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A Preliminary Bioinformatics Data Analysis of Single Nucleotide Polymorphisms of the PON1 Genes in Chronic Kidney Disease: In Silico Analysis

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Abstract

Serum paraoxonase (PON1), a glycoprotein synthesized in the liver, protects against oxidative stress and lipid peroxidation, potentially reducing the risk of chronic kidney disease (CKD). A study using bioinformatics methods, such as PROVEAN (Protein Variation Effect Analyzer), SIFT (Sorting Intolerant from Tolerant), Polyphen 2, and I-Mutant 2.0 analyzed non-synonymous single nucleotide polymorphisms (SNPs) of the PON1 gene. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis and Gene Ontology (GO) enrichment were used to identify biological processes and pathways. SIFT analysis of the PON1 gene's SNPs showed that 55 and 33 were tolerable, and 22 were harmful alterations. According to PROVEAN analysis, 22 mutations were neutral, and 33 were harmful. Polyphen 2 revealed that 26 were damaging and 32 were benign. Thirty-four SNPs on I-Mutant analysis showed decreased thermodynamic stability, while twenty-one showed enhanced stability. The study found that the structure and function of the PON1 gene are impacted by mutations, with decreased stability predicted. These mutations may affect CKD's pathobiology and risk for cardiovascular disease. A wet lab investigation on PON1 pathways could help link CKD pathophysiology and progression.

Keywords: In silico, Single nucleotide polymorphisms, Serum paraoxonase (PON1), chronic kidney disease (CKD).

Introduction

Health issues associated with chronic kidney disease (CKD) are widespread and negatively impact people worldwide. According to the 2010 Global Disease Burden Report, chronic renal disease was the 27th leading cause of death worldwide in 1990. However, chronic renal disease moved up to the 18th spot on the list in 2010 (1). CKD is the term used to describe abnormalities in kidney structure or function that have affected health and have been present for at least three months. Despite the aetiology, CKD is indicated by kidney damage or an estimated glomerular filtration rate (eGFR) of less than 60 mL/min/1.73 m² that persists for three months or more. According to GFR, CKD is classified into six groups by the Kidney Disease Improving Global Outcomes (KDIGO) 2012 classification (G1 to G5, with G3 being further subdivided into 3a and 3b). It is also recommended that the cause of CKD be determined. The urinary albumin-creatinine ratio (ACR; mg/g or mg/mmol) in an early morning "spot" urine sample is used to categorize each stage of CKD. It also includes staging based on the three albuminuria levels (A1, A2, and A3) (2).

Primary care doctors must detect and treat CKD in its early stages since CKD is linked to serious health issues such as cardiovascular disease, end-stage renal disease (ESKD), and mortality (3). Patients with CKD have a range of cardiometabolic diseases, including diabetes, insulin resistance, hypertension, and dyslipoproteinaemia, in addition to other physical abnormalities that may contribute to oxidative stress (4).

Highly reactive atoms or molecules known as free radicals possess one or more unpaired electron(s) in their outer shell and can be generated t hrough the interaction of oxygen with specific compounds (5). These radicals can be generated within cells and act as oxidants or reductants by either gaining or losing a single electron (6). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) refer to the reactive radical and non-radical derivatives of oxygen and nitrogen, respectively (7). Oxidative stress is defined by an imbalance in the production and elimination of reactive oxygen and nitrogen species (RONS), resulting from either their excessive generation or a diminished capacity to neutralize them and repair the resultant damage (8).

Free radicals and oxidative stress are recognized to be harmful to human health. Numerous studies demonstrate that free radicals do, contribute to the onset and progression of a variety of illnesses, including cardiovascular disease and cancer. The progression of CKD is primarily affected by oxidative stress, leading to glomerular damage, renal ischemia, and, in turn, contributing to inflammation, endothelial dysfunction, and arterial hypertension (9). Oxidative stress adversely affects the kidneys, which triggers the recruitment of inflammatory cells and the release of proinflammatory cytokines, culminating in an initial inflammatory phase.

Oxidative stress causes damaged cell components, including proteins, DNA, and lipids, to accumulate. Cells evolved several defense mechanisms, including detoxification, antioxidant enzymes, repair enzymes, and thiol-redox systems, to prevent the harmful effects of oxidative stress. The “antioxidant defense system” of the cells is generally understood to consist of cellular enzymatic and non-enzymatic antioxidant components that work together in a complex network to keep the generation and clearance of ROS/RNS in balance (10). Antioxidants are essential for shielding our bodies from the harm caused by free radicals. They cleanse excess free radicals and balance their production (11). The term “antioxidant enzymes” refers to the majority of the enzymatic elements of this antioxidant defense mechanism, such as glutathione peroxidase, superoxide dismutase, and catalase. Paraoxonase 1 (PON1) and other antioxidant enzymes are essential for combating oxidative stress and supporting HDL’s antiatherogenic properties (12). The objective is to investigate whether PON1 plays a mechanistic role in the development of cardiovascular disease associated with chronic renal illness.

Investigations indicate that the activity and concentration of PON1 are influenced by two prevalent polymorphisms located in the coding region (at positions 55 and 192). Variations in PON1 serum concentrations and the incidence of cardiovascular disease have been linked to the leucine/methionine polymorphism at position 55 of the amino acid sequence (L55M) (13). The Q192 isoform has been shown to hydrolyze paraoxon and metabolize oxidized LDL more efficiently than the R192 isoform, indicating that the glutamine/arginine polymorphism at position 192 (Q192R) impacts the PON1 function (14). PON1 serves a crucial role in physiological processes, highlighted by the association between diminished PON1 activity and a heightened risk of cardiovascular disease.

Single-nucleotide polymorphisms, or SNPs, are the most prevalent genetic variation (15). As a result of the advancements, the dbSNP database of technologies for next-generation sequencing currently contains over 950 million SNPs in the human genome -(http://www.ncbi.nlm.nih.gov/SNP/) (16). SNPs are polymorphisms in a single nucleotide that affect

the DNA sequence (A, T, C, or G). SNPs are estimated to occur at a frequency of 1 in 1,000 bp across the genome. These minute variations might be transient or transversional. About 25% of SNPs cause silent mutations, which do not change translated amino acids; 25% cause missense mutations, sometimes known as coding SNPs or cSNPs, and 50% occur in noncoding regions. Known as synonymous SNPs, these quiet SNPs are probably not influenced by natural selection (17). Conversely, natural selection may affect nonsynonymous SNPs (nSNPs, change-encoded amino acids), which can result in pathology. Both synonymous and nonsynonymous SNPs affect pre-mRNA conformation (or stability) and promoter activity. They also modify a protein's subcellular location and capacity to bind its substrate or inhibitors (SNPs) (18). Thus, they might be responsible for genome evolution, drug deposition, and disease susceptibility.

Thanks to bioinformatics techniques for in silico gene analysis, screening a large number of people is no longer necessary to find a statistically significant association between genes and illnesses. Stated differently, these methods facilitate SNP pre-selection (19). Separating disease-associated SNPs from neutral SNPs would be highly beneficial before using wet lab-based techniques. In silico analysis can be helpful when independent future research cannot establish the links between the illnesses (20). Thus, it may be possible to distinguish between true and false positives using independent proof of SNP functioning discovered by applying prediction algorithms.

The study intends to perform an in silico analysis of PON1 and its receptor gene using bioinformatics tools such as sorting the intolerant from tolerant (SIFT), Protein Variation Effect Analyzer (PROVEAN), Polyphen 2, I-mutant software, and protein-protein interactions by STRING database to ascertain the likely detrimental effects of mutations and protein-protein interactions of these genes. The present study may indicate that experimental research is necessary to investigate the possible role of PON1 gene alterations in pathobiology, progression, and risk of cardiovascular disease (CVD) in CKD.

This study aims to do a preliminary bioinformatics analysis of SNPs in the paraoxonase 1 (PON1) gene and investigate possible potential associations with CKD. The analysis was conducted using in silico techniques to identify functionally significant SNPs, predict the probable impact on PON1 gene expression or protein function, and explore their possible roles in the pathophysiology of CKD.

Materials and Methods

The analysis of the PON 1 gene using bioinformatic tools (SIFT, PROVEAN, Polyphen 2, and I-Mutant) is shown in Figure 1.

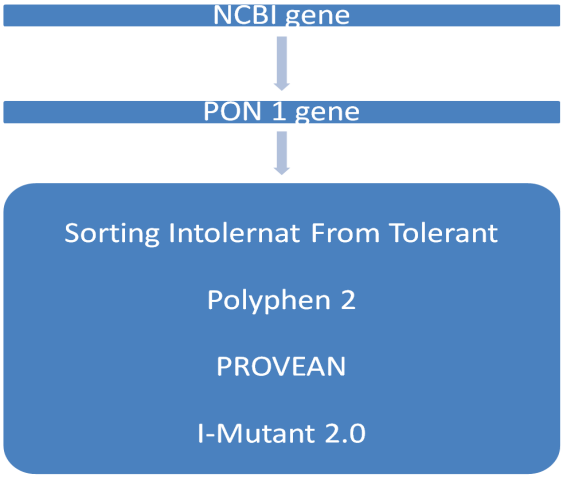

Figure 1: Illustrating the use of bioinformatics tools (SIFT, PROVEAN, Polyphen 2, and I-Mutant 2.0) for gene analysis.

Employing a bioinformatics program to analyze PON1 gene SNPs for stability, damage, and benignity. The main sources of information on the human PON1 gene were the National Center for Biological Information (http://www.ncbi.nlm.nih.gov/) (21). The polymorphism data on SNPs of the human PON1 gene and related metadata were obtained for the computational analysis from the publicly accessible online database dbSNP-NCBI (http://www.ncbi.nlm.nih.gov/SNP/) and protein sequence from FASTA(http://www.ncbi.nlm.nih.gov/SNP/).

Sorting intolerant from tolerant (sift) approach

The functional impact of damaging nsSNPs was evaluated by sorting intolerant from tolerant (SIFT), a sequence homology-based approach. To ascertain whether a change in an amino acid could affect protein function and, consequently, alter phenotype, SIFT (Sorting Intolerant From Tolerant) uses sequence homology (22). When used on human variation datasets, SIFT could differentiate between neutral polymorphisms and mutations implicated in disease. We applied SIFT to a database of missense substitutions linked to or involved in disease, assuming that amino acid alterations that cause disease are detrimental to protein function (23). SIFT calculates the normalized probability for each mutation in terms of the tolerance index (TI) score or SIFT score. SIFT scores can be classified as potentially intolerant (0.051-0.10), tolerant (0.201-1.00), borderline (0.101-0.20), or intolerant (0.00-0.05) (24). As the tolerance index rises, the probability that an amino acid substitution will have an effect falls.

Structural homology-based (PolyPhen) approach

The functional effects of coding nsSNPs were analysed using a structural homology-based approach (PolyPhen). A computational technique for identifying potentially useful nsSNPs is PolyPhen (25). By using fundamental physical and comparative concepts, this technique (Polymorphism Phenotyping 2) forecasts how modifications in amino acids may impact the structure and functionality of human proteins (26). Making use of PolyPhen 2 (http://genetics.bwh.harvard.edu/pph2). The possible effects of a change in an amino acid on the structure and functionality of the PON1 protein were examined. The protein sequence including the mutational site was submitted to the server along with two different amino acid variants. Predictions are based on a combination of structural, phylogenetic, and sequence annotation information that characterizes a substitution and where it occurs in the protein. The PolyPhen score assigns specificity and sensitivity values to nsSNPs and divides them into three primary categories: benign, perhaps harmful, and probably harmful.

Assess the functional impact of coding nsSNPs by PROVEAN

The function of the standalone PROVEAN software package distribution can be accessed online through the “PROVEAN Protein” interface. Its primary purpose is anticipating a protein sequence from any creature. The program generates PROVEAN scores after obtaining a protein sequence along with changes in amino acids. It then performs a BLAST search to identify homologous or supporting sequences (27). The Protein Variation Effect Analyzer or PROVEAN, predicts how each class of protein sequence variants—including insertions, deletions, and multiple substitutions in addition to single amino acid changes—will affect the alignment-based score (28). The score calculates how much a query sequence’s sequence similarity to a protein sequence homolog changes when an amino acid variant of the query sequence is added or removed. If the protein variation’s PROVEAN score is less than -2.5, it is predicted to have a “deleterious” effect; if it is larger than -2.5, it is predicted to have a “neutral” effect. The PROVEAN tool can be accessed at http://provean.jcvi.org (29).

Assessment of the functional impact of coding nsSNPs by I-Mutant 2.0

I-Mutant2.0 (http://folding.biofold.org/i-mutant/i-mutant 2.0.html), a support vector machine-based tool, is used to predict the impact of nonsynonymous mutations on protein stability. For the first time, I-Mutant2.0 can predict the extent to which a protein sequence mutation will or won’t affect the folded protein’s stability. Additionally, it can predict the changes in the stability of the altered protein structure (30). According to the technique, I-Mutant 2.0 scores larger than zero are assumed to indicate enhanced stability, whereas numbers less than zero will reflect lower stability.

STRING analysis

STRING refers to the Search Tool for the Retrieval of Interacting Genes (STRING). Finding knowledge about protein-protein interactions is a significant task in fundamental biological research and aids in the identification of new therapeutic targets for the treatment of a range of illnesses. Protein-protein interactions must be experimentally probed using time-consuming methods like affinity chromatography or co-immunoprecipitation. High-throughput testing methods include mass spectrometry and yeast two-hybrid screens. As a result of these developments, a variety of computer techniques have been created to forecast networks of protein-protein interactions by building databases like STRING (31). The STRING offers exceptionally thorough coverage and convenient access to information on both anticipated and experimental interactions. STRING assigns a confidence score to interactions within a stable and consistent identifier space, in addition to auxiliary information like as protein domains and three-dimensional structures. STRING version 9.0, accessible at http://string-db.org (32), covers over 1100 completely sequenced organisms. To find biological processes and pathways, KEGG pathway analysis and Gene Ontology (GO) enrichment were employed.

Results and Discussion

Identification of harmful and tolerant SNPs

Using the gene ID 5444, the dbSNP was used to get the SNPs in the human PON1 gene. Out of the 55 SNPs examined, 22 variants were determined to be harmful, and the remaining variants were to be tolerated when the SNPs were submitted to the SIFT tool to predict their impact on protein function. **Table 1** presents the comprehensive outcome.

Table 1: The number and percentage of SNPs damaging, tolerated, and decreased protein stability results of the PON1 gene.

Bioinformatic tools	PON1 gene (55 SNPs)	
SIFT	40% (22no's) Deleterious	60% (33no's) tolerated
Polyphen2	32% (18no's) damaged	37% (68no's) benign
PROVEAN	60% (33no's) (deleterious	40% (22no's) Neutral
I-Mutant 2.0	62% (34no's) decreased stability	48% (31 no's) increased stability

nsSNPs damaged by the PolyPhen 2 server

The PolyPhen 2 server received all 55 missense nsSNPs that were submitted to SIFT. Of the 55 SNPs, 26 were thought to be likely harmful. Table 2 shows that the results from the structurally based technique PolyPhen and the evolutionary-based approach SIFT showed a substantial correlation. Twenty -two of the SNPs identified by PolyPhen as likely harmful were also found to be harmful by SIFT, indicating that these nsSNPs may impair the structure and function of proteins.

Destructive nsSNPs discovered by I-Mutant 2.0

The stability of protein structural changes is predicted by using an online tool called I-Mutant 2.0. Table 2 displays the outcomes for each of the 55 missense SNPs' inputs. The free energy change upon mutation is anticipated to either increase or decrease. It was discovered that 34 of the 55 SNPs examined resulted in a drop in free energy.

Functional Characterization of PON1 by PROVEAN

PROVEAN predicts 33 out of 55 as deleterious and remaining as neutral mutations. Table 2 shows the outcomes for each of the 55 missense SNPs. The PROVEAN analysis yielded a greater number of harmful SNPs than the SIFT analysis did. This might be because, in addition to amino acid alterations, the PROVEAN tool can also evaluate insertions and deletions.

Table 2: PON1 gene SNP analysis using bioinformatic methods (SIFT, PROVEAN, Polyphen 2, and I-Mutant 2.0)

SNP	Amino acid change	SIFT score	SIFT median	SIFT prediction	POLYPHEN 2	Sensitivity	Specificity	PROVEAN score	Prediction (cut off = -2.5)	I-Mutant (2.0)
rs373190914	I48I	1	2.76	Tolerated				0.000	Neutral	0.19
rs372449149	T318I	0.052	2.75	Tolerated	0.993 (damaged)	0.70	0.97	-4.340	Deleterious	-2.13
rs372449149	T318I	0.058	2.63	Tolerated	0.993 (damaged)	0.70	0.97	-4.340	Deleterious	-213
rs371803280	V268M	0.005	2.75	Deleterious	0.452 (damaged)	0.89	0.90	-2.131	Neutral	-0.82
rs371803280	V268M	0.008	2.63	Deleterious	0.452 (damaged)	0.89	0.90	-2.131	Neutral	-0.82
rs371338407	P79R	0.004	2.79	Deleterious	0.705 (damaged)	0.86	0.92	-5.657	Deleterious	-1.21
rs371338407	P79R	0.004	2.83	Deleterious	0.705 (damaged)	0.86	0.92	-5.657	Deleterious	-1.21
rs370355032	P210S	0.187	2.75	Tolerated	0.761 (damaged)	0.85	0.92	-4.254	Deleterious	-1.67
rs370355032	P210S	0.292	2.63	Tolerated	0.761 (damaged)	0.85	0.92	-4.254	Deleterious	-1.67
rs369422555	W281C	0.001	2.63	Deleterious	1.000 (damaged)	0.00	1.00	-9.675	Deleterious	0.64
rs369422555	W281C	0.001	2.75	Deleterious	1.000 (damaged)	0.00	1.00	-9.675	Deleterious	0.64
rs368620674	F120S	0.001	2.76	Deleterious	1.000 (damaged)	0.00	1.00	-6.235	Deleterious	-0.60
rs368620674	F120S	0.007	2.67	Deleterious	1.000 (damaged)	0.00	1.00	-6.235	Deleterious	-0.60
rs368248410	I271V	0.22	2.63	Tolerated	0.081	0.93	0.85	-0.728	Neutral	-0.32
rs368248410	I271V	0.226	2.75	Tolerated	0.081	0.93	0.85	-0.728	Neutral	-0.32
rs368206333	G344C	0	2.63	Deleterious	1.000 (damaged)	0.00	1.00	-8.352	Deleterious	2.00
rs368206333	G344C	0	2.74	Deleterious	1.00 (damaged)	0.00	1.00	-8.352	Deleterious	2.00
rs367566813	M88T	0.008	2.76	Deleterious	0.594 (damaged)	0.87	0.91	-4.007	Deleterious	0.02
rs367566813	M88T	0.009	2.71	Deleterious	0.594 (damaged)	0.87	0.91	-4.007	Deleterious	0.02
rs202062288	M127I	0.341	2.75	Tolerated	0.000	1.00	0.00	1.652	Neutral	0.65
rs202062288	M127I	0.405	2.66	Tolerated	0.000	1.00	0.00	1.652	Neutral	0.65
rs201783178	R18G	0.434	3.02	Tolerated	0.000	1.00	0.00	2.879	Neutral	-1.10
rs199693212	F292S	0.167	2.75	Tolerated	0.828 (damaged)	0.84	0.93	-2.833	Deleterious	-1.11
rs199693212	F292S	0.178	2.63	Tolerated	0.828 (damaged)	0.84	0.93	-2.833	Deleterious	-1.11
rs199616322	P59S	0.412	2.76	Tolerated	0.023	0.95	0.81	-3.959	Deleterious	0.66

rs199616322	P59S	0.416	2.75	Tolerated	0.023	0.95	0.81	-3.959	Deleterious	0.66
rs189946844	E123V	0.129	2.67	Tolerated	0.036	0.94	0.82	-3.315	Deleterious	-0.71
rs189946844	E123V	0.129	2.76	Tolerated	0.036	0.94	0.82	-3.315	Deleterious	-0.71
rs185623242	S302L	0.001	2.63	Deleterious	1.000 (damaged)	0.00	1.00	-5.268	Deleterious	-1.28
rs185623242	S302L	0.001	2.75	Deleterious	1.000 (damaged)	0.00	1.00	-5.268	Deleterious	-1.28
rs150657027	A6V	0.858	2.96	Tolerated	0.000	1.00	0.00	-0.552	Neutral	0.01
rs149100710	E49K	0.021	2.71	Deleterious	0.995 (damaged)	0.68	0.97	-3.303	Deleterious	-0.11
rs148911901	M289K	0.174	2.79	Tolerated	0.000	1.00	0.00	-2.897	Deleterious	-0.90
rs148911901	M289K	0.323	2.71	Tolerated	0.000	1.00	0.00	-2.897	Deleterious	-0.90
rs148785172	A126T	1	2.66	Tolerated	0.000	1.00	0.00	2.009	Neutral	1.50
rs148785172	A126T	1	2.75	Tolerated	0.000	1.00	0.00	2.009	Neutral	1.50
rs147867887	T121I	0.506	2.67	Tolerated	0.000	1.00	0.00	0.938	Neutral	-1.14
rs147867887	T121I	0.525	2.76	Tolerated	0.000	1.00	0.00	0.938	Neutral	-1.14
rs146211440	S23A	1	2.94	Tolerated	0.000	1.00	0.00	0.621	Neutral	-0.70
rs145997673	G330S	0.009	2.63	Deleterious	0.982 (damaged)	0.75	0.96	-4.544	Deleterious	-0.29
rs145997673	G330S	0.009	2.75	Deleterious	0.982 (damaged)	0.75	0.96	-4.544	Deleterious	-0.29
rs144612002	I48V	0.517	2.76	Tolerated	0.001	0.99	0.15	-0.467	Neutral	0.19
rs144390653	M127R	0.001	2.66	Deleterious	0.005	0.97	0.74	-2.799	Deleterious	0.65
rs144390653	M127R	0.001	2.75	Deleterious	0.005	0.97	0.74	-2.799	Deleterious	0.65
rs141948033	N19D	0.514	3.02	Tolerated	0.000	1.00	0.00	0.008	Neutral	0.84
rs141665531	P40L	0.115	2.92	Tolerated	0.003	0.98	0.44	-5.480	Deleterious	0.03
rs141598837	K340R	0.234	2.75	Tolerated	0.036	0.94	0.82	-1.626	Neutral	-0.74
rs141598837	K340R	0.279	2.63	Tolerated	0.036	0.94	0.82	-1.626	Neutral	-0.74
rs138512790	C42R	0	2.78	Deleterious	1.000	0.00	1.00	-10.594	Deleterious	-0.47
rs112078575	K151R	0.804	2.79	Tolerated	0.000	1.00	0.00	0.285	Neutral	-2.35
rs112078575	K151R	0.833	2.69	Tolerated	0.000	1.00	0.00	0.285	Neutral	-2.35
rs80019660	A201V	0.202	2.75	Tolerated	0.771 (damaged)	0.85	0.92	-1.627	Neutral	1.72
rs80019660	A201V	0.475	2.62	Tolerated	0.771 (damaged)	0.85	0.92	-1.627	Neutral	1.72
rs72552788	L90P	0.001	2.71	Deleterious	1.000 (damaged)	0.00	1.00	-5.744	Deleterious	-1.69
rs72552788	L90P	0.001	2.76	Deleterious	1.000 (damaged)	0.00	1.00	-5.744	Deleterious	-1.69

Table 3 shows the accepted range of damaging and Tolerant SNPs using SIFT, Ploypheh, and PROVEAN bioinformatic tools and the accepted range of their stability through I-Mutant 2.0.

Figure 2 Shows the Percentage of deleterious and Tolerant SNPs by using Bioinformatic tools (SIFT, PROVEAN, Polyphen 2, and I-Mutant 2.0).

Table 3: Bioinformatic tools (SIFT, PROVEAN, Polyphen 2, and I-Mutant 2.0) showing damaging and tolerated ranges of the PON1 gene.

BIOINFORMATIC TOOL	Damaged/Deleterious	Tolerated
SIFT	0.0 – 0.05	0.05 – 1
POLYPHEN 2	0.5 – 1	0.00 – 0.5
PROVEAN	<-2.5	>-2.5
I-Mutant 2.0	<0 (decreased stability)	>0 (increased stability)

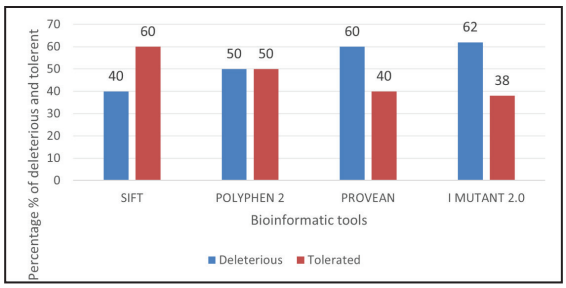

Figure 2: Comparison of deleterious and tolerated PON1 gene by bioinformatic tools (SIFT, PROVEAN, Polyphen 2, and I-Mutant 2.0).

Using STRING analysis, Figure 3 depicts the PON1 protein-protein interaction (PPI) network. The network has 50 edges and 11 nodes (proteins), with a clustering coefficient of 0.949 and a high average node degree of 9.09. The proteins are at least somewhat physiologically related to one another, according to the PPI enrichment p-value of 1.11e-16. The network's nodes are proteins. The edges represent the proposed functional links. Eight different colored lines representing the presence of the eight different categories of evidence that were taken into consideration while predicting the linkages were drawn on an edge in evidence mode (Fig. 3).

Bluish-green lines are derived from carefully selected databases; pink lines are experimentally determined; green lines indicate gene neighborhoods; violet lines indicate gene co-occurrences; reddish-green lines indicate text mining; black lines indicate co-expression; and blue lines indicate protein homology. Table 4 shows the predictions of the functional partner genes with the PON1 gene through the STRING Analysis. This table also explains the Co-expression and Experimental interaction of various genes and their combined score with the PON1 gene.

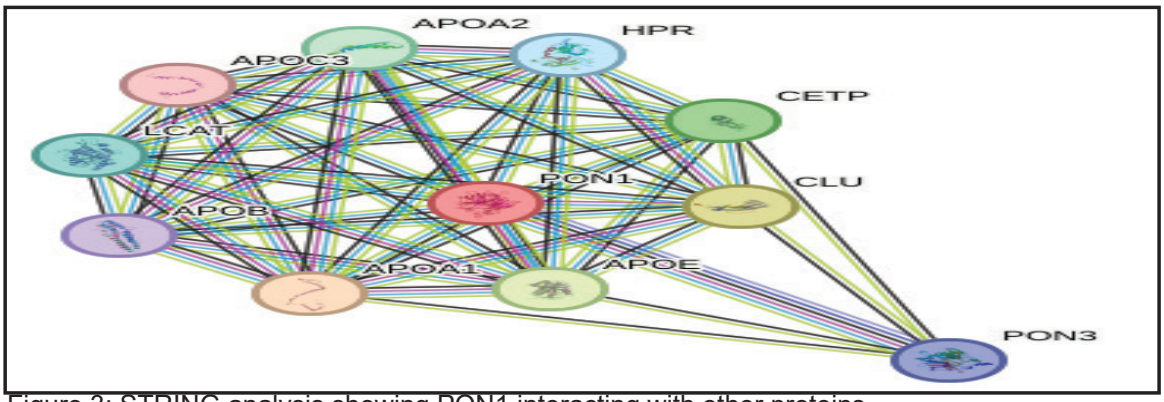

Figure 3: STRING analysis showing PON1 interacting with other proteins

Table 4: Predicted functional partner genes with PON1 gene

Nodes	Neighbour hood on chromosome	Gene fusion	Co-occurrence	Co-expression	Experimental interaction	Databases annotated	Automated text mining	Homology	Combined score
APA1	0	0	0	0.81	0	0.720	0.996	0	0.998
CLU	0	0	0	0	0.292	0.720	0.983	0	0.996
APOE	0	0	0	0.683	0	0.720	0.918	0	0.977
CETP	0	0	0	0.090	0.	0.720	0.890	0	0.968
APOA2	0	0	0	0.920	0	0.720	0.845	0	0.967
LCAT	0	0	0	0.138	0	0.720	0.874	0	0.965
HPR	0	0	0	0.784	0	0.720	0.833	0	0.962
PON3	0	0	0.068	0	0.817	0.700	0.090	0.964	0.957
APOB	0	0	0	0.378	0	0.720	0.818	0	0.952
APOC3	0	0	0	0	0	0.720	0.732	0	0.933

Figure 4 shows the k-means clustering of proteins involved in PON1 and associated metabolic pathways. The proteins have been grouped into clusters based on their biological roles. Cluster 1 (red) highlights high-density lipoprotein synthesis with nine genes, including LCAT and APOA1, which play a multifaceted role in triglyceride homeostasis and prevent stress-induced aggregation of blood plasma proteins. Cluster 2 (green) includes CETP synthesis, with one gene involved in the regulation of reverse cholesterol transport. Cluster 3 (blue) highlights PON 3 synthesis, which works similarly to hydrolyzing aromatic lactones and lactones with aliphatic substituents in rings of five or six, but not simple lactones or lactones with polar substituents.

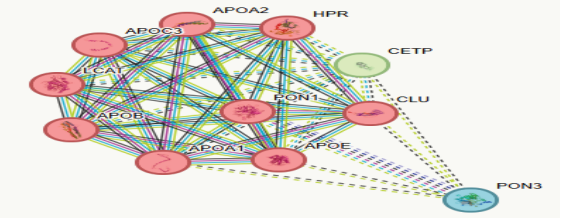

Figure 4: Showing K-means cluster analysis of PON1 with other proteins

Figure 5 shows the MCL clustering of proteins involved in PON1 and associated metabolic pathways. The proteins have been grouped into clusters based on their biological roles: MCL clustering shows only one cluster (red) highlighting high-density lipoprotein synthesis, eleven genes, including CETP, LCAT, APOA1, etc. (Fig. 5), which inhibits the aggregation of blood plasma proteins brought on by stress and has a variety of functions in maintaining triglyceride homeostasis.

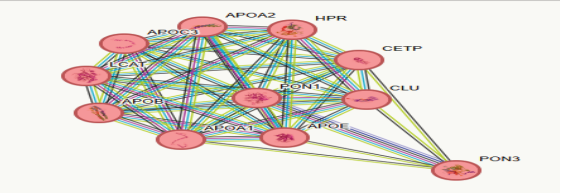

Figure 5: Showing MCL cluster analysis of PON1 with other proteins

Figure 6 shows the DBSCAN clustering of proteins involved in PON1 and associated metabolic pathways. The proteins have been grouped into clusters based on their biological roles: DBSCAN clustering shows only one cluster (Red) highlighting high-density lipoprotein synthesis, ten genes, including CETP, LCAT, APOA1, etc. (Fig. 6), which inhibits the aggregation of blood plasma proteins brought on by stress and has a variety of functions in maintaining triglyceride homeostasis.

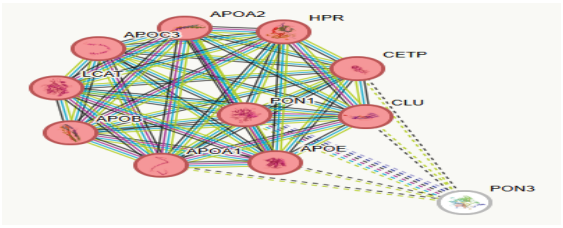

Figure 6: Showing DBSCAN cluster analysis of PON1 with other proteins

Figure 7 highlights the biological processes associated with PON1 and its interacting proteins. Notable terms include: Lipoprotein metabolism is involved in reverse cholesterol transport, plasma lipoprotein particle remodeling, high-density lipoprotein remodeling, and triglyceride-rich lipoprotein particle remodeling. Cholesterol transport and cholesterol efflux play a significant role in cholesterol homeostasis. The enrichment of these terms supports the theory that disruptions in PON1 or its network can impact lipid metabolic pathways, and its decreased levels may potentially lead to cardiovascular risk in CKD Patients. The KEGG pathway analysis identifies significant metabolic pathways involving PON1 gene.

Cholesterol metabolism is directly involved in absorbing, synthesizing, and transporting cholesterol in the body; the PPAR signaling pathway controls the expression of genes related to the intake, storage, oxidation, and metabolism of fatty acids. Participates in the process of vitamin absorption and digestion. These enriched pathways demonstrate PON1's centrality in maintaining cholesterol homeostasis, and the network can impact lipid metabolic pathways; its decreased potentiality leads to cardiovascular risk in CKD patients.

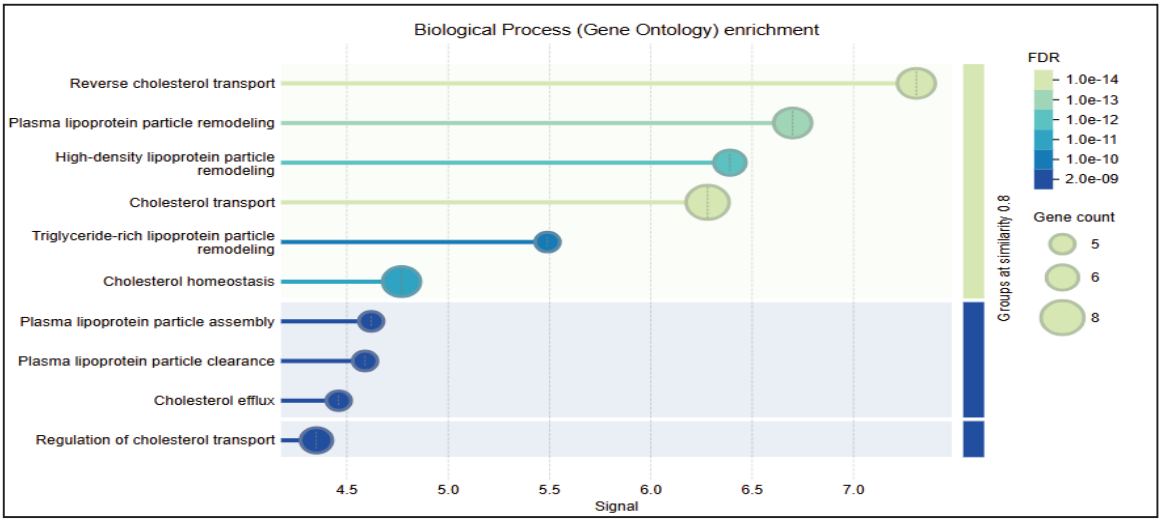

Figure 7: Gene Ontology (GO) biological processes enrichment of PON1 gene by STRING Analysis

The results across figures and tables collectively depict PON1 as a central enzyme in lipid metabolism. Its interactions with other proteins like apoproteins and lipoproteins and their biosynthesis pathways, as well as its relevance to various biological processes, highlight its significance. These findings provide a strong foundation for the decreased PON1

gene potentiality, which leads to cardiovascular risk in CKD Patients.

Discussion

The susceptibility of these patients to CVD often makes care more challenging, even with advancements in CKD management approaches. A possible key factor in the disproportionate morbidity and mortality from cardiovascular disease is the defective High-Density Lipoprotein (HDL). The PON-1 enzyme, which is produced by the liver, binds to High-Density lipoprotein (HDL) cholesterol and circulates. Reduced PON-1 activity has also been connected to adverse outcomes and a higher risk of CVD (33). The HDL-associated protein PON1 can hydrolyze oxidized LDL cholesterol, perhaps protecting against atherosclerosis. Additionally, PON1 can break phospholipid peroxidation adducts, which may have cytoprotective effects (34). The degree of coronary lesion was predicted by the decline in PON1 activity in serum, which was demonstrated to have a significant protective function in the development of atherosclerosis (35). Many inflammatory conditions, including systemic lupus erythematosus, rheumatoid arthritis, diabetes mellitus, and several hepatic and renal conditions, such as renal failure, psoriasis, and macular degeneration, are linked to low serum PON1. These disorders are likewise characterized by elevated rates of CHD and malfunctioning HDL, which is thought to be (though not confirmed) brought on by decreased PON1 activity (36). Multiple polymorphisms in the coding and promoter areas affect PON1 function and gene expression levels. The most prevalent polymorphisms in the area that codes for PON1 are Q192R, which contains a leucine (L) to methionine (M) substitution at codon 55 and a glutamine (Q) to arginine (R) change at codon 192 (35).

The PON1 gene was analyzed in silico using SIFT, PolyPhen-2, PROVEAN, and I-Mutant, which yielded important information about the structural and functional effects of its genetic variants. The outcomes of these instruments show how particular single nucleotide polymorphisms (SNPs) may affect PON1's stability and enzymatic activity, which are essential for its physiological function in detoxification and antioxidation. SIFT, PolyPhen-2, PROVEAN, and I-Mutant results indicate lower protein stability and higher harmful and detrimental scores. This may have important ramifications for oxidative stress-related diseases like cardiovascular and neurological problems.

The protein-protein interaction (PPI) network, biochemical pathways, and metabolic processes linked to PON1 are all thoroughly examined in the STRING ANALYSIS research. Every figure and table produced by this study emphasizes a distinct aspect of the biological network of PON1 and its relevance to human health and illness.

A comprehensive understanding of PON1's function in metabolism is offered by the combination of STRING analysis, clustering techniques, GO enrichment, and KEGG pathway mapping. The study confirms PON1's pivotal role in cholesterol metabolism and its subsequent impact on the risk of cardiovascular disease.

Conclusion

According to this preliminary bioinformatics research, single nucleotide polymorphisms (SNPs) in the PON1 gene may play a part in the aetiology of CKD. SIFT, PROVEAN, PolyPhen-2, I-Mutant, and STRING were used to determine the potential impacts of genetic variations on the structure, function, and interactions of the PON1 protein. Utilizing in silico tools, we identified key SNPs that may influence PON1 function, particularly in oxidative stress and lipid metabolism pathways, which are critical in developing CVD risk in CKD progression. Future research should concentrate on bridging computational predictions with functional studies to investigate the molecular significance of PON1 variations in CKD and evaluate their

potential as diagnostic or therapeutic targets.

Conflicts of Interest

The authors declared that they have no conflicts of interest.

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