ANALYSIS

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Clinical, histological, hematological, biochemical, genetic and pharmacogenomic study of human polycystic kidney disease population

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ABSTRACT

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 which progresses to end stage renal failure is indeed the most common genetic disorders affecting about one third of people with diabetes. The other causative factors include blood sugar, high blood pressure and inherited factors. This can be treated only by dialysis or kidney transplant. This is a first attempt study initiated in Indian scenario to clinically analyze the Histological, Hematological, Biochemical, Genetic and Pharmacogenomic parameters of Human Polycystic Kidney Disease. The study's main objective was to compare the Western data with the Indian data regarding the location, mutation and genetic alteration of PKD 1 gene and to trace for Novel mutations for the same. Selected subjects were screened using Biochemical, Ultrasonography, Serum Protein Analysis and Hematological analysis to detect and differentiate the different blood component levels between control and experimental subjects. A Pedigree chart / biogenetic tree were drafted to trace the linkage pattern and the rate of PKD gene expression from F1 to F2 and subsequently to F3 generation. PCR by SSCP method and DNA Sequencing was demonstrated for all 78 subjects for five Exons, namely Exon 15 (Nucleotide position 29039 – 29523), Exon 15 (Nucleotide position 29476 – 29847, Exon 18 – 20 (Nucleotide position 32835 – 33314), Exon 25 0 IVS 26 (Nucleotide position 38978 – 39675) and Exon 29 – 30 (Nucleotide position 41579 – 41915) to identify

established and de novo mutations at significant mucleotide positions. Finally 8 EGFr molecules from the Enzyme Database Brenda was selected 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. Statistical tools were incorporated to investigate the existence of significant amount of difference between normal and PKD subjects within the study variables and to identify the variables which significantly predicts the index variables such as Blood Urea and Serum Creatinine in the PKD subjects.

Keywords: Polycystic kidney disease, hereditary character, Autosomal dominant, End Stage Renal Failure and National Institute of Health.

1. INTRODUCTION

The formation of an organism requires coordination of cell behaviors and thus is highly dependent on communication among cells mediated by cell surface receptor proteins and their ligands on the adjacent cells or presented in the extracellular matrix (Clark and Brugge, 1995; Cunningham, 1995; Gumbiner, 1996 and Miller and Moon, 1996). Renal development involves reciprocal inductive interactions between an epithelial structure, the ureter bud (a caudal outgrowth of the mesonephric duct also called the Wolffian duct), and a surrounding mesenchyme, the metanephric blastema (csigal@jdrf.org). Signals from the tips of ureteric bud epithelium induce the metanephric mesenchyme to undergo a sequence of events leading to its transformation into an epithelial structure that gives rise to the glomerular and tubular epithelia of the mature kidney. On the other hand, the transdifferentiated mesenchyme induces branching morphogenesis of the ureter bud, leading to the development of the collecting duct (CD) system (Davies, 1996 and Vainio and Muller, 1997). As development proceeds, the CD epithelium itself turns from an empryonic inductor into an excretory epithelium composed of two intermingled functionally and morphologically different cell types, the principal (P) cells and the intercalated (IC) cells (Tisher and Madsen, 1996). In the last few years, studies on early stages of renal embryogenesis have revealed a complex cascade of inducting and signaling events implicating transcription factors, growth factors and their receptors, extracellular matrix constituents, and extracellular matrix degrading enzymes (Lechner and Dressler, 1997; Lelongt et al., 1997 and Wallner et al., 1997). By contrast, still very little is known about the molecular and cellular events that control later stages of renal development. These post0inductive stages include segmental organization and functional maturation of individual nephron segments, branching and growing of the CD, and generation of its cellular heterogeneity (Hanna Debiec, et al., 1998). 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. 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).

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. The disease is characterized by the progressive enlargement of a portion of renal tubule segments (proximal, distal, loop of Henle, collecting duct), (www.nlm.nih.gov/medlineplus/ency/encylopedia_K.html).

The tubules enlarge from a normal diameter of 40 um to several centimeters in diameter, causing marked gross and microscopic anatomic distortion (Almers and Stirling, 1984; Fish and Molitoris, 1994). 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. 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 (Watnic TJ, et al., 1997, Peral B, et al., 1997 and Thongoppakhun W, et al., 1999).

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 (www.emedicine.com/radio/autosomal-dominant-polycystic-kidney-disease.htm).

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. A landmark advance in PKD research occurred when scientists discovered the genes, known as PKD1 and PKD2, which, when mutated, are responsible for the most prevalent form of PKD. Since the discovery of these causative genes, investigators using three mouse models of the disease have made several advances in understanding PKD (www.umm.edu/urology/polycyst.htm).

Polycystins represent an expanding family of membrane proteins composed of two subfamilies, polycystin I-like and polycystin 2-like molecules. PC1, encoded by PKD1, is predicted to be a 460-kDa integral membrane glycoprotein with a very large extracellular amino terminus, 11 transmembrane domains, and a small intracellular carboxyl terminus (Anonymous, 1995 and Hughes, J, et al., 1995). PC1 is found in the plasma membrane or in the cell-cell junction of cultured cells and in tissues (Geng, L, et al., 1996, Ibraghimov-Beskrovnaya, O, et al., 1997 and Peters, D, et al., 1999).

PC1 has recently been shown to function as a G protein-coupled receptor (Delmas, P, et al., 2002), although its ligand(s) has not been identified. PC2, the product of PKD2, is predicted to encode an integral membrane protein of 110 kDa with an EF-hand domain at its carboxyl terminus (Mochizuki, T, et al., 1996). Sequence homology to other ion channels suggests a pore-forming capacity of PC2. Since mutations in PC1 and PC2 result in similar phenotypes and PC2 is able to interact with PC1 through its coiled-coil domain in vitro (Qian, F, et al., and Tsiokas, L, et al., 1997), it has been speculated that PC1 and PC2 form a functional complex. Both polycystins are predicted to be transmembrane proteins with cytoplasmic C-terminal tails (Chen, X, et al., 1999). Polycystin-1 is believed to play a role in cell-cell or cell-matrix interactions, renal tubulogenesis, and intracellular signaling pathways (via its C-terminal tail) (Delmas, P, et al., 2002, Foggensteiner, L, et al., 2000, Geng, L, et al., 1996, Gonzalez-Perret, S, et al., 2001 and Gonzalez-Perrett, S, et al., 2002). Polycystin-2 has also been implicated in intracellular signaling pathways and has been shown to have calcium channel activity (Guo, L, et al., 2000, Hamill, O, et al., 1981, Hanaoka, K, et al., 2000 and Hou, X, et al., 2002). A recent study in kidney epithelial cells suggests that both polycystins may also play a role in cilium mechanosensation (Hughes, J, et al., 1995).

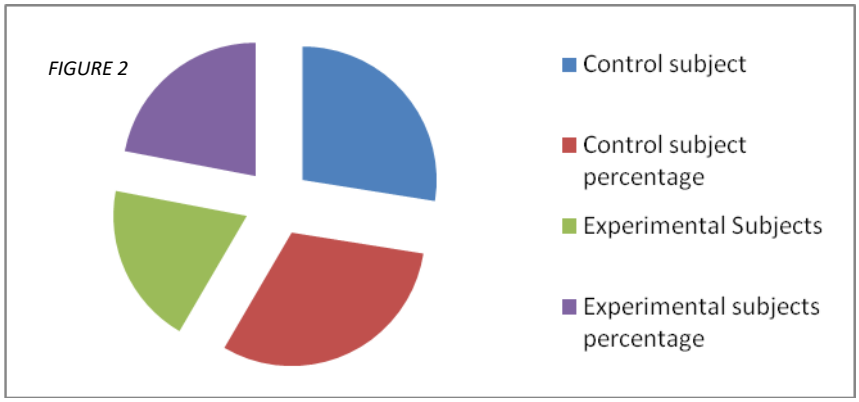
Various approaches exist in humans to determine whether a gene or genomic region influences a particular disease or phenotype. Recently it has been emphasized that in comparison to the traditional linkage approach, association studies might be more powerful to detect genes for polygenic diseases. To determine possible candidates genes, animal models provide valuable insights both in the pathogenic mechanism and genetic basis of diseases. The constantly increasing knowledge of nucleotide sequence and map position of genes, both in animals and humans, make it now possible to determine homologous regions in humans. In particular, the chromosomal localization of genes and their order tends to be conserved between mammalian species. By identifying a genetic link, the narrowing down of the regions of DNA and Nucleic acids in the body is made easy thus reducing the complication to a greater extent (www.brc.mcw.edu/projects/esrd.html). In certain cases, each of these genes may contribute little to the phenotypic expression of the trait, thus making the variant genes hard to detect. In addition, the ultimate expression of variant genes may depend on the ever-changing milieu within which genes operate. One factor that might contribute to changes in genetic background within the lifetime of an individual is acquired chromosomal aneuploidy (loss or gain of chromosomes) in somatic cells (Jeffrey P. Gardner, *et al.,* 2000). The various techniques employed for genetic analysis of PKD includes Karyotyping, PCR and DNA Sequencing (www.ncbi.nlm.nih.gov/entrez/7709805Abstract).

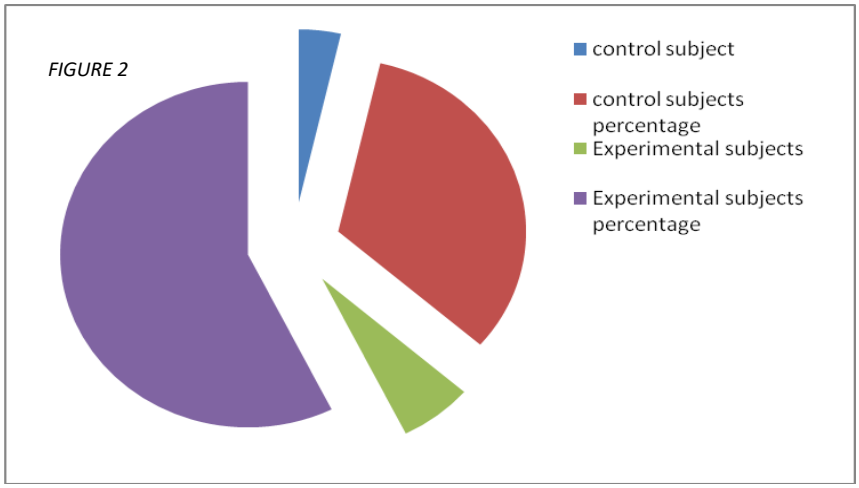
The need for ongoing development of new drugs needs no emphasis in light of the current global situation of health and disease. The shortcoming of traditional drug discovery as well as the allure of a more deterministic approach to combating disease has led to the concept of "Rational drug design." Rational 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 (www.healthtech.com/2012/sbd/). 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. On the other hand structure-based drug design helps to study the three dimensional structure of a drug target interacting with small molecules and thus 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). Ideally, these two techniques complement one another.

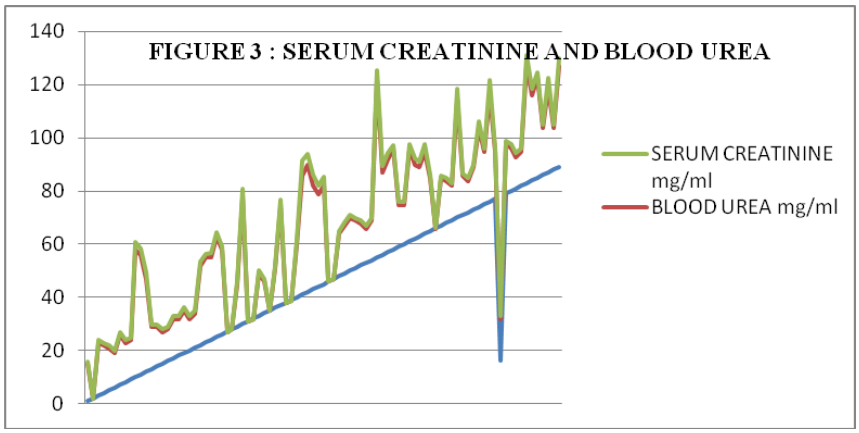
2. METHODOLOGY

The blood samples were collected using a heparanised vacutainer from 78 subjects of 8 families. After preliminary screening using Biochemical and Ultrasound scan studies they were categorized as PKD (experimental) or control subjects. The Subject Number, Life Status, Nature of Death, Family Number, Generation Number, Offspring Type, Clinical Status, Subject Name and Age were attributed

to the 78 subjects. Hydragel Protein (E) K20 Serum Protein Kit was used for the separation and quantification of serum protein by Agarose gel electrophoresis. Cell Dyne 6000 Analyzer was used to analyze the hematological parameters. Routine Cytogenetic analysis were made on the blood cells following 72 hours of incubation in RPM1 1640 culture medium containing a B-cell mitogen, the phytohaemagglutination (PHA). The standard Giemsa and Trypsin G banding technique of Sea bright (1971) was used for Cytogenetic analysis. Metaphase plates were photographed using an Applied Imaging Cyto Vision Automated Karyotyping System Version 3. PCR by SSCP using standardized method was demonstrated for all 78 subjects for five Exons, namely 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) of PKD 1 gene for critically excluding experimental subjects which showed a shift in the banding pattern for DNA Sequencing. DNA Sequencing for 12 samples, 10 experimental subjects [samples which showed a shift in the banding pattern] and two control subjects was done commercially from Bangalore Cenei Pvt. Ltd. For our current study, we researched 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. Statistical Analysis was implemented as an effective tool to analyze the significant difference between various study variables of the observed data between the normal and PKD subjects who were taken for the study.







3. RESULTS

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 consented to co-operate for the study amidst social and family constraints.

Biochemical Analysis

Biochemical Screening was done for all the 78 subjects in the hospital to detect the values for all the 18 biochemical components in the blood which served as a tool to differentiate the control subjects from the experimental subjects. 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. Table 3A & Figure 1 represents the percentage of live control and experimental subjects in the total sample population. Table 3B & Figure 2 represents the percentage of expired control and experimental parent subjects. Table 5 & Figure 3 comprise of the Blood Urea and Serum Creatinine level of control and experimental subjects analysed for the present study. The experimental subjects showed a significant increase in values above the normal

range (Blood Urea value > 20 mg/dl and Serum Creatine value > 1.4 mg/dl) thus helping to differentiate them from the control subjects in the sample population. Blood Sugar, Blood Cholesterol and Serum Total Protein values showed a marked variation between the normal subjects and the experimental subjects as depicted in Figure 4 thereby confirming the major primary role and contribution of these parameters in the expression of PKD gene. Significant increase in Sodium, Potassium, Chloride and Bicarbonate values than the normal range is witnessed in experimental subjects (Sodium value > 144 mEq/L, Potassium value > 5.2 mEq/L, Chloride value > 111 mmol/dl and Bicarbonate value > 20 mmol/dl) as depicted in Figure 7. An elevation is witnessed in the Serum Uric Acid, Serum Albumin, Serum Globulin and Bilirubin values above the normal range (Serum Uric Acid value > 8.8 mg/dl, Serum Albumin value > 5.0 g/dl, Serum Globulin value > 2.0 g/dl and Bilirubin value > 0.3 mg/dl) in the experimental subjects as depicted in Figure 5. The experimental subjects revealed an increase in the SGOT, SGPT and SAP values when compared to the control subjects in the sample population as depicted in Figure 8. Serum Calcium and Serum Phosphorous values showed a marked increase in the experimental subjects (Figure 9). Thus all the biochemical parameters showed a significant increase than the normal values in the experimental subjects thereby confirming the first screening test undertaken to confirm the positivity of the clinical status of the subjects of PKD.

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).

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 3a

Percentage of live control and experimental subjects

S.No.	Control Subjects	Control Subjects Percentage	Experimental Subjects	Experimental Subjects Percentage
1	52	58.43	37	41.57

Table 3b

Percentage of expired control and experimental parent subjects

S.No.	Control Subjects	Control Subjects Percentage	Experimental Subjects	Experimental Subjects Percentage
1	4	36.36	7	63.64

Table 4

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 5

Pedigree analysis and gene transfer percentage of control and experimental subjects from F1 to F2 and F3 Generation

Generations	Normal Expiry	PKD Expiry	Control Subjects	Experimental Subjects
F1	5	6	3	2
F2	-	1	28	14
F3	-	-	16	14
TOTAL	5	7	47	30
PERCENTAGE	5.61%	7.86%	52.83%	33.70%

Table 6

Sample selection for PCR analysis by SSCP method [78 subjects]

	CONTROL SUBJECTS	EXPERIMENTAL SUBJECTS	EXPERIMENTAL SUBJECTS SELECTED FOR DNA SEQUENCING *	CONTROL SUBJECTS SELECTED FOR DNA SEQUENCING *
TOTAL	**48**	**30**	**10**	**2**
PERCENTAGE	**61.53%**	**38.47%**	**83.33%**	**16.67%**

Table 7

Sample Selection for DNA Sequencing

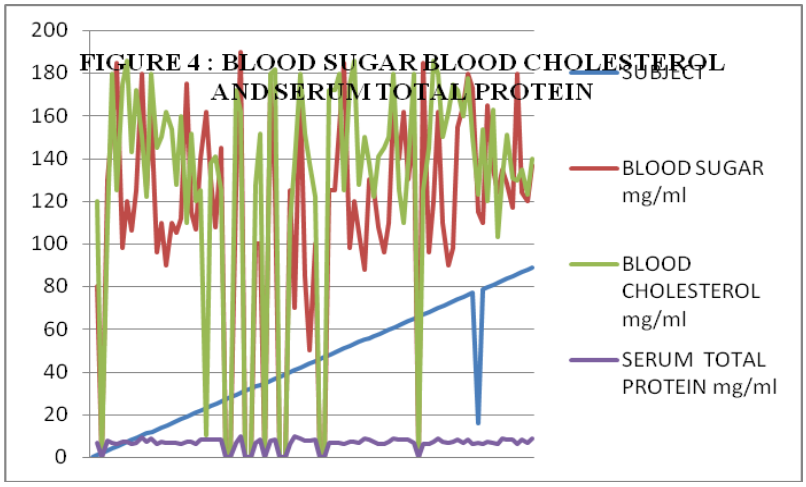
Family No.	Generation No.	Offspring Yes = 1 No = 0 2 = Parent	Status N = Normal P = PKD	Subject No.	Shift in SSCP Gel Y = Yes N = No
1	2	0	N	8	N
1	2	1	P	10	Y
1	2	1	P	11	Y
1	3	1	P	23	Y
2	2	1	P	30	Y
3	3	1	P	37	Y
3	2	1	N	34	N
4	2	1	P	41	Y
5	2	1	P	55	Y
6	2	1	P	70	Y
7	1	2	P	76	Y
8	1	2	P	87	Y

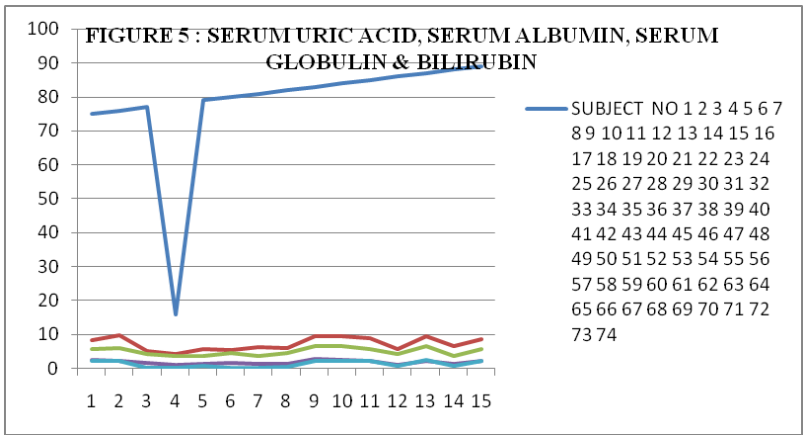
Ultrasound Scan Study

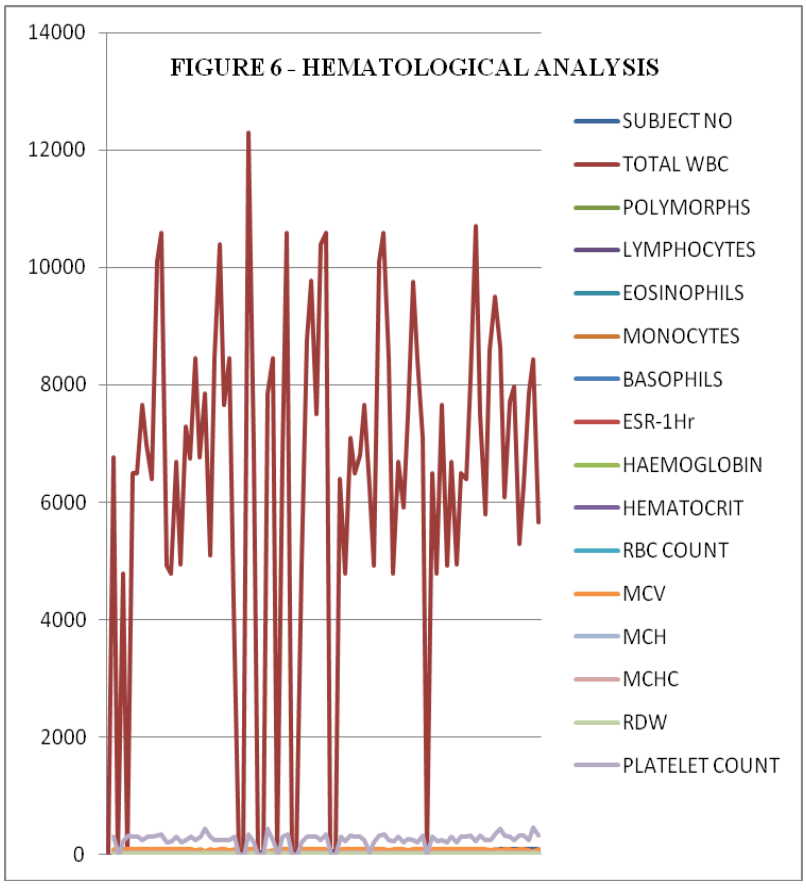
Ultrasound scan study was performed for all the subjects in Madras Scan Centre to confirm the first positivity of the clinical status as indicated by the Biochemical screening. The experimental subjects revealed three types of cysts in the right and left kidneys. Table 4 and Figure 10 categorizes the 30 subjects in F1, F2 and F3 generation under the three cystic types and reveals the percentage rate of each cyst type.

Serum Protein Electrophoresis

Serum Protein Electrophoresis was done commercially using Hydragel Protein € K20 Serum Protein Kit to counter check the data of biochemical analysis obtained from the hospital data with reference to the albumin / Globulin ration level. The results indicated a







significant increase in the Albumin and globulin level thereby validating the data received from the hospital. Furthermore, a marked increase in Total Protein value by Biuret Test was also noted in all the experimental subjects confirming the positivity of the clinical status of the experimental subjects (Figure 5).

Hematology

Cell Dyne 6000 analyzer used to analyze the hematological parameters revealed a marked variation in the experimental subject values than the normal range (Figure 6). Subject No 33 revealed a peculiar manifestation of Hematuria condition (Blood in the urine). [Plate 1 depicts the Hematuria condition in Subject No 33].

Pedigree Analysis

A pedigree chart / Biogenetic tree was constructed in accordance with the linkage analysis pattern of Roser Torra, et al., (1996) with slight modifications to update and suite the present study. 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 (Table 5 & Figure 11). Plate 2A and B gives an overall view of the pedigree chart of the 8 families comprising of 89 subjects taken for the study.

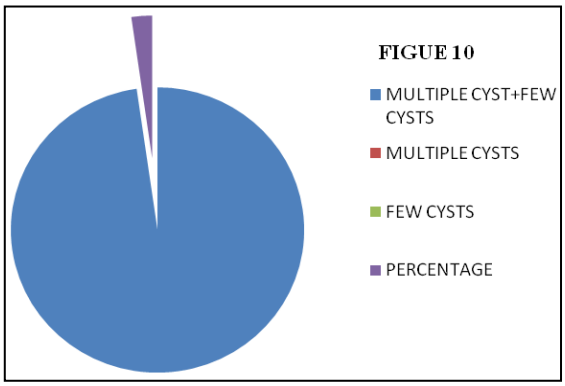
Karyology / Chromosomology

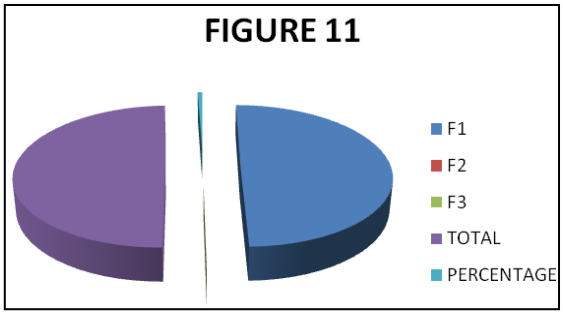
Karyotyping analysis was carried out for few chronic experimental subjects. Since PKD is characterized by the alteration at the gene level, contrasting variation was not encountered in the metaphase plate of the karyotype. Plate 3 depicts the Karyotype of a chronic experimental subject.

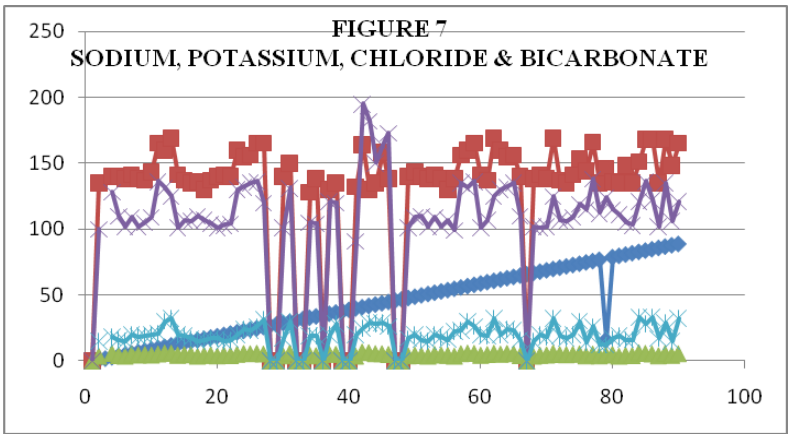
PCR analysis by SSCP

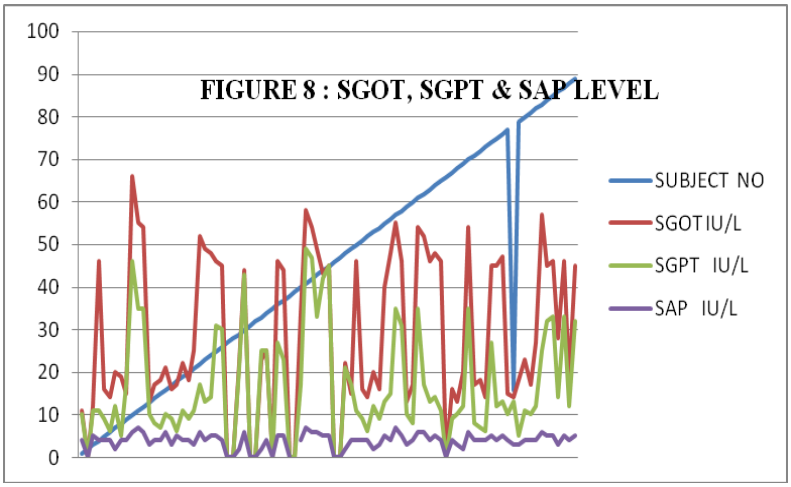
Table 6 depicts the percentage rate of control and experimental subjects selected for PCR analysis by SSCP method for Exon 15 (Nucleotide position 29039 – 29523), Exon 15 (Nucleotide position 29476 – 29847), Exon 18 – 20 (Nucleotide position 32835 – 33314), Exon 25 – IVS 26 (Nucleotide position 38978 – 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. Plate 4 reveals the Agarose gel showing high molecular weight genomic DNA isolates from blood samples. Plates 5 – 9 are Representative

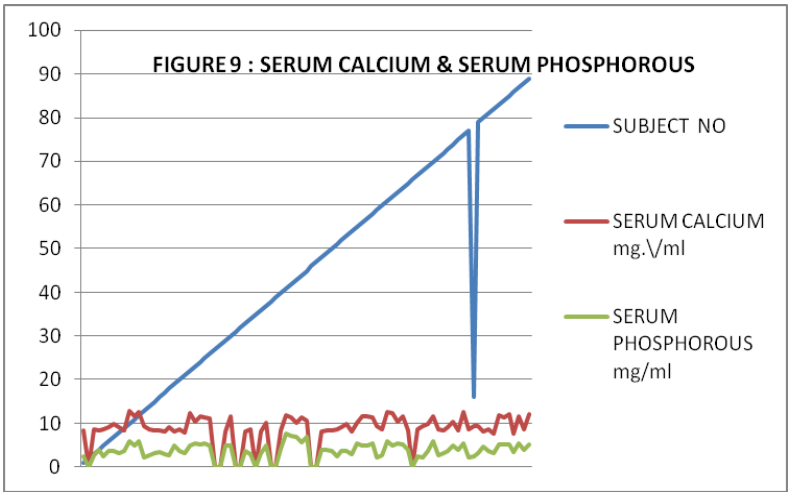
Agarose gels for PCR product of Exon 15 (Nucleotide position 29039 – 29523), Exon 15 (Nucleotide position 29476 – 29847), Exon 18 – 20 (Nucleotide position 32835 – 33314), Exon 25 – IVS 26 (Nucleotide position 38978 – 39675) and Exon 29 – 30 (Nucleotide position 41579 – 41915). Plates 10 – 14 are Representative SSCP gel analysis 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).











DNA sequencing

DNA Sequencing for 12 samples, 10 experimental subjects [samples which showed a shift in the banding pattern by SSCP] and two control subjects (Table 7) 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 15 – 20 shows the Transitions, Transversiion and Heterozygous condition identified by DNA sequencing of control subjects for Exon 25 – IVS 26 (Nucleotide position 38978 – 39675), Exon 18 – 20 (Nucleotide position 32835 – 33314) and Exon 29 – 30 (Nucleotide position 41579 – 41915).

Statistical tools incorporated in the present study revealed the existence of significant amount of difference between normal and PKD subjects within the study variables and helped to identify the variables which significantly influence the dependent variables such as Blood Urea and Serum Crreatinine in the PKD subjects which can pave the way and prevent the stress of undergoing laborious clinical tests to confirm the positivity of clinical status of PKD (Graphs 1 – 6).

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. 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. Results from research work undertaken in Han SPRD rat support a significant therapeutic potential of EGFR tyrosine kinase inhibition in ADPKD. Based on the above findings 8 molecules of epidermal growth factor receptor (EGFR) were taken from the Enzyme database Brenda. These 8 EGFR lead molecules 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 offspring's 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.

PLATE 1: HEMATURIA

PLATE 3: KARYOTYPE

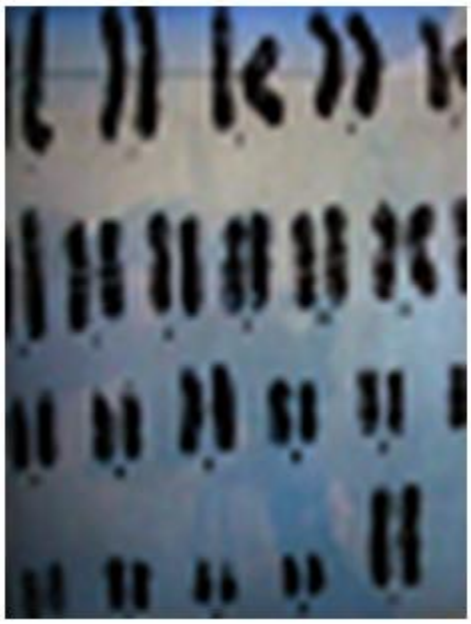


PLATE 2A: PEDIGREE CHART

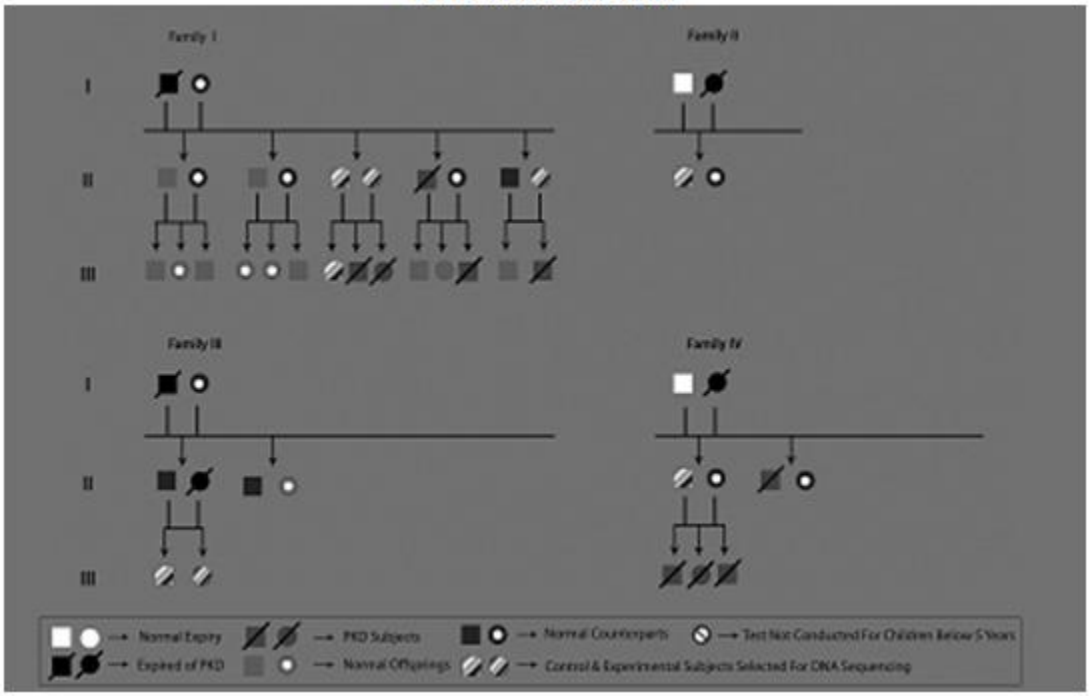


PLATE 2B: PEDIGREE CHART

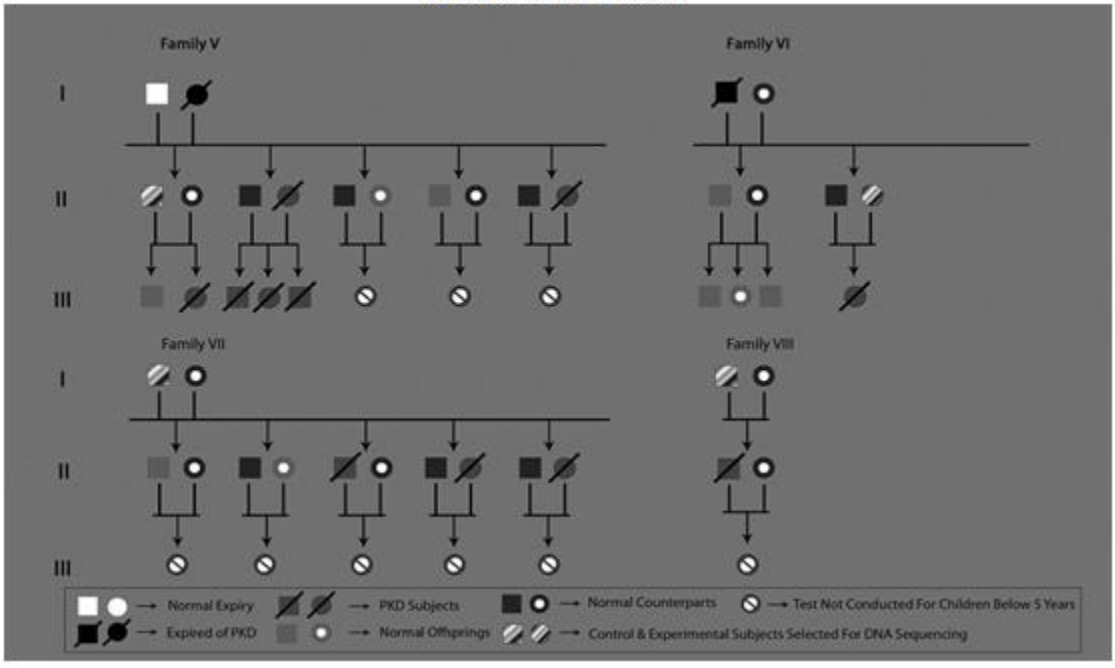
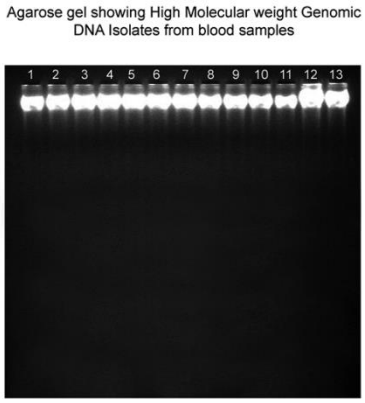
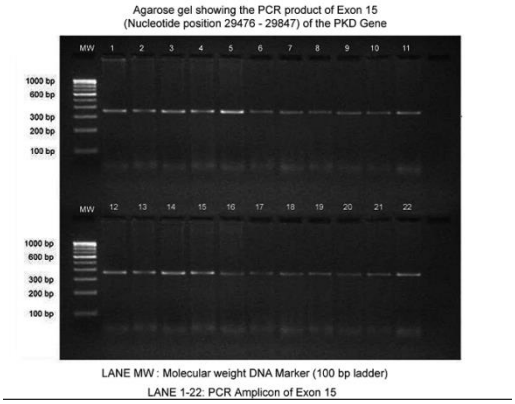


PLATE 4 - AGAROSE GEL SHOWING HIGH MOLECULAR WEIGHT GENOMIC DNA ISOLATES FROM BLOOD SAMPLES

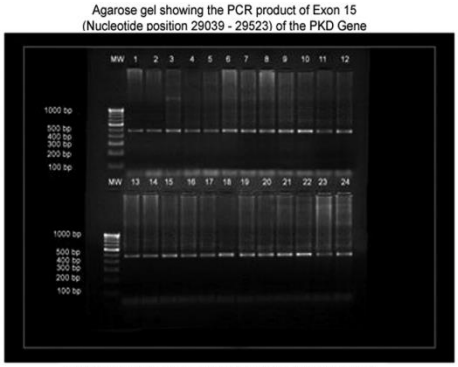


PLATES 5 - REPRESENTATIVE AGAROSE GELS FOR PCR PRODUCT OF EXON 15 (NUCLEOTIDE POSITION 29039 – 29523)



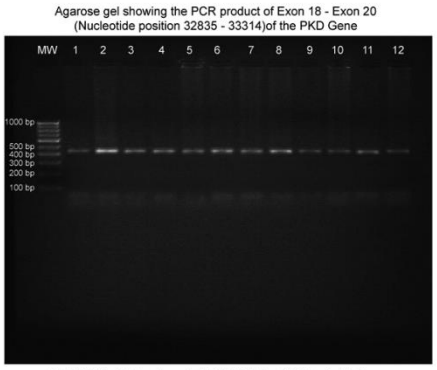
LANE MW : Molecular weight DNA Marker (100 bp ladder)
LANE 1-22: PCR Amplicon of Exon 15

PLATES 6 - REPRESENTATIVE AGAROSE GELS FOR PCR PRODUCT OF EXON 15 (NUCLEOTIDE POSITION 29476 – 29847)



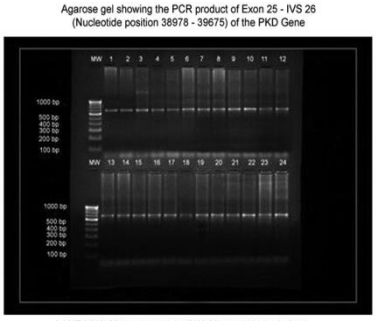
LANE MW : Molecular weight DNA Marker (100 bp ladder)
LANE 1-24: PCR Amplicon of Exon 15

PLATES 7 - REPRESENTATIVE AGAROSE GELS FOR PCR PRODUCT OF EXON 18 – 20 (NUCLEOTIDE POSITION 32835 – 33314)



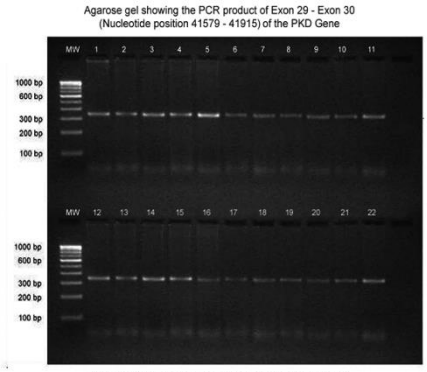
LANE MW : Molecular weight DNA Marker (100 bp ladder)
LANE 1 - 12 PCR Amplicon of Exon 18 - Exon 20

PLATES 8 - REPRESENTATIVE AGAROSE GEL FOR PCR PRODUCT OF EXON 25 – IVS 26 (NUCLEOTIDE POSITION 38978 – 39675)



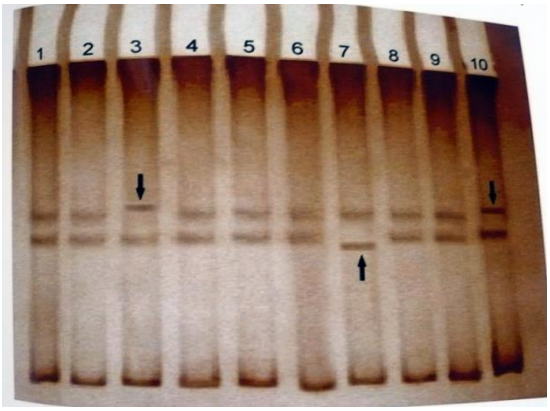
LANE MW : Molecular weight DNA Marker (100 bp ladder)
LANE 1-24: PCR Amplicon of Exon 25 - IVS 26

PLATES 9 - REPRESENTATIVE AGAROSE GELS FOR PCR PRODUCT OF EXON 29 - 30 (NUCLEOTIDE POSITION 41579 – 41915)

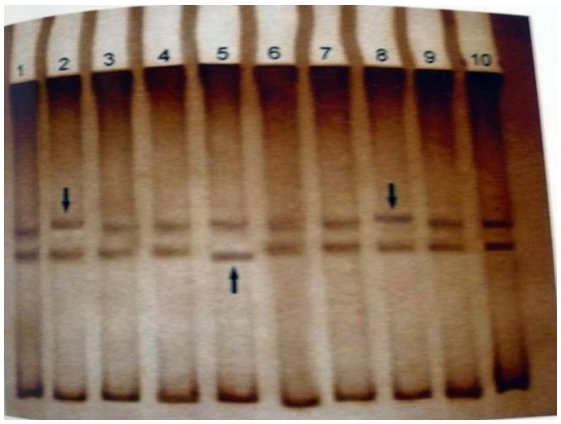


LANE MW : Molecular weight DNA Marker (100 bp ladder)

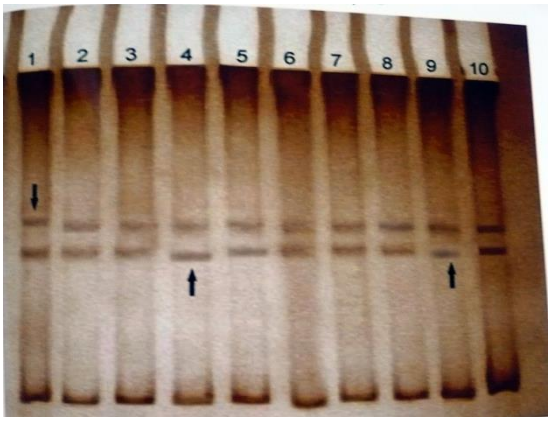
PLATES 10 : REPRESENTATIVE SSCP GEL ANALYSIS FOR PCR PRODUCT OF EXON 15 (NUCLEOTIDE POSITION 29039 – 29523)



PLATES 11 : REPRESENTATIVE SSCP GEL ANALYSIS FOR PCR PRODUCT OF EXON 15 (NUCLEOTIDE POSITION 29476 – 29847)



PLATES 12 : REPRESENTATIVE SSCP GEL ANALYSIS FOR PCR PRODUCT OF EXON 18 - 20 (NUCLEOTIDE POSITION 32835 - 33314)



PLATES 13 : REPRESENTATIVE SSCP GEL ANALYSIS FOR PCR PRODUCT OF EXON 25 IVS 26 (NUCLEOTIDE POSITION 38978 – 39675)

PLATE 14 : REPRESENTATIVE SSCP GEL ANALYSIS OF EXON 29 – 30 (NUCLEOTIDE POSITION 41579 – 41915)

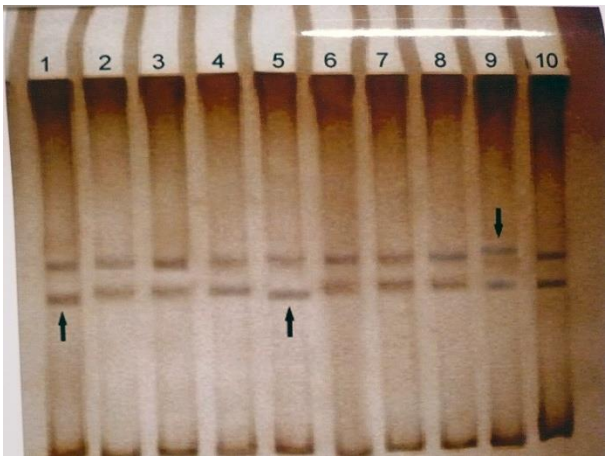
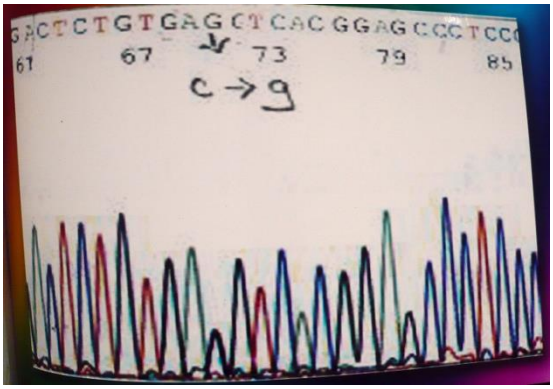


PLATE 15 : TRANSITIONS AND TRANSVERSIONS OBSERVED IN DNA SEQUENCING FOR EXON 18 – 20 (NUCLEOTIDE POSITION 32835 – 33314)



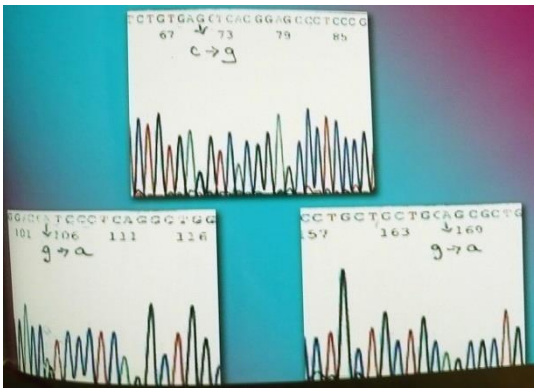


PLATE 16 : TRANSVERSIONS OBSERVED IN DNA SEQUENCING FOR EXON 25 – IVS 26 (NUCLEOTIDE POSITION 38978 – 39675)

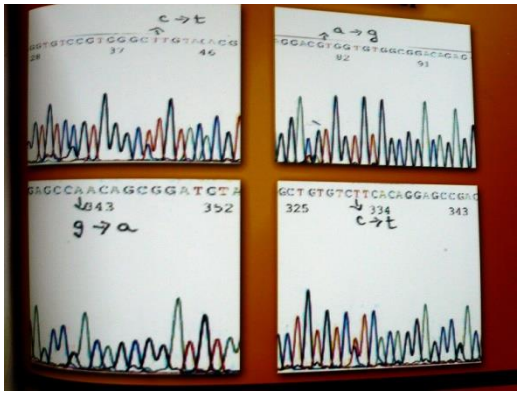
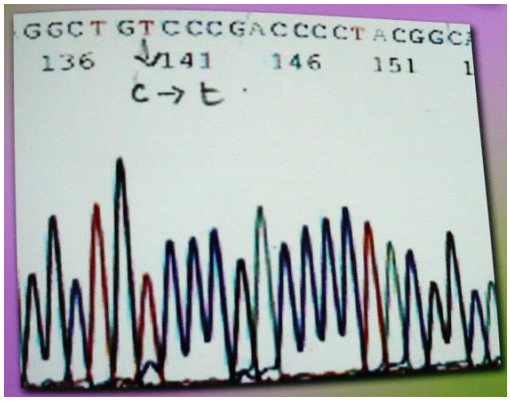
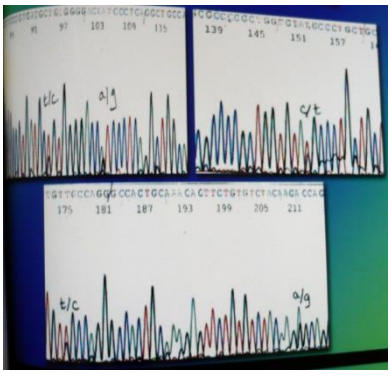
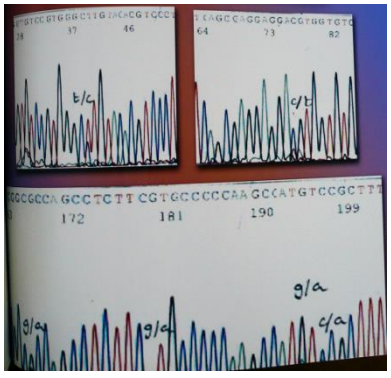


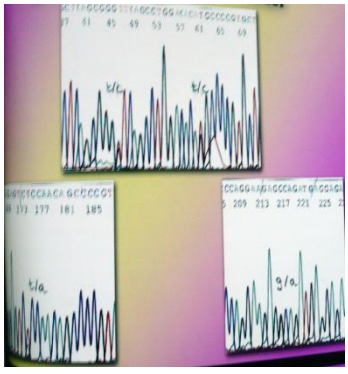
PLATE 17 : TRANSVERSIONS OBSERVED IN DNA SEQUENCING FOR EXON 29 – 30 (NUCLEOTIDE POSITION 41579 – 41915)



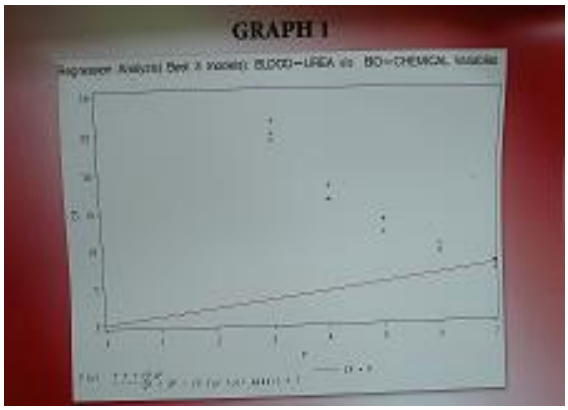
PLATES 18 - 20 : HETEROZYGOUS CONDITION OBSERVED IN DNA SEQUENCING FOR EXON 18 – 20 (NUCLEOTIDE POSITION 32835 – 33314), EXON 25 – IVS 26 (NUCLEOTIDE POSITION 38978 – 39675) AND EXON 29 – 30 (NUCLEOTIDE POSITION 41579 – 41915)

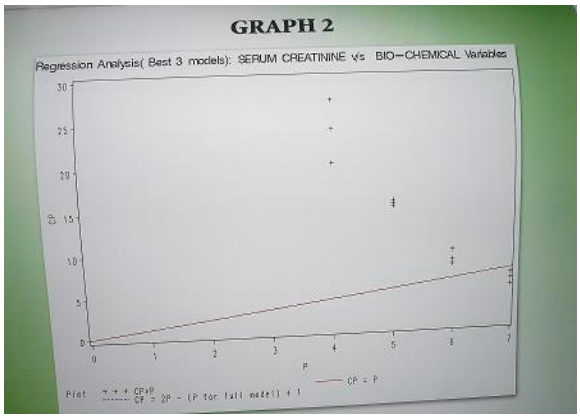


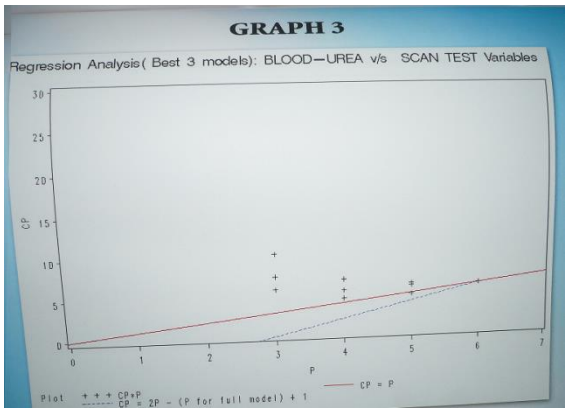


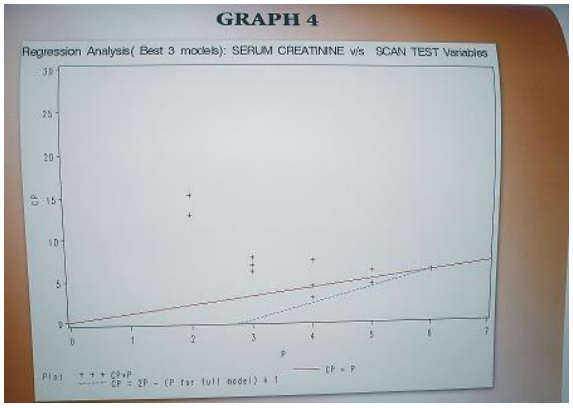


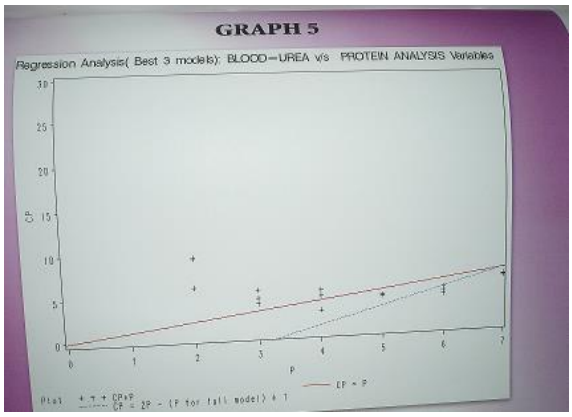
Graphs 1 – 6

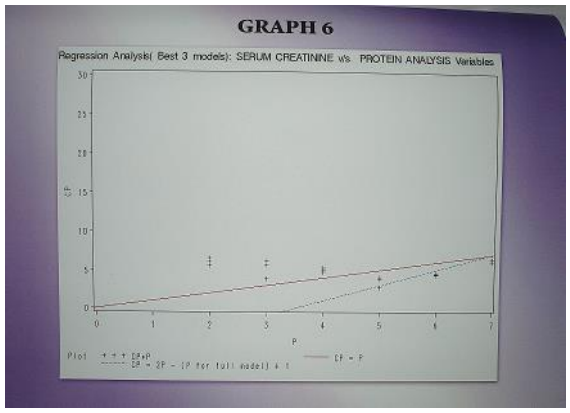












4. SUMMARY

The significant advances in understanding the molecular basis of adult dominant Polycystic 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 are likely to generate, in the foreseeable future, a variety of possible strategies for molecular interventions in clinical research. 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.

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