Network Pharmacology of rostafuroxin

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Abstract- The present study employs an integrated network pharmacology approach to investigate the gene and receptor associations of lead compounds, with a particular focus on the antihypertensive agent rostafuroxin. Molecular structures were retrieved from the PubChem database in SMILES format and analyzed using Swiss Target Prediction to obtain corresponding UniProt IDs. Further gene identification was conducted using the Similarity Ensemble Approach (SEA) and cross-referenced with bioinformatics resources including Mala Cards, OMIM, and Disgenet. Overlapping gene targets were identified using artificial intelligence tools and visualized with Venny 2.1.0. Pathway enrichment analysis was performed using Reactome, while protein–protein interaction networks were constructed and analyzed via Cytoscape's STRING plugin to determine key network nodes based on centrality metrics. This comprehensive workflow highlights the utility of network pharmacology in elucidating multi-target drug mechanisms. By integrating computational tools with systems biology, the study provides novel insights into the pharmacological actions of rostafuroxin and supports the broader application of network-based drug discovery strategies in treating complex diseases such as hypertension.

Keywords: Network pharmacology, Rostafuroxin, SwissTargetPrediction, Hypertension, Cytoscape, STRING, Bioinformatics, Drug discovery, Multi-target therapy, Systems biology

INTRODUCTION OFNETWORK PHARMACOLOGY

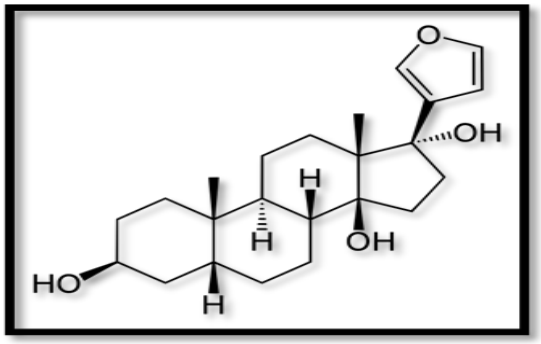
A novel method of drug creation using system biology and network analysis—analysis of network topology, node connection, redundancy, and multidirectionality—network pharmacology.[1] In order to examine the interaction between the drug and the node or network module in the network, the idea is to combine the biological nature network with the drug action network. After that, the focus will shift from searching for a specific target to conducting a thorough network analysis. Network pharmacology offers a novel perspective on drug action analysis[2]. Using computational biological tools to integrate genomic technology with system biology, network pharmacology (NP) is a new field that is helpful in drug discovery. Network pharmacology is a method that can explain intricate connections between medications, disorders, and biological systems.[3] A new field of study termed network pharmacology (NP) tries to grasp pharmacological effects and interactions with several targets.[4] It employs processing capacity to methodically catalog a drug molecule's molecular interactions within a living cell. NP emerged as a crucial instrument for comprehending the intricate connections between the entire body and botanical formula.[4] Hopkins, a British pharmacologist, first proposed network pharmacology as a new field in Nature Biotechnology in 2007. It is founded on the theories of pharmacology, bioinformatics, and other fields and is predicated on the idea that network databases are developing quickly. The effective mechanism of treating TCM diseases can be further explored by using visualization technology, high-throughput technology, network analysis, and other techniques. From a macro-explain Chinese medicine perspective, the interaction mechanism between the disease and the single medicine or Chinese medicine compound reflects many components, including the characteristics of targets and pathways.[5][6] Systematic biomedical technology is integrated with network pharmacology. The special benefits of TCM in treating illnesses are integrated with individual or compound medications from traditional Chinese medicine through computer software and the TCM database website. It offers a better resource for the development of clinically useful new medications and the treatment of illnesses.

The holistic idea put out by traditional Chinese medicine aligns with network pharmacology's "drug–target–gene–disease" hypothesis and its comprehensive examination of biological systems

(Hopkins, 2008).[7]The following steps are the main focus of network pharmacology procedures: (1) mapping drug targets and disease phenotypic targets together in the biomolecular network; (2) determining the mechanism of disease-drug association; and (3) analyzing the network to analyze the mechanism between network targets and the regulation of the system. This affects the balance of network targets and interferes with the phenotype at all levels.[8][9] So, we outlined network pharmacology-related databases and approach for network visualization and analysis. Numerous academics and professionals have created databases pertaining to network pharmacology, which incorporate pertinent medical data and serve as a foundation for network pharmacology research .[10]Multi-target and combinatorial drug therapy offer a novel network-based approach to drug discovery given the intricate signaling systems of illnesses.[11] Networks not only increase the therapeutic efficacy of pharmaceuticals by forecasting undesirable side effects but also offer a more wide selection of illness targets, hence transforming the definition and treatment of diseases.[12] A commonly used approach for the identification of new targets for complex TCM formulae, network pharmacology is a thorough technique combining computer-aided algorithms and virtual models to forecast multi-target.[13]

A novel approach to pharmaceutical research is the use of network pharmacology in conjunction with sophisticated detection technology to investigate illness and symptom biomarkers. Some scholars support the combined application of metabolomics and network pharmacology.[14] One study approach that focuses on "network targets" is network pharmacology. Therefore, it makes sense to employ integrated and systematic biology methodologies like epigenomics, transcriptomics, proteomics, and metabolomics to describe the manifestation as well as the underlying cause of disorders. [15]By combining multi-omics with network pharmacology, it is possible to systematically uncover the underlying mechanisms through disease or syndrome biomarker. Network pharmacology relies on algorithmic scores in addition to data collecting.[16]

Molecular introduction-
Rostrafuroxin



Structure of Rostafuroxin
Synonyms:PST2238
M. Wt 374.51
Formula C23H34O4
Storage Store at +4°C
Purity ≥98% (HPLC)
Chemical Name: 3β, 5β, 14β)-21, 23-Epoxy-24-norchola-20, 22-diene-3, 14, 17-triol
Bioavailability: 50% to 60%
Protein Binding: 70% to 80 %

A digitoxigenin analogue, rostafuroxin has been demonstrated to reduce blood pressure in an animal model of hypertension.[17] It alters the actions of the Na+/K+-ATPase enzyme, which preserves the gradients of potassium and sodium ions across plasma membranes . Clinical trials are being conducted to investigate the treatment of essential hypertension using rostafuroxin[18]."The efficacy of Rostafuroxin in the Treatent of Essential Hypertension".[19]

The digitoxigenin derivative rostafuroxin (PST2238: 17beta-(3-furyl)-5beta-androstan-3beta, 14beta, 17alpha-triol-hydrate) has an IC50 of 2 μM and replaces ouabain binding from the dog kidney Na+–K+ATPase without interfering with other receptors or enzymatic activities known to be involved in the regulation of blood pressure or hormonal steroid control .[20]The antihypertensive effect of rostafuroxin is long-lasting since it is still present 24 h after oral dosing .[21]Rostafuroxin is characterized by a highly safe profile, as evidenced by acute and chronic toxicological and pharmacological safety studies**.** In developed nations, 30–40% of adults suffer from primary hypertension, a complex polygenic illness that is the primary cause of difficulties with the cardiovascular (CV), renal, and cerebral organs .[22]It has been demonstrated in cultured renal cells that have either been transfected with the mutant α-adducin variants or exposed to nanomolar ouabain for an

extended period of time that it can neutralize the molecular effects of EO/ouabain and mutant α-adducin on the Na+–K+ pump function. When compared to the matching control cells, the activity of the Na+ K+ pump is significantly increased in both situations. Rostafuroxin can restore it to physiological levels at concentrations between 10−10 and 10−8 M without changing the Na+–K+ pump activity in control cells.[23][24]

MATERIAL AND METHODS-

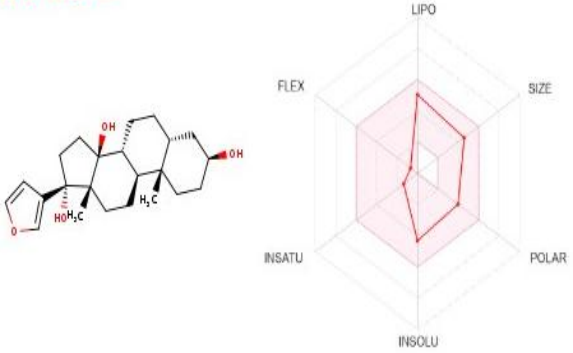
1) Selection of Phytochemical-

SMILIES OF ROSTAFUROXIN

C[C@]12CC[C@@H](C[C@H]1CC[C@@H]3[C@@H]2CC[C@]4([C@@]3(CC[C@@]4(C5=COC=C5)O)O)C)O

SWISS ADME

Molecule 1



SMILES O[C@H]1CC[C@]2([C@@H](C1)CC[C@@H]1[C@@H]2CC[C@]2([C@]1(O)CC[C@]2(O)c1ccoc1)C)C

Physicochemical Properties	
Formula	C23H34O4
Molecular weight	374.51 g/mol
Num. heavy atoms	27
Num. arom. heavy atoms	5
Fraction Csp3	0.83
Num. rotatable bonds	1
Num. H-bond acceptors	4
Num. H-bond donors	3
Molar Refractivity	104.63
TPSA	73.83 Å²

Lipophilicity	
Log $P_{o/w}$ (iLOGP)	3.03
Log $P_{o/w}$ (XLOGP3)	3.29
Log $P_{o/w}$ (WLOGP)	3.88
Log $P_{o/w}$ (MLOGP)	2.70
Log $P_{o/w}$ (SILICOS-IT)	3.42
Consensus Log $P_{o/w}$	3.26

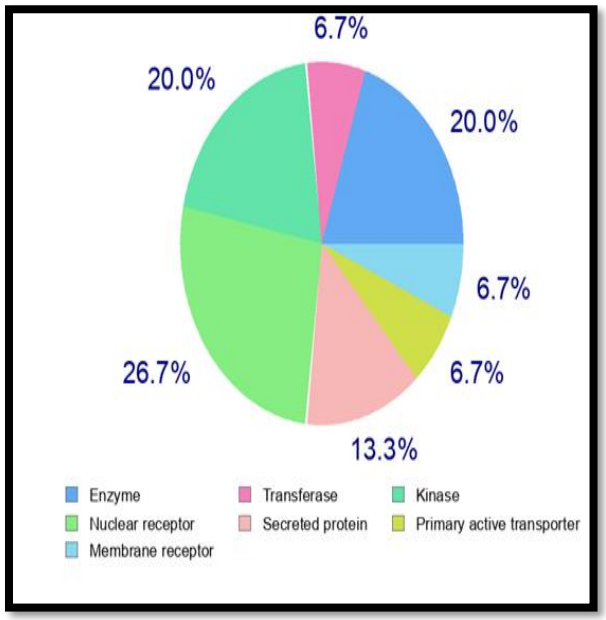
Water Solubility	
Log S (ESOL)	-4.31
Solubility	1.85e-02 mg/ml ; 4.95e-05 mol/l
Class	Moderately soluble
Log S (Ali)	-4.52
Solubility	1.14e-02 mg/ml ; 3.05e-05 mol/l
Class	Moderately soluble
Log S (SILICOS-IT)	-4.35
Solubility	1.67e-02 mg/ml ; 4.46e-05 mol/l
Class	Moderately soluble

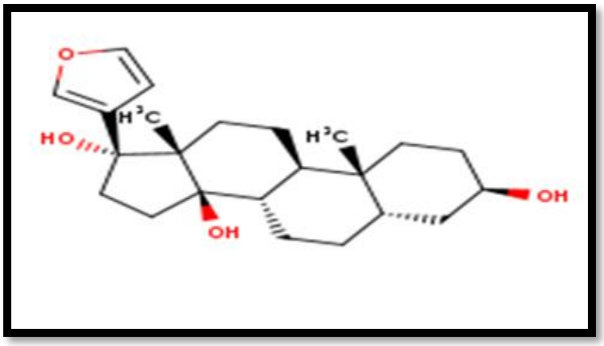
Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	Yes
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-6.25 cm/s

Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55

Medicinal Chemistry	
PAINS	0 alert
Brenk	0 alert
Leadlikeness	No; 1 violation: MW>350
Synthetic accessibility	5.50

2) Selection of Target Identification -





3) DISEASE GENE – DISGENET

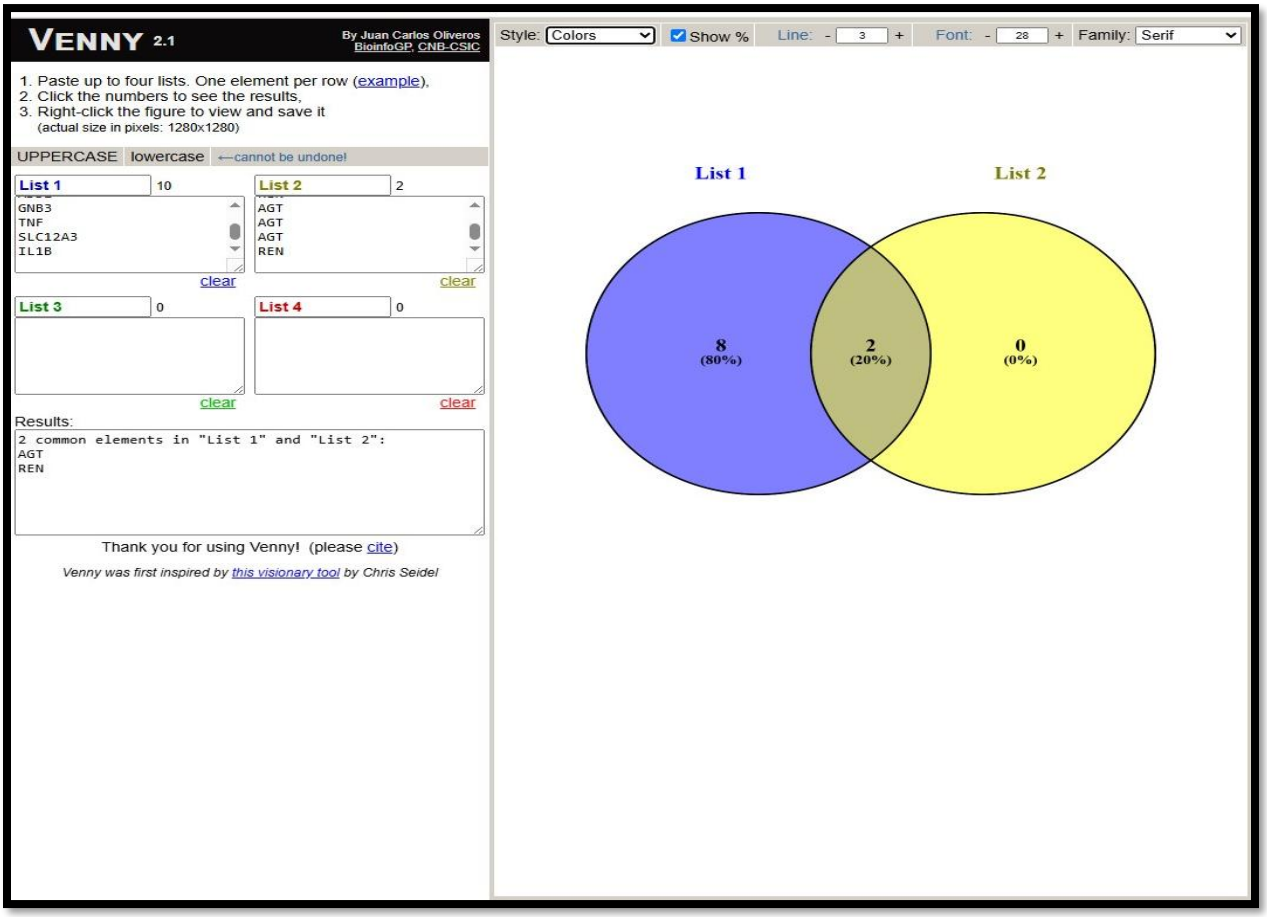
DISGENET
AGT
REN
NOS3
GRK4
AGTR1
ADD1
GNB3
UMOD
TNF
IL1B

SWISS TARGET

Gene	Gene Full Name	N diseases$_g$	N variants$_g$	Score$_{gda}$	N PMIDs	N Chemicals	N PMIDs Chemic
AGT	angiotensinogen	1230	99	1	262	18	26
REN	renin	821	51	1	191	28	81
GRK4	G protein-coupled receptor kina...	76	11	0.9	14	0	0
NOS3	nitric oxide synthase 3	1003	45	0.9	72	2	5
AGTR1	angiotensin II receptor type 1	618	79	0.85	77	4	7
ADD1	adducin 1	106	18	0.8	52	2	2
GNB3	G protein subunit beta 3	203	9	0.8	32	4	3
TNF	tumor necrosis factor	4015	21	0.8	18	3	3
SLC12A3	solute carrier family 12 member 3	324	412	0.75	9	0	0
IL1B	interleukin 1 beta	2641	24	0.75	6	1	1

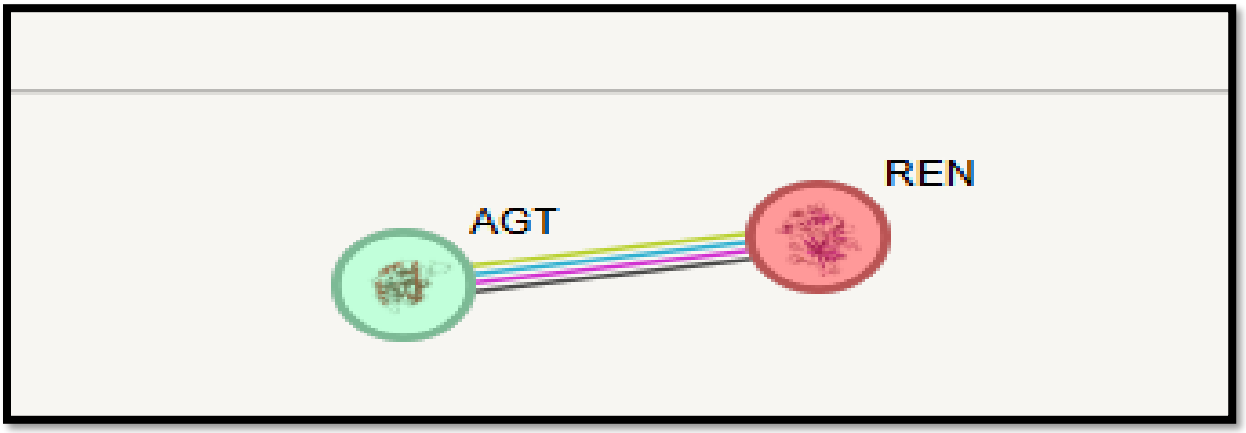
Gene	Score$_{gda}$	Association Type	Sentence	PMID	Reference Year
AGT	1	Genetic Variation	To test this hypothesis, we studied CypD acetylation in patients with	38639088	2024
AGT	1	Genetic Variation	Five variants (CYP11B2 rs179998, AGT rs5051 and rs699, AGTR1 rs5186, a...	38541065	2024
AGT	1	Genetic Variation	AGT, CYP11B2 & ADRB2 gene polymorphism & essential hypertension (...	39382462	2024
AGT	1	Genetic Variation	The Association of M235T Genetic Polymorphism in Angiotensinogen	38541014	2024
REN	1	Biomarker	Endothelial Function in Prehypertension		2024
REN	1	Biomarker	Effects of Exogenous Ketosis on Renal Function, Renal Perfusion, and ...		2024
AGT	1	Biomarker	Safety, Tolerability, PK and PD of ADX-850 in Participants With Hypert...		2024
AGT	1	Genetic Variation	Association of Angiotensin II Type 1 Receptor (AT1R) Gene Polymorphi...	36684488	2023
AGT	1	Causalor Or Contrib...	The success of Angiotensin II receptor blockers, specifically Angioten...	38158508	2023
REN	1	Altered Expression	Supressed plasma renin in patients with primary hypertension is tho...	35414109	2023

4) Selection of Common Gene –
VENNY
AGT
REN

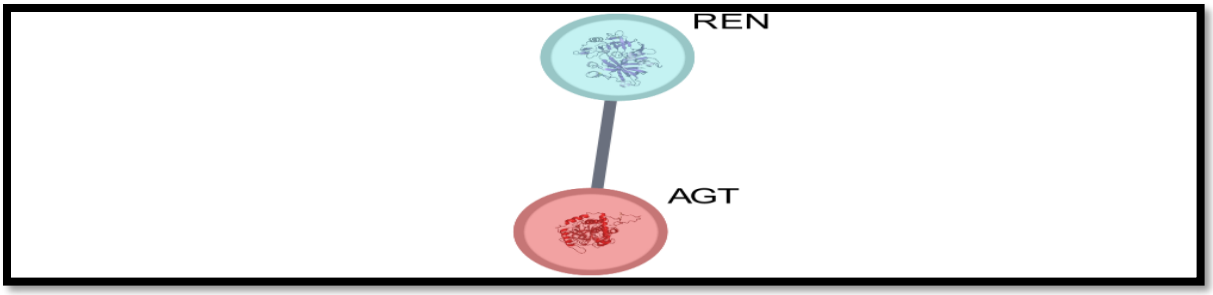


5) PPI Interaction and Ranking

STRING DATA-



6) CYTOSCAPE-CENTRALITY MEASURES



Results Panel

Result 1

Result List(2 in total)

Sorting in select nodes Sorting in whole network

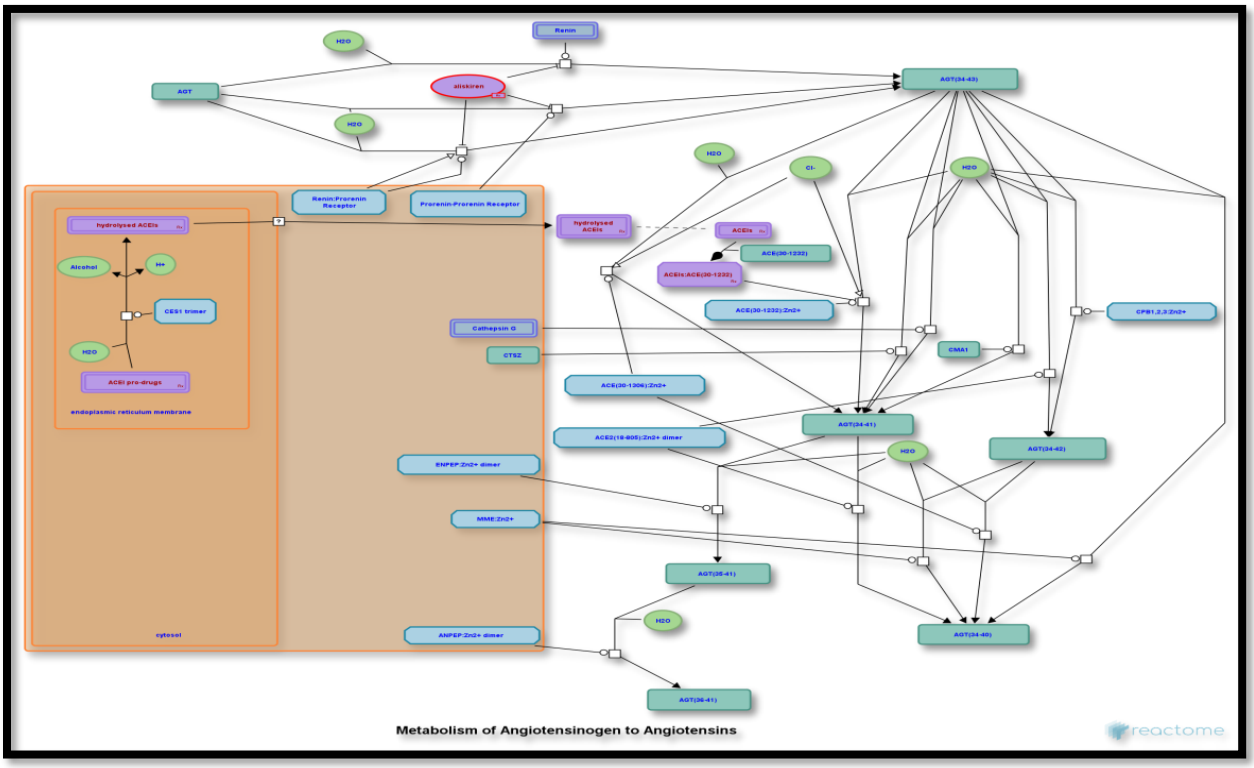
No.	Name	Degree	Betweenness	Closeness
1	9606.ENSP00000355627	1.0	0.0	1.0
2	9606.ENSP00000272190	1.0	0.0	1.0

7) PATHWAY ANALYSIS REPORT

REACTOME -

PATHWAT DETAILS –

1. Metabolism of Angiotensinogen to Angiotensins (R-HSA-2022377)

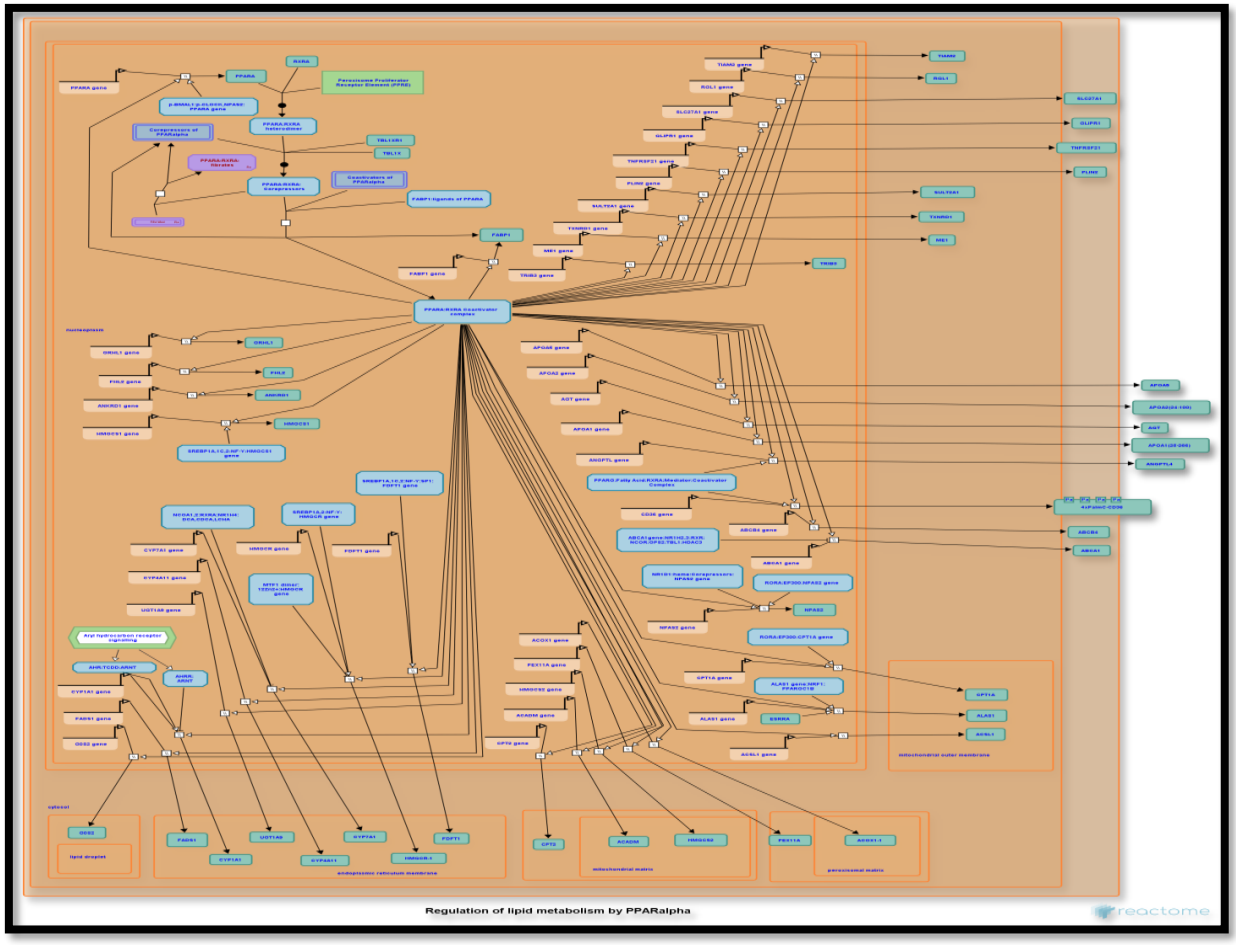


Angiotensinogen, a prohormone, is synthesized and secreted mainly by the liver but also from other tissues. Renin, an aspartyl protease specific for angiotensinogen, is secreted into the bloodstream by juxtaglomerular cells of the kidney in response to a drop in blood pressure. Renin cleaves angiotensinogen to yield a decapaptide, angiotensin I. Circulating renin can also bind the membrane-localized renin receptor (ATP6AP2) which increases its catalytic activity. After cleavage of angiotensinogen to angiotensin I by renin, two C-terminal amino acid residues of angiotensin I are removed by angiotensin-converting enzyme (ACE), located on the surface of endothelial cells, to yield angiotensin II the active peptide that causes vasoconstriction, resorption of sodium and chloride, excretion of potassium, water retention, and aldosterone secretion.

More recently other, more tissue-localized pathways leading to angiotensin II and alternative derivatives of angiotensinogen have been identified. Chymase, cathepsin G, and cathepsin X (cathepsin Z) can each cleave angiotensin I to yield angiotensin II. Angiotensin-converting enzyme 2 (ACE2) cleaves 1 amino acid residue from angiotensin I to yield angiotensin which can be cleared by ACE to yield angiotensin-ACE2 can also cleave angiotensin II to yield angiotensin-. Neprilysin can cleave either angiotensin- or angiotensin I to yield angiotensin-. Angiotensin binds the MAS receptor and, interestingly, produces effects opposite to those produced by angiotensin II.

Aminopeptidase A (APA, ENPEP) cleaves angiotensin II to yield angiotensin III, which is then cleaved by aminopeptidase N (APN, ANPEP) yielding angiotensin IV. Angiotensin IV binds the AT4 receptor Inhibitors of renin and ACE are currently used to treat hypertension.[25][26][27][28][29]

2.Regulation of lipid metabolism by PPARalpha (R-HSA-400206)



Cellular compartments: cytosol, nucleoplasm.

Peroxisome proliferator-activated receptor alpha (PPAR-alpha) is the major regulator of fatty acid oxidation in the liver. PPARalpha is also the target of fibrate drugs used to treat abnormal plasma lipid levels.

PPAR-alpha is a type II nuclear receptor (its subcellular location does not depend on ligand binding). PPAR-alpha forms heterodimers with Retinoid X receptor alpha (RXR-alpha), another type II nuclear receptor. PPAR-alpha is activated by binding fatty acid ligands, especially polyunsaturated fatty acids having 18-22 carbon groups and 2-6 double bonds.

The PPAR-alpha:RXR-alpha heterodimer binds peroxisome proliferator receptor elements (PPREs) in and around target genes. Binding of fatty acids and synthetic ligands causes a conformational change in PPAR-alpha such that it releases the corepressors and binds coactivators (CBP-SRC-HAT complex, ASC complex, and TRAP-Mediator complex) which initiate transcription of the target genes.

Target genes of PPAR-alpha participate in fatty acid transport, fatty acid oxidation, triglyceride clearance, lipoprotein production, and cholesterol homeostasis.[30][31]

RESULT AND DISCUSSION-

- In the presence investigation, genes associated with the lead and receptor molecules were analyzed.
- The new study also uses the Pubchem to determine the grins of chemicals. By incorporating these smiles into the complex procedure called as "swiss target prediction," uniport ids for the genes and receptors were obtained.
- The present study then found that additional genes were extracted from the A Rostafuroxin molecule and from the SEA tool using a number of tools, such as Swiss target prediction, Malacards, Omim, and Disgenet. Additionally, an Al tool was used to identify common genes.
- The common gene was obtained using Venny 2.1.0.
- In the current Network Pharmacology investigation, a string tool from Cytoscape was used for centrality measurements, and Reactome software was used to obtain disease pathways.
- Therefore, network pharmacology provides fresh understanding into drug action study.

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