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Pathogenetic and Molecular Study of Human Polycystic Kidney Population

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Abstract;- Morphogenesis of the kidney is regulated by reciprocal tissue interactions between the epithelial ureteric bud and the metanephric mesenchyme. Polycystin is expressed in the ureteric bud but is least abundant at the tip of the bud in 14-18-wk fetal kidney suggesting that polycystin is not primarily an inductive signal for mesenchyme-epithelial conversion and it reaches its peak expression in maturing proximal tubules and collecting ducts. Its expression declines thereafter but is maintained at a low level in mature tubules through adulthood. 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. 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 candidate 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. 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.

Keywords: **Morphogenesis, Polycystic kidney disease, Polycystic, Candidate genes and Genetic Link.**

I. 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 legends on the adjacent cells or presented in the extra cellular 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. 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 Tran differentiated 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 embryonic 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, extra cellular matrix constituents, and extra cellular 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 post inductive 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 most common form of polycystic kidney 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). The tubules enlarge from a normal diameter of 40 um to several centimeters in diameter, causing marked gross and microscopic anatomic distortion. The cause of the cystic change in the tubules is unknown, but current possibilities include obstruction of tubule fluid flow by hyper plastic 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. 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

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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 (Table 1). **Autosomal recessive PKD** is a rare, inherited form. Symptoms of Autosomal recessive PKD begin in the earliest months of life, even in the womb. **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).

Adult polycystic kidney disease is transmitted as an auto somal dominant trait and affects approximately 1 in 1000 people. Cysts arise from the nephrons and the collecting tubules. Islands of normal parenchymal renal tissue are interspaced between the cysts. Micro dissection reveals that the cysts communicate directly with the nephrons and collecting tubules in patients present with hypertension and progressive renal failure after their third decade of life. Uncommonly, autosomal dominant polycystic kidney disease (ADPKD) appears in children, and it is rarely seen in neonates. Of patients with ADPKD, 25-50% have associated hepatic cysts, 9% have associated pancreatic cysts, and 5% have associated splenic cysts; pulmonary cysts occur uncommonly. These extra-renal manifestations are not found in neonates and children. (www.emedicine.com/radio/autosomal-dominant-polycystic-kidney-disease.htm).

PDK1 and PKD2 are two recently identified genes that are responsible for the vast majority of autosomal polycystic kidney disease, a common inherited disease that causes progressive renal failure. Mutations in the PKD-1 gene are responsible for 85% of cases of ADPKD, which affects 500,000 patients in the U.S. and leads to ESRD. Polycystin-1 is highly expressed in focal adhesions at the basal surface of migrating ureteric bud epithelial cells of normal developing kidneys. In normal adult kidneys, polycystin-1 is down regulated and seen in the cell-cell adherent junctions of medullary collecting tubules. The developmental state can be modeled in vitro in sub confluent cultures (csigal@jdrf.org). The replicated portion of PKD1, which comprises nearly 70% of the length of the gene, is predicted to harbor at least 85% of the mutations present in affected autosomal dominant polycystic kidney disease type 1 pedigrees. The relative paucity of reported mutations involving this segment is attributable to the significant technical challenges posed by the genomic structure of the gene. The gene has several features that are major impediments to its quick analysis. It encodes a 14-kb mRNA, it is very GC-rich, and >70% of its length is replicated in multiple copies with very high sequence identity. Very few positions within the replicated segment are suitable for the design of PKD1-specific primers; therefore, all strategies that have been used to analyze the 5' end have relied on the use of long-range (LR) PCR methods (Watnic TJ, *et.al.*, 1997, Peral B, *et.al.*, 1997 and Thongoppakhun W, *et.al.*, 1999). In the most common form of PKD, the severity of the mutation was directly related to whether the animals died before birth or has decreased life span. It is concluded that the presence of polycystin-2 is essential for normal development of parts of the kidney, heart and pancreas. A second research which examined kidney cysts from two patients discovered 71% of the cysts to have mutations in the PKD1 gene. The finding suggest that PKD1 mutations may be modifiers of disease severity, and that independent disturbances in the production of the polycystin proteins by the PKD genes may be sufficiently disruptive to cause cyst formation. Thus the identification of mutations in human disease genes, especially those that encode proteins of unknown function, can be instructive for identification of functionally important domains and can serve as guideposts for experimental investigation into the pathogenesis of disease. (www.niddk.gov/health/kidney/pubs/polycyst.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). Some of the genetic tools incorporated in the present study to detect genetic disorders at various levels areas follows

Karyotype is a photomicrograph of chromosomes arranged according to a standard classification (http/karyotyping.tripod.com/). It is a test used to identify and evaluate the size, shape and number of chromosomes in a sample of body cells. This test gives clues to problems associated with a person's growth, development, and body function. However, genetic alterations by way of mutations are not identified at this

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chromosomal level test hence more powerful molecular tools like PCR analysis and DNA Sequencing are employed to detect alterations at the genetic level (www.nlm.nih.gov/medlineplus/ency/article/003935/ht

PCR is used in molecular biology and genetic disease research to identify new genes. Due to the high sensitivity and particularity of PCR, scientists have employed it as an essential tool for improving human health and human life. Medical research and clinical medicine are profiting from PCR mainly in two areas: detection of infectious disease organisms, and detection of variations and mutations in genes, especially human genes. Since PCR can amplify unimaginably tiny amounts of DNA, even that from just one cell, physicians and researchers can examine a single sperm, or track down the elusive source of a puzzling infection. These PCR-based analyses are proving to be just as reliable as previous methods-sometimes more so-and often much faster and cheaper (www.gmotesting.com/docs/Sensitivity50cycles.doc). By distinguishing tiny variations in DNA, this method is also leading to new kinds of genetic testing. These tests diagnose not only people with inherited disorders, but also people who carry deleterious variations, known as mutations, which could be passed to their children. These carriers are usually not themselves affected by the mutant gene, but can pass it to the next generation. (www.ornl.gov/sci/1sm/dnaper.html). It is not worthy of the results of the Human Genome Project, the huge international effort to identify and study all human genes that many new genetic tests are emerging. 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 researcher's essential requirement in reading and deciphering the DNA base-sequence is the precision, speed, reliability, and low cost of such operation (www.ncbi.nlm.nih.gov/entrez/7709805Abstract). 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.

II. OBJECTIVES

The main objective of the present study 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 PKD 1 gene using PCR by SSCP and DNA Sequencing Technique. 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.

III. MATERIALS AND METHODOLOGY

DNA ISOLATION

Reagents Required

To Prepare The Non-Nucleated Cell Lysis Buffer – Red Blood Cell Lysis Buffer – RCLB (TKM-1 Buffer) The Following Reagents Were Used. 10 M M Tris – Hcl, Ph 7.5, 100 M M $Mgcl_2$, 10 M M KC1, 2 M M EDTA (SODIUM SALT) Ph 8.0), 100 M M Tritonx 100. The Total Ph Should Be 7.5. For 100 Ml RCLB Preparation, 1 Ml (1000 Ul) Tris HC1,1 Ml (1000 Ul) $Mgc1_2$,1 Ml (1000 Ul) KC1,0.4 Ml (400 Ul) 0.5 EDTA Was Used With The Remaining As Autoclaved Water. To Prepare Nucleated Cell Lysis Buffer – NLB (TKM-II Buffer) The Following Reagents Were Used. 10 M M Tris – HC1, Ph 7.5, 100 M M $Mgc12_2$, 10 M M KC1, 2 M M Na_2 EDTA, 400 M M Nac1, 1% SDS (Sodium Do-Decyl Sulphate), 5M Nac1 And Ethanol 70% And 100%. 5ml Of Blood From The Subject Was Collected And Stored In EDTA. The Volume Was Doubled By Adding RCLB [Red Cell Lysis Buffer]. 4 – 5 Drops Of Tritonx 100 Detergent Was Then Added For The Lysis Of RBC. The Solution Was Incubated At 37°c For 5 Minutes. After Incubation, The Solution Was Centrifuged At 2000xg For 15 Minutes. The Supernatant Was Discarded. To The Pellet 10 Ml Of RCLB Was Added Again.The Pellet Was Mixed And Centrifuged. The Same Process Was Repeated Till A White Pellet Was Obtained. 1 Ml Of NLB [Nucleated Cell Lysis Buffer] Was Added To Lyse The WBC. 0.2% SDS [Sodium Do-Decyl Sulphate] Was Added After NLB Addition. The Solution Was Incubated At 55°c For ½ To 3 Hrs. 2 M (0.4 Ml Or 400 Ul) Of Nac1 Was Added After The Incubation Process. The Solution Was Centrifuged At 10000xg For 15 Minutes. The Supernatant Was Transferred To A New Tube. Double The Volume Of Ice Cold Ethanol Was Added To The Supernatant. DNA Precipitated Out In The Form Of A Slender Strand. The DNA Strand Was Then Transferred To A New Micro Centrifuge Tube. 0.5 Ml Or 500 Ul Of 70% Ethanol Was Added. The Solution Was Centrifuged At 2000xg For 5 Minutes. Ethanol Was Drained Off. The Pellet Was Air

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Dried In Vaccum. 0.5 Ml Or 500 Ul Of TE Buffer (Tris EDTA) Was Added To Store The DNA Strand (Table 1).

PCR ANALYSIS

Working Volume

S.NO.	REAGENTS	WORKING CONCENTRATION	WORKING VOLUME	10 SAMPLES
1	10x PCR buffer	1x2 ul	2 ul	20 ul
2	dNTP'S	200 um	0.4 ul	4 ul
3	Primer F	30-50 pm	0.3 ul	3 ul
4	Primer R	30-50 pm	0.3 ul	3 ul
5	Tag polymerase 3 ul	1.5 units	0.5 ul	5 ul
6	Sterile water	-	15.5 ul	155 ul 190 ul Master Mix
7	Template DNA	100 ng	1 ul	-

Table 1 : Working Volume For Pcr Analysis

Working volume = 19 ul of master mix and 1 ul Template DNA = 20 ul (Table 2)

IV. SAMPLE PREPARATION FOR PCR ANASYSIS

Working volume of 20 ul was transferred to 2 ml eppendorf tube to which the following reagents were added. To this the following reagents were added. Triple volume of buffer = 20 x 3 = 60 ul, PCR product 20 ul, Sodium Acetate = 1/10 of this volume = 80/10 = 8 ul, PCR product = 88 ul and double the volume of 100% Ethanol = 176 ul. The Total = 175 ul + 88 ul = 263 ul. 263ul of PCR was freezed overnight. The sample was centrifuged at 10,000 rpm for 30 minutes. The supernatant was discarded and 100 ul of 70% ethanol was added. The sample was centrifuged at 10,000 rpm for 30 minutes. The supernatant was discarded and the pellet was carefully air dried. 20 ul of TE buffer was added to the pellet and was then stored in a deep freezer.

PCR Program

The PCR program was carried out for the following Exons (Table 3)

1. PCR PROGRAM FOR EXON 15 (Nucleotide position 29039 – 29523), EXON 15 (Nucleotide position 29476 – 29847) and EXON 25 – IVS 26 (Nucleotide position 38978 – 39675).
2. PCR PROGRAM FOR EXON 18 – 20 (Nucleotide position 32835 – 33314) and EXON 29 – 30 (Nucleotide position 41579 – 41915)

STEP NUMBER	STEPS IN PROGRESS	TEMPERATURE	TIME PERIOD
1	INITIAL DENATURATION	94°C	5 minutes
2	DENATURATION	94°C	45 seconds
3	ANNEALING	63°C	1 minute
4	EXTENSION	72°C	1 minute
GO TO STEP 2, REPEAT 29 TIMES			
5	FINAL EXTENSION	72°C	5 minutes

TABLE 2 : PCR PROGRAM

PURIFICATION OF PCR PRODUCT BY AGAROSE GEL PREPARATION

	AGAROSE POWDER	1 X TAE BUFFER	ETHIDIUM BROMIDE
1	1.0 gm	100 ml	12 ul
	0.5 gm	50 ml	6 ul
	0.25 gm	25 ml	3 ul
2	1.5 gm	100 ml	18 ul
	0.75 gm	50 ml	9 ul

TABLE 3: WORKING CONCENTRATION PROTOCOL

Agarose gel powder of 0.25 gms was dissolved in 25 ml TAE buffer. The solution was boiled for 1 minute for the Agarose to dissolve. The solution was cooled and 3ul of Ethidium Bromide was added to it. The solution was poured into the gel tray after fixing the comb. The comb was gently removed after the setting of the gel (Table 3).

SSCP ACRYLAMIDE GEL PREPARATION (20 ml DNA SSCP)

37% Acrylamide 6% 3.2 ml (33200 ul)	10 x TBE 0.5 X 1 ml (1000 ul)	40% Glycerol (5000 ul) (Since 99% concentration is available 2 ml was added (200 ul)	100% TEMED 0.2% 0.04 ml 40 ul (Brown in color and activator for APS and the whole reaction	10% APS 0.05% 0.1 ml (Catalyst) Acrylamide bis Polyacrylamide
1	**2**	**3**	**4**	**5**

Table 4: Reagents for SSCP

1 – 3 reagents are mixed and made up to 20 ml with sterile water. The gel slides are cleaned and the sides are gelled with old gel liquid. After the fifth addition of APS, the gel is poured carefully without air bubbles and the combs are fixed. The gel is left undisturbed to set. The buffer solution is added to the Gel Apparatus. The combs are removed gently. The wells are cleansed with a syringe. The gel apparatus is pre – run for 20 – 30

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minutes with the buffer solution (Table 5). Equal volume of sample and Equal volume of loading dye is mixed. The sample is maintained at 94 / 95°C for 4 minutes. It is immediately deep freezed for 1 minute in ice. The samples are loaded in wells and are run at 50 Volts current for 12 – 16 hrs. The gel is removed and taken for silver staining.

Silver Staining For DNA – PAGE and Photo Documentation

1. 100% Acetone
2. 50% Acetone
3. TCA stock solution
4. 37% Formaldehyde
5. Silver Nitrate stock solution
6. Sodium Carbonate
7. Sodium Thio-sulphate

50% Acetone - 60 ml, TCA stock solution 1.5 ml and 37% Formaldehyde -25 ul was poured into plastic box and the gel was placed and gently rocked for 5 minutes after which the solution was drained off. The gel was rinsed with sterile distilled water, rocked gently for 1 minute and the solution was drained of f. 60 ml of 50% Acetone was added to the gel and was gently rocked for 5 minutes after which the solution was drained off. 100 ml sodium thio-sulphate in 60 ml of distilled water was added to the gel and was gently rocked for 1 minute after which the solution was drained off. The gel with rinsed with distilled water, rocked gently for 1 minute and the solution was drained off. Silver Nitrate stock solution - 0.8 ml and 37% Formaldehyde - 0.6 ml were made up to 60 ml with sterile distilled water and this solution was poured on gel and gently rocked for 8 – 10 minutes after which the solution was drained off. The gel was rinsed with distilled water, rocked gently for 1 minute after which the solution was drained off. Sodium Carbonate - 1.4 gm, 37% Formaldehyde - 25 ul and Sodium Thio-sulphate -12.5 ul were made up to 60 ml with sterile distilled water. This solution was poured on the gel and was gently mixed till the band appears. The gel was rinsed with sterile water, rocked gently for 1 minute after which the solution was drained off. 1% glacial acetic acid was poured in sterile distilled water and rinsed for 1 minute. The solution was drained off. The gel was allowed to stand in sterile distilled water for 10 minutes and then was viewed under white light Tran illuminator. The gels with the bands were Photo documented.

V. 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 Genei Pvt. Ltd.

VI. STATISTICAL METHOD

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. The Statistical tools incorporated to study the marked significance between the normal and PKD subjects for the present study are as under.

VII. 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 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 6 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 7 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 (Table 8). Subject No 33 revealed a peculiar manifestation of Hematuria condition (Blood in the urine). 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 1A 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 2 depicts the Karyotype of a chronic experimental subject.

PCR Analysis by SSCP and DNA Sequencing

Table 9 depicts the percentage rate of control and experimental subjects selected for PCR analysis by SSCP

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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 3 reveals the Agarose gel showing high molecular weight genomic DNA isolates from blood samples. Plates 4 – 8 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 9 – 13 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 for 12 samples, 10 experimental subjects [samples which showed a shift in the banding pattern by SSCP] and two control subjects (Table 10) 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 Swiss port. 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 14 – 19 & Tables 11 -16). 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 Creatinine 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).

VIII. SUMMARY

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. 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. The present study reveals mutations located in the reiterated region which was confirmed by analyses of *PKD1*-genomic DNA using SSCP-PCR method and Direct Sequencing. Notably, we found several disease-associated C-T or G-A mutations that led to charge or hydrophobicity changes in the corresponding amino acids. This suggests that the mutations cause conformational alterations in the PKD1 protein products that may impact the normal protein functions and may hinder the normalcy of the functioning of the experimental subjects. The study is the first report of screen able mutations in the full-length PKD1 gene for Exon 25 – IVS 26, Exon 18 – 20 and Exon 29 – 30 of the Indian population (Table 27). Mutations in a majority group of patients are still unknown, and thereby requires further characterization in the future. 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 in the future to understand the phenotype/genotype correlation and disease phenotypes in different genetic backgrounds, i.e., gender, race, and ethnicity. Thus 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.

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