mechanisms that lead to podocyte loss and injury have been reported in various studies. Multiple other mechanisms have been identified in which HDAC inhibitors have been involved and are associated with attenuating podocyte injury. These are MAPK pathway, ROS pathway, NF-κB/iNOS signalling pathway, TGF-β1/Smad pathway and apoptosis signalling pathway (Varghese and Majumdar, 2021) (Van Benedenet al, 2013) (Van Benedenet al, 2011). ATRA has been identified to exert protective effects on podocytes through mechanisms of inhibition of NF-κB gene and c-AMP mediated podocyte differentiation

(Sierra-Mondragon et al., 2018). It has also been identified to act by down-regulating TGF-β1/Smad pathway, by reduction of ROS generation and inhibiting protein kinase C activity (Sierra-Mondragonet al, 2019) (Kim et al., 2015) (Liu et al., 2011). Even though many mechanisms are proposed and identified for HDAC inhibitors and ATRA there is still a need to understand the exact mechanism on how these compounds exerts their protective effects in renal dysfunction. Further clinical and preclinical studies are therefore crucial.

Although no clinical studies have been conducted on the combination of ATRA and VPA for treating renal diseases, there are several clinical studies that are ongoing with different HDACi and specifically VPA (Varghese and Majumdar, 2021). Recently, Veterans Aging Cohort Study was conducted by Inoue et al., in patients exposed to VPA treatment. The study noted that VPA exposure significantly brought down the decline in estimated glomerular filtration rate. Also, the beneficial effect of VPA was more distinct in proteinuric patients (Inoueet al, 2019a). Our present study will surely unlock the possibilities for conducting more preclinical studies on the drug combinations of ATRA and VPA which would eventually pave way to clinical studies to cross potential barriers in the area of nephrotic syndrome.

5. Conclusion

Our study revealed that ATRA and VPA were commonly involved in multiple biological pathways associated with NS. NS is a complex kidney condition and there is no specific treatment available so far to achieve complete remission. Repurposing of drugs is a strategy that is greatly explored by pharmaceutical industry. Our results from network pharmacology analysis unravelled multiple targets that were common for the two drugs, that act in unison in biological pathways of mitochondrial biogenesis, ER stress and pathways associated with renal fibrosis and sclerosis. These pathways can contribute in understanding the complex disease mechanisms of NS. We therefore identify the potential of ATRA and VPA acting synergistically in treating NS. The synergism of ATRA and VPA thus converges to be effective in treating renal fibrosis, sclerosis, inflammation and proteinuria which are implicated in the pathogenesis and tissue re-engineering witnessed in NS. The study gave rise to identification of pathways and mechanisms associated with our drug candidates, which were less explored in treating nephrotic syndrome. Our analyses revealed that pathways of mitochondrial biogenesis and ER stress played a part in nephrotic syndrome and are critical pathways that need intervention to treat the condition along with other crucial pathways of inflammation, sclerosis and fibrosis. These two pathways have not been investigated considerably with regards to NS.

Overall, our work points towards a new prospect for treating the chronic renal condition of nephrotic syndrome with the help of network pharmacology analysis. We identify network pharmacology as an initial inherent approach in identifying drug candidates for repurposing and synergism. Further additional preclinical and clinical studies are undeniably requisite for validation and affirmation. Our study outcome will thus undoubtedly invigorate further preclinical and clinical explorative studies.

CRediT authorship contribution statement

Rini Varghese: Conceptualization, Resources, Writing – original draft, Preparation, Investigation, Methodology, Formal analysis. **Anuradha Majumdar:** Project administration, Visualization, Conceptualization, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crphys.2021.12.004.

References

Abraham, G., et al., 2016. Chronic kidney disease hotspots in developing countries in South Asia. Clin. Kidney J. 9 (1), 135–141.

Avner, N.Y.E., Harmon, W., Niaudet, P., 2009. Pediatric Nephrology, vol. 1.

Bairoch, A., et al., 2005. The Universal Protein Resource (UniProt). Nucleic Acids Res. 33, 154–159 no. DATABASE ISS.

Berdasco, M., Esteller, M., 2013. Genetic syndromes caused by mutations in epigenetic genes. Hum. Genet. 132 (4), 359–383.

Boor, P., Šebeková, K., Ostendorf, T., Floege, J., 2007. Treatment targets in renal fibrosis. Nephrol. Dial. Transplant. 22 (12), 3391–3407.

Bug, G., et al., 2005. Clinical trial of valproic acid and all-trans retinoic acid in patients with poor-risk acute myeloid leukemia. Cancer 104 (12), 2717–2725.

Catania, J.M., Chen, G., Parrish, A.R., 2007. Role of matrix metalloproteinases in renal pathophysiologies. Am. J. Physiol. Ren. Physiol. 292 (3).

Chandran, U., Mehendale, N., Tillu, G., Patwardhan, B., 2015a. Network pharmacology: an emerging technique for natural product drug discovery and scientific research on ayurveda. Proc. Indian Natl. Sci. Acad. 81 (3), 561–568.

Chandran, U., Mehendale, N., Tillu, G., Patwardhan, B., 2015b. Network pharmacology of ayurveda formulation triphala with special reference to anti-cancer property. Comb. Chem. High Throughput Screen. 18 (9), 846–854.

Cimino, G., et al., 2006. Sequential valproic acid/all-trans retinoic acid treatment reprograms differentiation in refractory and high-risk acute myeloid leukemia. Cancer Res. 66 (17), 8903–8911.

Cinatl Jr., Jindrich, et al., 1997. Sodium Valproate Inhibits in vivo growth of Human neuroblastoma cells. Anti Cancer Drugs 8, 958–963.

Corona, J.C., Duchen, M.R., 2016. PPARγ as a therapeutic target to rescue mitochondrial function in neurological disease. Free Radic. Biol. Med. 100, 153–163.

dan Xiong, D., et al., 2018. A network pharmacology-based analysis of multi-target, multi-pathway, multi-compound treatment for ovarian serous cystadenocarcinoma. Clin. Drug Invest. 38 (10), 909–925.

de Jong, H., Geiselmann, J., Hernandez, C., Page, M., 2003. Genetic network analyzer: qualitative simulation of genetic regulatory networks. Bioinformatics 19 (3), 336–344.

de Zeeuw, D., et al., 2013. Bardoxolone methyl in type 2 diabetes and stage 4 chronic kidney disease. N. Engl. J. Med. 369 (26), 2492–2503.

Deu-pons, J., Bauer-mehren, A., Baron, M., Sanz, F., Furlong, L.I., 2015. Database tool DisGeNET : a Discovery Platform for the Dynamical Exploration of Human Diseases and their Genes, pp. 1–17.

Di, S., et al., 2018. A network pharmacology approach to uncover the mechanisms of Shen-Qi-Di-Huang decoction against diabetic nephropathy. Evid. base Compl. Alternative Med. 2018, 1–14, 2018.

Dong, Y., Zhao, Q., Wang, Y., 2021. Network pharmacology-based investigation of potential targets of astragalus membranaceous-angelica sinensis compound acting on diabetic nephropathy. Sci. Rep. 11 (1), 1–16.

Doucet, A., Favre, G., Deschênes, G., 2007. Molecular mechanism of edema formation in nephrotic syndrome: therapeutic implications. Pediatr. Nephrol. 22 (12), 1983–1990.

Duenas-Gonzalez, A., Candelaria, M., Perez-Plascencia, C., Perez-Cardenas, E., de la Cruz-Hernandez, E., Herrera, L.A., 2008. Valproic acid as epigenetic cancer drug: preclinical, clinical and transcriptional effects on solid tumors. Cancer Treat Rev. 34 (3), 206–222.

Eremina, V., et al., 2003. Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. J. Clin. Invest. 111 (5), 707–716.

Feng, D., et al., 2012. Combination of valproic acid and ATRA restores RARβ2 expression and induces differentiation in cervical cancer through the PI3K/Akt pathway. Curr. Mol. Med. 12 (3), 342–354.

Gao, L., Wang, K.X., Zhou, Y.Z., Fang, J.S., Qin, X.M., Du, G.H., 2018. Uncovering the anticancer mechanism of Compound Kushen Injection against HCC by integrating quantitative analysis, network analysis and experimental validation. Sci. Rep. 8 (1), 1–15.

Gee, H.Y., et al., 2015. KANK deficiency leads to podocyte dysfunction and nephrotic syndrome. J. Clin. Invest. 125 (6), 2375–2384.

Glassock, R.J., Warnock, D.G., Delanaye, P., 2017. The global burden of chronic kidney disease: estimates, variability and pitfalls. Nat. Rev. Nephrol. 13 (2), 104–114.

Hadden, M.J., Advani, A., 2018. Histone deacetylase inhibitors and diabetic kidney disease. Int. J. Mol. Sci. 19 (9).

Hall, G., et al., 2018. The human FSGS-causing ANLN R431C mutation induces dysregulated PI3K/AKT/mTOR/Rac1 signaling in podocytes. J. Am. Soc. Nephrol. 29 (8), 2110–2122.

Hasegawa, S., Inagi, R., 2020. Organelle stress and crosstalk in kidney disease. Kidney 1 (10), 1155–1162, 360.

Hopkins, A.L., 2008. Network pharmacology: the next paradigm in drug discovery. Nat. Chem. Biol. 4 (11), 682–690.

Il Goh, K., Cusick, M.E., Valle, D., Childs, B., Vidal, M., Barabási, A.L., 2007. The human disease network. Proc. Natl. Acad. Sci. U. S. A. 104 (21), 8685–8690.

Inoue, K., et al., 2019a. Podocyte histone deacetylase activity regulates murine and human glomerular diseases. J. Clin. Invest. 129 (3), 1295–1313.

Inoue, K., et al., 2019b. Podocyte histone deacetylase activity regulates murine and human glomerular diseases Podocyte histone deacetylase activity regulates murine and human glomerular diseases, 129 (3), 1295–1313.

Iwano, M., Plieth, D., Danoff, T.M., Xue, C., Okada, H., Neilson, E.G., 2002. Evidence that fibroblasts derive from epithelium during tissue fibrosis. J. Clin. Invest. 110 (3), 341–350.

Jensen, L.J., et al., 2009. STRING 8-A global view on proteins and their functional interactions in 630 organisms. Nucleic Acids Res. 37 (Suppl. 1), 412–416.

Kanehisa, M., 2002. The KEGG Database, vol. 247, pp. 91–103.

Khan, S., Jena, G., Tikoo, K., Kumar, V., 2015. Valproate attenuates the proteinuria, podocyte and renal injury by facilitating autophagy and inactivation of NF-κB/iNOS signaling in diabetic rat. Biochimie 110, 1–16.

Kim, C.S., Park, J.S., Ahn, C.W., Kim, K.R., 2015. All-trans retinoic acid has a potential therapeutic role for diabetic nephropathy. Yonsei Med. J. 56 (6), 1597–1603.

Korbet, S.M., 2012. Treatment of primary FSGS in adults. J. Am. Soc. Nephrol. 23 (11), 1769–1776.

Kuendgen, A., et al., 2005. Results of a phase 2 study of valproic acid alone or in combination with all-trans retinoic acid in 75 patients with myelodysplastic syndrome and relapsed or refractory acute myeloid leukemia. Ann. Hematol. Suppl. 84 (13), 61–66.

Li, S., Zhang, B., 2014. Traditional Chinese medicine network pharmacology: theory, methodology and application. Chin. J. Nat. Med. 11 (2), 110–120.

Li, Y., et al., 2021. Based on network pharmacology tools to investigate the molecular mechanism of Cordyceps sinensis on the treatment of diabetic nephropathy. J. Diabetes Res. 2021, 1–12, 2021.

Liu, Y., 2010. New insights into epithelial-mesenchymal transition in kidney fibrosis. J. Am. Soc. Nephrol. 21 (2), 212–222.

Liu, T., Lin, Y., Wen, X., Jorissen, R.N., Gilson, M.K., 2007. BindingDB: a web-accessible database of experimentally determined protein-ligand binding affinities. Nucleic Acids Res. 35 (Suppl. 1), 198–201.

Liu, X., Lü, L., Tao, B.B., Zhu, Y.C., 2008. All-trans retinoic acid inhibits the increases in fibronectin and PAI-1 induced by TGF-β1 and Ang II in rat mesangial cells. Acta Pharmacol. Sin. 29 (9), 1035–1041.

Liu, X., Lü, L., Tao, B.B., Zhou, A.L., Zhu, Y.C., 2011. Amelioration of glomerulosclerosis with all-trans retinoic acid is linked to decreased plasminogen activator inhibitor-1 and α-smooth muscle actin. Acta Pharmacol. Sin. 32 (1), 70–78.

Liu, M., Qiao, Z., Zhang, Y., Zhan, P., Yi, F., 2020. Histone Deacetylases Take Center Stage on Regulation of Podocyte Function, pp. 236–246.

Liu, F., et al., 2016. Silencing of histone deacetylase 9 expression in podocytes attenuates kidney injury in diabetic nephropathy. Sci. Rep. 6, 1–11 no. January.

Luo, F., Gu, J., Chen, L., Xu, X., 2014. Systems pharmacology strategies for anticancer drug discovery based on natural products. Mol. Biosyst. 10 (7), 1912–1917.

Manning, B.D., Cantley, L.C., 2007. AKT/PKB signaling: navigating downstream. Cell 129 (7), 1261–1274.

Massy, Z.A., et al., 2005. Impact of inflammation and oxidative stress on vascular calcifications in chronic kidney disease. Pediatr. Nephrol. 20 (3 SPEC. ISS), 380–382.

Mekahli, D., et al., 2009. Long-term outcome of idiopathic steroid-resistant nephrotic syndrome: a multicenter study. Pediatr. Nephrol. 24 (8), 1525–1532.

Mezzano, S.A., et al., 2001. Tubular NF-κB and AP-1 activation in human proteinuric renal disease. Kidney Int. 60 (4), 1366–1377.

Nangaku, M., 2004. Mechanisms of tubulointerstitial injury in the kidney: final common pathways to end-stage renal failure. Intern. Med. 43 (1), 9–17.

Pathan, M., et al., 2015. FunRich: an open access standalone functional enrichment and interaction network analysis tool. Proteomics 15 (15), 2597–2601.

Qian, Y., et al., 2021. Mechanism of Cordyceps Cicadae in treating diabetic nephropathy based on network pharmacology and molecular docking analysis. J. Diabetes Res. 2021, 1–10, 2021.

Qi, Q., et al., 2016. Identification of the anti-tumor activity and mechanisms of nuciferine through a network pharmacology approach. Acta Pharmacol. Sin. 37 (7), 963–972.

Qi, G., et al., 2019. Up-regulation of TIF1γ by valproic acid inhibits the epithelial mesenchymal transition in prostate carcinoma through TGF-β/Smad signaling pathway. Eur. J. Pharmacol. 860 (9677), 172551.

Raman, K., Damaraju, N., Joshi, G.K., 2014. The organisational structure of protein networks: revisiting the centrality-lethality hypothesis. Syst. Synth. Biol. 8 (1), 73–81.

Ren, Q., You Yu, S., 2016. CD2-associated protein participates in podocyte apoptosis via PI3K/Akt signaling pathway. J. Recept. Signal Transduct. 36 (3), 288–291.

Rouka, E., Hatzoglou, C., Gourgoulianis, K.I., Zarogiannis, S.G., 2020. nteractome networks between the human respiratory syncytial virus (HRSV), the human metapneumovirus (HMPV), and their host: in silico investigation and comparative functional enrichment analysis. Microb. Pathog. 141, 104000.

Ruiz, S., Pergola, P.E., Zager, R.A., Vaziri, N.D., 2013. Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease. Kidney Int. 83 (6), 1029–1041.

Saleem, M., Bierzynska, A., 2017. Recent advances in understanding and treating nephrotic syndrome. F1000Research 6, 1–7.

Senft, D., Ronai, Z.A., 2015. UPR, autophagy, and mitochondria crosstalk underlies the ER stress response. Trends Biochem. Sci. 40 (3), 141–148.

Shannon, P., et al., 2003. Cytoscape: a software Environment for integrated models of biomolecular interaction networks. Genome Res. 13 (11), 2498–2504.

Sierra-Mondragon, E., Molina-Jijon, E., Namorado-Tonix, C., Rodríguez-Muñoz, R., Pedraza-Chaverri, J., Reyes, J.L., 2018. All-trans retinoic acid ameliorates inflammatory response mediated by TLR4/NF-κB during initiation of diabetic nephropathy. J. Nutr. Biochem. 60, 47–60.

Sierra-Mondragon, E., et al., 2019. All-trans retinoic acid attenuates fibrotic processes by downregulating tgf-β1/smad3 in early diabetic nephropathy. Biomolecules 9 (10).

Singh, A.K., et al., 2013. Epidemiology and risk factors of chronic kidney disease in India - results from the SEEK (screening and early evaluation of kidney disease) study. BMC Nephrol. 14 (1), 1–10.

Song, G., Ouyang, G., Bao, S., 2005. The activation of Akt/PKB signaling pathway and cell survival. J. Cell Mol. Med. 9 (1), 59–71.

Soriano, A.O., et al., 2007. Safety and clinical activity of the combination of 5-azacytidine, valproic acid, and all-trans retinoic acid in acute myeloid leukemia and myelodysplastic syndrome. Blood 110 (7), 2302–2308.

Steenhard, B.M., et al., 2010. Deletion of von Hippel-Lindau in glomerular podocytes results in glomerular basement membrane thickening, ectopic subepithelial deposition of collagen α1α2α1(IV), expression of neuroglobin, and proteinuria. Am. J. Pathol. 177 (1), 84–96.

Van Beneden, K., et al., 2011. Valproic acid attenuates proteinuria and kidney injury. J. Am. Soc. Nephrol. 22 (10), 1863–1875.

Van Beneden, K., et al., 2013. Comparison of trichostatin A and valproic acid treatment regimens in a mouse model of kidney fibrosis. Toxicol. Appl. Pharmacol. 271 (2), 276–284.

Varghese, R., Majumdar, A., 2021. Current research in biotechnology current therapies in nephrotic syndrome : HDAC inhibitors, an emerging therapy for kidney diseases. Curr. Res. Biotechnol. 3, 182–194 no. May.

Wang, X., et al., 2014. Histone deacetylase 4 selectively contributes to podocyte injury in diabetic nephropathy. Kidney Int. 86 (4), 712–725.

Yu, G., et al., 2016. Network pharmacology-based identification of key pharmacological pathways of yin–huang–qing–fei capsule acting on chronic bronchitis. Int. J. COPD 12, 85–94.

Yu, M., et al., 2019. Identify the key active ingredients and pharmacological mechanisms of compound XiongShao capsule in treating diabetic peripheral neuropathy by network pharmacology approach. Evid. base Compl. Alternative Med. 2019, 1–11, 2019.

Zhang, C., 2020. Integrated Network Pharmacology Analysis and Pharmacological Evaluation to Explore the Active Components and Mechanism of Abelmoschus Manihot (L.) Medik. on Renal Fibrosis.

Zhang, G.B., Li, Q.Y., Chen, Q.L., Su, S.B., 2013. Network pharmacology: a new approach for Chinese herbal medicine research. Evid. base Compl. Alternative Med. 2013, 1–9, 2013.

Zhang, L., Chen, X.P., Qin, H., Jiang, L., Qin, Y.H., 2018. Atra attenuate proteinuria via downregulation of TRPC6 in glomerulosclerosis rats induced by adriamycin. Ren. Fail. 40 (1), 266–272.

Zhou, Y., Yang, J., 2008. Chronic kidney disease, 97 (17).

Zimmermann, G.R., Lehár, J., Keith, C.T., 2007. Multi-target therapeutics: when the whole is greater than the sum of the parts. Drug Discov. Today 12 (1–2), 34–42.