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Pharmacogenomic Study of Human Polycystic Kidney Disease

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Abstract

We are now entering an era of the mass-analysis of genetic information, which will signal the beginning of the study of living organisms on the basis of their most detailed plan: the DNA base-sequences. Throughout the 20th century, the chief epidemiologic impression of polycystic kidney disease (PKD) has been what an early observer described as its outstandingly hereditary character. An Autosomal dominant form of the disease is indeed among the most common genetic disorders, with an incidence of roughly one person in every thousand. Overall, the disease accounts for as much as 5% of all cases of chronic renal failure.. The researcher's essential requirement in reading and deciphering the DNA base-sequence is the precision, speed, reliability, and low cost of such operation. In the early days of genetics, scientists did not have the resources to look at more than a few genes at a time. This made the process of understanding the influence of genetics on an organism slow and arduous. Scientists were faced with the enormous task of attempting to understand Genetics with little information to complete the task. The understanding of genes would have been very helpful in solving this problem. With the announcement in June of 2001 that the first draft of the human genome had been completed, scientists' approach to biology completely changed. This is a first attempt study in Indian scenario initiated to conduct a Pharmacogenomic study of Human Polycystic Kidney Disease.

Keywords: Polycystic kidney disease, Genetic information, DNA base-sequence, Human genome and Pharmacogenomic study

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1. Introduction

The kidney is an organ that functions to reabsorb essential fluid and ions, and this is facilitated by the strictly polarized distribution of numerous transporters, enzymes, and antigens distributed along the 10 distinct segments of the nephron in an epithelial cell type-specific fashion (Peral *et.al.,* 1997). The polarization of membrane proteins is a critical component in the differentiation of renal tubule epithelial cells and is largely established in the human metanephric kidney before birth (Patricia D. Wilson, *et. al.,* 2000). Each nephron in the kidney is composed of a variety of cell types that are organized in a distinctive order so as to sequentially reabsorb ions and solutes from the glomerular ultrafiltrate and to concentrate the urine. Little is known about mechanisms that lead to the development

of discrete cell types or the ordering of cells into specialized segments along the nephron (Howie *et.al.,* 1993). However, physiologists have identified ion channel, exchanger, and transporter functions in different nephron segments, leading to detailed insights into how ion and solute transport is regulated (Almers and Stirling, 1984). For proper function of renal epithelial cells, ion channels, exchangers, and transporters must be localized to functionally and structurally distinct plasma membrane domainas, termed apical and basolateral, that face the nephron lumen and interstitial space, respectively (Almers and Stirling, 1984; Fish and Molitoris, 1994). Studies have shown that both cell-cell and cell-substrate contacts are required to initiate formation of these plasma membrane domains. Subsequently, targeting to and retention of proteins in these domains establishes and maintains the functional polarity of these cells. Cytoskeletal proteins play essential roles in the development and maintenance of structural and functional organization of polarized epithelial cells (Hanaoka and Guggino, 2000). Significantly, recent studies of several kidney diseases show changes in the polarized distribution of membrane and cytoskeletal proteins (Fish and Molitoris, 1994).

Polycystic kidney disease

Polycystic kidney disease is a bilateral disorder that affects approximately 200,000-400,000 persons in the United States. The most common form of the disease is inherited as an Autosomal dominant trait (ADPKD). It typically causes renal insufficiency by the fifth or sixth decade of life (Delmas *et.al.,* 2002). The disease is characterized by the progressive enlargement of a portion of renal tubule segments (proximal, distal, loop of Henle, collecting duct). The tubules enlarge from a normal diameter of 40 um to several centimeters in diameter, causing marked gross and microscopic anatomic distortion (Foggensteiner *et. al.,* 2000**)**. The cause of the cystic change in the tubules is unknown, but current possibilities include obstruction of tubule fluid flow by hyperplastic tubule cells, increased compliance of the tubule basement membranes, and/or increased radial growth of cells in specific portions of the renal tubule (Gonzalez Perret *et.al.,* 2001). Several studies show that the epithelia of the cysts continue to transport Na+, K+, Cl-, H+, and organic cations and anions in a qualitative fashion similar to that of the tubule segment from which they were derived. ADPKD, then, is a disease in which some gigantic renal tubules, over a period of several decades, impair the function of non-affected nephrons and thereby lead to renal failure.

Types of PKD

There are three different types of PKD, which vary according to the way people can get the different form. One of the inherited forms is dominant, meaning that those who have the gene from either their mother or father will have the disease. The other inherited form, which is recessive, only develops in those individuals who have copies of the gene from both parents; otherwise, they carry the gene and may pass it to their children, but they themselves may not have kidney disease. Autosomal dominant PKD is the most common, inherited form. Symptoms usually develop between the ages of 30 and 40, but they can begin earlier, even in childhood. About 90 percent of all PKD cases are Autosomal dominant PKD. Autosomal recessive PKD is a rare, inherited form. Symptoms of Autosomal recessive PKD begin in the earliest months of life, even in the womb. Table 1 enumerates the characteristics of Autosomal dominant polycystic kidney disease (ADPKD) and Autosomal recessive polycystic kidney disease (ARPKD). Acquired cystic kidney disease (ACKD) develops in association with long-term kidney problems, especially in patients who have kidney failure and who have been on dialysis for a long time. Therefore it tends to occur in later years of life. It is not an inherited form of PKD (www.nlm.nih.gov/medlineplus/ency/article/003611.html). It is not unusual for cysts to also develop in the liver and within the systemic vasculature. Recent evidence indicates that besides the documented cyst enlargement and interstitial fibrosis, apoptotic loss of non-cystic nephrons is a significant component of the pathology of PKD and may contribute to the progressive loss of renal function (www.nlm.nih.gov/medlineplus/ency/article/007135.html).

Table 1. Characteristic Features of Autosomal Dominant Polycystic Kidney Disease (ADPKD) and Autosomal Recessive Polycystic Kidney Disease (ARPKD)

Characteristic	ADPKD	ARPKD
Inheritance	**Autosomal dominant**	**Autosomal recessive**
Incidence	1/500 to 1/1000	1/6000 to 1/40000
Gene (chromosome)	*PKDI* (Chr 16); *PKD2* (Chr 4)	PKHD1 (Chr 6)
Age of onset of ESRD	53 yr (*PKD1*); 69 yr (*PKD2*)	Infancy/Childhood usually
Location of renal cysts	All nephron segments	Collecting ducts[a]
Extrarenal manifestations	Hepatic cysts/pancreatic cysts	Biliary dysgenesis
	Cerebral & aortic aneurysms	Hepatic fibrosis
	Cardiac valvular abnormalities	Portal hypertension
	Systemic hypertension	Systemic hypertension
Protein name	Polycystin-1; Polycystin-2	Fibrocystin/Polyductin
Protein size	Polycystin-1; 4302 amino acids	4074 amino acids and alternative shorter forms

	Polycystin-2; 968 amino acids	
Protein structure	Polycystin-1; Integral membrane protein, multiple Ig-like domains, similar to egg jelly receptor	Transmembrane protein (and possible secreted forms), multiple TIG/IPT domains, as occur in hepatocyte growth factor receptor and plexins
	Polycystin-2; Integral membrane protein, similar to TRP channel	
Tissue distribution	Polycystin-1 and -2: Widespread	Kidney, pancreas, and liver
Subcellular localization	Polycystin-1: Plasma membrane, cilia[b]	Unknown
	Polycystin-2: Endoplasmic reticulum, cilia	
Function	Polycystin-1: ? Receptor, forms ion channel when co expressed with polycystin-2	? Receptor
	Polycystin-2: Calcium-activated cation channel	
[a] Cysts appear transiently in proximal tubules during fetal development (90). [b] Based on localization in male-specific sensory neurons in *C. elegans* (66).		

Bioinformatics

We are now entering an era of the mass-analysis of genetic information, which will signal the beginning of the study of living organisms on the basis of their most detailed plan: the DNA base-sequence. The entire set of human genes is now available. This represents an irresistible amount of data that breached the bioinformatics gap that lay between biologists and their understanding of genetics. DNA is the "genetic blueprint" that determines the genotypic make-up of each organism. In its barest form, DNA consists of two strings of nucleotides, or bases (abbreviated A, C, G, and T), wound around each other. The bases composing DNA have specific binding capabilities: A always binds to T, and C always binds to G. These binding capabilities are useful for scientists to understand since, if the nucleotide sequence of one DNA strand is determined, complementary binding allows the sequence of other strand to be deduced. (www.DNAsequencing.html). Thus, DNA sequencing reveals crucial variations in the nucleotides that constitute genes and these mutational changes can produce disease and even death by forcing the genes to produce abnormal proteins, or sometimes no proteins at all.

The need for ongoing development of new drugs needs no emphasis in light of the current global situation of health and disease. Traditionally, the process of drug development has revolved around a screening approach, as nobody knows which compound or approach could serve as a drug or therapy. Such almost blind screening approach is very time-consuming and laborious. The shortcoming of traditional drug discovery; as well as the allure of a more deterministic approach to combating disease has led to the concept of "Rational drug design." Nobody could design a drug before knowing more about the disease or infectious process than past. For "rational" design, the first necessary step is the identification of a molecular target critical to a disease process or an infectious pathogen. Then the important prerequisite of "drug design" is the determination of the molecular structure of target, which makes sense of the word "rational". In fact, the validity of "rational" or "structure-based" drug discovery rests largely on a high-resolution target structure of sufficient molecule detail to allow selectivity in the screening of compounds. (www.mdl.ipc.pku.edu.cn/Drug_Design.html)

Rational drug design

Rational drug design is a more focused approach, which uses information about the structure of a drug receptor or one of its natural ligands to identify or create candidate drugs. The three-dimensional structure of a protein can be determined using methods such as X-ray crystallography or nuclear magnetic resonance spectroscopy. Armed with this information, researchers in the pharmaceutical industry can use powerful computer programmes to search through databases containing the structures of many different chemical compounds. The computer can select those compounds that are most likely to interact with the receptor, and these can be tested in the laboratory. If an interacting compound cannot be found in this manner, other programmes can be used that attempt, from first principles, to build molecules that are likely to interact with the receptor. Further programmes can search databases to identify compounds with similar properties to known ligands. The idea is to narrow down the search as much as possible to avoid the expense of large-scale screening. The first drug produced by rational designing was Relenza, which is used to treat influenza. Relenza was developed by choosing molecules that were most likely to interact with neuraminidase, a virus-produced enzyme that is required to release newly formed viruses from infected cells. Many of the recent drugs developed to treat HIV infections (e.g. Ritonivir, Indinavir) were designed to interact with

the viral protease, the enzyme that splits up the viral proteins and allows them to assemble properly (www.newdrugdesign.com/Rachel_Theory_03.html).

Structure-based drug design

In structure-based drug design, the three dimensional structure of a drug target interacting with small molecules is used to guide drug discovery. Structure-based drug design represents the idea of seeing exactly how the molecule interacts with its target protein. This structural information can be obtained with X-ray crystallography or nuclear magnetic resonance spectroscopy (NMR). However, most companies that are specializing in structure-based drug design focus on only one method of structure determination, at least initially. Originally, structure-based drug design was equated with de novo design or building a molecule from the ground up. The active site of the protein was a space to be filled with a molecule that complemented it in terms of shape, charge, and other binding components. (http://www.healthtech.com/2012/sbd/). Combinatorial chemistry is the best thing that ever happened to structure-based drug design. Purely random combinatorial chemistry has evolved into focused combinatorial libraries, which can be considered an abstract form of structure-based drug design. Researchers realized that specific knowledge of the target could guide the power of combinatorial chemistry to rapidly make many compounds. (www.anadyspharma.com/science/structure.htm). Docking is the computational procedure in which a potential drug molecule or ligand is placed in the active site of the target molecule. Using models of the binding energy of various parts of the molecules, the interaction between the two can be evaluated to find if there is likely to be a good 'fit'. (www.dock.compbio.ucsf.edu) The procedure can also be used to find the best location for a ligand in the active site, and this can guide the addition of side chains that can improve the fit, a process known as structure-based drug design. (www.answers.com/topic/quantum-pharmaceuticals). Despite advances in technology and understanding of biological systems, drug discovery is still a long process with low rate of new therapeutic discovery. Information on the human genome, its sequence and what it encodes has been hailed as a potential windfall for drug discovery, promising to virtually eliminate the bottleneck in therapeutic targets that has been one limiting factor on the rate of therapeutic discovery. However, data indicates that "new targets" as opposed to "established targets" are more prone to drug discovery project failure in general. This data corroborates some thinking underlying a pharmaceutical industry trend beginning at the turn of the twenty-first century and continuing today which finds more risk aversion in target selection among multi-national pharmaceutical companies (http://www.healthtech.com/2002/sbd/).

Objective

The present study was aimed at first screening the population through Biochemical and Molecular Techniques like PCR and DNA Sequencing to confirm the clinical status of PKD and further move on to screen and select appropriate EGF molecules from the Enzyme Database Brenda based on their inhibition characteristic to cyst growth in cancer which might parallelly prove to be a promising tool in the future to reduce the cyst growth in PKD as well.

2. Materials and methods

15 families were initially selected for the present study. After personal counseling to seek their consent, 8 families comprising of 78 subjects of F1, F2 and F3 generation were screened for Biochemical and Histological parameters to confirm their clinical status. PCR by SSCP method and DNA Sequencing were carried out for the positive samples. With the help of Enzyme Database Brenda 8 EGF molecules were selected to be a promising tool to reduce cyst growth for PKD.

3. Results and Discussion

Biochemical Screening done for all the 78 subjects in the hospital to detect the values for all the 18 biochemical components in the blood served as a tool to differentiate the control subjects from the experimental subjects (Graphs 1-6). The experimental subjects showed a significant increase or decrease in value than the normal range with special reference to Blood Urea and Serum Creatinine level, the index factors thereby confirming the positivity of the clinical status of the subject. Based on the results received from hospital by Biochemical screening, the subjects were segregated as control subjects (48) and experimental subjects (30) from a total live sample size of 78. Table 2 comprises of the normal values of all the 18 Biochemical parameters analyzed in the blood for a confirmatory test. Ultrasound scan study was performed for all the subjects in Madras Scan Centre to confirm the first positivity of the clinical status as indicated by the Biochemical screening. The experimental subjects revealed three types of cysts in the right and left kidneys. Table 3 categorizes the 30 subjects in F1, F2 and F3 generation under the three cystic types and reveals the percentage rate of each cyst type. Cell Dyne 6000 analyzer used to analyze the hematological parameters revealed a marked variation in the experimental subject values than the normal range Graph 7. Subject No 33 revealed a peculiar manifestation of Hematuria condition (Blood in the urine – Plate 1). Pedigree chart was mainly constructed to trace the linkage pattern and the rate of PKD gene expression from F1 to F2 and subsequently to F3 generation. Assuming the positivity of PKD condition to one of the parent in the F1 generation, the PKD gene transfer rate was calculated for the F2 and F3 generation.

Plate 2A and B gives an overall view of the pedigree chart of the 8 families comprising of 89 subjects taken for the study. Karyotyping analysis was carried out for few chronic experimental subjects. Since PKD is characterized by the alteration at the gene level, contrasting variation was not encountered in the metaphase plate of the karyotype. Plate 3 depicts the Karyotype of a chronic experimental subject. Plates 4–9 are Representative Agarose gels for PCR product of Exon 15 (Nucleotide position 29039– 29523), Exon 15 (Nucleotide position 29476 – 29847), Exon 18 – 20 (Nucleotide position 32835 – 33314), Exon 25 – IVS 26 (Nucleotide position 38978 – 39675) and Exon 29 – 30 (Nucleotide position 41579 – 41915). PCR Analysis by SSCP method was carried out for both the control and experimental subjects for Exon 15 (Nucleotide position 29039 – 29523), Exon 15 (Nucleotide position 29476 – 29847), Exon 18 – 20 (Nucleotide position 32835 – 33314), Exon 25 – IVS 26 (Nucleotide position 38978 – 39675) and Exon 29 – 30 (Nucleotide position 41579 – 41915). Among the 30 experimental subjects, 10 showed a shift in the banding pattern which formed the criteria for selection for DNA Sequencing (Table 4). DNA Sequencing for 12 samples, 10 experimental subjects [samples which showed a shift in the banding pattern by SSCP] and two control subjects was done commercially from Bangalore Genei Pvt. Ltd, for Exon 25 – IVS 26 (Nucleotide position 38978 – 39675), Exon 18 – 20 (Nucleotide position 32835 – 33314) and Exon 29 – 30 (Nucleotide position 41579 – 41915). Comparison of the analyzed PKD 1 regions with standard sequence of PKD 1 gene and its present protein was performed by using the basic BLAST search and blastn in nr database of Genebank and blastz in Swissport. Substitution mutations of Transition and Transversion type and Heterozygous condition were observed in both the experimental and control subjects at the same nucleotide positions common to all and also at different positions in the nucleotide within the selected exons (Plates 10 – 20 & Tables Graphs 8-13).

Research work undertaken in Han: SPRD rat support a significant therapeutic potential of EGFR tyrosine kinase inhibition in ADPKD. Based on the above findings, molecules for epidermal growth factor receptor (EGFr) were taken from the Enzyme database Brenda. This database maintains a list of numerous enzymes with their characteristic properties which are experimentally derived ones. For our current study, we researched for (EGFr) and found the following 8 EGR inhibitors from the Enzyme Database Brenda which might prove to be a promising tool for the future in combating ADPKD. These inhibitors are usually shown in two dimensional structures, but since enzymes are three dimensional structure and their active sites are also being three dimensional entity we converted these two dimensional molecules into three dimensional structures using DS viewer.

Drug Trials Are Underway for PKD

Tolvaptan

Polycystic Kidney Disease or PKD destroys a person's kidneys and there is no cure, but the Food and Drug Administration has just fast-tracked a new drug that is being tested at the Mayo Clinic in Rochester, Minn. Gary DeGrande's family legacy is a frightening one. He inherited PKD from his father, who got it from his mother. "Then the result is either dialysis or transplantation," DeGrande said. "There's really no treatment for PKD. There is no cure for PKD." The Mayo Clinic is hoping to change that. It is one of several sites testing what could be the first drug to control the cysts of PKD. The drug is called Tolvaptan. Maras has taken the drug in a preliminary trial and is now enrolled in a three-year study at Mayo. Dr. Vincente Torres, a PKD expert, said he has seen the drug work on mice. "So the fact that it works in animals with mutations in the same genes that cause the disease in humans makes us be cautiously optimistic that we will have good results." While the drug is not a cure, it holds the promise of a healthier future for more than 500,000 Americans and 12 million people worldwide. It is too late for DeGrande. Six months ago, his wife gave him one of her kidneys, but the drug may spare his son from the ravages of PKD. Tolvaptan appears to be safe and it is already approved by the FDA for other conditions. The only side-effect reported is thirstiness (http://wcco.com/specialreports/local_story_060094436.html).

Neugene

Most human diseases arise from the increased function or dysfunction of genes within the body, either that of pathogens, such as viruses, or of one's own genes. The Human Genome Project has paved the way toward identifying the genes associated with most major human diseases and determining the sequence of their genetic codes. Using modern methods of chemical synthesis, compounds can be prepared that recognize target gene sequences in a pathogen or pathogenic process. When these compounds bind tightly to the disease-causing sequence, the genetic process is inhibited, thus disabling the pathogen or pathogenic process. This is called antisense technology because the strands of genetic material that get translated into a protein are called "sense" strands, and so "antisense" drugs are meant to stop that translation process. In clinical studies, NeuGene antisense compounds have displayed advantageous pharmaceutical properties in stability, specificity, efficacy, delivery and safety (www.avibio.com/neugene.html). The Phase 1b clinical trial represented the first time NEUGENES had been given to adult patients with substantial impairment of kidney function, which is important for drug clearance and metabolism. The single-center study, performed at Legacy Good Samaritan Hospital in Portland, Oregon, showed that significant renal impairment in trial participants did not substantially impact how patients distributed and cleared NEUGENES from the body.

This supports the use of Neugenes as a potential safe and effective treatment for the PKD disease (Figure 2). In this clinical trial, three groups of eight patients with PKD and various degrees of kidney function impairment were exposed to increasing doses of AVI's proprietary Neugene antisense drug. The primary purpose of the study was to compare the way the drug is distributed in, and cleared from, the blood in PKD patients compared to normal healthy volunteers. A second important objective was to determine the safety of this drug in this clinical setting. Side effects were noted as minimal and not related to the study drug. In addition, kidney function was not adversely affected (Figures 1 – 6). Further, blood distribution in patients was not significantly different from that of normal volunteers (http://medtech.info/news/20021121ma075928@751)

EGF Inhibition Factor – The Great Boon

Epidermal growth factor (EGF) has an important role in the expansion of renal cysts. Epithelial cells from cysts from patients with the autosomal dominant form and from those with the autosomal recessive form are unusually susceptible to the proliferative stimulus of EGF. Moreover, cyst fluids from the former group of patients contain mitogenic concentrations of EGF, and this EGF is secreted into the lumens of cysts in amounts that can induce cellular proliferation (Du, J. and Wilson, PD, 1995). The over expression and abnormal location of EGF receptors on the apical (luminal) surface of cyst-lining epithelia creates a sustained cycle of autocrine-paracrine stimulation of proliferation in the cysts (Du, J. and Wilson, PD, 1995).

The clinical variability in the rate of progression of autosomal dominant polycystic kidney disease (ADPKD) has been attributed to genetic heterogeneity, though environmental factors and modifying genes very likely play an important role as well. The association examined between clinical outcome, defined by age at onset of end-stage renal disease (ESRD) in 46 ADPKD patients, revealed a polymorphism in the epidermal growth factor receptor (EGFR) gene, a candidate modifying gene. EGFR is a key element in renal tubular proliferation. Studies carried out in 46 unrelated patients with ADPKD and ESRD and 58 healthy controls confirmed the prevalence of PKD 1 mutations in patients. Results revealed the allelic frequencies of the EGFR polymorphism to be different in the ADPKD sample and the control population (G2=17.19; P=0.009). In particular, the frequencies of the 122 and 118bp length alleles had a different distribution (P=0.010 and P=0.047 respectively). Patients with the 122bp length polymorphism had ESRD at an earlier age, but this finding was not statistically significant. Thus these findings suggest an association between the EGFR microsatellite polymorphism and ADPKD.

However, it is difficult to establish which alleles are protective and which harmful. Based on the above findings molecules for epidermal growth factor receptor (EGFR) were taken from the Enzyme database Brenda. This database maintains a list of numerous enzymes with their characteristic properties which are experimentally derived ones. For our current study, we researched for (EGFR) and found the following EGR inhibitors.These inhibitors are usually shown in two dimensional structures, but since enzymes are three dimensional structure and their active sites are also being three dimensional entity we converted these two dimensional molecules into three dimensional structures using DS viewer. (www.brenda.uni-koeln.de). With this in context, these 8 EGFR molecules are selected based on their inhibitory role in cyst formation in cancer whereby they might also work parallel for PKD in reducing cyst growth. Thus, these 8 EGFR lead molecules which are derived from this study if taken to the next level of drug testing and clinical trial practices may serve to be a promising tool to reduce the cyst growth in PKD in Indian population thereby delaying the onset of ESRD and may spare the offsprings or future generations from the ravages of PKD. A larger, multicenter study may help to clarify these results and replicate preliminary finding of an association between ADPKD and the EGFR polymorphism thereby proving the efficacy of these lead molecules which are derived from this study.

Bioinformatics of PKD

The need for ongoing development of new drugs needs no emphasis in light of the current global situation of health and disease. Traditionally, the process of drug development has revolved around a screening approach, as nobody knows which compound or approach could serve as a drug or therapy. Such almost blind screening approach is very time-consuming and laborious. The shortcoming of traditional drug discovery; as well as the allure of a more deterministic approach to combating disease has led to the concept of "Rational drug design". Nobody could design a drug before knowing more about the disease or infection process than past. The first necessary step is the identification of a molecular target critical to a disease process or an infectious pathogen. Then the important prerequisite of "drug design" is the determination of the molecular structure of target, which makes sense of the word "rational". In fact, the validity of "rational" or "structure-based" drug discovery rests largely on a high-resolution target structure of sufficient molecule detail to allow selectivity in the screening of compounds. Docking was a computational procedure incorporated to find the reaction of a potential drug molecule or ligand when placed in the active site of the target molecule. Using models of the binding energy of various parts of the molecules, the interaction between the two can be evaluated to find if there is likely to be a good 'fit'. This procedure used to find the best location for a ligand in the active site, and thus can guide the addition of side chains that can improve the fit,

a process known as structure-based drug design. Increasing evidence supports an important role for the epidermal growth factor (EGF) / transforming growth factor-alpha (TGF-alpha) / EGF receptor (EGFR) axis in promoting tubular epithelial cell proliferation and cyst formation in polycystic kidney disease (PKD). Based on the above findings molecules for epidermal growth factor receptor (EGFR) were taken from the Enzyme database Brenda database which maintains a list of numerous enzymes with their characteristic properties which are experimentally derived ones. These 8 EGFR lead molecules which are derived from this study if taken to the next level of drug testing and clinical trial practices may serve to be a promising tool to reduce the cyst growth in PKD in Indian population thereby delaying the onset of ESRD and may spare the offsprings or future generations from the ravages of PKD. A larger, multicenter study may pave the way to clarify these results and replicate preliminary finding of an association between ADPKD and the EGFR polymorphism thereby proving the efficacy of these lead molecules discovered.

Table 2. Normal Values of Biochemical Components in Blood

S.NO.	Normal Values of Biochemical Components in Blood	Key to Abbreviations
1	BLOOD UREA NITROGEN [BUN] : - 20 mg/dl	IU – International Unit
2	SERUM CREATININE : 0.8 – 1.4 mg/dl	L – Liter
3	BLOOD GLUCOSE TEST : 64 – 128 mg/dl	dl – Decilitre = 0.1 litre
4	TOTAL CHOLESTEROL : 100 – 240 mg/dl	g/dl – gram per deciliter
5	SERUM TOTAL PROTEIN : 6.3 – 7.9 g/dl	mg – milligram
6	SERUM SODIUM : 136 – 144 meq/L	mmol – millimole
7	POTASSIUM TEST : 3.7 – 5.2 meq/L	mEq – milliequivalents
8	SERUM CHLORIDE : 101 – 111 mmol/L	
9	SERUM BICARBONATE : 15 – 20 mmol/L	
10	SERUM URIC ACID : 4.1 – 8.8 mg/dl	
11	SERUM ALBUMIN : 3.9 – 5.0 g/dl	
12	SERUM GLOBULIN : 1.4 – 2.0 g/dl	
13	TOTAL BILIRUBIN : 0.2 – 1.9 mg/dl	
14	SGOT [Serum Glutamic Oxaloacetic Transaminase] – Normal Range Varies, iu/l	
15	SGPT [Serum Glutamate Pyruvate Transaminase] – Normal Range Varies, iu/l	
16	SAP [Serum Acid Phosphatase] – Normal Range Varies, iu/l	
17	SERUM CALCIUM : 8.5 – 10.9 mg/dl	
18	SERUM PHOSPHOROUS : 2.4 – 4.1 mg/dl	

Table 3. Cystic Types Encountered In Experimental Subjects of F1, F2 and F3 generation

S.No	Type of Cists	F1 Generation	F2 Generation	F3 Generation
1	Multiple Cyst + Few Cysts	2	11	4
2	Multiple Cysts	-	3	1
3	Few Cysts	-	-	9
4	Percentage	4.66%	46.67%	46.67%

Table 4. Sample selection for DNA Sequencing

Family No.	Generation No.	Offspring Yes = 1 No = 0 2 = Parent	Status N = Normal P = PKD	Subject No.	Shift in SSCP Gel Y = Yes N = No
1	**2**	**0**	**N**	**8**	**N**
1	**2**	**1**	**P**	**10**	**Y**
1	**2**	**1**	**P**	**11**	**Y**
1	**3**	**1**	**P**	**23**	**Y**
2	**2**	**1**	**P**	**30**	**Y**
3	**3**	**1**	**P**	**37**	**Y**
3	**2**	**1**	**N**	**34**	**N**
4	**2**	**1**	**P**	**41**	**Y**
5	**2**	**1**	**P**	**55**	**Y**
6	**2**	**1**	**P**	**70**	**Y**
7	**1**	**2**	**P**	**76**	**Y**
8	**1**	**2**	**P**	**87**	**Y**

FIGURE 1: PHARMACOPHORE

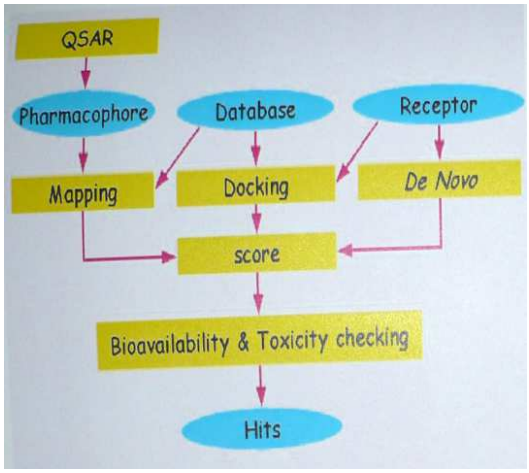


FIGURE 2 : BREAKUP OF PHASES IN CLINICAL TRIALS

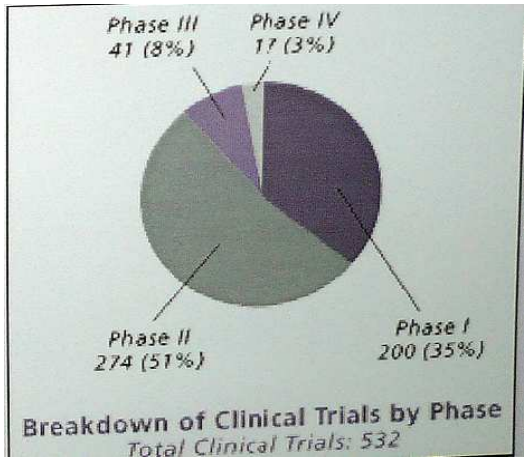


FIGURE 3 : CLINICAL TRIAL CHART

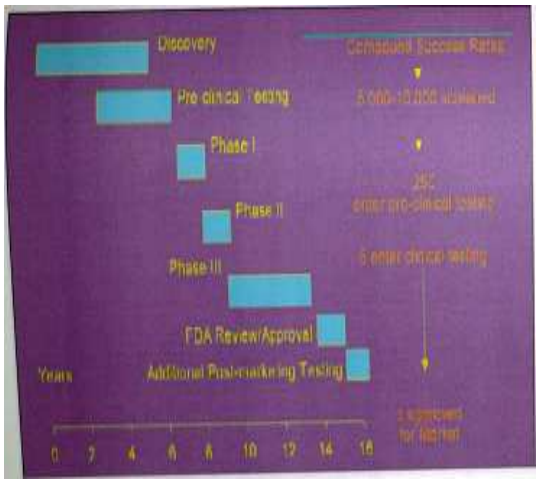


FIGURE 4 : PHASES OF REGULATORY CLINICAL TRIALS

FIGURE 5 : RANDOMIZATION

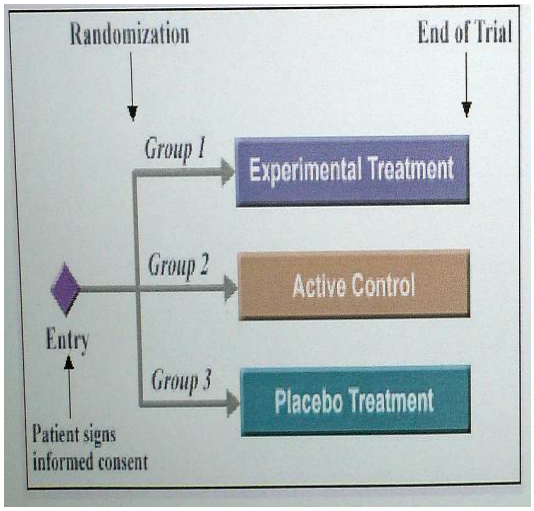


FIGURE 6 : NEUGENE ANTISENSE CLINICAL DEVELOPMENT PROGRAM

Plate 1 . Hematuria

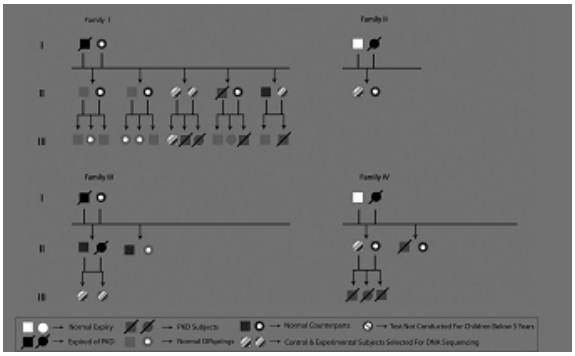


Plate 2A – Pedigree chart of 8 Families Comprising of 89 Subjects

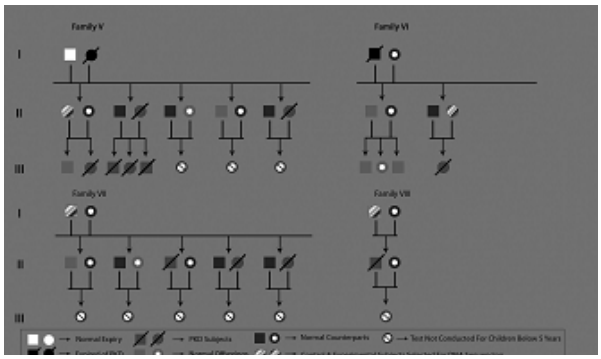


Plate 2B–Pedigreechart of 8 Families Comprising of 89 Subjects

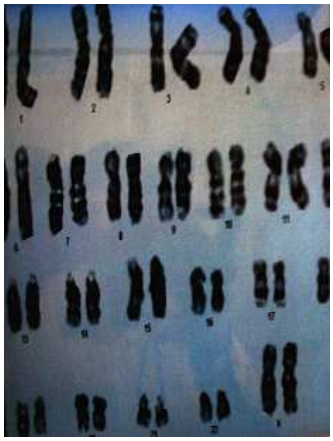


Plate 3–Karyotype of an Experimental Subject

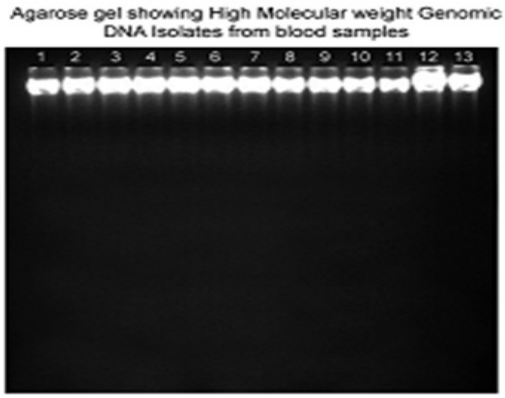
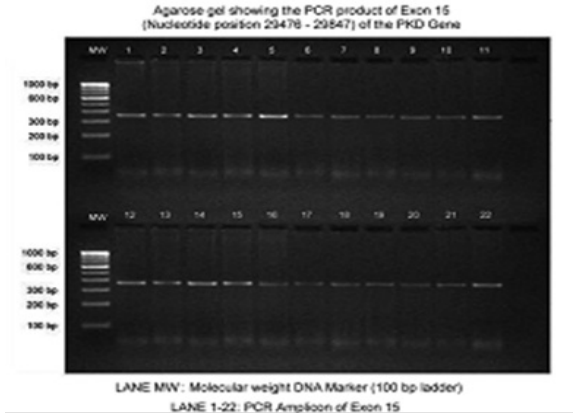
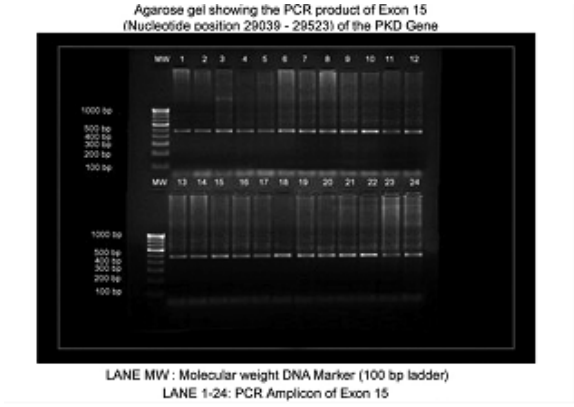


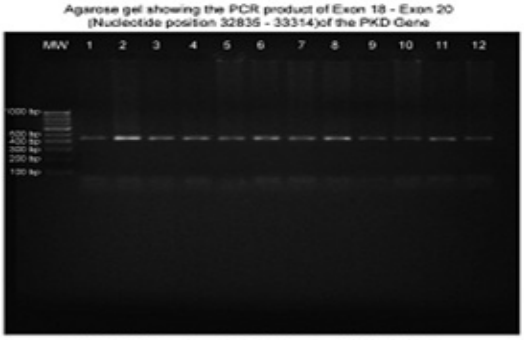
Plate 4 - Agarose Gel Showing High Molecular weight Genomic DNA Isolates from Blood Samples



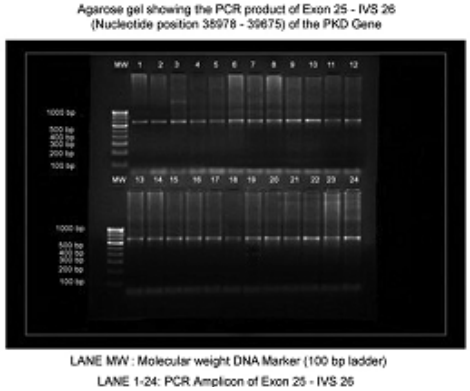
Plates 5. Representative Agarose Gels for Pcr Product of Exon 15 (Nucleotide Position 29039 – 29523)



Plates 6. Representative Agarose Gels for Pcr Product of Exon 15 (Nucleotide Position 29476 – 29847)

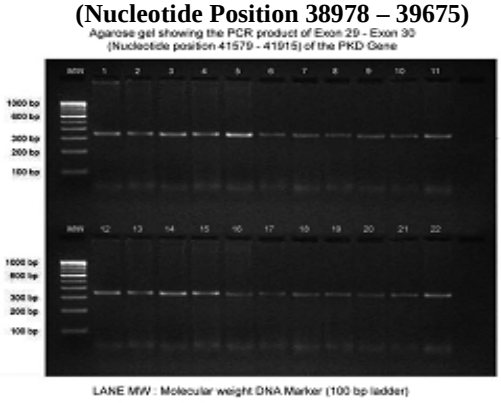


Plates 7. Representative Agarose Gels for PCR Product of Exon 18 – 20 (Nucleotide Position 32835 – 33314)

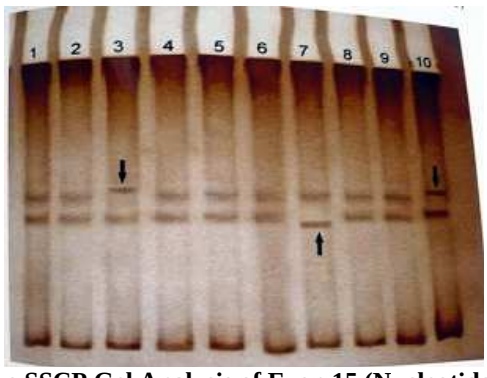


Plates 8. Representative Agarose Gels for PCR Product of Exon 25 – IVS 26

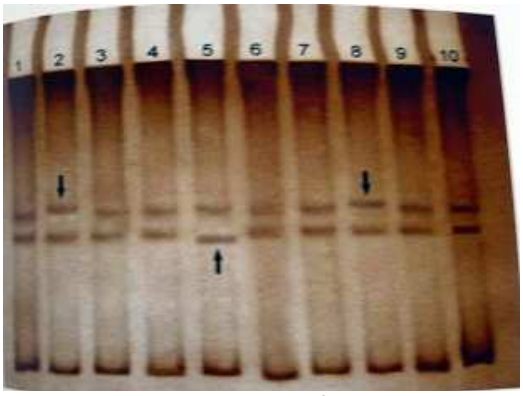
(Nucleotide Position 38978 – 39675)



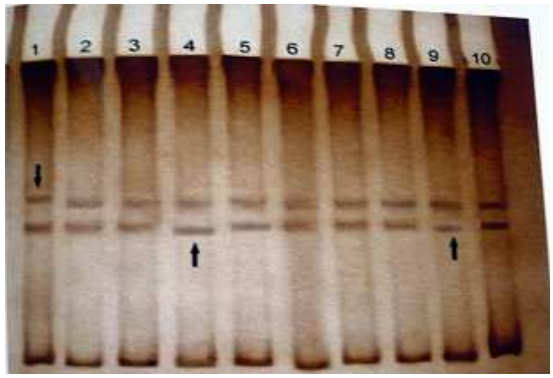
Plates 9. Representative Agarose Gels for PCR Product of Exon 29–30 (Nucleotide Position 41579 – 41915)



Plates 10: Representative SSCP Gel Analysis of Exon 15 (Nucleotide Position 29039–29523)



Plates 11. Representative SSCP GEL Analysis of Exon 15 (Nucleotide Position 29476 – 29847)



Plates 12. Representative SSCP Gel Analysis of Exon 18–20 (Nucleotide Position 32835–33314)

Plate 13: Representative SSCP Gel Analysis of Exon 25–IVS 26 (Nucleotide Position 38978 – 39675)

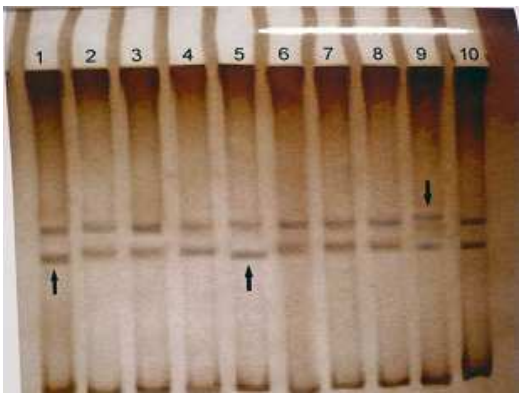


Plate 14. Representative SSCP Gel Analysis of Exon 29 – 30 (Nucleotide Position 41579 – 41915)

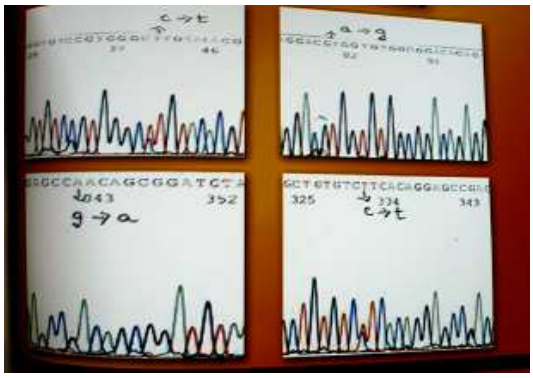


Plate 15. Transitions and Transversions Observed in DNA Sequencing for Exon 18 – 20 (Nucleotide Position 32835 – 33314)

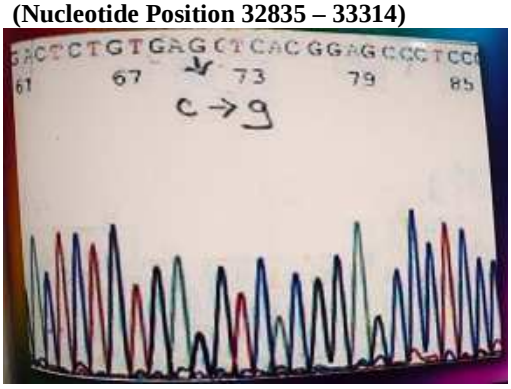


Plate 16A. Transitions Observed in DNA Sequencing for exon 25 – IVS 26 (Nucleotide Position 38978 –39675)

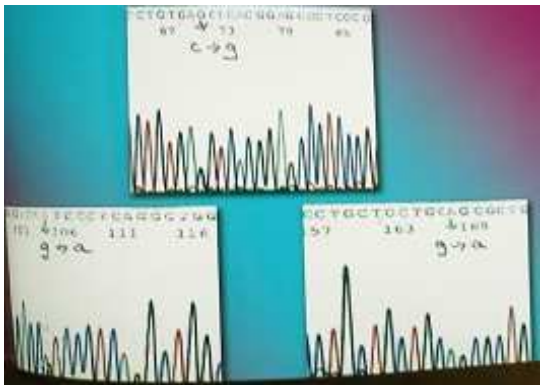


Plate 16B. Transversions Observed in DNA Sequencing for Exon 25–IVS 26 (Nucleotide Position 38978 – 39675)

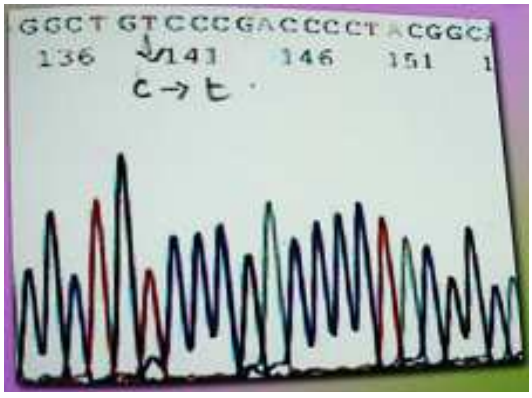


Plate 17. Transversions Observed in DNA Sequencing For Exon 29 – 30 (Nucleotide Position 41579 – 41915)

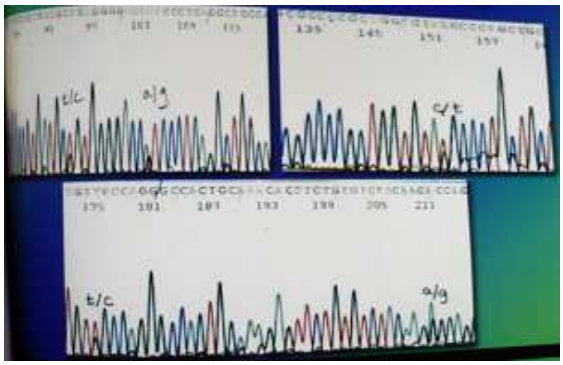


Plate 18. Heterozygous Condition Observed in DNA Sequencing for Exon 18–20 (Nucleotide Position 32835–33314)

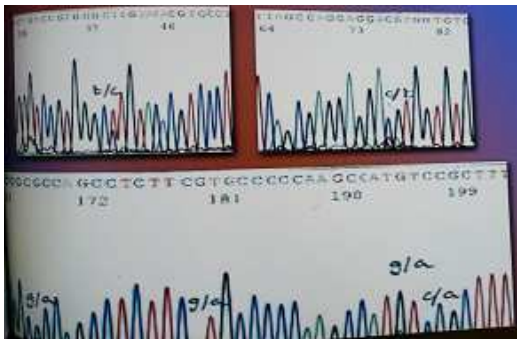


Plate 19 : Heterozygous Condition Observed in DNA Sequencing for Exon 25 – IVS 26 (Nucleotide Position 38978–39675)

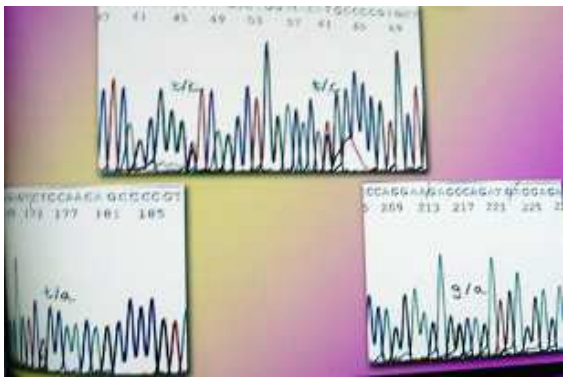
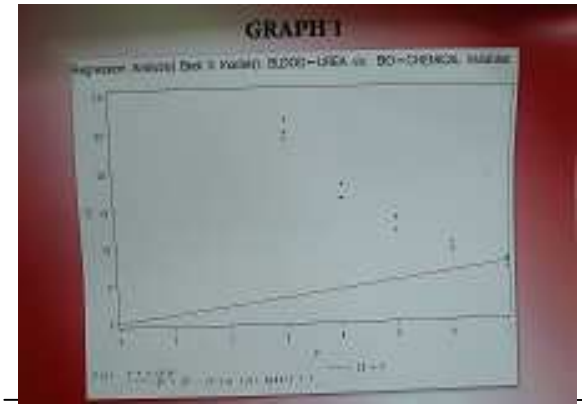
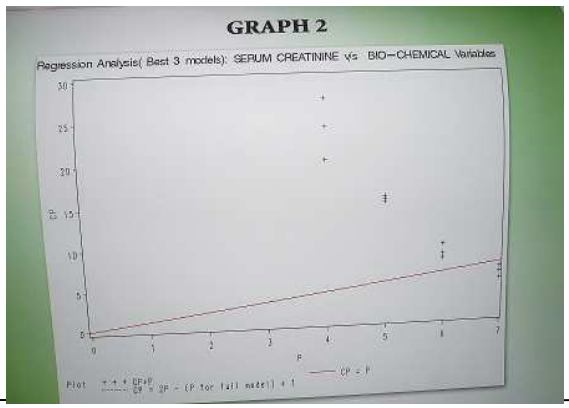


Plate 20. Heterozygous Condition Observed in DNA Sequencing for Exon 29–30 (Nucleotide Position 41579–41915)

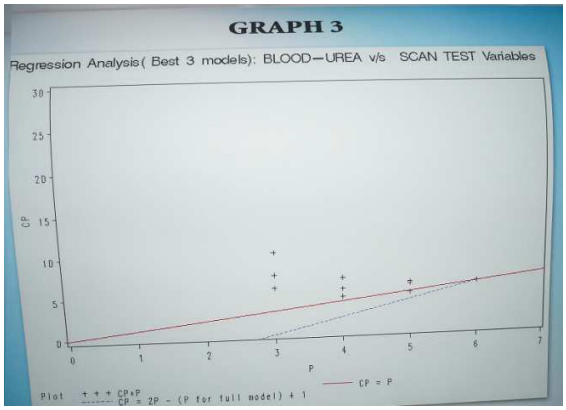
GRAPH 1



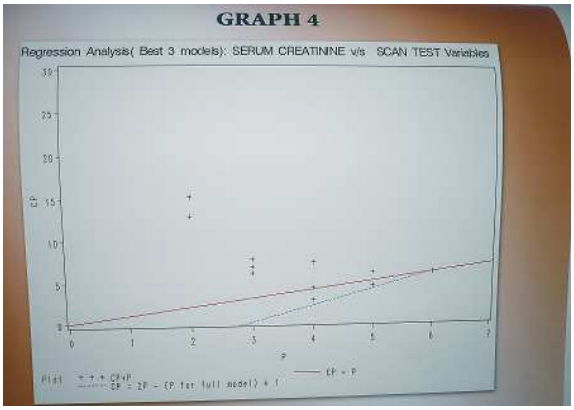
GRAPH 2



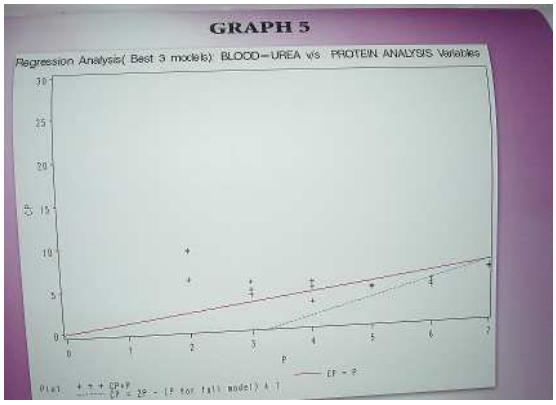
GRAPH 3



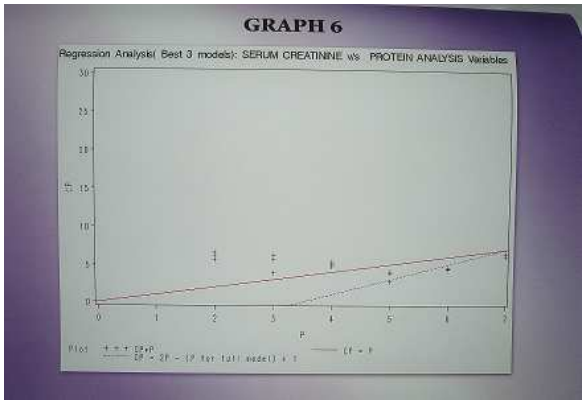
GRAPH 4

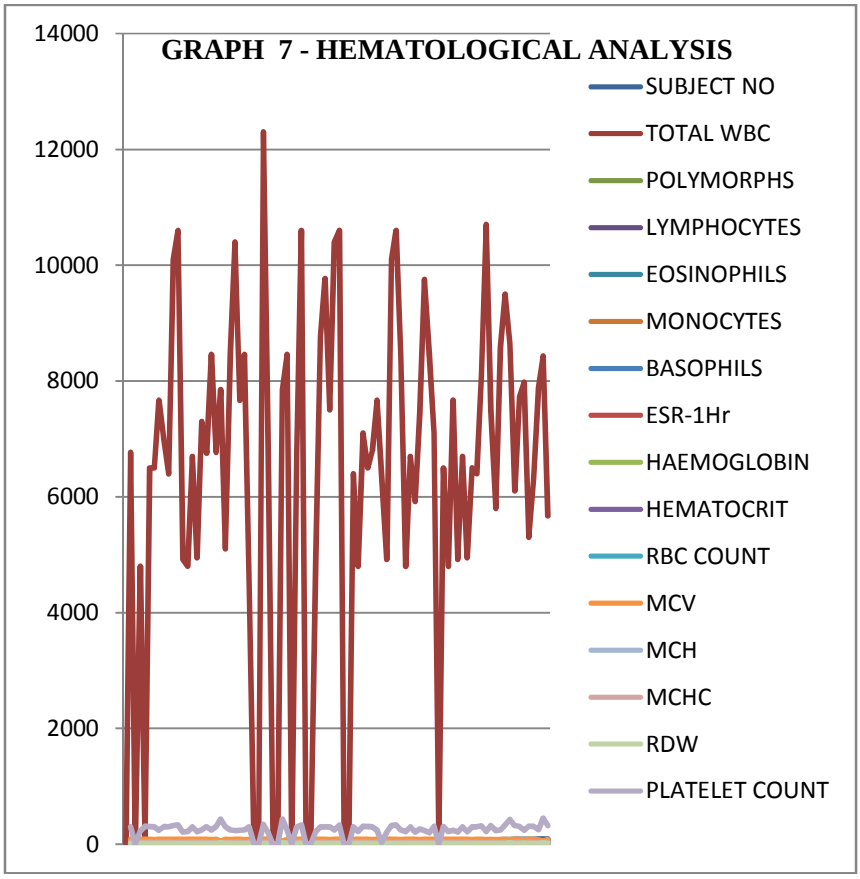


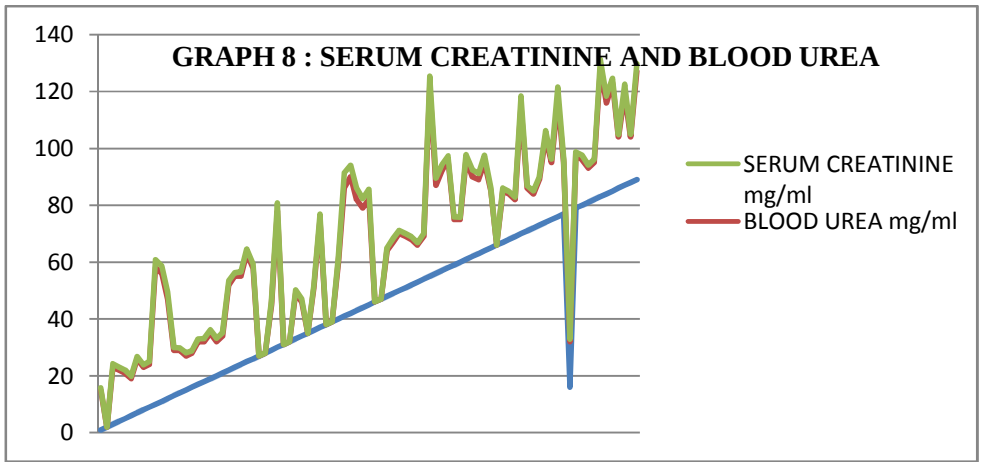
GRAPH 5

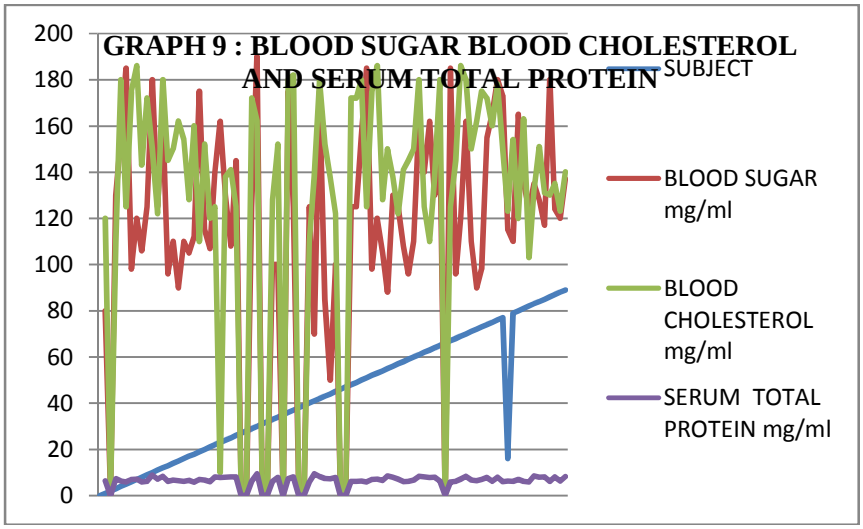


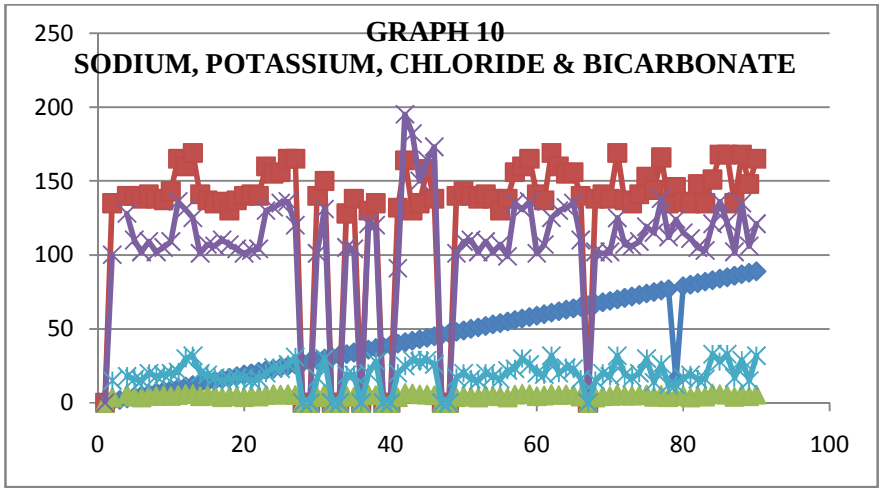
GRAPH 6

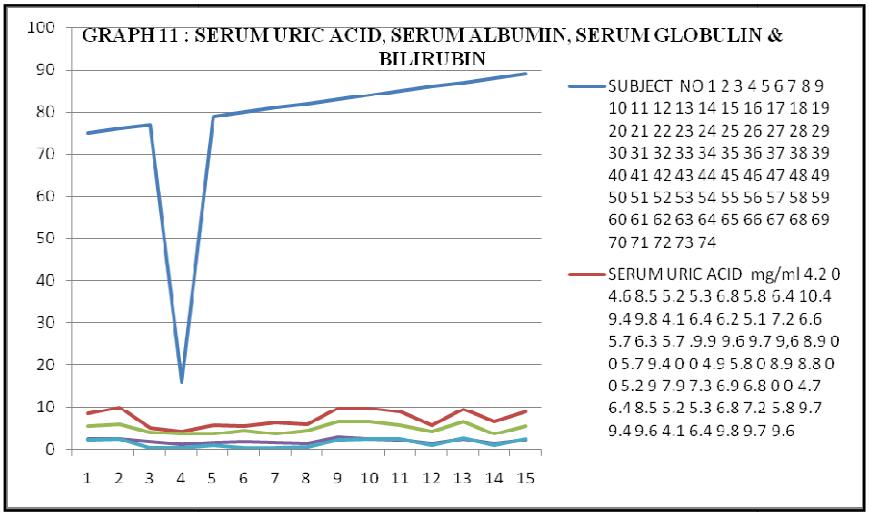


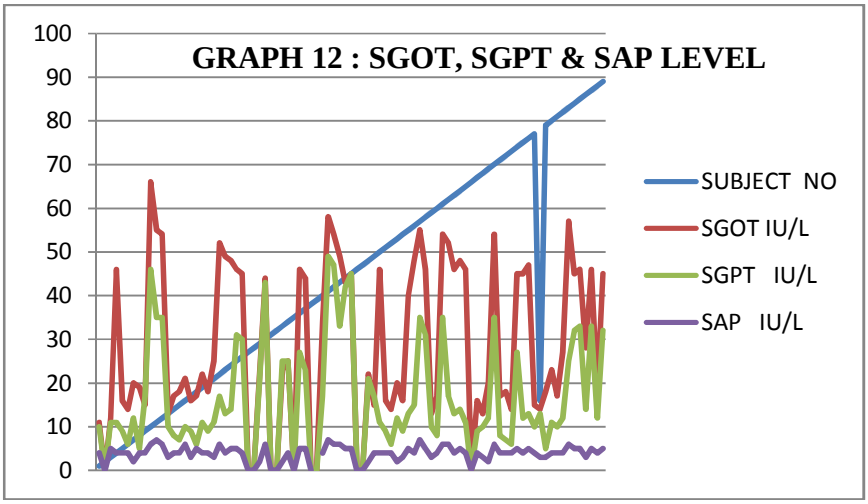

GRAPH 7 - HEMATOLOGICAL ANALYSIS
14000
12000
10000
8000
6000
4000
2000
0
SUBJECT NO
TOTAL WBC
POLYMORPHS
LYMPHOCYTES
EOSINOPHILS
MONOCYTES
BASOPHILS
ESR-1Hr
HAEMOGLOBIN
HEMATOCRIT
RBC COUNT
MCV
MCH
MCHC
RDW
PLATELET COUNT

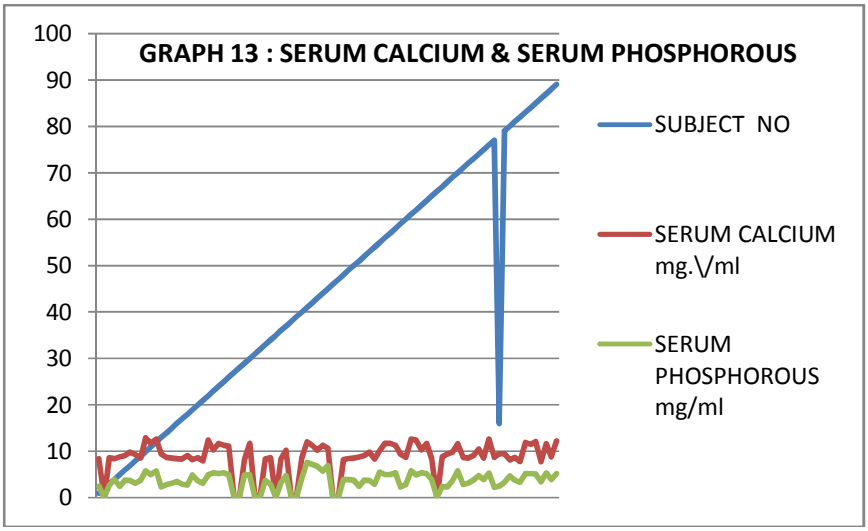

GRAPH 8 : SERUM CREATININE AND BLOOD UREA
140
120
100
80
60
40
20
0
SERUM CREATININE mg/ml
BLOOD UREA mg/ml


GRAPH 9 : BLOOD SUGAR BLOOD CHOLESTEROL AND SERUM TOTAL PROTEIN
200
180
160
140
120
100
80
60
40
20
0
SUBJECT
BLOOD SUGAR mg/ml
BLOOD CHOLESTEROL mg/ml
SERUM TOTAL PROTEIN mg/ml


GRAPH 10
SODIUM, POTASSIUM, CHLORIDE & BICARBONATE
250
200
150
100
50
0
0
20
40
60
80
100


GRAPH 11 : SERUM URIC ACID, SERUM ALBUMIN, SERUM GLOBULIN & BILIRUBIN
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
SUBJECT NO 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74
SERUM URIC ACID mg/ml 4.2 0 4.6 8.5 5.2 5.3 6.8 5.8 6.4 10.4 9.4 9.8 4.1 6.4 6.2 5.1 7.2 6.6 5.7 6.3 5.7 .9.9 9.6 9.7 9,6 8.9 0 0 5.7 9.4 0 0 4.9 5.8 0 8.9 8.8 0 0 5.2 9 7.9 7.3 6.9 6.8 0 0 4.7 6.4 8.5 5.2 5.3 6.8 7.2 5.8 9.7 9.4 9.6 4.1 6.4 9.8 9.7 9.6





4. Summary & Conclusion

The significant advances in understanding the molecular basis of adult dominant Polcystic Kidney Disease have generated intense interest and have provided investigators with important research opportunities. The present investigation, largely fundamental and a pioneering study in Indian population is likely to generate, in the foreseeable future, a variety of possible strategies for molecular interventions in clinical research. The development of statistical models proved to be an important tool to predict the index variables, Blood Urea and Serum Creatinine for PKD without undergoing the strenuous procedure of clinical testing. The important area of investigation was the identification of novel mutations of human ADPKD1 which elucidated the interactions between PKD susceptibility loci, genetic determinants and cellular and molecular mechanisms which resulted in disrupted normal kidney function.

Although certain areas of research in PKD are already receiving careful study, the timely opportunities to discover more about the etiology and pathogenesis in particular, and the related cellular and molecular mechanisms that determine kidney function in general, need to be addressed in a more elaborative manner. Future studies can be undertaken to understand the phenotype/genotype correlation and disease phenotypes in different genetic backgrounds, i.e., gender, race, and ethnicity. Specific role of genes in determining severity of disease and extra-renal manifestations and predicted impact of mutations on proteins could be the immediate next endeavor to be taken up. Substantial difficulties exist in the translation of fundamental insight into therapeutic methods, including the slow rate of progression of the disease in patients and the difficulty of monitoring cyst growth. The present research study aimed at the identification of lead molecules which could pave the way for therapeutic opportunities and gene targeted strategies to prevent the progressive rate of cyst growth. Thus, with the help of the identification of lead or target molecules to combat PKD, innovative therapeutic interventions is not beyond our reach.

5. Reference

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