[Short title + Author Name - P&H title] 31 (2024) 104028

HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

الجمعية السعودية لعلوم الحياة
SAUDI BIOLOGICAL SOCIETY

Original article

Identification of renal protective gut microbiome derived-metabolites in diabetic chronic kidney disease: An integrated approach using network pharmacology and molecular docking

G.R. Saranya , Pragasam Viswanathan *

Renal Research Lab, Pearl Research Park, School of Bioscience and Technology, Vellore Institute of Technology, Vellore 632 014, Tamil Nadu, India

A R T I C L E I N F O

Keywords:
Gut microbes
Diabetes mellitus
Chronic kidney disease
Gut metabolites
Network pharmacology

A B S T R A C T

Metabolites from the gut microbiota define molecules in the gut-kidney cross talks. However, the mechanistic pathway by which the kidneys actively sense gut metabolites and their impact on diabetic chronic kidney disease (DCKD) remains unclear. This study is an attempt to investigate the gut microbiome metabolites, their host targeting genes, and their mechanistic action against DCKD. Gut microbiome, metabolites, and host targets were extracted from the gutMgene database and metabolites from the PubChem database. DCKD targets were identified from DisGeNET, GeneCard, NCBI, and OMIM databases. Computational examination such as protein–protein interaction networks, enrichment pathway, identification of metabolites for potential targets using molecular docking, hubgene-microbes-metabolite-samplesource-substrate (HMMSS) network architecture were executed using Network analyst, ShinyGo, GeneMania, Cytoscape, Autodock tools. There were 574 microbial metabolites, 2861 DCKD targets, and 222 microbes targeting host genes. After screening, we obtained 27 final targets, which are used for computational examination. From enrichment analysis, we found NF-KB1, AKT1, EGFR, JUN, and RELA as the main regulators in the DCKD development through mitogen activated protein kinase (MAPK) pathway signalling. The (HMMSS) network analysis found *F.prausnitzi, B.adolescentis,* and *B.distasonis* probiotic bacteria that are found in the intestinal epithelium, colonic region, metabolize the substrates like tryptophan, other unknown substrates might have direct interaction with the NF-kB1 and epidermal growth factor receptor (EGFR) targets. On docking of these target proteins with 3- Indole propionic acid (IPA) showed high binding energy affinity of -5.9 kcal/mol and -7.4kcal/mol. From this study we identified, the 3 IPA produced by *F. prausnitzi A2-165* was found to have renal sensing properties inhibiting MAPK/NF-KB1 inflammatory pathway and would be useful in treating CKD in diabetics.

1. Introduction

Globally, the type2 diabetic population has a high incidence of developing chronic kidney disease (CKD)(Rossing et al., 2023). It is estimated, about 40 % of a diabetic individual may be prone to develop CKD at some point during their lives (Fenta et al., 2023)(Afkarian et al., 2016). However, the percentage of CKD development in the diabetes population is not known precisely due to other causes like obesity, dyslipidemia, atherosclerosis, hypertension and aging (Hoogeveen, 2022). In diabetic indivduals, the onset of CKD progression is associated with long-term exposure to hyperglycemia, glycosylation of end products, reactive oxygen species, and cytokines response; infection that

Abbreviations: NF-KB1, Nuclear factor kappa −B subunit1; EGFR, Epidermal Growth Factor Receptor gene; JUN, JUN, protooncogene, transcription factor subunit; AKT1, AKT serine/Threonine kinase1; RELA, v-rel avian reticuloendotheliosis viral oncogene homolog A; KEGG, Kyto Encyclopedia of Genes and Genomes; DAVID, Database for Annotation, Visualization, and Integrated Discovery; FDR, False Discovery Rate; NCBI, National Centre for Biotechnology Information; OMiM, Online Mendelian Inheritance in Man; SMILES, Simplified Molecular Input Line Entry System; HDAC1, Histone deacetylase 1; GSK3B, Glycogen synthase kinase-3 beta; PPARG, Peroxisome Proliferator Activated Receptor Gamma; CYP1A1, Cytochrome P450 1A1; CXCL8, C-X-C Motif Chemokine Ligand 8; CASTp, Computed Atlas of Surface Topography of Proteins; RCSB PDB, RCSB Protein Data Bank; NFE2L2, Nuclear Factor Erythroid 2-Like 2; IL2, Interleukin-2; PTGS2, Prostaglandin-endoperoxide synthase 2; HMOX1, Heme oxygenase 1; IL6, Interleukin-6; MAPK, Mitogen Activated Protein Kinase; DCKD, Diabetic Chronic kidney disease; SEA, Similarity Ensemble Approach; STP, Swiss Target Prediction; SCFA, Short chain fatty acids; eGFR, estimated glomerular filtration rate.

* Corresponding author.
E-mail addresses: saranya.gr@vit.ac.in (G.R. Saranya), pragasam.v@vit.ac.in (P. Viswanathan).

https://doi.org/10.1016/j.sjbs.2024.104028
Received 26 February 2024; Received in revised form 14 May 2024; Accepted 19 May 2024
Available online 23 May 2024

affects kidney glomerular filtering system that leads to end-stage renal disease (ESRD) (Fotheringham et al., 2022) through various number of pathways, like endocrine, inflammatory, hemodynamic and neurologic pathway (Rukavina Mikusic et al., 2020).

The primary hallmark for pathogenesis of DCKD is facilitated through inflammation of the kidneys caused by the metabolic action of gut dysbiosis or pathogenic infection. It has been shown that individuals with diabetic kidney disease shown to have a higher composition of gut microbes from the phylum Proteobacteria. Positive correlations of gut microbes have been found between the renal functions. At genus level, *Bacteroidetes* and *Elusimicrobia*, at species-level, *Syntrophaceticus schinkii Citrobacter farmeri* (Zhao, 2022) is associated with albumin/creatinine ratio in urine, where as *Lachnospiraceae_NC2004_group*, *Verrucomicrobia, Subdoligranulum* associated with eGFR (estimated glomerular filtration rate), *Abiotrophia*, *Peptococcaceae* with serum creatinine. On the other hand, *Abiotrophia*, *Peptococcaceae*, and *Ruminococcus* have negative correlation with eGFR whereas *Faecalibacterium, Lachnoclostridium, Roseburia, Oscillospirales, Ruminococcus* with HbA1C is also observed in DKD patients (Zhao et al., 2023). The main features of gut dysbiosis is leaky gut formation, is facilitated by relative abundance of four phyla Anaplasma, Aspergillus, Verruciformes, and Clostridium in DKD patients (Tian et al., 2023), results in endothelial dysfunction, oxidative stress that increases mitochondria dysfunction in the early stage of CKD (Al Khodor and Shatat, 2017)(Harlacher et al., 2022). Shang et al. studied the composition of gut dysbiosis pattern of diabetic kidney disease patients in a mouse model. They found 35 enriched gut genera specific to diabetic kidney disease (DKD) were from the phyla *Proteobacteria, Actinobacteriota, Synergistota, Euryarchaeota, Patescibacteria, Verrucomicorbiota,* and *Cyanobacteria* in DKD (Shang et al., 2022). In individuals with DKD and diabetes mellitus, a different study found an enhanced composition of *Fusobacteria*, which is correlated negatively with elevated blood glucose and appears positively associated with epidermal growth factor receptor (EGFR) with the *Verrucomicrobiota* species (Tao et al., 2019). Thus, determining the gut microbiome-based target for inflammation-mediated organ failure holds great promise for showing the advancement of the kidney injury in diabetic individuals.

It is believed that the gut microbes of a healthy individual maintain homeostasis by interacting with distant organs via metabolites obtained through fermentation of the substrate from the host diet (Zheng et al., 2020). Despite this scenario, understanding how inflammation drives the progression of DCKD is attainable through leaky gut formation in two ways: first, development of DCKD alters gut microbes that influence uremic toxin accumulation; second, accumulation of protein-bound uremic toxins obtained through microbial metabolism of aromatic amino acids from host diet (Wojtaszek et al., 2021). A study by Pavan et al. identified gut microbial changes caused by CKD perpertuate, the accumulation of uremic toxic metabolites like p-cresol sulfate, indoxyl sulfate in serum/plasma, elevated systemic inflammation intensified the kidney disease (Pavan, 2016). Conversely, consumption of short-chain fatty acid (SCFA) metabolites (acetate, propionate and butyrate) produced by beneficial gut microbes, reduced kidney damage by protecting the proximal tubular cells in renal transplanted patients (Huang et al., 2012). Acetate, a type of SCFA metabolite treatment in ischemia–reperfusion (I/R) kidney-injured mice model, lowered inflammation, reactive oxygen species, immune infiltration and increased epithelial cell proliferation in damaged kidneys (Ramezani et al., 2016). Furthermore, in children with chronic kidney disease, plasma-acetate appeared to reduce hypertension (Lu et al., 2021). Another study by Fabian et al. suggest diet supplements with propionate-SCFA to ESRD patients impede systemic elevation by expanding peripheral regulatory T-cells (Meyer et al., 2020). Dietary precursor of tryptophan, trimethylamine-N oxide (TMAO), a protein bound uremic toxin metabolite influence the intestinal barrier integrity damage, play a role in insulin resistance and DKD development (Lv et al., 2022). Tryptophan metabolism by intestinal bacteria such as *Enterobacteriaceae* results in the production of indoxyl sulfate, which damages kidneys by activating the aryl hydrocarbon receptor (AhR)(Hui et al., 2023). Conversely, *Lactobacillius sp.* metabolize tryptophan to indole derivatives like indole 3 lactic acid and inhibit the AhR pathway, which reduces leaky gut in diabetic rat model (Miao et al., 2024).

However, all of the validated research describes the composition of gut microorganisms, related metabolites, and the effects of inflammatory response due to gut dysbiosis associated with diabetic chronic kidney disease and none describes about the pathway mechanistic action of gut microbes and metabolite against disease progression. On further, the mechanism of gut microbiota in mediating inflammation in kidney organs and its significant through metabolite accumulation is still complex and need to be explored. Despite this, we hypothesised that studying gut metabolites with renal protective properties could be a promising target to combat inflammation-mediated chronic kidney disease (CKD) progression in diabetics. Our research employs the network pharmacology and molecular docking approach that focuses on identifying the gut microbiome-metabolite(s)-substrate-sample source network architecture, a key signaling pathway that targets the DCKD progression would bring a base platform for future research.

2. Methodology

Several web-based database like Swiss target prediction (STP), Similarity ensemble approach (SEA), GutmGene were used to identify the targets of gut microbiota-derived metabolites. Disease-specific targets related to diabetic chronic kidney disease (DCKD) were retrieved from GeneCards, DisGenet, OMiM database by using the key words " Type2 diabetes mellitus", "Chronic kidney disease", "Diabetic chronic kidney disease". In addition, the National Centre for Biotechnology Information (NCBI) database was also searched for disease targets based on the category "Homosapiens". Thirdly, genes overlapping between disease-specific targets and gut microbiome-derived metabolite key targets were identified. These genes then interacted with host genes specific to the microbiome and were deemed as final targets. Gene ontology and enrichment analysis, gene-gene interaction, gene-disease association, and HMMSS network analysis were performed using final key targets to determine the pathways and biological and molecular mechanisms. Finally, molecular docking was performed to identify the gut microbiota-derived metabolites against DCKD.

2.1. Targets collection

2.1.1. Collection of gut microbial metabolites and its potential targets

Human-specific gutmicrobiota and its metabolites were collected from the gutMGene v1.0 database (https://bio-annotation.cn/gutmgene/)(Cheng et al., 2022). The canonical SMILES of each metabolite were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and identified through Swiss Target Prediction (STP) (https://www.swisstargetprediction.ch/)(Daina et al., 2019) and Similarity Ensemble Approach (SEA) (https://sea.bkslab.org/) (Wang et al., 2016). The overlapping potential metabolite gene targets between SEA and STP (SEA Vs. STP) were identifed using InteractiVenn (https://www.interactivenn.net/)(Heberle et al., 2015) web tool.

2.1.2. Collection of diabetic chronic kidney disease (DCKD) targets

Human disease-specific databases such as DisGeNET (v7.0)(Piñero et al., 2015), GeneCard (Safran et al., 2022), OMIM (Amberger et al., 2015), and NCBI (Geer et al., 2009) were used to screen the disease targets of diabetic chronic kidney disease. The InteractiVenn web tool was utilized to find cross-referencing disease targets by combining the targets from the four databases and SEA-STP targets (SEA-STP Vs. Disease targets).

2.1.3. Collection of gut microbe targets against diabetic chronic kidney disease

About 224 gut microbial genes from 1500 microbial species related

to humans were retrieved from the gutmGene online repository (Cheng et al., 2022). The targeted genes were identified by intersecting the cross- referencing and gut microbial targets (SEA-STP −Disease targets Vs. gut genes) to find the gut microbe-based therapeutic target for treating diabetic chronic kidney disease. Further, the final targets were used for downstream computational analysis. The wholesome collection and the targets screened are represented in **(**Supplementary sheet 1**).**

2.2. Protein-Protein interaction (PPI) network analysis

We attempted PPI analysis on the final targets to predict specific insights and interactions between genes-proteins and each gene linked to DCKD. We imported the final 27 targets into the STRING 12.0 online tool (https://string-db.org/) (Szklarczyk et al., 2021). By choosing 'Homosapiens 9606′ as an organism with a medium confidence threshold score as > 0.400. We obtained strong PPI network interaction and the output of the string was visualised using cytoscape 3.10.0 (Otasek et al., 2019). We collected the top 15 hub targets from Cytoscape to find the molecular protein–protein network interactions using NetworkAnalyst 3.0(Zhou et al., 2019) a webtool based on STRING Interactome as the reference database. The target with higher degree scores represent the most valuable protein −coding target against DCKD. Further, we evaluated the hub targets with three different PPI interactions: i) Host/microbiome PPI interaction to check the microbial hub target's interaction with the host proteins using Network Analyst 3.0 with parameter set, 'microbiome' in species and microbiolink (Domain-Domain) ii) physical interaction and co-expression analysis of hub targets are done using GeneMANIA software (Franz et al., 2018) to identify the inter-relational and functional analysis of hub targets and iii) tissue-specific PPI interaction of hub targets to find the hub targets interaction specific to tissue (whole blood) concerning Differential Net database in Network Analyst 3.0.

2.3. Functional similarity and pathway enrichment evaluation

Computational gene ontology (CGO) analysis or functional similarity analysis study is performed for the hub target attributes to define the biological and molecular functional mechanism of genes inside the host cell. For gene ontology analysis, the hub gene targets were submitted to ShinyGo 0.77 (https://bioinformatics.sdstate.edu/go/)(Ge et al., 2020), a graphical web tool used to find the gene ontology features differentiating the hub targets from other set of background gene targets. We customized the setting parameters to match species as 'homosapiens' and we kept a false discovery rate (FDR < 0.05) to determine the hierarchial clustering of gene type, chromosomal position, percentage of GC content and transcript length between gene targets **(**Supplementary sheet 2**).** Also, the study of gene functional features, biological and molecular way of protein interaction CGO, was performed for final targets with significant p value < 0.05. Secondly, gene enrichment and pathway assement of hub targets are done using DAVID (Database for Annotation, Visualization and Integrated Discovery) v6.8 web tool (https://david.ncifcrf.gov/)(Sherman et al., 2022) concerning the KEGG (Kyto Encylcopedia of Genes and Genomes) database. This study is done to find the relative abundance of hub targets in specific biological pathways aggravating DCKD conditions. DAVID tool is used for pathway enrichment analysis of any set of gene lists. We imported the hub targets in the DAVID tool, and the output (.Tsv) file from DAVID was imported into the science and research plot (SR) tool (https://www.bioinformatics.com.cn/en), a statistical tool for bioinformatics, with manual alignment, the data is uploaded and based on −log(10) fold enrichment, gene counts and false discovery rate < 0.05, the blubble plot graph is plotted and visualised.

2.4. Gene-disease association prediction

Predicting gene-disease associations enables us to identify genes that change biological processes linked to disease conditions. We conducted a *meta*-analysis of gene-disease association prediction for the hub targets concerning the DisGenet database specific to DCKD using the network analyst (Zhou et al., 2019) web application tool. The target genes of interest are mapped to the disease-specific to the kidney system. The degree of disease is calculated for each hub targets with nodes and edges. Nodes represent hub targets and diseases; edges represents the degree of interaction with the diseases. The higher the interaction, the higher the edges, and vice versa.

2.5. Hubgenes-Microbes-Metabolite-Substrate-Sample source (HMMSS) network analysis

The degree of interaction of target proteins of an enriched key signalling pathway in DCKD interacts with gut microbes, substrate, key microbial metabolites derived from the substrate, and richness of metabolites in fecal, serum, blood, intestinal epithelial cells and the colonic region was obtained from gutMgene (Cheng et al., 2022) using HMMSS network interaction architecture to identify the potential metabolite based theraupetic target against DCKD; and identified the degree of interaction by constructing HMMSS network architecture. The target proteins obtained from the key signalling pathway are called hubtarget. The degree of ineraction between hub target-substrate, hub target-metabolite, metabolite-sample source, hub target-microbes collected manually in (.xls) file format and imported into Cytoscape 3.10.0 (Otasek et al., 2019) tool. The hubtarget is the source, and metabolite, microbes, substrate and sample source are nodes. The hub target is defined as green color node, the substrate in violet, microbes in skyblue, metabolites in yellow and sample richness in red colour. The interaction of edges is represented in a dotted blue color. The illustrated network architecture was constructed based on interaction degree score using Cytoscape 3.10.0.

2.6. Drug-likeness and toxicology evaluation of gut microbial derived metabolites

We screened 203 metabolites from gutmGene, including metabolites screened from the HMMSS network for drug-likeness and toxicology evaluation. Insilico evaluation of physiochemical properties of drug-likeness of each metabolites done using SwissADME (https://www.swissadme.ch/), based on the following criteria: Topological polar surface area $< 140A^{0}2$; molecular weight(>180–400 kD); bioavailability score (0.55–0.85 or 30 %); druglikeness 0.48; satisfying five Lipinski's rule **(**Supplementary sheet 3**).**

2.7. Molecular docking mechanism

Molecular docking was used to predict the pharmacology network and molecular complex interaction between gut metabolites and target genes to treat DCKD. The docking method is described stepwise: **Step1:** The 3D structure of screened target genes was retrived using the RCSB-PDB database (Burley et al., 2023). The protein surface features of screened targets were quanitified based on active pocket sites, delineation of atoms, and concave surface regions using the CASTp web tool (Tian et al., 2018). **Step 2:** The 2D structure of the ligand, microbiome-derived metabolite molecules was downloaded as (.Sdf) format from PubChem and converted to (.Pdb) file format using molegro molecular viewer software (Bitencourt-Ferreira and de Azevedo, 2019) **Step3:** The target protein and the ligand molecules were docked to check the high affinity binding using Autodock 1.57 software (Seeliger and De Groot, 2010), by adding polar hydrogen and water molecules removal from the target, followed by adding kollman and compute gasteiger charges. **Step 4:** The grid box with grid center and dimensions (X,Y,Z) set the docking site after the target protein and ligand's active sites were chosen. The grid dimensions used for each target protein are given in **(**Supplementary sheet 4**). Step 5**: To predict the conformation of the ligand and

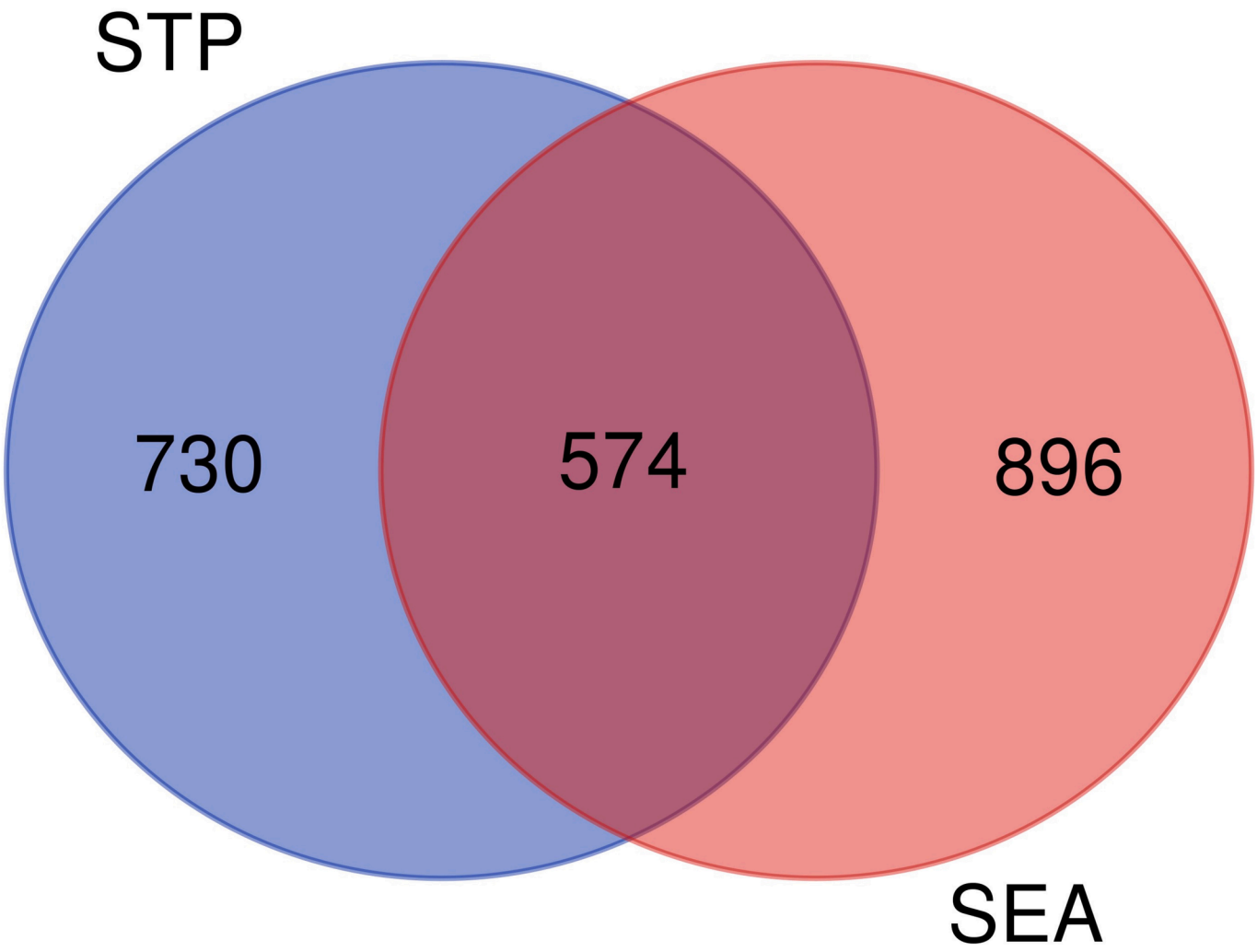


Fig. 1. Collection of common targets: SEA Vs. STP. Venn diagram illustrating the (574) overlapping targets for gut metabolites in STP and SEA.

protein molecule, docking was carried out using an algorithm based on the Lamarckian genetic model. Using Biovia Discovery Studio visualiser software (Jejurikar and Rohane, 2021), three-dimensional (3D) image of protein and ligand interaction was studied and visualised. The Ligplot + V.2.2.8 (Wallace et al., 1995) tool visualizes two-dimensional (2D) ligand and protein interactions. The negative affinity binding scores determine the conformation. Higher negative scores generally indicate more interactive conformation between the protein molecule and the ligand.

2.8. Prediction of ADME and toxicity screening for gut metabolite targets

Docked metabolites with high binding energy efficiency with target protein are selected for toxicity screening. We used ADMETlab2.0, to conduct a toxicology analysis for the docked metabolites that might be used as a probiotic target to treat DCKD (Dong et al., 2018). Using the above-mentioned software, computationally, docked metabolites were screened for additional ADMET properties such as intestinal adsorption rate, plasma −protein binding, toxicity analysis like Ames test, hepatotoxicity test, hERG channel blockers and rate of elimination by the kidney system.

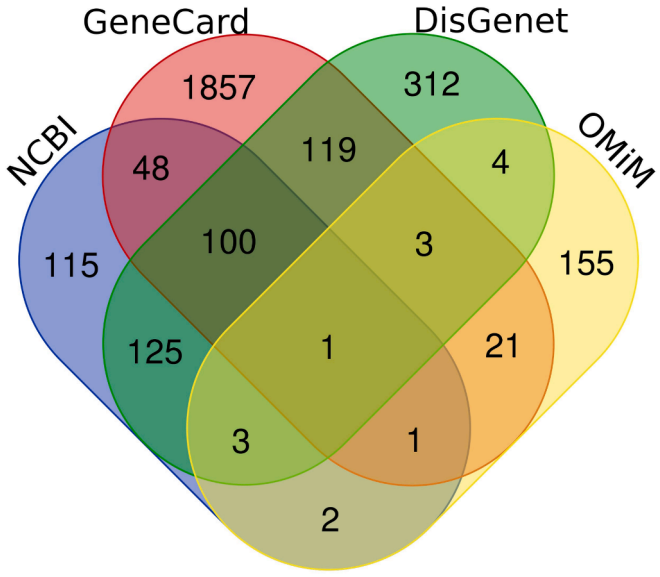


Fig. 2. Collection of DCKD targets: GeneCard Vs.NCBI Vs.DisGenet Vs.OmiM. Venn diagram illustrating the number of genes shared and distinct among DCKD target databases from DISGENET, OMiM, NCBI, and GeneCard.

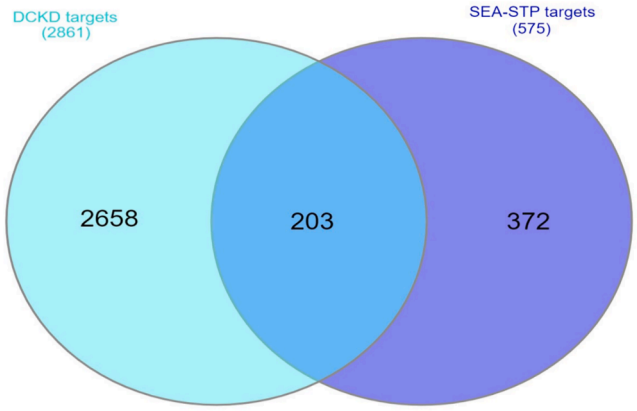


Fig. 3. Collection of coretargets: DCKD targets Vs SEA-STP targets. Venn diagram showing the amount of genes that SEA-STP targets and DCKD targets have in common and different from each other. Both had 203 common targets and it is represented as core targets.

3. Results

3.1. Collection of gut microbial metabolites and its potential targets

To find the renal sensing property of gut- derived metabolites against diabetic CKD, we collected a total of (205) human gut microbiome derived metabolites from the gutMgene database, of which (1304) targets were obtained from Swiss Target Prediction (STP) database, and (1470) targets from Similarity Ensemble Approach (SEA) **(**Fig. 1**).** We obtained (574) common potential targets against DCKD from the overlap of SEA and STP targets. The details are illustrated in **(**Supplementary sheet 1**).**

3.2. Collection of diabetic chronic kidney disease (DCKD) targets

For the disease targets specific to diabetic chronic kidney disease conditions, we obtained (2865) genes from four different human gene databases such as NCBI, OMiM, Genecards, and DisGenet **(**Fig. 2**).** We finalised (203) intersecting targets between SEA –STP targets and DCKD targets (SEA-STP Vs DCKD) **(**Fig. 3**).**

3.3. Collection of gut microbe derived metabolite targets against DCKD

We collected the metabolite-targeting gut genes of about (222) genes from the GutmGene database and found the final targets by intersecting with (203) SEA-STP and DCKD core targets. We finalised (27) targets as a final targets of gut microbiome-derived metabolite against DCKD **(**Fig. 4**)** and used for the computational analysis.

3.4. Protein-Protein interaction (PPI) network of gut microbial gene targets and DCKD

The final 27 targets were added to the STRING tool to identify the direct and indirect interaction of target genes and proteins with a minimum confidence threshold of 0.400. The STRING output is visualised in Cytoscape 3.7.1 (Fig. 5**A).**

Based on the degree ranking score method, the top 15 genes called hub targets were identified using Cytoscape. The ranking scores for each target is given in **(**Supplementary Table 1**).** The list of 15 hub targets are HDAC1, AKT1, EGFR, JUN, NF-KB1, RELA, GSK3B, PPARG, CYP1A1, CXCL8, NFE2L2, IL2, PTGS2, HMOX1, IL6. Further these hub targets (15) were added to the Network Analyst web server to identify the generic protein–protein interaction network invovled in the onset and progression of DCKD. Based on STRING interactome as a reference database, we identified 13 targets that formed a relatively large network

Fig. 4. Final targets of gut microbiome metabolite Vs. DCKD targets. Venn diagram representing the core targets (203) in pink colour and (222) gutMgene targets in greeen colur and intersection represent final targets (27) of microbes metabolite targeting host targets. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

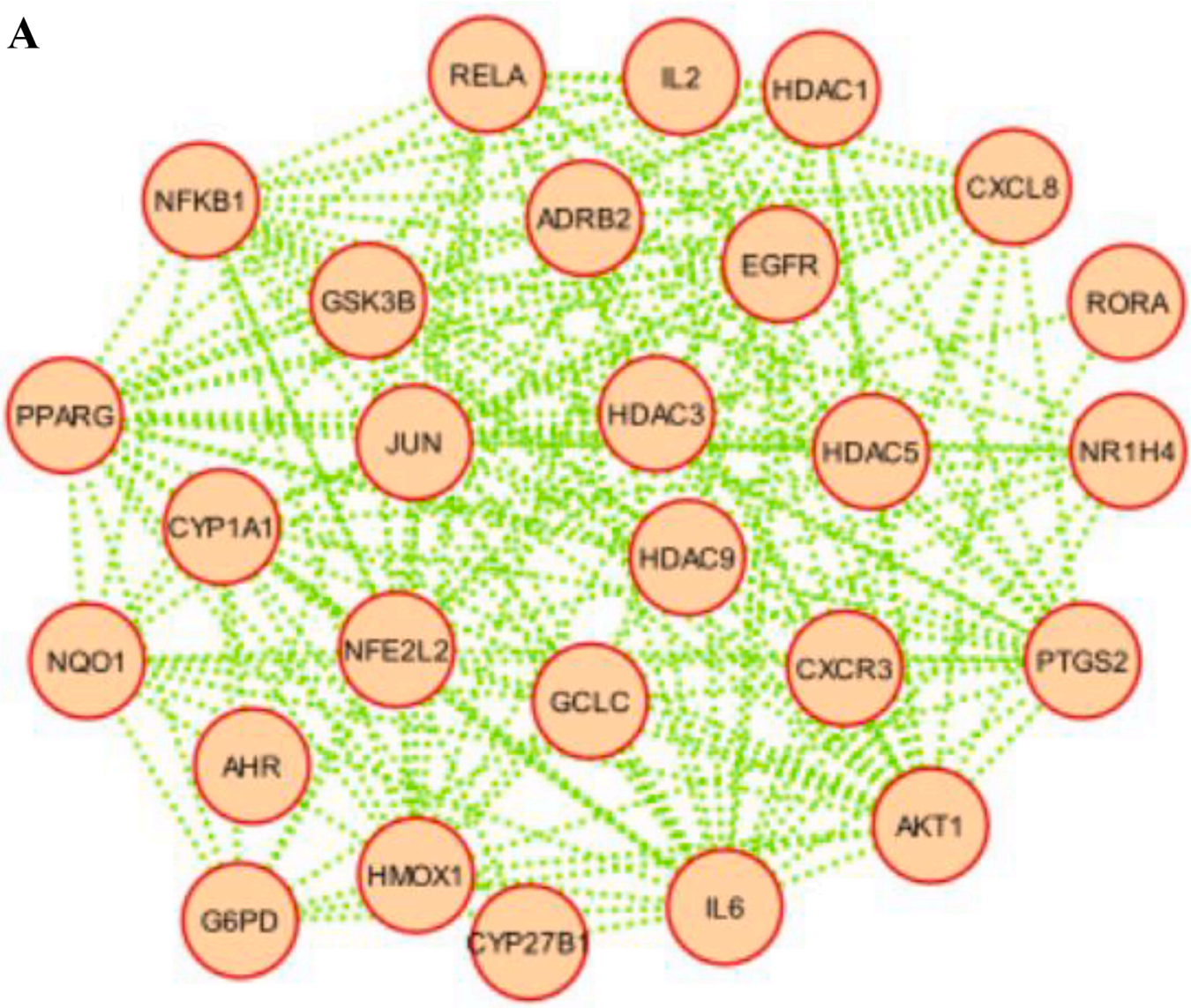


Fig. 5. Protein-Protein network interaction analysis of targets. **(A).** Protein-Protein network interaction of final targets (27) using STRING. PPI interactions of 27 final targets obtained from STRING database. The orange node circles represent the genes and green dotted lines represents the degree of interaction. All 27 genes found to have interaction with one another. **(B).** Protein-protein network interaction of top (15) hub targets using NetworkAnalyst. The PPI of top 15 hub genes is shown in the network pattern graph. This network is large with 852 nodes, 1181 edges, and 14 seeds. Nodes in green circles represent hub genes and the red edges represent connections between the hub genes. Bigger size of nodes indicate significant gene interaction. **(C).** Physical interaction and co-expression prediction of hubtargets. Physical and coexpression analysis of hub genes using GeneMania tool. Physical interaction is represented in red color lines and co– expression is indicated in violet color lines. **(D).** Tissue-specific (Whole blood) PPI interaction of hub targets. The tripartite graph displays the interaction of top hub genes in whole blood. The level of interaction is shown with blue lines and genes are represented by purple circles. The genes EGFR, NFkB1, and HDAC1 have higher expression in blood.

with 852 nodes, 1181 edges, and 14 seeds (final targets or genes of interest). Network analyst calculate the highly significant interaction based on the seed size. The huge edges circle represents higher gene interaction, and the small ones represents less interaction against DCKD (Fig. 5**B).** The network result shows HDAC1 (Histone Deacetylase Enzyme 1) has a higher significant level of interaction with a degree score of 237, followed by AKT1 (AKT serine/theronine kinase 1) (219), EGFR (Epidermal growth factor receptor) (176), JUN (Jun proto-oncogene)(124), NF-KB1 (Nuclear factor- kappa B subunit 1), (115), RELA (Transcription Factor p65, part of the NF-kB complex) (100). Two seeds, PTGS2 (Prostoglandin- endoperoxide synthase 2) and HMOX1 were found to not correlate with other targets. Highly interactive 13 genes based on seed size are represented in (Table1**).**

3.5. Physical interaction and coexpression analysis

Identified hub targets from PPI interaction network analysis was added to GeneMANIA, an online bioinformatics tool to find gene's physical interaction and co-expression. We found hub targets accounted for 29.74 % of physical interactions, 36.27 % of coexprression, 10.39 % of co-localization, 4.83 % of genetic interactions, pathway analysis of 4.70 % and 1.11 % of shared protein domains in DCKD conditions. The interaction image developed from GeneMANIA is represented in (Fig. 5**C).**

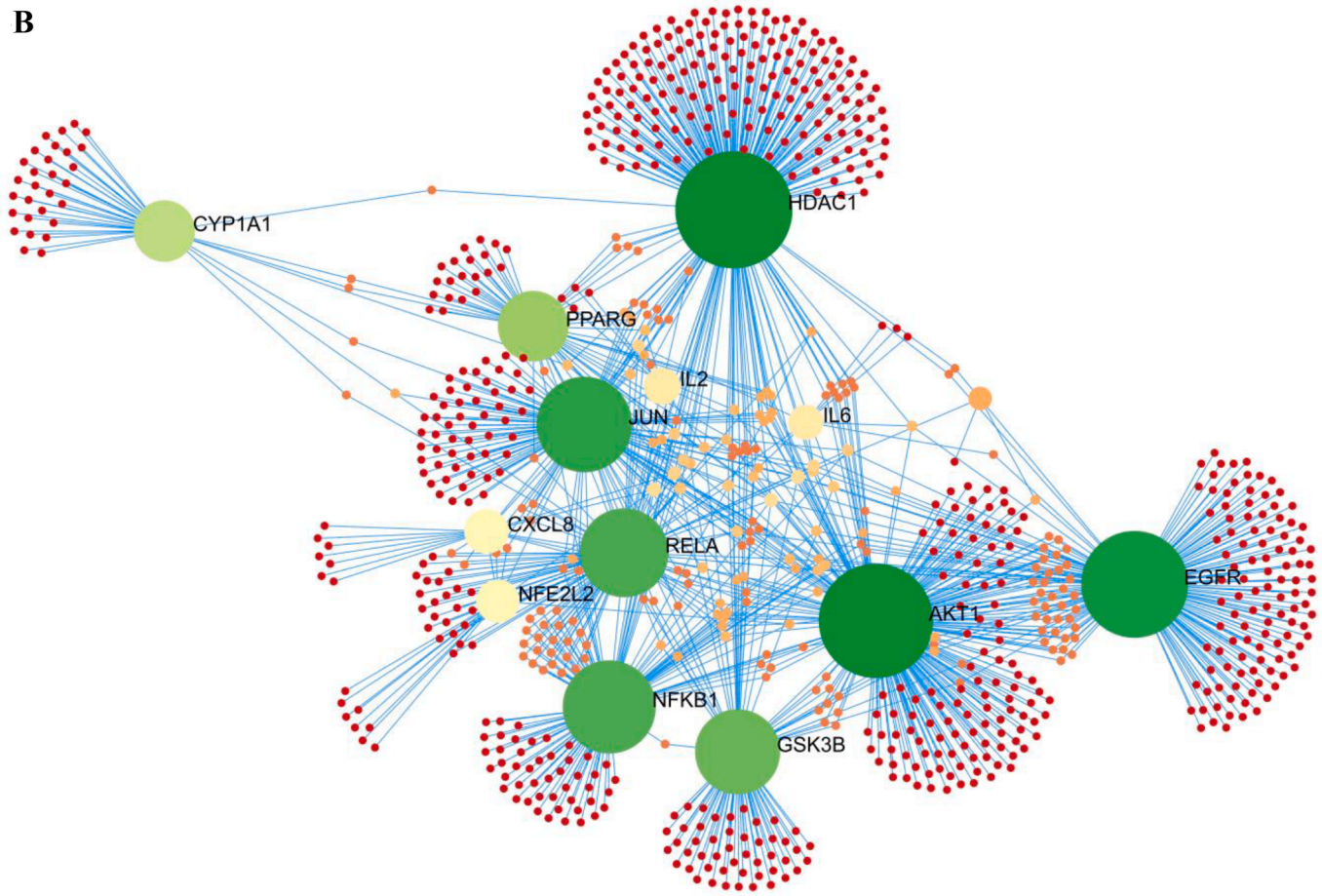


Fig. 5. (*continued*).

3.6. Tissue-specific (Whole blood) PPI analysis

A relationship between toxic metabolites derived from the gut and kidney axis is achieved through the circulatory system. Tissue-specific PPI interaction is performed to check whether the hub targets are assigned to interact or expressed throughout the blood. The study was performed using the Network Analyst server using DifferentialNet database as a reference. The hub targets gene interaction networks form 1461 nodes connected with 1771 edges. The result shows the interaction of EGFR in whole blood is higher with degree score (873), followed by HDAC1 (227). Other genes, RELA (150), AKT1 (107), NF-KB1(101) and JUN (79), form a stable interaction but to a lesser extent than those previously stated targets across whole blood. The tissue-specific PPI interactions of hub targets are represented in the linear Bi/Tripartite graphical layout. The bigger circle represents a huge interaction and viceversa **(**Fig. 5**D).**

3.7. Gene ontology (GO) and pathway enrichment analysis

Functional annotation of 15 hub targets was identifed using ShinyGo 0.77 online server. Gene ontology(GO) classifications in terms of processes like biological process (BP), molecular functions (MF) were analysed. Hub targets with FDR < 0.05 based on nominal p-values are considered enriched. The hierarchial clustering tree chart was plotted to represent the (GO) classification of hub genes enriched. The bigger dots in the tree represents more significant p-values. The tree results for the top 20 pathways of GO: the biological process of hub targets in our rapid response to abiotic stimulus, mechanical stimulus (GO: 0009612), molecule of bacterial origin (GO:0071219), lipopolysaccharides (GO: 0032496), reactive oxygen species(GO:0034614) and positive regulation of transcription by RNA polymerase II (GO:0045944) (**Supplementary** Fig. 1A). The GO: molecular process shows the involvement of hub targets in transcription factor binding (GO:0008134), NF-KappaB binding(GO:0051059), DNA-binding transcription activator activity, RNA polymerase II-specific (GO: 0051059), chromatin (GO: 0003682) and actinin binding (GO: 0042805) (Supplementary Fig. 1B). The functional annotations of gene ontology predicted the hub genes NF-KB1, EGFR, JUN, RELA and AKT1 which predominately participate in the above- mentioned biological process and molecular functions related to DCKD conditions. The functional annotation of gene ontology analysis of hub genes is given in (Supplementary Table 2). Secondly, KEGG pathway enrichment evaluation is done for 15 hub targets using DAVID to determine the key signalling pathways against DCKD (Fischer exact *p*-value(EASE score < 0.05; FDR < 0.05; threshold count-2). Using the SR plot tool, a bubbleplot graph of the key signaling pathways was drawn from the DAVID output **(**Fig. 6**).** DAVID results predicted the MAPK (mitogen activated protein kinase) signalling pathway with FDR 0.01 by the target genes NF-KB1, EGFR, JUN, RELA and AKT1 might be found to regulate the pathophysiology of DCKD **(**Supplementary Table 3**).**

3.8. Gene-disease association network prediction

Gene-disease association network analysis was conducted to predict the relationship between the hub genes (NF-KB1, EGFR, JUN, RELA, AKT1) of a key signaling pathway that has been associated with the onset and progression of chronic kidney disease in diabetics. This study is conducted on a network analyst web server concerning the DisGenet database specific to human disease. We obtained a subnetwork as an

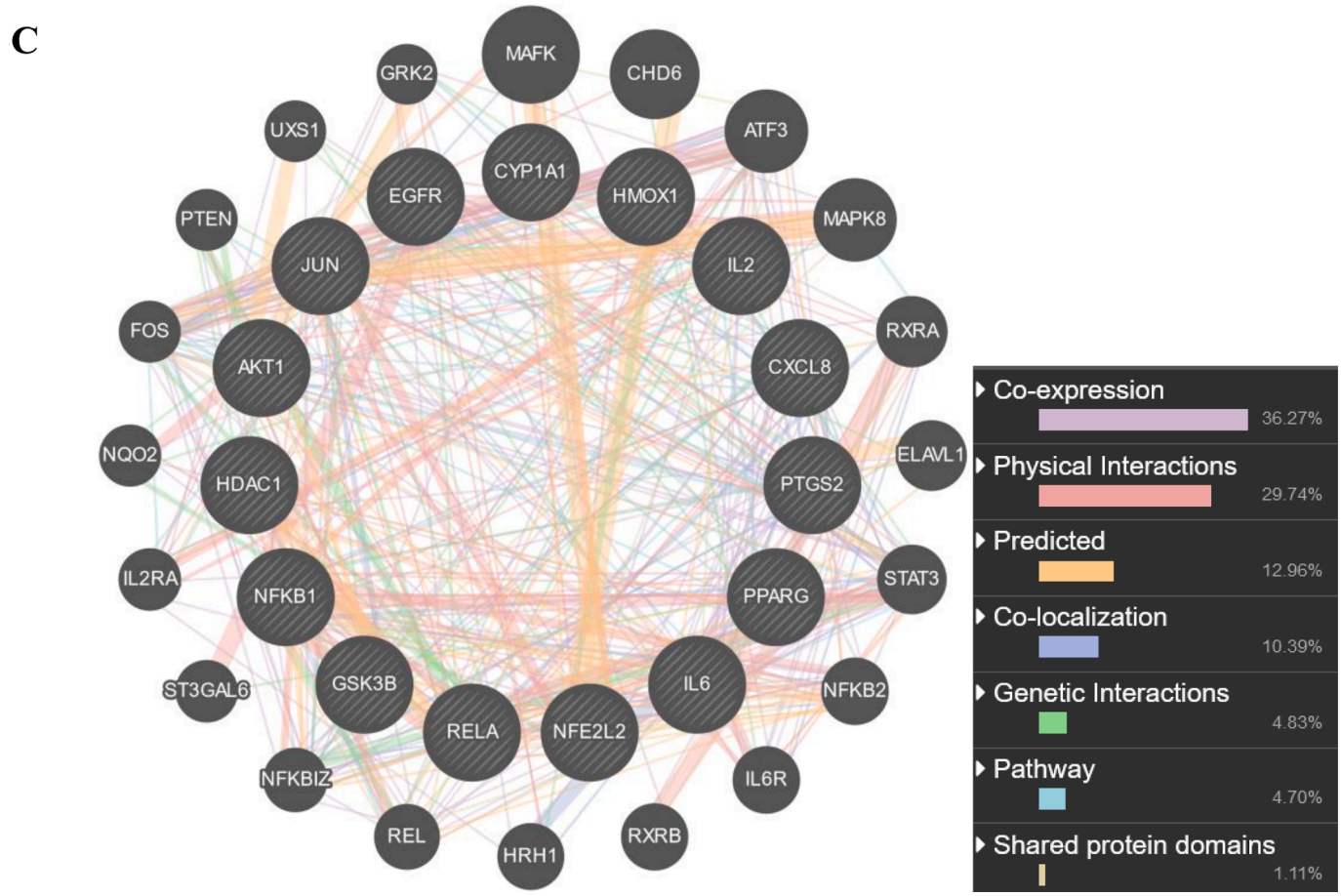


Fig. 5. (*continued*).

output with three seeds (JUN, NF-KB1 and EGFR); the big sub- network is generally termed "continents", they have huge interaction of genes associated with disease conditions, and the small sub-network is generally termed "Islands" have lesser interaction with disease. Three seeds formed a relatively large network with 123 nodes and 134 edges. Of the three seeds, we found both NF-KB1 and EGFR hub proteins are highly expressed in type2 diabetes mellitus conditions. Independently, the NF-KB1 gene is expressed in kidney-related diseases like chronic kidney failure, hyperoxaluria and hypogammaglobulinemia, where the condition leads to decreased antibody levels in the blood. Specific expression of EGFR has been linked with acute kidney injury, insulin resistance, increased blood pressure,cardiomyopathy dilation and decreased body weight. Also, the JUN gene is independently expressed in the kidney's reperfusion injury. Thus, from our study, we identified that the hub genes NF-KB1 and EGFR get highly expressed in high blood glucose level which might be responsible for CKD development in the human host. The gene-disease association network in concentric circle layout is represented in **(**Fig. 7**).**

3.9. Hubgenes-microbes-metabolite-substrate-samplesource (HMMSS) network analysis

The three hub targets of the key signaling pathway (NF-KB1, EGFR, and JUN) that were screened in DCKD-related gene-disease association network analysis are used to assess the degree of interaction with 14 gut microbes, three substrates (tryptophan and two unknown), seven metabolites, and 6 sample source from gutmGene to construct HMMSS network. Based on the HMMSS network, we found that the expression of NF-KB1 activation induces the activation of EGFR, JUN and AKT1 target genes in DCKD condition during gut dysbiosis. The metabolite 3-indole propionic acid, which is produced from the fermentation of unknown and tryptophan substrate by hindgut microbes like *Faecalibacterium prausnitzii A2-165, Bifidobacterium adolescentis, Fecalibacterium prausnitzi, Bacteroides distonis*, and *Bacteroides vulgatus*, can inhibit the activation of NF-KB1 in gut region epithelial cells, as demonstrated by the network architecture in **(**Fig. 8**).** Also, the metabolite is found in all types of sample sources like feces, blood and colonic region which are linked to regulating the intestinal homeostasis in diabetics individuals. The other strain, *Streptococcus salivarius, Streptococcus salivarius K12 strain,* has a dual effect with NFKB-1 expression against DCKD development. The hub protein EGFR expression was found to interact with uremic toxin metabolites like indoxyl sulfate and p-cresol sulfate, which are responsible for the development of CKD in the type2 diabetes population.

The *Lachnospiraceae* metabolize unknown substrate and produce phenylacetylglutamine which can suppress the activation of EGFR and the metabolite found in feces and urine. Similarly, strains like *Fusobacteria, L.acidophilus ATCC 4357,* and *Firmicutes* found in the colonic region metabolize unknown substrates and produce butyrate metabolite which tends to inhibit the action of JUN. The increased composition of *Escherichia coli* metabolizes tryptophan to produce indole and vancomycin to induce the expression of hub protein AKT1. It is found that all the hub targets are highly induced by the action of NF-KB1 with degree score (11) and may prominently suppresed by the Firmicutes phylum, *Faecalibacterium prausnitzii A2-165* with higher degree of interaction (33) by metabolites butyrate and 3-indole propionic acid and helps to maintain barrier integrity in DCKD condition **(**Table 2**).**

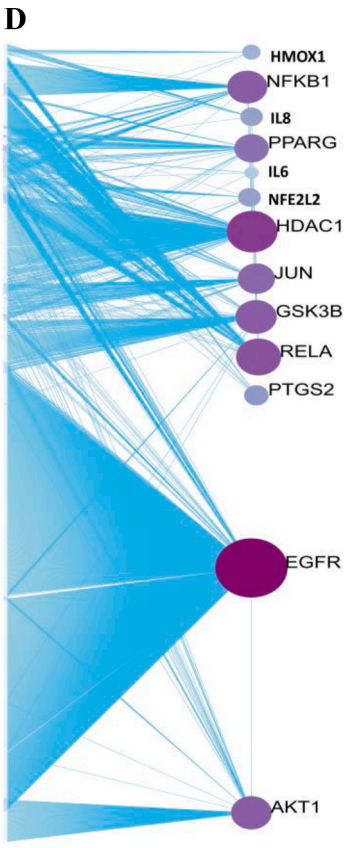


Fig. 5. (*continued*).

Table 1
PPI network analysis of hub targets based on topological ranking method.

S. No	Gene Id	Label	Gene Abbrevation	Degree	Betweenness
1.	3065	HDAC1	Histone Deacetylase Enzyme 1	237	150290.3
2.	207	AKT1	AKT serine/theronine kinase 1	219	148453.7
3.	1956	EGFR	Epidermal growth factor receptor	176	103,840
4.	3725	JUN	Jun proto-oncogene	124	79500.1
5.	4790	NF-KB1	Nuclear factor kappa Bsubunit 1	115	67366.55
6.	5970	RELA	Transcription Factor p65, part of the NF-kB complex	100	49028.06
7.	2932	GSK3B	Glycogen synthase kinase 3 beta	88	50282.41
8.	5468	PPARG	peroxisome proliferator activated receptor gamma	52	24515.21
9.	1543	CYP1A1	Cytochrome p450 family 1 subfamily A member 1	36	26742.57
10.	3576	CXCL8	C-X-C motif chemokine ligand 8	16	6854.26
11.	4780	NFE2L2	NFE2 like bZI transcription factor 2	15	6021.58
12.	3558	IL2	Interleukin −2	10	4002.67
13.	5743	PTGS2	Prostoglandin-endoperoxide synthase 2	3	941

3.10. Screening for druglikeness of metabolites produced from gut microbes

All 203 gutmGene metabolites were screened using the SwissADME webserver for physiochemical characteristics, druglikeness, and ADME properties. About 30 metabolites out of the 203, including 3-Indole propionic acid were screened via HMMSS network analysis. Docking is not performed for other metabolites that interact in the network, such as indoxyl sulfate and p-cresolsulfate, which are uremic toxins that stimulate the expression of hub target proteins in DCKD conditions. Due to its low molecular weight, drug-likeness, butyrate and other metabolites, Indole is similarly not considered for docking analysis. Therefore 3-Indole propionic acid and 29 metabolites were checked for physiochemical properties in SwissADME. Each metabolite met the Lipinski rule of violation criteria, and each metabolite's screen details are given in (Supplementary sheet 3). The screened metabolites underwent docking tests against JUN, EGFR, and NF-KB1 target proteins to verify the binding conformation of the metabolite-protein complex used as a potential therapeutic target against DCKD.

3.11. Molecular docking mechanism

Three target proteins—NF-KB1, EGFR, JUN— associated with MAPK key signalling pathway were subjected to a molecular docking test to determine their effectiveness in binding affinity with 3-Indole propionic acid (3IPA) which was chosen based on degree of interaction via HMMSS network architecture. Negative score values determine the efficacy and stability of binding affinity between protein-metabolite complexes. Higher negative scores form the most stable complex. For the three target conformers, EGFR −3IPA (PDB-4uv7), JUN-3IPA (PDB-4 h39), and NF-KB1-3IPA (PDB-8TKM), we ran a molecular docking test using Autodock tool. EGFR showed the most stable complex among the three targets, measuring −7.4 kcal/mol. NF-KB1 and JUN followed closely behind with −5.91 kcal/mol and −5.38 kcal/mol, respectively (Table 3).

Although NF-KB1 and JUN binding affinity appear llower than the

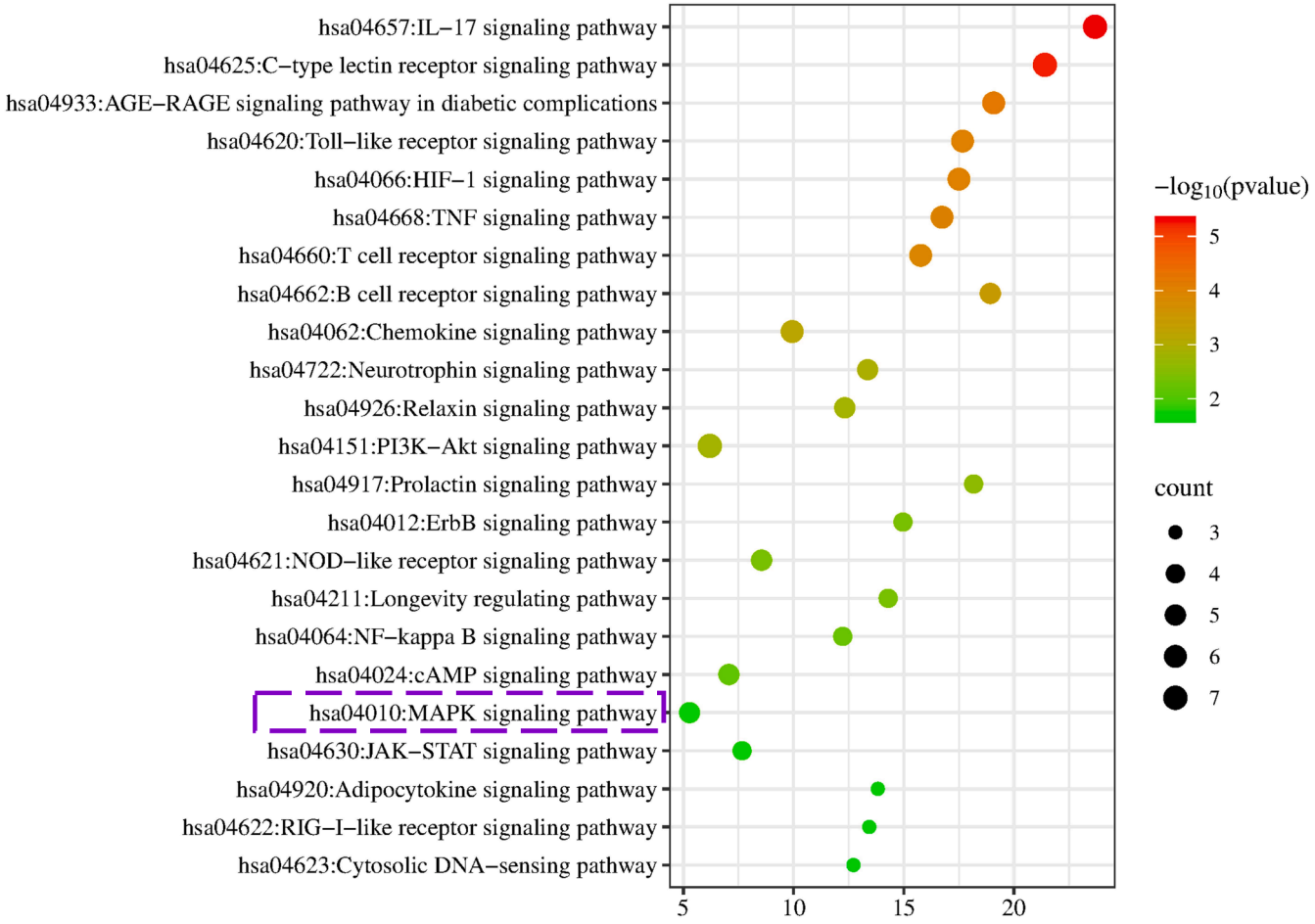


Fig. 6. Pathway enrichment analysis of hub targets against DCKD. The bubble plot graph shows the important signaling pathway of hub genes.

EGFR target, both targets inevitably form a stable complex affinity binding because energy binding efficacy is greater than −4 kcal/mol. (Fig. 9). Furthermore, we are interested in identifying additional possible targets of metabolites produced by gut microbes. Consequently, screening them for drug-likeness characteristics, we docked 29 metabolites with three target proteins. Interestingly, the protein NF-KB1 forms a complex with 11 methoxycuruvularin, exhibiting a binding energy of −6.84 kcal/mol. JUN-Icaritin with a binding energy of −10.06 kcal/mol, while EGFR forms a stable complex with naringenin at −9.13 kcal/mol. The docked results were given in (Supplementary excel sheet 4).

3.12. *Prediction of ADME and toxicity screening of gut metabolite targets*

ADMET screening for the four stabled complex metabolites—3-indole propionic acid, 11-methoxycuruvularin, icaritin, and naringenin—reported the following results. The adsorption potential of these metabolites in the gastrointestinal tract was predicted; the findings indicated that all four of the metabolites showed high permeability rates (Supplementary Table 4). Plasma-protein binding (PPB) was predicted to assess the binding potential of the metabolites in blood. The results show that the metabolites 3-indole propionic acid and 11-methoxycuruvularin have higher percentages of binding—88.8 % and 65.8 %, whereas icaritin and naringenin show less binding with > 90 % respectively. According to the toxicity analysis results, 3-indole propionic acid and 11-methoxy curuvularin tested negative for all tests, while icaritin and naringenin failed the Ames toxicity test for mutagenicity.

Thus, computational prediction of toxicity screening of gut metabolite shows the metabolite 3- Indole propionic acid attributed by *Faecalibacterium prausnitizi* AT 165 inhibits the target protein NF-KB1, primarily activated target gene by lipopolysaccharide injury in DCKD patients. Interestingly, this metabolite was also found to subside the expression of EGFR and JUN which is associated with DCKD development via gut dysbiosis. The other possible metabolite, 11- methoxycuruvularin attributed by *Bacillus* sp. *46,* has the potential to inhibit NF-KB1 independently but not effectively with JUN and EGFR. This might still work to reduce the immunomodulatory response action of EGFR and JUN because it targets the primary activator NF-KB1, which triggers EGFR and JUN. However, to use these microbes or metabolites as a probiotic target, either alone or combined formulations, clinical validation is necessary to treat DCKD.

4. Discussion

The release of metabolites by gut microbes through fermentation of the host substrate source is attributed to gut eubiosis (balanced microbial community inside the gut region) or dysbiosis that could inhibit or stimulate immuno-inflammation inside the host. Recent research breif about the gut microbes and their metabolites linked with gut kidney cross-talks in the progression of renal disease (Gong et al., 2019) (Saranya and Viswanathan, 2023) developement. However, active renal sensing bacterial metabolites, substrate source, microbes, host genes response and its interplay mechanism in the context of diabetic chronic kidney disease is not explored much. Utilizing a network pharmacology and molecular docking approach, we planned to initiate the investigations on the scenario above to find a probiotic producing metabolite targeting the key signalling pathway against DCKD. For this study, we collected metabolites of intestinal gut flora, a list of microbes present in the host gut, and a list of host-targeting genes by microbes

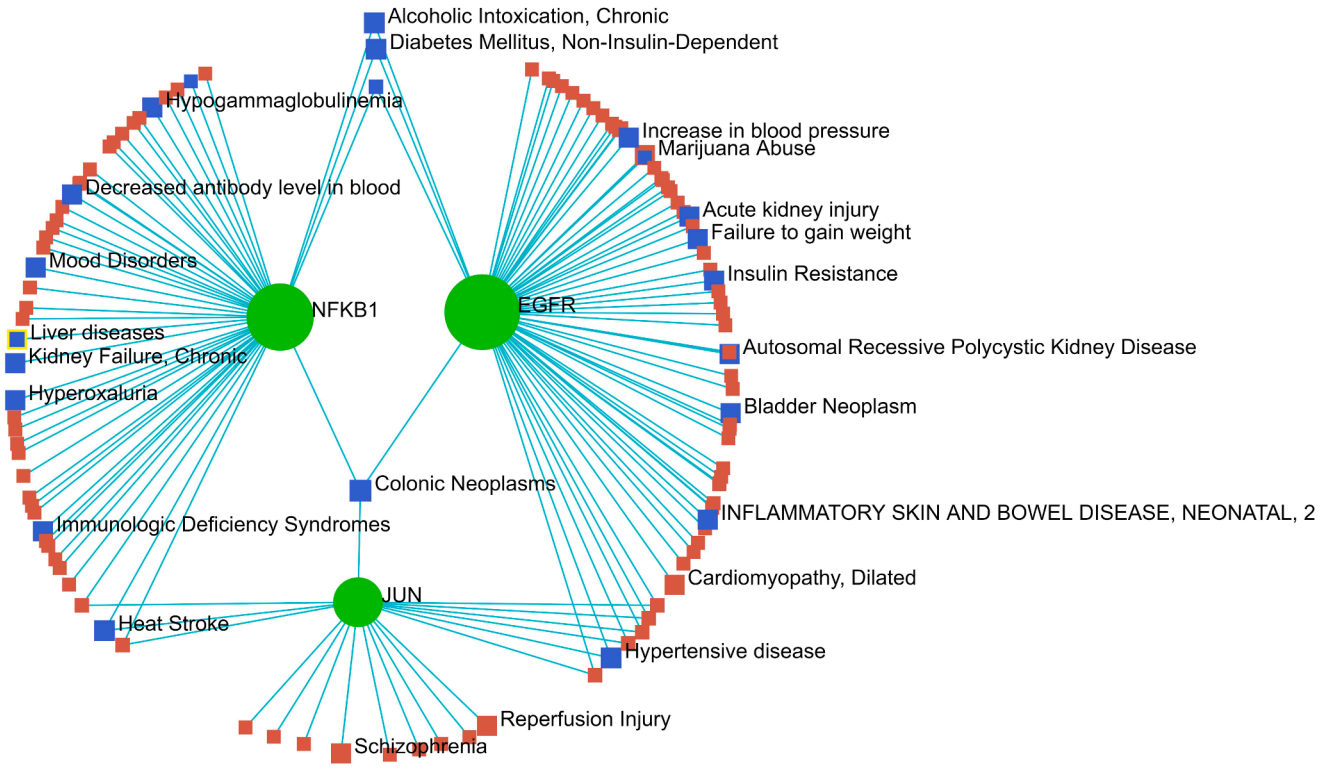


Fig. 7. Gene- Disease association of significant pathway oriented hub targets specific to DCKD condition. A network of genes linked to kidney disease is connected to three central genes. Diseases are represented by colored boxes, with kidney-specific diseases indicated by blue colons. Genes are depicted as green circles, and their interactions are shown by sky blue lines. In DCKD conditions, the EGFR and NF-KB1 gene targets interact significantly.

from a comprehensive database that included information on the human gut microbiome from 400 research publications (Cheng et al., 2022).

The molecular protein–protein interaction (PPI) of the top 15 hub genes using network analysis, revealed HDAC1, AKT1, EGFR, JUN, NF-KB1, and RELA are the most important key targets linked to the pathophysiology of DCKD. A study on epidermal growth factor receptor (EGFR) reported to undergo phosphorylation in high glucose conditions, resulting in accelerated kidney disease and enlargement of kidney size in diabetic animal models (Saad et al., 2005)(Advani et al., 2011). The expression of transcription factor NF-KB1 and its proteins like RELA highly involve in insulin resistance, vascular cell damage and renal inflammation (Guijarro and Egido, 2001). In addition, AKT1 signalling is also associated with mitochondrial dysfunction in kidney damage (Lan and Du, 2015). This illustrates the involvement of these hub targets and their influence on the progression of diabetic kidney disease. Tissue specific protein–protein interaction (PPI) network analysis of hub targets across whole blood confirmed the higher interaction of tissue-specific proteins were found between NF-KB1, EGFR, RELA, HDAC1, GSK3B and JUN. In addition, hub genes physical interaction and co expression analysis prediction are defined, to identify the main functional features of hub genes in the treatment of DCKD.

The development of chronic kidney disease is also significantly influenced by metabolic process like nutrient factors, aging (de Góes et al., 2023), hypertension (Weldegiorgis and Woodward, 2020) and hyperglycemia (Triozzi et al., 2021); according to the results of gene ontology analysis of hub targets from our study we predicted these targets were also associated with alteration based on nutrient metabolism, abiotic stimulus, reactive oxygen species, additionally responds to external stimuli like lipopolysaccharides or response to bacterial origin. According to one study, NFKB1 directly correlated with diet-induced insulin resistance, activates reactive oxygen species levels, impacts brain endothelial dysfunction (Arcambal et al., 2019), and tubular epithelial cellular damage in kidney tissue. Subsequently, oxidative stress brought on by reactive oxygen species due to high glucose level leads to the onset of renal fibrosis, a main pathophysiology feature of CKD, is marked by activation of NFKB inflammatory signalling pathway (Ren et al., 2023). This phenomena act as a vital factor in triggering the cascade action of TLR4, a receptor for lipopolysaccharides from bacteria, which mediate tubulointerstitial inflammation in DCKD patients. Also, the activation of NFKB modifies proteins and peptides, the binding of chemokine proteins to their receptors (monocyte chemoattractant protein −1–chemokine receptor 2; MCP1-CCR2 complex), aid the development of renal fibrosis (He et al., 2023) and substanstial action of inflammation induce the expression of cyclooxygenase (COX) 2 gene, which exacerbates renal fibrosis to end stage renal disease (Zhang and Li, 2009). Thus there is a clear evidence that the action of inflammation inudced by NFKB proliferate the renal fibrosis which affects the function of kidneys. Therefore, it is abundantly evident that the sequential action of inflammation promotes renal fibrosis, thereby impairing kidney function (Fig. 10).

From the enrichment bubble plot analysis for the hub targets we found the key enriched pathway against DCKD due to the inflammatory action of external stimuli. Based on a false discovery rate < 0.05, the lowest rich factor, we identified the key targets were associated with MAPK: mitogen- activated protein kinase pathway that regulates the gene expression by translational modification of proteins resulting to cause adverse effects in kidney tissues like renal fibrosis, inflammation, oxidative stress and glycosylation of end products accumulation related to the pathogenesis of DCKD (Barletto Sousa Barros et al., 2019). Gene-disease association network analysis is further done for the key targets associated with MAPK signalling pathway against DCKD. The resulting network obtained based on degree ranking method, confirmed three targets (NF-KB1, EGFR, JUN) are highly interactive in developing kidney disease. Of the three, NF-KB1 and EGFR are directly linked with hyperglycemic-induced renal impairments and associated with disease conditions like diabetic mellitus (Coto et al., 2018)(Ledeganck et al.,

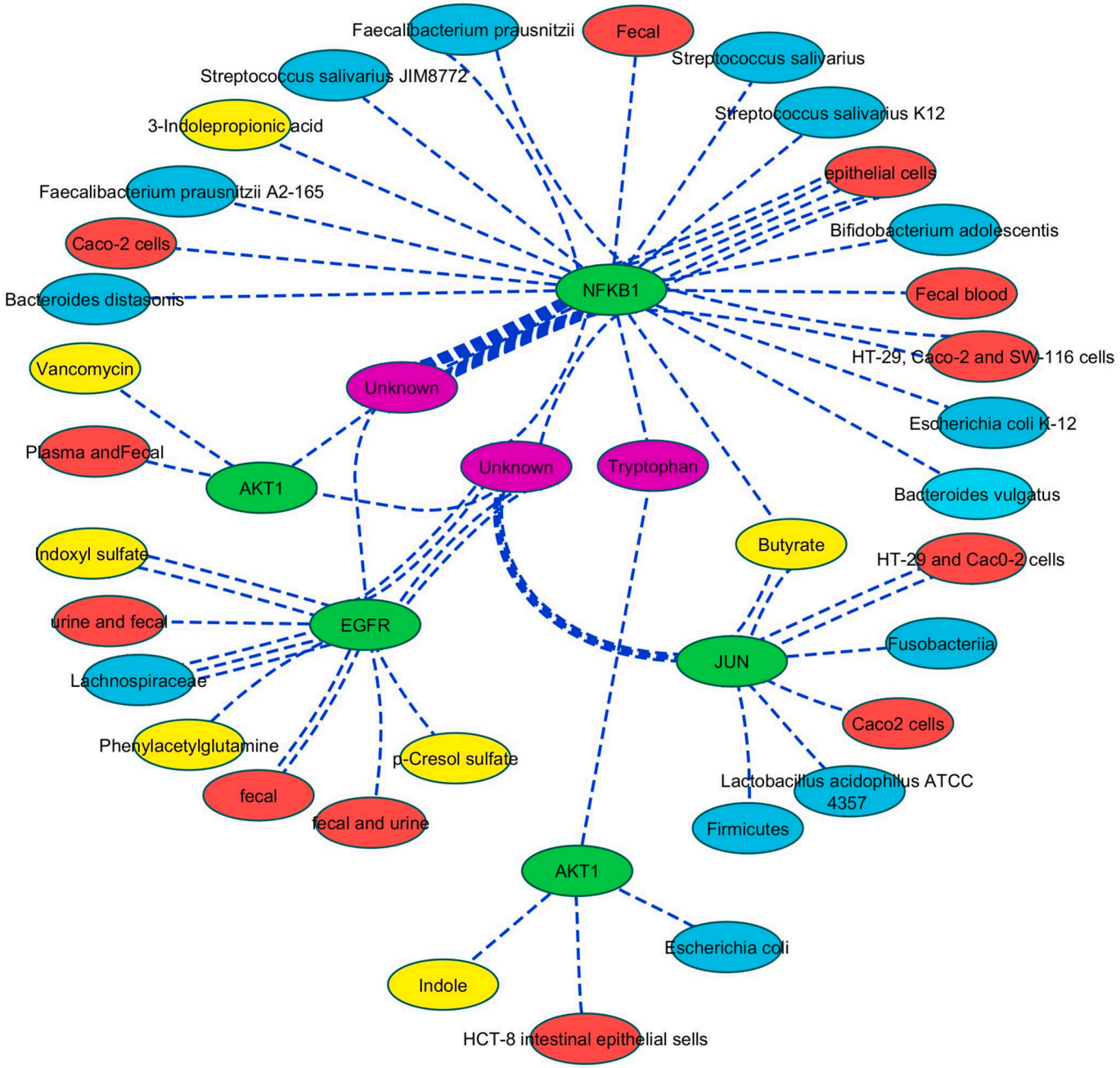


Fig. 8. HMMSS network architecture. The HMMSS network architecture visually demonstrates the interplay between hub genes, microorganisms, metabolites, sample source, and substrate within the MAPK signaling pathway, with nodes depicted as elliptical circles and the intensity of interaction indicated by blue-colored dotted lines, wherein green ellipse circles depict hub genes, red circles represent sample source richness, violet circles symbolize substrate, sky blue represents gut microbe data, and yellow signifies microbe-produced metabolites.

2021), chronic kidney failure (Sheng et al., 2021)(Harskamp et al., 2016), acute kidney failure, hyperoxaluria (Wu et al., 2021)(Tozawa et al., 2008) etc. development due to lipopolysaccharides or bacterial induced injury associated with leaky gut. Thus, from enrichment and gene-disease association network analysis, we predicted three targets (NF-KB1, EGFR, JUN) and the MAPK signalling pathway might act as antagonist and be shown to be a significant targets against DCKD.

The results of hubgenes-microbes-metabolite-substrate-sample source (HMMSS) network architecture of the three hub targets showed that the activation of NF-KB1 with degree score 11 found to interact strongly with *Faecalibacterium prausnitzii A2-165* that involved in fermentation of tryptophan and two unknown substrates to produce 3-indole propionic acid (IPA) metabolite. This metabolite is found to bound with intestinal epithelial cells, CaCo2 cells, and the fecal and blood system of the host. IPA has garnered attention due to its potential anti inflammatory, anti hyperglycemic and metabolic regulatory properties in restoring kidney functions (Peron et al., 2022). While *F. prausnitzii* is widely investigated as a butyrate producer (Li et al., 2022), evidence suggests it produce indole and its derivatives, including indole propionic acid (Menni et al., 2019). Studies have linked IPA to beneficial effects on gut health and the immune system (Mani and Li, 2015)(Tuomainen et al., 2018).

The quantitiy of IPA produced through bacterial metabolism can be influenced by the specific group of gut microbes and the diet rich in tryptophan. Studies conducted in vitro, demonstrate that IPA directly regulate GLUT5, a glucose/fructose membrane transporter that

Table 2
HMMSS network architecture.

Type	Term	Degree of Interaction
	NF-KB1	11
	EGFR	4
Hub Genes	JUN	3
	AKT1	1
Microbiome	*Faecalibacterium prausnitzii A2-165*	33
	Faecalibacterium prausnitzii	30
	Bifidobacterium adolescentis	20
	Bacteroides distasonis	18
	Streptococcus salivarius	18
	Escherichia coli	18
	Streptococcus salivarius JIM8772	14
	Streptococcus salivarius K12	14
	Escherichia coli K-12	12
	Lachnospiraceae	9
	Lactobacillus acidophilus ATCC 4357	6
	Bacteroides vulgatus	6
	Firmicutes	1
	Fusobacteria sp.	1
Metabolite	3 Indolepropionic acid	3
	Butyrate	2
	Indole	1
	Phenylglutamine	1
	vancomycin	1
	p-cresol sulfate	1
	Indoxylsulfate	1

promotes glucose absorption (Jennis et al., 2018). Invivo Studies in diabetic rat models using IPA supplements for two weeks mitigated the high glucose-induced oxidative stress, mitochondrial dysfunction, and endoplasmic reticulum stress in the diabetic test group. (Gundu et al., 2022). The gut microbes that have been identified in humans are mostly responsible for converting tryptophan into indole derivatives, such as indole acetic acid (IAA) and indole propionic acid (IPA). 34 strains create the majority of IAA when compared to IPA — *Bacteroidetes, Fusobacteria, Escherichia, Shigella, Staphylococcus, and Klebsiella* have been reported to produce the most IAA (Kaur et al., 2019). In the event of an infection, these gut flora may support pathogenic colonization, biofilm association, or spore development. Only three strains—*Lactobacillus, Bifidobacterium, and Clostridium* sp.—participate in the IPA enrichment production route. Our study revealed, *Fecalibacterium prausnitzi A-125,* a gram-positive probiotic bacteria that is an efficient generator of butyrate, contributes to the IPA production route. This pathway has the potential to avoid leaky gut and have anti-inflammatory properties against diabetic kidney disease (Fig. 10). However, further invitro and invivo research is necessary to fully understand its mechanism and potential health implications related for kidney health.

Secondly, the network architecture infers that protien-bound uremic toxins, such as p-cresol sulfate and indoxyl sulfate, a product of gut dysbiosis, are found to have a strong interaction with the epidermal growth factor receptor (EGFR) associated with CKD condition. A study has been implicated that in CKD, inflammation-induced EGFR-mediated NF-KB1 transactivation due to uremic toxins is associated to cause glomerular sclerosis and tubular interstitial fibrosis (Rayego-Mateos et al., 2018), which leads to end stage renal disease. Thirdly, we found upregulation of c-JUN transcription factor involved in the transactivation of NF-KB1 and EGFR, which causes reperfusion injury in renal proximal tubules causing acute kidney injury (Grynberg et al., 2021). Therefore, we conclude HMMSS network results that there is a cyclical relationship between JUN and the transcription factor EGFR in high glucose conditions that trigger the expression of the immuno-inflammatory gene NF-KB1 and speed up the development of chronic kidney disease (CKD).

According to the molecular docking results, 3-indole propionic acid exhibited good energy binding efficacy with all three targets—NF-KB1, EGFR, and JUN. Thus, our research investigated the possibility of 3 indole propionic acids produced by *F. prausnitizi AT165* would act as a probiotic renal protective metabolite that targets NF-KB1, EGFR and JUN mediated transactivation of NF-KB1 inhibition via MAPK signalling pathway to lessen the severity of CKD in the diabetic population.

5. Limitations

There is still much to learn about different diet-oriented metabolites and how changes in gut microbiota may impact key regulators in the development of diabetic kidney disease (DCKD). These details will help to validate further through molecular simulation, invitro and invivo study for extensive understandings of metabolites, gut microbes interaction and key signalling pathways that may be the cornerstone for subsequent investigations.

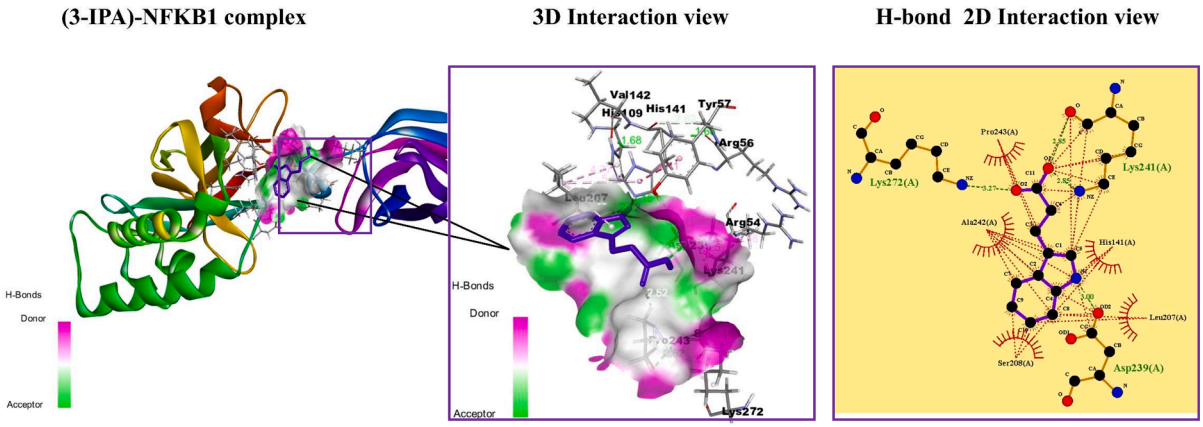
6. Conclusion

In a nut shell, our study examined the microbial-based metabolite that aid in promoting intestinal homeostasis and mitigating the effects of diabetic chronic kidney disease through network pharmacology

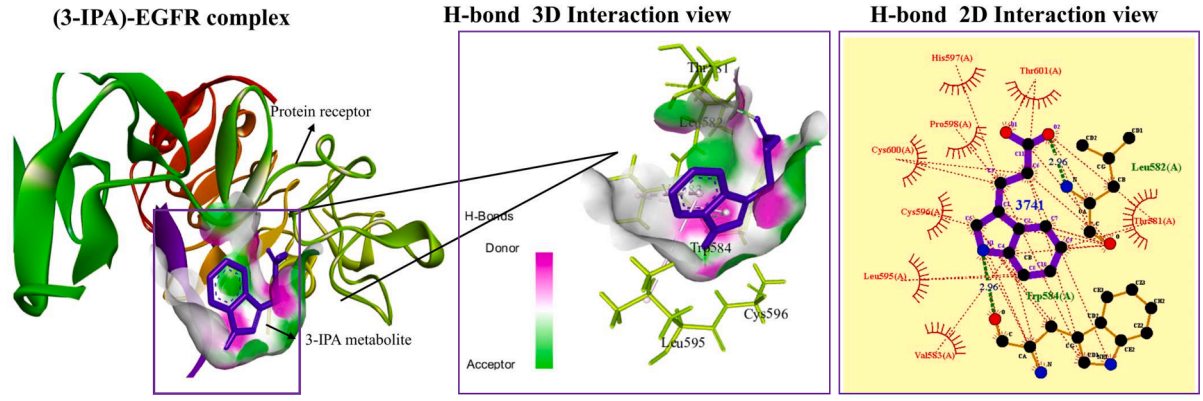
Table 3
Binding efficacy of target protein with gut microbial derived metabolites.

Target protein-metabolite complex	PDB ID of target protein	PubChem ID of gut metabolite	Metabolites produced by gut microbe ID	Energy binding efficiency in (−Kcal/mol)	Total no of hydrogen bond	H-bond interaction	Steric interaction
Target protein −metabolite complex from HMMSS network against DCKD							
EGFR-3 Indole propionic acid	4uv7	3744		−7.4	3	Thr 598, Leu 582, Trp 584	Leu 582, Pro 598
NF-KB1-3 Indole propionic acid	8TKM	3744	*Faecalibacterium prausnitzii* (853, gm0327)	−5.91	3	Lys 241, Lys 272, Asp 239	His 141, Lys 241
c-JUN-3 Indole propionic acid	4 h39	3744		−5.38	2	Gly 280, Cys 283	Leu 340, Ile 342, Gly 280
Target protein with other possible gut metabolite targets against DCKD							
EGFR- Naringenin	4uv7	439,246	*Eubacterium ramulus* (39490, gm0737)	−9.13	2	Trp 584, Pro 598	Cys 596, Thr 601, Pro 598, Leu 582, Leu 595
NF-KB1-11 Methoxycuruvularin	8TKM	10,381,440	*Bacillus* sp. *46* (1266601, gm1132)	−6.84	4	Lys 334(A), Ile278 (A), Phe 298 (A), Asp 297(A)	Gly 296 (A), Phe 298 (A), Phe 295 (A)
JUN-Icaritin	4 h39	5,318,980	*Bacterium MRG-PMF-1* (1477104, gm0861)	−10.06	4	Ala 305,Leu 307, Ile342, Leu 340	Ala 305, Leu 307, Ile342, Leu 340, Leu 279,Val 341, Asp 343,Thr 281, Leu 307,Phe 309

A . NF-KB1-3IPA (PDB- 8TKM)



B. EGFR-3IPA (PDB- 4uv7)



C. JUN-3IPA (PDB- 4h39)

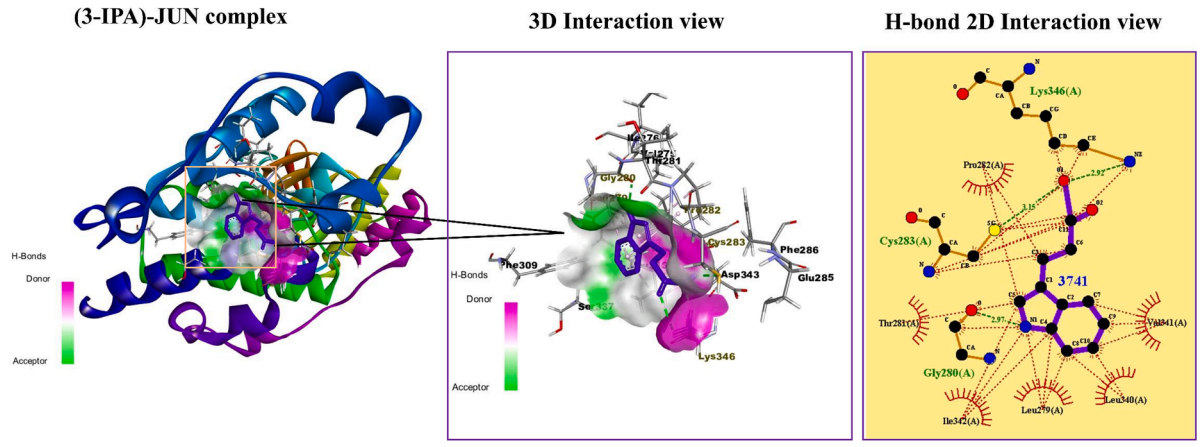


Fig. 9. Molecular docking mechanism of target protein with gut microbial derived metabolite.

approach. This article provides a computational proof of approach to use *Fecalibacterium Prausnitzii AT165,* a member of the phylum Firmicutes, ferments the tryptophan rich diet substrate to produce 3-indole propionic acid as a end product, which has renal sensing or protective properties in downregulating the expression of inflammatory genes NF-KB1, JUN and eGFR via MAPK/NF-KB signaling pathway, and may be used as a better probiotic formulation, to treat DCKD. Comparatively the other metabolites 11-methoxycuruvularin produced by *Bacillus* sp. 46 targets NFk-B1, naringenin by *E. ramulus* targets EGFR, Icaritin by *Bacterium MRG-PMF-1* targets JUN was also found to cascade NF-KB activation solely in an individual manner. Thus, these probiotic metabolite formulation might have renal protective sensing properties that down-regulate the inflammatory genes and maintain the slow progression of CKD in diabetic individuals. However, due to the shifting occurences of gut microbes and metabolites, this static dynamics cannot be identified by computational approach, we need clinical pre validation to investigate further to prove either active probiotic consortia or its secondary metabolites (3-IPA) have renal protective sensing properties to treat CKD in diabetes individuals.

Moreover, the metabolites used in the investigation were not

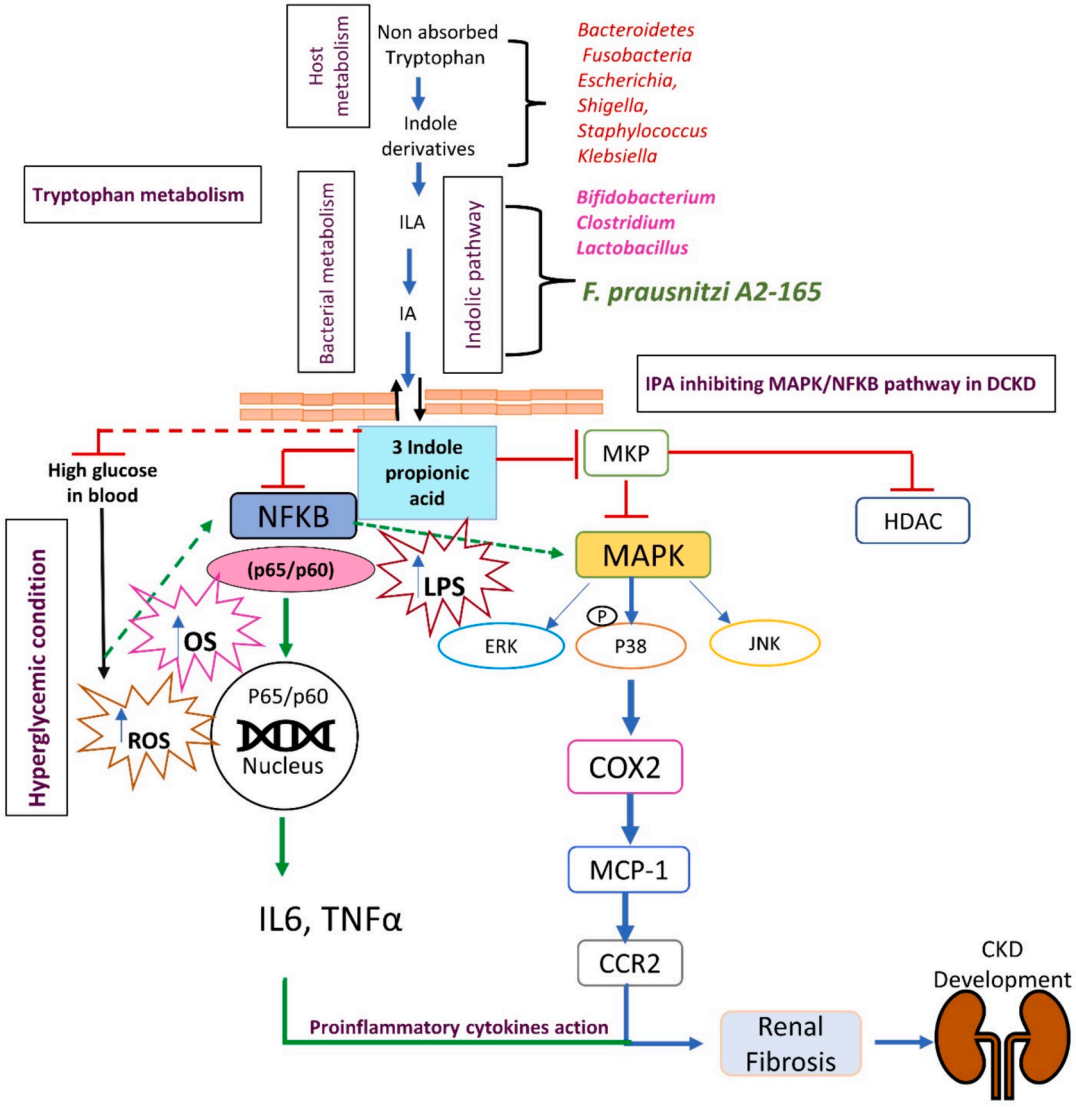


Fig. 10. Mechanism of IPA produced by gut microbe inhibiting MAPK/NFKB in DCKD The figure illustrates the three ways in which IPA blocks external stimuli: 1. 1) Green color arrows indicate direct inhibition of NFKB activation caused by high glucose level-induced reactive oxygen species (ROS), oxidative stress (OS) activation; 2) The inhibition of mitogen activated protein kinase (MAPK) prevents the activation of monocyte chemotactic protein (MCP-1) by blocking the binding of MPK-activated protein kinase, which inhibits the phosphorylation of p38 and reduces MCP-1 activation. This, in turn, limits the binding of CCR2, chemokine receptor 2, which restricts MCP-1 activation and contributes to renal fibrosis is indicated by red colour lines.3) The lipopolysaccharide (LPS)-induced activation of the MAPK pathway caused by NFKB is indicated by green dots.

empirically confirmed by mass spectrometric methods; rather, they were obtained from database searches and literature. Therefore, to reduce trial-and-error methods, dynamic molecular modeling could be employed in the future in tandem with clinical data, based on tryphtophan based diet modification on gut microbes and metabolite validation. All together, our findings suggest, the metabolites generated by specific bacteria with respect to diet substrate have renal protective sensing effects in mitigating DCKD, although more research is needed to confirm this.

Funding

This work was supported by Vellore Institute of Technology-Vellore 632014 and Indian Council of Medical Research (ICMR-BMI/11(21)/2022), New Delhi.

CRediT authorship contribution statement

G.R. Saranya: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Pragasam Viswanathan:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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.

Acknowledgement

The authors grateful to thank Indian Council of Medical Research

(ICMR-BMI/11(21)/2022), New Delhi for financial support and Vellore Institute of Technology -Vellore 632014 for providing encouragement and full support for completion of the project.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2024.104028.

References

Advani, A., Wiggins, K.J., Cox, A.J., Zhang, Y., Gilbert, R.E., Kelly, D.J., 2011. Inhibition of the epidermal growth factor receptor preserves podocytes and attenuates albuminuria in experimental diabetic nephropathy. Nephrology. https://doi.org/10.1111/j.1440-1797.2011.01451.x.

Afkarian, M., Zelnick, L.R., Hall, Y.N., Heagerty, P.J., Tuttle, K., Weiss, N.S., De Boer, I. H., 2016. Clinical manifestations of kidney disease among US adults with diabetes, 1988–2014. Am. Med. Assoc JAMA - J. https://doi.org/10.1001/jama.2016.10924.

Al Khodor, S., Shatat, I.F., 2017. Gut microbiome and kidney disease: a bidirectional relationship. Pediatr. Nephrol. https://doi.org/10.1007/s00467-016-3392-7.

Amberger, J.S., Bocchini, C.A., Schiettecatte, F., Scott, A.F., Hamosh, A., 2015. OMIM.org: Online Mendelian Inheritance in Man (OMIM®), an Online catalog of human genes and genetic disorders. Nucleic Acids Res. https://doi.org/10.1093/nar/gku1205.

Arcambal, A., Taïlé, J., Rondeau, P., Viranaïcken, W., Meilhac, O., Gonthier, M.P., 2019. Hyperglycemia modulates redox, inflammatory and vasoactive markers through specific signaling pathways in cerebral endothelial cells: Insights on insulin protective action. Free Radic. Biol. Med. https://doi.org/10.1016/j.freeradbiomed.2018.10.430.

Barletto Sousa Barros, J., da Silva Santos, R., Adamski da Silva Reis, A., 2019. Implication of the MAPK signalling pathway in the pathogenesis of diabetic nephropathy. EMJ Diab. https://doi.org/10.33590/emjdiabet/10311665.

Bitencourt-Ferreira, G., de Azevedo, W.F., 2019. Molegro virtual docker for docking, in: Methods in Molecular Biology. https://doi.org/10.1007/978-1-4939-9752-7_10.

Burley, S.K., Bhikadiya, C., Bi, C., Bittrich, S., Chao, H., Chen, L., Craig, P.A., Crichlow, G. V., Dalenberg, K., Duarte, J.M., Dutta, S., Fayazi, M., Feng, Z., Flatt, J.W., Ganesan, S., Ghosh, S., Goodsell, D.S., Green, R.K., Guranovic, V., Henry, J., Hudson, B.P., Khokhriakov, I., Lawson, C.L., Liang, Y., Lowe, R., Peisach, E., Persikova, I., Piehl, D. W., Rose, Y., Sali, A., Segura, J., Sekharan, M., Shao, C., Vallat, B., Voigt, M., Webb, B., Westbrook, J.D., Whetstone, S., Young, J.Y., Zalevsky, A., Zardecki, C., 2023. RCSB Protein Data Bank (RCSB.org): delivery of experimentally-determined PDB structures alongside one million computed structure models of proteins from artificial intelligence/machine learning. Nucleic Acids Res. https://doi.org/10.1093/nar/gkac1077.

Cheng, L., Qi, C., Yang, H., Lu, M., Cai, Y., Fu, T., Ren, J., Jin, Q., Zhang, X., 2022. GutMGene: a comprehensive database for target genes of gut microbes and microbial metabolites. Nucleic Acids Res. https://doi.org/10.1093/nar/gkab786.

Coto, E., Díaz-Corte, C., Tranche, S., Gómez, J., Alonso, B., Iglesias, S., Reguero, J.R., López-Larrea, C., Coto-Segura, P., 2018. Gene variants in the NF-KB pathway (NFKB1, NFKBIA, NFKBIZ) and their association with type 2 diabetes and impaired renal function. Hum. Immunol. https://doi.org/10.1016/j.humimm.2018.03.008.

Daina, A., Michielin, O., Zoete, V., 2019. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Res. https://doi.org/10.1093/nar/gkz382.

de Góes, C.R., Vogt, B.P., Biruete, A., Wilkinson, T.J., Snelson, M., 2023. Editorial: Nutrition and metabolism in kidney diseases. Nutr. Front. https://doi.org/10.3389/fnut.2023.1088977.

Dong, J., Wang, N.N., Yao, Z.J., Zhang, L., Cheng, Y., Ouyang, D., Lu, A.P., Cao, D.S., 2018. Admetlab: A platform for systematic ADMET evaluation based on a comprehensively collected ADMET database. J. Cheminform. https://doi.org/10.1186/s13321-018-0283-x.

Fenta, E.T., Eshetu, H.B., Kebede, N., Bogale, E.K., Zewdie, A., Kassie, T.D., Anagaw, T.F., Mazengia, E.M., Gelaw, S.S., 2023. Prevalence and predictors of chronic kidney disease among type 2 diabetic patients worldwide, systematic review and meta-analysis. Diabetol. Metab. Syndr. https://doi.org/10.1186/s13098-023-01202-x.

Fotheringham, A.K., Gallo, L.A., Borg, D.J., Forbes, J.M., 2022. Advanced glycation end products (AGEs) and chronic kidney disease: does the modern diet AGE the kidney? Nutrients. https://doi.org/10.3390/nu14132675.

Franz, M., Rodriguez, H., Lopes, C., Zuberi, K., Montojo, J., Bader, G.D., Morris, Q., 2018. GeneMANIA update 2018. Nucleic Acids Res. https://doi.org/10.1093/nar/gky311.

Ge, S.X., Jung, D., Jung, D., Yao, R., 2020. ShinyGO: A graphical gene-set enrichment tool for animals and plants. Bioinformatics. https://doi.org/10.1093/bioinformatics/btz931.

Geer, L.Y., Marchler-Bauer, A., Geer, R.C., Han, L., He, J., He, S., Liu, C., Shi, W., Bryant, S.H., 2009. The NCBI biosystems Database. Nucleic Acids Res. https://doi.org/10.1093/nar/gkp858.

Gong, J., Noel, S., Pluznick, J.L., Hamad, A.R.A., Rabb, H., 2019. Gut Microbiota-kidney cross-talk in acute kidney injury. Semin. Nephrol. https://doi.org/10.1016/j.semnephrol.2018.10.009.

Grynberg, K., Ozols, E., Mulley, W.R., Davis, R.J., Flavell, R.A., Nikolic-Paterson, D.J., Ma, F.Y., 2021. JUN Amino-Terminal Kinase 1 Signaling in the Proximal Tubule Causes Cell Death and Acute Renal Failure in Rat and Mouse Models of Renal Ischemia/Reperfusion Injury. Am. J. Pathol. https://doi.org/10.1016/j.ajpath.2021.02.004.

Guijarro, C., Egido, J., 2001. Transcription factor-kappa B (NF-kappa B) and renal disease. Kidney Int.

Gundu, C., Arruri, V.K., Sherkhane, B., Khatri, D.K., Singh, S.B., 2022. Indole-3-propionic acid attenuates high glucose induced ER stress response and augments mitochondrial function by modulating PERK-IRE1-ATF4-CHOP signalling in experimental diabetic neuropathy. Arch. Physiol. Biochem. https://doi.org/10.1080/13813455.2021.2024577.

Harlacher, E., Wollenhaupt, J., Baaten, C.C.F.M.J., Noels, H., 2022. Impact of uremic toxins on endothelial dysfunction in chronic kidney disease: a systematic review. Int. J. Mol. Sci. https://doi.org/10.3390/ijms23010531.

Harskamp, L.R., Gansevoort, R.T., Van Goor, H., Meijer, E., 2016. The epidermal growth factor receptor pathway in chronic kidney diseases. Nat. Rev. Nephrol. https://doi.org/10.1038/nrneph.2016.91.

He, S., Yao, L., Li, J., 2023. Role of MCP-1/CCR2 axis in renal fibrosis: Mechanisms and therapeutic targeting. Med. (united States). https://doi.org/10.1097/MD.0000000000035613.

Heberle, H., Meirelles, V.G., da Silva, F.R., Telles, G.P., Minghim, R., 2015. InteractiVenn: A web-based tool for the analysis of sets through Venn diagrams. BMC Bioinform. https://doi.org/10.1186/s12859-015-0611-3.

Hoogeveen, E.K., 2022. The epidemiology of diabetic kidney disease. Kidney Dial. https://doi.org/10.3390/kidneydial2030038.

Huang, S.T., Shu, K.H., Cheng, C.H., Wu, M.J., Yu, T.M., Chuang, Y.W., Chen, C.H., 2012. Serum total p-cresol and indoxyl sulfate correlated with stage of chronic kidney disease in renal transplant recipients. Transplant. Proc. https://doi.org/10.1016/j.transproceed.2011.11.023.

Hui, Y., Zhao, J., Yu, Z., Wang, Y., Qin, Y., Zhang, Y., Xing, Y., Han, M., Wang, A., Guo, S., Yuan, J., Zhao, Y., Ning, X., Sun, S., 2023. The role of tryptophan metabolism in the occurrence and progression of acute and chronic kidney diseases. Mol. Nutr. Food Res. https://doi.org/10.1002/mnfr.202300218.

Jejurikar, B.L., Rohane, S.H., 2021. Drug Designing in Discovery Studio. Asian J. Res. Chem.

Jennis, M., Cavanaugh, C.R., Leo, G.C., Mabus, J.R., Lenhard, J., Hornby, P.J., 2018. Microbiota-derived tryptophan indoles increase after gastric bypass surgery and reduce intestinal permeability in vitro and in vivo. Neurogastroenterol. Motil. https://doi.org/10.1111/nmo.13178.

Kaur, H., Bose, C., Mande, S.S., 2019. Tryptophan Metabolism by Gut Microbiome and Gut-Brain-Axis: An in silico Analysis. Front. Neurosci. https://doi.org/10.3389/fnins.2019.01365.

Lan, A., Du, J., 2015. Potential role of Akt signaling in chronic kidney disease. Nephrol. Dial. Transplant. https://doi.org/10.1093/ndt/gfu196.

Ledeganck, K.J., den Brinker, M., Peeters, E., Verschueren, A., De Winter, B.Y., France, A., Dotremont, H., Trouet, D., 2021. The next generation: Urinary epidermal growth factor is associated with an early decline in kidney function in children and adolescents with type 1 diabetes mellitus. Diabetes Res. Clin. Pract. https://doi.org/10.1016/j.diabres.2021.108945.

Li, H.B., Xu, M.L., Xu, X.D., Tang, Y.Y., Jiang, H.L., Li, L., Xia, W.J., Cui, N., Bai, J., Dai, Z.M., Han, B., Li, Y., Peng, B., Dong, Y.Y., Aryal, S., Manandhar, I., Eladawi, M. A., Shukla, R., Kang, Y.M., Joe, B., Yang, T., 2022. Faecalibacterium prausnitzii Attenuates CKD via Butyrate-Renal GPR43 Axis. Circ. Res. https://doi.org/10.1161/CIRCRESAHA.122.320184.

Lu, P.C., Hsu, C.N., Lin, I.C., Lo, M.H., Yang, M.Y., Tain, Y.L., 2021. The association between changes in plasma short-chain fatty acid concentrations and hypertension in children with chronic kidney disease. Front. Pediatr. https://doi.org/10.3389/fped.2020.613641.

Lv, Q., Li, Z., Sui, A., Yang, X., Han, Y., Yao, R., 2022. The role and mechanisms of gut microbiota in diabetic nephropathy, diabetic retinopathy and cardiovascular diseases. Front. Microbiol. https://doi.org/10.3389/fmicb.2022.977187.

Mani, S., Li, H., 2015. Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and toll-like receptor 4. FASEB J. https://doi.org/10.1096/fasebj.29.1_supplement.575.17.

Menni, C., Hernandez, M.M., Vital, M., Mohney, R.P., Spector, T.D., Valdes, A.M., 2019. Circulating levels of the anti-oxidant indoleproprionic acid are associated with higher gut microbiome diversity. Gut Microbes. https://doi.org/10.1080/19490976.2019.1586038.

Meyer, F., Seibert, F.S., Nienen, M., Welzel, M., Beisser, D., Bauer, F., Rohn, B., Westhoff, T.H., Stervbo, U., Babel, N., 2020. Propionate supplementation promotes the expansion of peripheral regulatory T-Cells in patients with end-stage renal disease. J. Nephrol. https://doi.org/10.1007/s40620-019-00694-z.

Miao, H., Wang, Y.N., Yu, X.Y., Zou, L., Guo, Y., Su, W., Liu, F., Cao, G., Zhao, Y.Y., 2024. Lactobacillus species ameliorate membranous nephropathy through inhibiting the aryl hydrocarbon receptor pathway via tryptophan-produced indole metabolites. Br. J. Pharmacol. https://doi.org/10.1111/bph.16219.

Otasek, D., Morris, J.H., Bouças, J., Pico, A.R., Demchak, B., 2019. Cytoscape automation: empowering workflow-based network analysis. Genome Biol. https://doi.org/10.1186/s13059-019-1758-4.

Pavan, M., 2016. Influence of prebiotic and probiotic supplementation on the progression of chronic kidney disease. Minerva Urol. e Nefrol.

Peron, G., Meroño, T., Gargari, G., Hidalgo-Liberona, N., Miñarro, A., Lozano, E.V., Castellano-Escuder, P., González-Domínguez, R., del Bo', C., Bernardi, S., Kroon, P. A., Cherubini, A., Riso, P., Guglielmetti, S., Andrés-Lacueva, C., 2022. A polyphenol-rich diet increases the gut microbiota metabolite indole 3-propionic acid in older adults with preserved kidney function. Mol. Nutr. Food Res. https://doi.org/10.1002/mnfr.202100349.

Piñero, J., Queralt-Rosinach, N., Bravo, À., Deu-Pons, J., Bauer-Mehren, A., Baron, M., Sanz, F., Furlong, L.I., 2015. DisGeNET: A discovery platform for the dynamical exploration of human diseases and their genes. Database. https://doi.org/10.1093/database/bav028.

Ramezani, A., Massy, Z.A., Meijers, B., Evenepoel, P., Vanholder, R., Raj, D.S., 2016. Role of the gut microbiome in Uremia: A potential therapeutic target. Am. J. Kidney Dis. https://doi.org/10.1053/j.ajkd.2015.09.027.

Rayego-Mateos, S., Rodrigues-Diez, R., Morgado-Pascual, J.L., Valentijn, F., Valdivielso, J.M., Goldschmeding, R., Ruiz-Ortega, M., 2018. Role of epidermal growth factor receptor (EGFR) and its ligands in kidney inflammation and damage. Mediators Inflamm. https://doi.org/10.1155/2018/8739473.

Ren, N., Wang, W.F., Zou, L., Zhao, Y.L., Miao, H., Zhao, Y.Y., 2023. The nuclear factor kappa B signaling pathway is a master regulator of renal fibrosis. Front. Pharmacol. https://doi.org/10.3389/fphar.2023.1335094.

Rossing, P., Baeres, F.M.M., Bakris, G., Bosch-Traberg, H., Gislum, M., Gough, S.C.L., Idorn, T., Lawson, J., Mahaffey, K.W., Mann, J.F.E., Mersebach, H., Perkovic, V., Tuttle, K., Pratley, R., 2023. The rationale, design and baseline data of FLOW, a kidney outcomes trial with once-weekly semaglutide in people with type 2 diabetes and chronic kidney disease. Nephrol. Dial. Transplant. https://doi.org/10.1093/ndt/gfad009.

Rukavina Mikusic, N.L., Kouyoumdzian, N.M., Choi, M.R., 2020. Gut microbiota and chronic kidney disease: evidences and mechanisms that mediate a new communication in the gastrointestinal-renal axis. Pflugers Arch. Eur J. Physiol. https://doi.org/10.1007/s00424-020-02352-x.

Saad, S., Stevens, V.A., Wassef, L., Poronnik, P., Kelly, D.J., Gilbert, R.E., Pollock, C.A., 2005. High glucose transactivates the EGF receptor and up-regulates serum glucocorticoid kinase in the proximal tubule. Kidney Int. https://doi.org/10.1111/j.1523-1755.2005.00492.x.

Safran, M., Rosen, N., BarShir, R., Twik, M., Iny Stein, T., Dahary, D., Fishilevich, S., Lancet, D., 2022. GeneCards – the human gene database. GeneCards Suite Chapter, Pract. Guid. to Life Sci. Databases.

Saranya, G.R., Viswanathan, P., 2023. Gut microbiota dysbiosis in AKI to CKD transition. Biomed. Pharmacother. https://doi.org/10.1016/j.biopha.2023.114447.

Seeliger, D., De Groot, B.L., 2010. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. J. Comput. Aided. Mol. Des. https://doi.org/10.1007/s10822-010-9352-6.

Shang, J., Cui, W., Guo, R., Zhang, Y., Wang, P., Yu, W., Zheng, X., Wang, T., Dong, Y., Zhao, J., Ding, S., Xiao, J., Ren, Z., Zhao, Z., 2022. The harmful intestinal microbial community accumulates during DKD exacerbation and microbiome–metabolome combined validation in a mouse model. Front. Endocrinol. (lausanne). https://doi.org/10.3389/fendo.2022.964389.

Sheng, L., Bayliss, G., Zhuang, S., 2021. Epidermal Growth Factor Receptor: A Potential Therapeutic Target for Diabetic Kidney Disease. Front. Pharmacol. https://doi.org/10.3389/fphar.2020.598910.

Sherman, B.T., Hao, M., Qiu, J., Jiao, X., Baseler, M.W., Lane, H.C., Imamichi, T., Chang, W., 2022. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res. https://doi.org/10.1093/nar/gkac194.

Szklarczyk, D., Gable, A.L., Nastou, K.C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N. T., Legeay, M., Fang, T., Bork, P., Jensen, L.J., von Mering, C., 2021. The STRING database in 2021: Customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res. https://doi.org/10.1093/nar/gkaa1074.

Tao, S., Li, L., Li, L., Liu, Y., Ren, Q., Shi, M., Liu, J., Jiang, J., Ma, H., Huang, Z., Xia, Z., Pan, J., Wei, T., Wang, Y., Li, P., Lan, T., Tang, X., Zeng, X., Lei, S., Tang, H., Ma, L., Fu, P., 2019. Understanding the gut–kidney axis among biopsy-proven diabetic nephropathy, type 2 diabetes mellitus and healthy controls: an analysis of the gut microbiota composition. Acta Diabetol. https://doi.org/10.1007/s00592-019-01316-7.

Tian, W., Chen, C., Lei, X., Zhao, J., Liang, J., 2018. CASTp 3.0: Computed atlas of surface topography of proteins. Nucleic Acids Res. https://doi.org/10.1093/nar/gky473.

Tian, E., Wang, F., Zhao, L., Sun, Y., Yang, J., 2023. The pathogenic role of intestinal flora metabolites in diabetic nephropathy. Front. Physiol. https://doi.org/10.3389/fphys.2023.1231621.

Tozawa, K., Yasui, T., Okada, A., Hirose, M., Hamamoto, S., Itoh, Y., Kohri, K., 2008. NF-κB activation in renal tubular epithelial cells by oxalate stimulation. Int. J. Urol. https://doi.org/10.1111/j.1442-2042.2008.02131.x.

Triozzi, J.L., Parker Gregg, L., Virani, S.S., Navaneethan, S.D., 2021. Management of type 2 diabetes in chronic kidney disease. BMJ Open Diabetes Res. Care. https://doi.org/10.1136/bmjdrc-2021-002300.

Tuomainen, M., Lindström, J., Lehtonen, M., Auriola, S., Pihlajamäki, J., Peltonen, M., Tuomilehto, J., Uusitupa, M., De Mello, V.D., Hanhineva, K., 2018. Associations of serum indolepropionic acid, a gut microbiota metabolite, with type 2 diabetes and low-grade inflammation in high-risk individuals. Nutr. Diabetes. https://doi.org/10.1038/s41387-018-0046-9.

Wallace, A.C., Laskowski, R.A., Thornton, J.M., 1995. Ligplot: a program to generate schematic diagrams of protein-ligand interactions. Protein Eng. Des. Sel. https://doi.org/10.1093/protein/8.2.127.

Wang, Z., Liang, L., Yin, Z., Lin, J., 2016. Improving chemical similarity ensemble approach in target prediction. J. Cheminform. https://doi.org/10.1186/s13321-016-0130-x.

Weldegiorgis, M., Woodward, M., 2020. The impact of hypertension on chronic kidney disease and end-stage renal disease is greater in men than women: a systematic review and meta-analysis. BMC Nephrol. https://doi.org/10.1186/s12882-020-02151-7.

Wojtaszek, E., Oldakowska-Jedynak, U., Kwiatkowska, M., Glogowski, T., Malyszko, J., 2021. Uremic toxins, oxidative stress, atherosclerosis in chronic kidney disease, and kidney transplantation. Oxid. Med. Cell. Longev. https://doi.org/10.1155/2021/6651367.

Wu, Y., Zhang, J., Li, C., Hu, H., Qin, B., Wang, T., Lu, Y., Wang, S., 2021. The activation of ROS/NF- κ B/MMP-9 pathway promotes calcium-induced kidney crystal deposition. Oxid. Med. Cell. Longev. https://doi.org/10.1155/2021/8836355.

Zhang, R., Li, S., 2009. COX-2 as a novel target of CRF family peptides' participating in inflammation. Biochem. Biophys. Res. Commun. https://doi.org/10.1016/j.bbrc.2009.03.064.

Zhao, H., Yang, C.E., Liu, T., Zhang, M.X., Niu, Y., Wang, M., Yu, J., 2023. The roles of gut microbiota and its metabolites in diabetic nephropathy. Front. Microbiol. https://doi.org/10.3389/fmicb.2023.1207132.

Zhao, Y.Y., 2022. Recent advances of gut microbiota in chronic kidney disease patients. Explor. Med. https://doi.org/10.37349/emed.2022.00090.

Zheng, D., Liwinski, T., Elinav, E., 2020. Interaction between microbiota and immunity in health and disease. Cell Res. https://doi.org/10.1038/s41422-020-0332-7.

Zhou, G., Soufan, O., Ewald, J., Hancock, R.E.W., Basu, N., Xia, J., 2019. NetworkAnalyst 3.0: A visual analytics platform for comprehensive gene expression profiling and meta-analysis. Nucleic Acids Res. https://doi.org/10.1093/nar/gkz240.