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IN SILICO ANALYSIS OF THE IMPACT OF SNPS/SNP HAPLOTYPES ON PROTEIN STRUCTURE AND FUNCTION IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

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ABSTRACT

Autosomal dominant polycystic kidney disease (ADPKD) is considered as the most common single-gene disorder that results in renal failure. Prevalence polycystic kidney disease in Asia accounts to 2%. Single nucleotide polymorphism (SNPs) is common in autosomal dominant polycystic kindey disease (ADPKD) which occurs in PKD1 and PKD2 genes. The non synonymous single nucleotide polymorphism (nsSNP) of genes introduces amino acid changes in proteins that play an important role in providing genetic functional diversity. In order to treat the genetic disease, the recent research is focused on DNA based designing and natural therapeutic agents to treat genetic diseases. Therefore, this present work is undertaken with an aim to understand the occurrence of SNPs in PKD genes and to employ the existing natural products as a prime solution for the treatment ADPKD- Silent killer. The genotype and allele data of PKD1 from SNP database demonstrated the occurrence of A/G and C/T polymorphisms are increased when compounded with other 762 SNPs available for PKD1 gene. The emerging computational techniques have provided a platform to explore ADPKD disease at the molecular level to understand the variations in the protein structure and the mechanism of ADPKD. The predicted structure of this protein revealed the key residues of the protein which elucidate their structure/function characteristics with special emphasis on invariant conserved residues.

Keywords: Genetic marker, Mutation, Renal failure, Polycystin-1, Polymorphism.

INTRODUCTION

Autosomal dominant polycystic kidney disease found to be occurs worldwide and in all races. ADPKD disease is one of the most commonly inherited conditions in human with an incidence of 1:500 to 1:1000 (Grantham *et al.*, 2006; Grantham and Calvet, 2001; Persu *et al.*, 2002). It is genetically heterogeneous with two genes identified, PKD1 (16P13.3) and PKD2 (4q21) (Igarashi and Somlo,2002; Hoefele *et al.*, 2011) The proteins encoded by the PKD1 and PKD2 genes are polycystin 1 and polycystin 2 and they interact with each other in the primary cilia of renal epithelial cells and participate in complex signal transduction pathways, which seems to be involved in chemosensory/mechanic functions and have some role in cell proliferation and maturation (Igarashi and Somlo, *et al.*, 2002; Hogan *et al.*, 2010; Hoefele *et al.*, 2011).

The majority of mutations are unique to a single family, recurrent mutations account for 30% of the total. Currently, 978 sequence variants of the PKD1 gene and 193 sequence variants of the PKD2 gene have been published in the Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk). The specialized Autosomal Dominant Polycystic Kidney Disease Mutation Database (PKDB) presents 1,923 different sequence variants of PKD1 and 241 sequence variants of the PKD2 gene (http://pkdb.mayo.edu; Gout *et al.*, 2007; Losekoot e*t al.*, (2012). Losekoot *et al.*, (2012) suggested that the gene linkage studies of PKD1 and PKD2 allow accurate presymptomatic diagnosis of the disease which is due to PKD1.

A Single nucleotide polymorphism (SNP) is generally defined as a stable substitution of a single base with a frequency of more than 0.01 in at least one population (Taylor *et al.*, 2001). In human genetic studies, SNPs are simply referred to as bi-alleleic SNPs, which are very rare in the human genome. SNPs have been recognized as an important tool in human genetics and medicine (Miller *et al.*, 2001, Gray *et al.*, 2000).

As SNP data for Indian population is found to be limited there is a need for studying this PKD1 and PKD2 gene. In this context, this study focussed towards unravelling the SNP (Veeramuthumari and Isabel, 2013; Veeramuthumari *et al.*, 2013).

MATERIALS AND METHODS

The human C/T polymorphism at position 4058 in exon 45 polymorphic and

wild sequences were analyzed and confirmed with SNP data base, Online Mendelian Inheritance in Man (OMIM) available at National Centre for Biological Information (NCBI) website.

Servers used for the Study:

The sequence search analysis were performed at NCBI- BLASTp and BLASTn (Altschul *et al.,* 1997a; 1997b). The multiple alignments were generated using CLUSTALW (Thompson et al., 1994), the secondary structure was predicted through the SABLE. The fold recognition analysis was carried out by PHYRE (http://www.sbg.bio.ic.ac.uk/phyre/) and the Transmembrane analysis was performed using the TMHMM server (Sonnhammer *et al.,* 1998). The tools used for mutations and disease analysis are nsSNP analyzer polyPhen, SIFT, PhenoPred, Variant effect Predictor, SNPeffect, PROVEAN, PhenoPred, SNP & GO, SNPs3D, ModBase, PolyDoms, I-Mutant, MUSTER.

RESULTS AND DISCUSSION

The Polycystic kidney disease -1 and -2 (pkd-1 and pkd-2) gene, function, accession number, name, protein name, number, taxonomic identifier number, taxonomic lineage, the total number amino acid, sequence status, disease, binary interaction, variant number were found in Uniprot and SNP web database **(Table:1).** The single nucleotide polymorphism in PKD1 and PKD2 are important role in cause of the disease, polycystic kidney disease 1 and polycystic kidney disease 2. The present study demonstrated a single nucleotide polymorphism (SNP) in PKD1 gene at position (amino acid) 4058, the amino acid change is alanine (A) to valine (V), the (SNPs) is found as missense mutation and the deletrious effect of the mutation site **(Table:2).**

Table:1 Report of PKD1 gene using uniprot /NCBI search:

Gene Name	**PKD1**
Protein Name	Polycystin-1 Alternative name(s): Autosomal dominant polycystic kidney disease 1 protein
Organism	Homo sapiens (Human)
Taxonomic identifier	9606 [NCBI]
Taxonomic lineage	Eukaryota › Metazoa › Chordata › Craniata › Vertebrata › Euteleostomi › Mammalia › Eutheria › Euarchontoglires › Primates › Haplorrhini › Catarrhini › Hominidae › Homo
Amino Acid	4303
Sequence status	Complete
Disease	Polycystic kidney disease 1 (PKD1) [MIM:173900]
Binary interaction	NPHP1 O15259 PKD2 Q13563
Variant number	VAR_010095, VAR_005547
Domain	14
Protein size	460KD
Exon	46

(http://www.uniprot.org/uniprot/Q13563#ref11);(Hughes *et al.*, 1995; Mochizuki *et al.*, 1996)

Table: 2 Summary of PKD1 (A/V) gene sequence based prediction

Web Server	**Effect**
SNP database	Missense
Variant effect predictor	Polycystic kidney disease 1 (autosomal dominant
Uniprot/Swiss-prot	Single nucleotide polymorphism
(DM)2 – Domain Mapping & Disease mutation	Polymorphism
SIFT	Tolerated (Score: 0.09)
PolyPhen	Benign (Score:0.007)
PolyDoms	Nonsynonymous & Synonymous
MuSTAB	Disease
PROVEAN	Neutral (Score:-0.739)
nsSNP analysis	Nonsynonymous
Signal P4.1 Prediction	Signal peptide present

The multiple sequence alignment of polcystin-1 and polcystin-2 was carried out by CLASTALW. The evolutionary conservation at the position in which the missense mutation was observed revealed that this particular polymorphism is conserved across the species. Hence they reported that *in silico* analysis using powerful software tools can facilitate predicting the phenotypic effect of non-synonymous coding SNPs on the physico-chemical properties of the concerned protein such information is critical for genotype –phenotype correlation and also to understand disease biology

Bycroft *et al*., (1999) also stated that the PKD domain was first identified in the polycystic kidney disease protein PKD1, and contains an Ig-like fold. PKD1 is involved in adhesive protein-protein and protein-carbohydrate interactions. Most of these domains are present in the extracellular parts of proteins involved in interactions with other proteins. The domain is most often found in proteins archaebacteria and some vertebrates. Polycystic kidney disease gene 1 protein contains 14 repeats and it is the major locus of the common genetic disorder autosomal dominant polycystic kidney disease. The PKD domain motif search predicted to be a globular domain that contains an antiparallel β sheet. The domains do not contain conserved cysteines, and they are extracellular domains.

The BLAST search for proteins with similar sequences and known 3D structure was performed (Altschul *et al.*, 1997a; 1997b). Nucleotide sequence of pkd1 showed that 200 blast hits, wild and mutant showed 100% and 99% of sequence similarities. The BLASTp search of polcystin-1 found that 100 blast hits on query sequence. Both the search showed to be 100 and 99% identity. The current study also focused on modelling of polycystin-1 hence the study did protein structure database search which was observed to be less query coverage on both the sequence of these proteins. It was found to be low E-vlaue (<0.001) and hence very significant **(Table:3a & 3b).**

Table:3a NCBI-BLAST against Nucleotide Sequence of Polycystin-1 wild and mutant (BLASTn)

Acc.No.	Source	Length (bp)	Score (bits)	Query cover	Sequence Identity (%)	E-Value
Wild						
NM_000296.3	Homo sapiens polycystic kidney disease 1 (autosomal dominant) (PKD1), transcript variant 2, mRNA	14135	26103	100%	100%	0.0
NM_001009944.2	Homo sapiens polycystic kidney disease 1 (autosomal dominant) (PKD1), transcript variant 1, mRNA	14135	26088	100%	99%	0.0
Mutant						
NM_000296.3	Homo sapiens polycystic kidney disease 1 (autosomal dominant) (PKD1), transcript variant 2, mRNA	14135	26097	100%	99%	0.0
NM_00100994	Homo sapiens polycystic kidney disease 1 (autosomal dominant) (PKD1), transcript variant 1, mRNA	14135	26083	100%	99%	0.0

E-value must be low as possible, E-value <0.001; very significant
Source: https://blast.ncbi.nlm.nih.gov/Blast.cgi

Table: 3b NCBI-BLAST against Protein Sequence of Polycystin-1 cation channel wild and mutant (BLASTp):

Acc.No.	Source	Length (bp)	Score (bits)	Query cover	Sequence Identity (%)	E-Value
Wild						
AAC34211.1	PKD1 [Homo sapiens]	402	791	100%	99%	0.0
XP_005255427.1	PREDICTED: polycystin-1 isoform X5 [Homo sapiens]	402	799	100%	99%	0.0
Mutant						
AAC34211.1	PKD1 [Homo sapiens]	402	793	100%	100%	0.0
XP_005255427.	PREDICTED: polycystin-1 isoform X5 [Homo sapiens]	402	801	100%	100%	0.0

E-value must be low as possible, E-value <0.001; very significant
Source: http://www.ncbi.nlm.nih.gov/blast/Blast.cgi

The protein encoded by PKD1 (polycystin1) and PKD2 (polcystin2) interact in the plasma membrane to participate in signalling pathways that regulate renal tubular cell maturation. That is the coil structure located in exon 12 denotes the calcium binding EF hand domain. The coil-coil domain mediates the interaction between polcystin1 and polycystin2 is located in exon 12 and exon 13 (Hateboer *et al.,* 2000). Hateboer *et al.,* studied the mutation, which is predicted to in EF hand domain , this leads to protein truncation, certain frameshift or nonsense mutations in PKD2 may affect mRNA stability, resulting in null allele. This particularly likely when the mutation occurs close to the 5' end (Pei *et al.,* 1999)

The study will be focused on interaction of polycystin-1(PC-1) and polcystin-2 (PC-2) between them and other proteins; and docking as future perspectives. It leads to DNA based drug designing for the polycystic kidney disease and for other hereditary diseases. The initial part of the work the current study found that there is an interaction of PC-1 and PC-1. It also found that the interaction happens in the C- terminal tail of PC-1 interacts with the corresponding region of PC-2. They also interacts with other proteins like nephronophthisis 1(NPHP1), cadherin 1, type 1(CDH1), cadherin-associated protein), beta 1 (CTNNB1), paxillin (PXN), tuberous sclerosis (TSC2), paired box 2(PAX2), transient receptor potential cation channel (RPV1), cAMP responsive element binding protein 1(CREB1). Hence the study concluded that the polcystin-1 and polycystin-2 play an important role in human and needed more work on it.

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