Chapter 11

Discovery of Single Nucleotide Polymorphism in Polycystic Kidney Disease among South Indian (Madurai) Population

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.71201

Abstract

The kidneys serve an essential regulatory role in most of the animals, including vertebrates and some invertebrates. They are important in the urinary system and also serve homeostatic functions like regulation of electrolytes, maintenance of acid-base balance and regulation of blood pressure (via maintaining salt and water balance). They also serve as natural filter of the blood and remove wastes that are diverted to the urinary bladder. By producing urine, the kidneys excrete wastes such as urea and ammonia. The kidneys are responsible for reabsorption of water, glucose, amino acids and trace elements. They also produce hormones including calcitriol, renin and erythropoietin. The kidney is approximately 11–14 cm long, 6 cm wide and 4 cm thick. Each adult kidney weighs between 125 and 170 g in males and between 115 and 155 g in females. The left kidney is typically slightly larger than the right kidney. Each kidney is made up of about 1 million microprocessor units called nephrons.

Keywords: polycystic kidney disease, renal failure, single nucleotide polymorphism, polymerase chain reaction, disease complications

1. Introduction

The kidneys play an essential regulatory role in animals and are responsible for reabsorption of water, glucose, amino acids and trace elements. They also produce hormones including calcitriol, renin and erythropoietin. The kidney is approximately 11–14 cm long, 6 cm wide and 4 cm thick. Each adult kidney weighs between 125 and 170 g in males and between 115 and 155 g in females. The left kidney is typically slightly larger than the right kidney [1] **(Figure 1).** Each kidney is made up of about 1 million microprocessor units called nephrons.

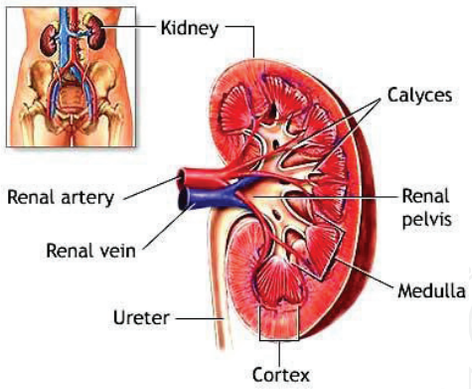

Figure 1. Structure and location of kidney. Source: [2] (http://www.sugarbp.org/kidneystucture_diabetes.htm).

The nephron is the basic structural and functional unit of a kidney [3]. Each nephron has an initial filtering component composed of a glomerulus and Bowman's capsule, which is connected to a long convoluted tubule lined by transporting epithelia.

Sodium chloride, potassium and glucose are filtered and reabsorbed along with water in the nephron back into the bloodstream. This maintains a correct balance of trace element within the blood, which assists in blood pressure regulation and normal levels of blood sugars. Hence, the kidneys are found to play a crucial role in regulating the amount of water and chemicals (electrolytes) in the body such as sodium, potassium and phosphorus [4].

1.1. Different types of kidney diseases

Usually both the kidneys are affected by various forms of diseases and then the waste products and excess fluid build up, causing severe swelling and symptoms of uremia (kidney failure). They are congenital kidney disease, hereditary kidney disorders and acquired kidney diseases.

1.1.1. Congenital disease

It involves malformation of the genitourinary tract, usually leading to some type of obstruction that subsequently produces infection and/or destruction of kidney tissue, which may eventually progress to chronic kidney failure. For example, horseshoe kidney, also known as *ren arcuatus* (in Latin), renal fusion or super kidney, is a congenital disorder affecting about 1 in 500 people [5, 6].

1.1.2. Hereditary disorders

Hereditary diseases are Alport's syndrome or hereditary nephritis, primary hyperoxaluria, cystinuria and polycystic kidney disease (PKD). The chapter found that polycystic kidney disease is more common among the population.

1.1.2.1. Polycystic kidney disease (PKD)

Epithelial cell polarity is vitally important for correct function of different tubule segments [3]. Cell polarity defects have been linked to a number of hereditary kidney diseases including polycystic kidney diseases (PKDs) characterized by the accumulation of fluid-filled cysts in the cortex and medulla [7–10]. There are two types of PKDs. They are autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD) [10].

1.1.3. Acquired kidney diseases

These diseases are numerous and are generally known as nephritis (inflammation of the kidney). The most common type of nephritis is glomerulonephritis and has many causes. The acquired kidney diseases are renal agenesis, multicystic dysplastic kidney, renal dysplasia, diabetic nephropathy, glomerulonephritis, hydronephrosis, interstitial nephritis, kidney stones, kidney tumors: Wilms tumor and renal cell carcinoma, lupus nephritis, minimal change disease (MCD), nephrotic syndrome, pyelonephritis and renal failure.

2. Renal failure

In renal failure, the kidneys lose their normal function due to various factors including infections, autoimmune diseases, diabetes and other endocrine disorders, cancer, and toxic chemicals [11]. Genetic variability on the development of renal failure is becoming clearer and emphasizes the need to elucidate the genetic basis for renal diseases and associated complications. Studies on genetic variability in renal failure would lead to better understanding of different phenotypes observed in polycystic kidney disease and would enable us to determine whether a patient is genetically predisposed to such complications.

2.1. Acute renal failure

Acute kidney injury (AKI), previously called acute renal failure (ARF), is a rapid loss of kidney function due to low blood volume from any cause, exposure to substances harmful to the kidney and obstruction of the urinary tract [12, 13]. Elevated blood urea nitrogen and creatinine or inability of the kidneys to produce sufficient amounts of urine is noted in these patients.

2.2. Stage 5 chronic kidney diseases

Stage 5 CKD is often called **end stage renal disease (ESRD).** The symptoms of Stage 5 CKD are:

- Increase in serum creatinine or protein in the urine are observed.
- The patients develop hypertension or congestive heart failure due to fluid overload and production of vasoactive hormones created by the kidney via the RAS (renin-angiotensin system).

- Urea accumulates, leading to azotemia and ultimately uremia. Urea is excreted by sweating and crystallizes on skin ("uremic frost") (http://en.wikipedia.org/wiki/Chronic_kidney_disease; "Chronic Kidney Disease". medscape.) [14].
- Later this progresses to secondary hyperparathyroidism, renal osteodystrophy and vascular calcification that further impair cardiac function.
- Metabolic acidosis, due to accumulation of sulfates, phosphates, uric acid, etc., leads to excitability of cardiac and neuronal membranes by promoting hyperkalemia [15].

People with chronic kidney disease (hyperlipidemia) suffer from accelerated atherosclerosis and are likely to develop cardiovascular disease than the general population [16].

3. Polycystic kidney disease (PKD)

There are two forms of **PKD:**

(i) Autosomal dominant polycystic kidney disease (ADPKD)

(ii) Autosomal recessive polycystic kidney disease (ARPKD)

3.1. Autosomal dominant polycystic kidney disease (ADPKD)

Autosomal dominant polycystic kidney disease occurs worldwide and in all races. ADPKD is one of the most commonly inherited conditions in humans with an incidence of 1:500 to 1:1000 [17, 18]. It is genetically heterogeneous with two genes identified: PKD1 (16p13.3) and PKD2 (4q21) [9, 19, 20].

3.2. Autosomal recessive polycystic kidney disease (ARPKD)

ARPKD is uncommon and occurs primarily in neonates and children. The gene responsible for ARPKD (*PKHD1*) has recently been identified on chromosome 6. Fibrocystin is defective in ARPKD [21, 22]. The occurrence of ADPKD is most common when compared to ARPKD and the mean age of onset is between 30 and 40 years. Both men and women are equally affected [23]. Hence the present study is also focused on PKD1 and PKD2 gene polymorphism in autosomal dominant polycystic kidney disease subjects and control subjects among South Indian population.

Tables 1 and **2** show polymorphism study in PKD1 and PKD2 gene among various populations on both national and international level.

3.3. Pathogenesis and genetics of polycystic kidney disease (PKD)

Abnormalities in gene expression, cell polarity, fluid secretion, apoptosis and extracellular matrix have also been described in PKD [17, 34–36]. ADPKD is one of the most common Mendelian disorders in humans [37, 38] and the most frequent genetic cause of renal failure

Author and year	Gene	Population	Mutation
Sumathy [24]	PKD1	Indian	PKD1 C-T or G-A, SSCP
Nair et al. [25]	ACE,	Nellore, Andhra Pradesh	I/D polymorphism
Elumalai et al. [26]	eNOS, VNTR	South Indian	a/b polymorphism
Veeramuthumari and Isabel [27]*	PKD1	South Indian (Madurai)	Ala/Val (C/T) polymorphism
Veeramuthumari et al. [28]*	PKD2	South Indian (Madurai)	Arg/Pro (G/C) polymorphism

*Current study.

Table 1. National reports of PKD1 and PKD2 gene polymorphism.

Author and Year	Gene	Population	Mutation
Hateboer et al. [29]	PKD2	Spain, Netherlands, UK, Bulgaria, Australia	C-T substitution, deletion, nonsense mutation, frameshift, missense, splice mutation
Koptides et al. [30]	PKD1 & PKD2	Cyprus	Mutation in exon 24, mutation in exon 1
	PKD1 & PKD2	Slovenia	Frameshift/missense mutation; nonsense mutation
Son et al. [31]	PKD1	Devis, USA	CT transversion (SNP)
	PKD1 & 2	US	Mutation
Lee et al. [32]	PKD1	Taiwan	C → A transversion
Galeano et al. [33]	PKD1	Belgium	SNP

Table 2. International reports of PKD1 and PKD2 gene polymorphism.

in adults. ADPKD is a genetically heterogeneous condition [39], which is caused by mutations in one of the three genes: PKD1 on chromosome 16 accounts for 85% of cases, whereas PKD2 on chromosome 4 accounts for 15% and mutations in the PKD3 gene are rare [40]. Hence the present study is focused on PKD1 and PKD2 genes in patients with ADPKD among South Indian (Madurai) population.

3.4. Chromosomal location of PKD1 and PKD2 gene

PKD1 has been mapped to the short arm of the 16th (16p13.3) chromosome, which encodes a protein called polycystin-1. The PKD1 gene is very large in size, consisting of 46 exons distributed over 52 kb of genomic DNA [41]. The gene encodes a 14.1-kb mRNA transcript to be translated into a protein composed of 4302 amino acids transcript with an open reading frame (ORF) of 12,909 bp [42] (**Figure 2**). The PKD2 gene maps to chromosome 4q21–23 (**Figure 3**). The PKD2 gene encodes a protein, polycystin-2, which is composed of 968 amino acids [45]. The interaction of polycystin-1 and polycystin-2 in renal tubules promotes normal development and function of the kidneys [46].

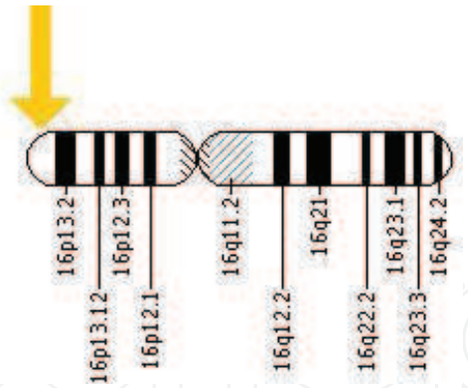

Figure 2. Chromosomal location of PKD1 gene. Source: [43] http://ghr.nlm.nih.gov/gene/PKD1.

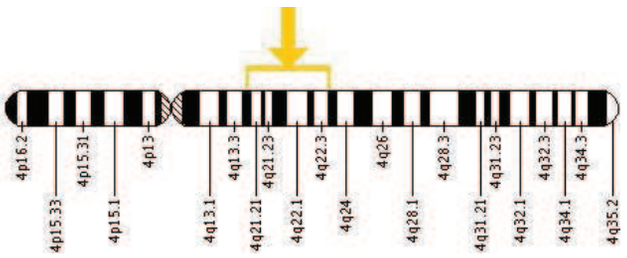

Figure 3. Chromosomal location of PKD2 gene. Source: [44] http://ghr.nlm.nih.gov/gene/PKD2.

3.4.1. Polycystin-1

The PKD1 gene codes for polycystin-1 (PC-1) and plays a vital role in cell-cell and cell-matrix interaction [41]. Thus, a defect in polycystin-1 leads to the alteration in the differentiation of epithelial cells and abnormal phenotypic expression of autosomal dominant polycystic kidney disease (ADPKD). The proteins encoded by the PKD1 and PKD2 genes define a new family. The polycystins play an important role in a variety of biological processes including fertilization, ion transduction and mechanosensation. Polycystin-1 is an integral membrane protein, which is predicted to contain an array of distinct protein motifs, including two leucine-rich repeats flanked by cystine-rich domains. Many of these motifs are involved in protein-protein or protein-carbohydrate interaction, which raises the possibility of polycystin-1, as a receptor for a yet unidentified ligand. The carboxyl terminus of polycystin-1 is located in cytoplasm and contains coil-coil domains and mediates the protein-protein interaction as well as several potential sites of phosphorylation. Polycystin-1 is expressed in many tissues, including the kidney, brain, heart, bone and muscles [47]. Foggensteiner et al. [48] have reported that several studies have identified polycystin-1 in the plasma membrane of tubular epithelial cells, in the distal nephron and in the collecting duct. The defect of polycystin-1 might lead to alteration in differentiation of epithelial cells and abnormal phenotypic expression of ADPKD [49].

3.4.2. Polycystin-2

Polycystin-2 is also widely expressed in many tissues, particularly the kidney, heart, ovary, testis, vascular smooth muscle and small intestine [47]. In the kidney, polycystin-2 like

polycystin-1 is expressed in all nephron segments, with the possible exception of the thin limbs but absent from glomeruli. Several studies have shown that the polycystin-2 channel conducts divalent cations including calcium and that this activity can be stimulated by calcium on the cytosolic side.

3.4.3. Fibrocystin/polyductin

The protein encoded by the *PKHD1* gene has been named polyductin or fibrocystin and is composed of 4074 amino acids [22]. Polyductin/fibrocystin is predicted to be a membrane protein consisting of a large extracellular domain, a single transmembrane segment and a short carboxyl-terminal tail. Polyductin is a novel protein, although it has some similarities to other proteins in the database.

3.5. Mechanism of cyst formation

Cysts will form in these patient's kidneys and several studies suggest that the cells that line these cysts will have lost both functional copies of a polycystin gene [50, 51]. Defects in the genes encoding PC1 or PC2 lead to aberrant gene transcription, cell proliferation and ion secretion, which in turn result in the formation of fluid-filled cysts. These cysts lead to the displacement of the normal renal parenchyma and the formation of a cyst-filled kidney with reduced functional capacity (**Figure 4**).

3.6. ADPKD-associated common complications

Common complications associated with ADPKD are hypertension, hematuria, urinary tract infection, renal calculi, cardiac valve abnormalities, diabetes, hernia of the anterior abdominal wall and cerebral berry aneurysms [29, 53]. Hematuria is the presence of red blood cells (RBCs) in the urine. In microscopic hematuria, the urine appears normal to the naked eye, but examination with a microscope shows a high number of RBCs [54]. Diabetic nephropathy (*neuropatia diabetica*) also known as Kimmelstiel-Wilson syndrome, or nodular diabetic glomerulosclerosis [55] and intercapillary glomerulonephritis, is a progressive kidney disease. Anterior abdominal wall hernias, also known as ventral hernias, are involved in the protrusion of part of the peritoneal sac through a defect in the muscle layers of the anterior abdominal wall [56]. A cerebral or brain aneurysm is a cerebrovascular disorder in which weakness in the wall of a cerebral artery or vein causes a localized dilation or ballooning of the blood vessel [57].

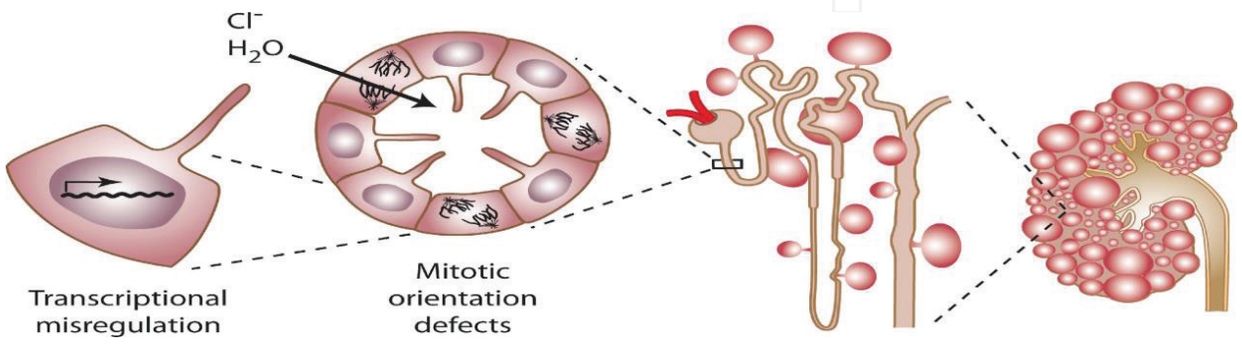

Figure 4. Cyst formation in nephron, kidney and at cellular level [52].

Clinically, PKD is characterized by progressive formation and enlargement of cysts leading to end-stage renal disease (ESRD) in late middle age. Overall, ADPKD accounts for approximately 5–10% of ESRD [58]. Hypertension occurs in 50–75% of patients prior to renal insufficiency and it is thought to accelerate the decline in renal function [59, 60]. Systemic hypertension is also very common, occurring in more than 75% of patients. Increased blood pressure (BP) has been attributed to activation of the renin-angiotensin system, but a primary defect in blood vessels may also exist [61].

3.7. Method of diagnosis and screening

Nowadays, ADPKD is studied by ultrasound, CT or MRI with multiple cysts that are generally visible that increase in size and number with age [62]. ADPKD is typically diagnosed in adults by the detection of bilaterally enlarged polycystic kidneys using transabdominal ultrasound scanning. The diagnosis of ADPKD is established primarily by imaging studies of the kidney [53]. For diagnosis of ADPKD, computer tomography (CT) has been used effectively, which has also revealed multiple cysts in kidneys and left ovary and aneurysm in the brain [53].

3.7.1. Treatment

When renal function, measured by glomerular filtration rate, is persistently poor, dialysis and kidney transplantation could be done. Cotran et al. [63] have stated that a common symptom of kidney stones is a sharp pain in the medial/lateral segments of the lower back. Approximately 50% of afflicted individuals have been shown to develop end-stage renal disease requiring dialysis or kidney transplantation before the age of 60 [8].

3.7.2. Trends in potential therapies and clinical trials

Until now, therapy for ADPKD has been directed toward limiting its complications. Cardiovascular complications, related to hypertension, are a major cause of morbidity and mortality. A major problem in therapeutic interventions in ADPKD is that this is a very slowly evolving condition, and GFR is well maintained until relatively late in the course of the disease at the age of 40. Better understanding of signaling pathways and cellular changes associated with ADPKD has suggested possible therapies to directly inhibit the development or growth of cysts, some of which are now being tested in clinical trials [64]. A stable somatostatin analogue, octreotide, has been shown to be effective at limiting progression in liver and kidney cystic disease in a rat model of PKD [65].

Advanced-stage ADPKD patients frequently receive a renal transplant without removal of the affected cystic kidneys, without side effects. Rapamycin is often used to prevent transplant rejection. The absence of polycystin permits excessive kinase activity in the mTOR pathway and the development of renal cysts [66]. Patients treated with rapamycin have been reported to show a statistically significant reduction in native polycystic kidney size over a period of 24 months compared with patients treated with other antirejection drugs. Other targets for therapy include triptolide, a compound derived from a traditional Chinese herbal therapy, which blocks glycosyl ceramide synthesis [67].

http://dx.doi.org/10.5772/intechopen.71201

4. Methodology to be followed for the discovery of single nucleotide polymorphism in polycystic kidney disease

Genomic DNA preparation: [68, 69].

Reagents required: phosphate buffer saline (PBS), red blood cell (RBC) lysis buffer, cell lysis buffer (CLB), ammonium acetate, isopropyl alcohol, 70% ethanol, TE buffer

Procedure: the blood samples were thawed at room temperature and 300 μl of blood was transferred to centrifuge tubes. Equal volume of PBS was added to it and incubated for 20 min and centrifuged at 3000 rpm for 5 min. The supernatant was removed and the pellet was resuspended in 900 μl of RBC lysis buffer and mixed thoroughly. This was centrifuged at 3000 rpm for 5 min and the supernatant was discarded. To the pellet 600 μl of ice-cold cell, lysis buffer was added and mixed well, and then 200 μl of ammonium acetate was added to the mixture to precipitate the proteins and centrifuged at 3000 rpm for 7 min. The supernatant was separated and 1000 μl of isopropanol was added and the tube was inverted till the DNA was precipitated and centrifuged at 7000 rpm for 2 min. The precipitated genomic DNA was washed with 600 μl of 70% ethanol and allowed to air dry. The DNA was resuspended in TE buffer and stored at −20°C.

Electrophoretic analysis of genomic DNA: the isolated DNA was confirmed by 0.7% agarose gel electrophoresis [68, 69].

Reagents required: Tris-boric acid EDTA buffer (TBE), gel loading dye, ethidium bromide (ETBR).

Equipment required: electrophoresis tank, power pack, voltage (100 V), gel documentation apparatus, UV-transilluminator.

Principle: electrophoresis refers to the separation of macromolecules of different size by application of a constant electric field (100 V) onto the DNA fragments placed in a matrix of polymerized agarose. As the DNA molecule is negatively charged and travels toward the anode, it is loaded at the cathode end. The speed of migration of the fragments has an inverse relation with the size of DNA. The separated fragments are visualized by staining the gels with an intercalating dye (ethidium bromide), which fluoresces under UV light. Acrylamide gels are used for separation of small fragments of DNA (5–500 bp). Agarose gels can resolve DNA fragments varying in size from 200 bp to about 50 kb depending upon the concentration of agarose in the gel.

Procedure: electrophoresis tank was filled with the 1× TBE buffer and the gel was immersed into the tank containing the buffer. Agarose gel (0.7%) was prepared with ethidium bromide and the gel was allowed to run for 1 hour at 80–100 V as pulse voltage. 20 μl of DNA sample was loaded with loading dye (bromophenol blue) in the wells. When bromophenol blue dye reached three fourth of the gel length, the power was shut down, and DNA bands were observed using gel documentation apparatus and photographed.

4.1. Genetic analysis

4.1.1. Polymerase chain reaction (PCR) for PKD1 gene (C/T polymorphism)

Amplification of isolated DNA using the following primers 5′-AGCTGTACGCCCTCACTGG-3′ (forward) and 5′-GTGACAGGTGCCAGGACTC-3′-(reverse). PCR was performed using genomic DNA (50 ng), *Taq* polymerase (1 U), dNTPs (10 mM) and each primers (10 μM) [37, 69, 70].

PCR condition used:

Initial denaturation	94°C	5 min	
Denaturation	94°C	30 s	
Annealing	61°C	30 s	35 cycles
Extension	72°C	30 s	
Final extension	72°C	7 min	

The PCR product (298 bp) was confirmed by 1.8% of agarose gel electrophoresis. The amplified product was subjected to RFLP analysis.

4.1.1.1. Restriction fragment length polymorphism (RFLP)

Amplified PCR product is digested with restriction enzyme *AvaII*, incubated the reaction mixture at 37°C for 3 hours and inactivated by incubation at 64°C for 15 min. The enzyme cuts the sequence if "T" was at position 4058. The digested fragments (298, 225 and 73bp) were confirmed by 1.2% agarose gel [37, 69, 70].

Restriction fragment length polymorphism (RFLP)

Restriction site for *AvaII*	5′….G↓GWCG ….3′
	3′….CCWG↑G….5′

Source: *E. coli* strain that carries the *AvaII* gene from *Anabaena variabilis*

4.1.2. Polymerase chain reaction (PCR) for PKD2 gene (G/C polymorphism)

Amplification of PKD2 gene using the following primers 5′-CGCGCCGGACGCCAGTGACC-3′ (forward) and 5′-GCCGGCCGTTCTGGTTCGT-3′ (reverse). PCR was performed using genomic DNA (50ng), *Taq p*olymerase (1U) and dNTPs (10mM) [69, 71].

PCR condition used:

Initial denaturation	94°C	5 min	
Denaturation	94°C	30 s	
Annealing	61°C	30 s	35 cycles
Extension	72°C	30 s	
Final extension	72°C	7 min	

The PCR product (279 bp) is confirmed by 1.8% of agarose gel electrophoresis. The amplified product was subjected to RFLP analysis.

4.1.2.1. Restriction fragment length polymorphism (RFLP)

Amplified product is digested with restriction enzyme *BanII*, incubated the reaction mixture at 37°C for 2 hours and inactivated by incubation at 65°C for 20 min. The enzyme cuts the sequence if "C" was at position 28. The digested segments were confirmed by 1.2% agarose gel [69–71]).

Restriction site for *BanII*:	5′ G RGCY↓ C 3′
	5′ C↑YCGR G 5′

Source: *E. coli* strain that carries the cloned *BanII* gene from *Bacillus aneurinolyticus*.

Sequencing: PKD1 gene (C/T) and PKD2 gene (G/C) single nucleotide polymorphism was sequenced by automated sequencer (Chromous Biotech, Chennai).

Allelic frequency calculation: allelic frequency was calculated by using *Hardy-Weinberg Equilibrium*. The phenotype and genotypic frequencies in sexually reproducing, diploid organisms could be determined by applying simple algebraic expression.

$$p + q = 1 \quad (1)$$

where p is the frequency of dominant allele and q is the frequency of recessive allele.

Statistical analysis:

- Pearson Chi-square (χ^2) test was performed to find the statistical significance of genotypes and the gene frequency between the control group and ADPKD patients.
- Odds ratio (OR) was calculated for allelic frequency.
- Heterozygosity was calculated for the control subject and PKD patients.

5. Identifications of single nucleotide and polymorphisms and discussions

5.1. Genetic analysis

ADPKD is one of the most common genetic diseases in humans, affecting all ethnic groups with a prevalence of 1 in 500 to 1000 individuals [9, 18, 19, 72]. The disease is characterized by the progressive formation and enlargement of fluid-filled cysts in both kidneys due to mutations in PKD1 (85%), PKD2 (15%) and PKD3 (rare) that leads to renal failure [73]. Cyst development involves impairments in a wide range of cellular processes including increased proliferation of the renal epithelial cells, fluid transport defects, alterations in tubular basement membrane, altered cell polarity and increased apoptosis [9, 74].

Genomic DNA was isolated from frozen blood of control and ADPKD patients; it was confirmed by agarose gel electrophoresis (0.7%). After confirming the presence of genomic DNA, most of the prepared gDNA of the 260/280 ratio was found to be 1.8 or 1.9; in a few subjects, the DNA showed 2.0, which might be RNA or protein contamination. To avoid that, RNase, protease was added. Then, it was confirmed and used for PCRanalysis.

5.1.1. Analysis of C/T polymorphism in PKD1 gene

Prepared gDNA when subjected to polymerase chain reaction (PCR), 298bp fragment was obtained. The amplified PCR product was subjected to RFLP analysis using *AvaII* enzyme. When "T" allele was present at position 4058, 225bp and 73bp (homozygous mutant -TT), heterozygous mutant (CT) 298bp, 225bp and 73bp was obtained, and for homozygous normal allele (CC), 298bp fragments were identified **(Figure 5).** The PCR and RLFP products were detected and confirmed by 1.2% agarose gel electrophoresis.

5.2. Genotype and allelic frequency analysis of PKD1 (C/T) gene

The study group comprised 300 ADPKD patients and an equal number of age- and sex-matched control group. Among them, the C/C genotype was observed in 131 (43.67%) control group and in 58 (19.33%) ADPKD patients; C/T genotype in 82 (27.33%) control group and in 99 (33%) ADPKD patients; T/T genotype was found in 87 (29%) control group and in 143 (47.67%) ADPKD patients. The allelic frequency was calculated by using *Hardy-Weinberg equation* ($p + q = 1$) and the study group showed the mutant "T" allele frequency (0.642) to be significantly higher in ADPKD patients than in the control group (0.425) and the normal "C" allele frequency was observed to be significantly decreased in ADPKD patients (0.358) than in the control group (0.575). The significant difference ($P < 0.05$, 9.488, χ^2 calculated value = 14.048) (**Table 3**) was noted both in genotype and in allelic frequency between the ADPKD patients and control group among South Indian (Madurai) population by using chi-square (χ^2) test. This work, which coincides with the work done by Constantinides [70] among Caucasian and Japanese population, also has revealed the association of C/T4058 polymorphism with ADPKD. The PKD1 gene is responsible for causing autosomal dominant polycystic kidney disease and it has been recently cloned and sequenced [75]. ADPKD is reported to be a very

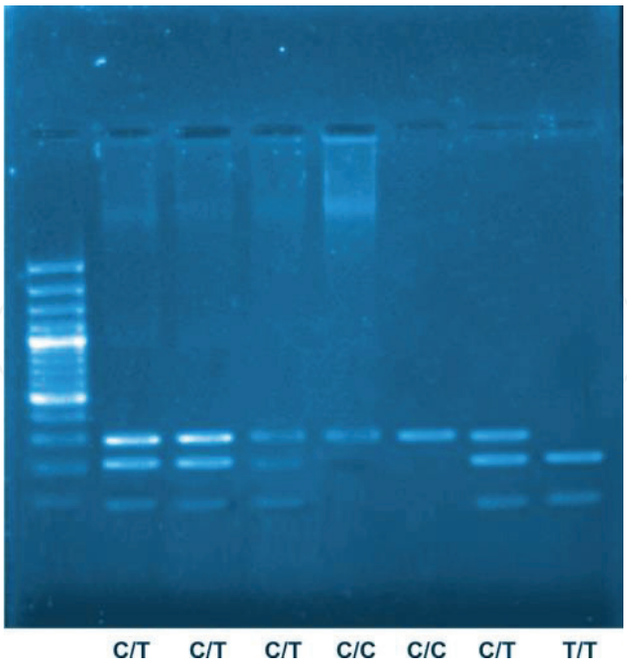

Figure 5. Confirmation of PKD 1 gene polymorphism using 1.2% agarose gel electrophoresis. T/T: homozygous mutant (225, 73 bp); C/T: heterozygous mutant (298bp, 225bp, 73bp) ; C/C: homozygous normal (298bp).

frequent disorder among Caucasian population with an estimated incidence of approximately 1:100. It has been shown to be characterized by genetic heterogeneity and three genes have been implicated in its pathogenesis called PKD1, PKD2 and PKD3 [76, 77].

The study found that the identification of DNA variation at nucleotide position at 12173 of PKD1 gene and C or T allele variation in the second position of amino acid codon at 4058 of polycystin-1 observed in 44 Japanese subjects, leading to suggest that these polymorphic alleles would be useful for linkage analysis only in specific ethnic groups [41]. It has been also reported that the PKD2 gene provides instructions for making a protein called polycystin-2, which is found in the kidneys before birth and in many adult tissues. It is also stated that the polycystin could be regulated by a larger and somewhat similar protein called polycystin-1, which is encoded by PKD1 gene [78].

5.2.1. Analysis of G/C polymorphism in PKD2 gene

Prepared gDNA was subjected to polymerase chain reaction (PCR) and 279bp fragment was amplified. The amplified PCR product is digested with the enzyme *Ban II*. The enzyme acts on the "C" variation but not on the "G" variation. If a "C" allele was present at position 83, 170bp and 109bp were obtained. If it was a homozygous mutant (CC), 170bp and 109bp; heterozygous mutant (GC), 279bp, 170bp and 109bp and homozygous normal (GG), 279bp fragments were identified**.** One such variation was at position 83 of PKD2, which was occupied by either G or C at exon 1. Hence, the amino acid residue was changed from arginine to proline.

The study also found that *BSP12861* restriction enzyme *also* acts on the "C" variation. This enzyme was added to 10 ADPKD patients of amplified PKD2 gene product (279bp). The

results showed to be like *Ban II* restriction digestion gene products. If a "C" allele was present at position 83, 170bp and 109bp were obtained. If it was homozygous mutant (CC), 170bp and 109bp; heterozygous mutant (GC), 279bp, 170bp and 109bp and homozygous normal (GG), 279bp fragments were identified **(Figure 6).** The study was supported by the work of Koptides et al., [30]. Koptides et al. demonstrated that both G/C transversion mutation and six 'Cs" insertion mutation in exon 1 of the PKD2 gene of three separate cysts. This mutation is expected to cause a translation frameshift, leading to the incorporation of 20 novel amino acids before a new stop codon is encountered.

5.3. Genotype and allelic frequency analysis of PKD2 (G/C) gene

The G/G genotype was observed in 137 (45.67%) control group and in 55 (18.33%) ADPKD patients, G/C genotype in 84 (28%) control group and in 93 (31%) ADPKD patients and C/C genotype in 79 (26.33%) control group and in 152 (50.67%) ADPKD patients. The allelic frequency was calculated by using *Hardy-Weinberg equation* ($p + q = 1$) and the study group showed the mutant "C" allele frequency (0.662) to be significantly higher in ADPKD patients than in the control group (0.403) and the normal "G" allele frequency to be significantly decreased in ADPKD patients (0.338) than in the control group (0.597). Significant difference ($P < 0.005$, 14.860, χ^2 calculated value = 20.451) (**Table 4**) was noted in genotype and allelic frequency between the ADPKD patients. G/C polymorphism at position 83 in exon 1 of PKD2 gene among South Indian (Madurai) population with ADPKD revealed the "CC" and "GC" genotype and the frequency of "C" allele was found to be significantly higher in the ADPKD patients compared to the control group. The study has revealed higher frequency of "C" allele and lower frequency of "G" allele in ADPKD patients. These results coincide with the work of Koptides et al., [30], who identified a polymorphism at position 83, which was occupied by either G or C encoding either arginine or proline (R28P).

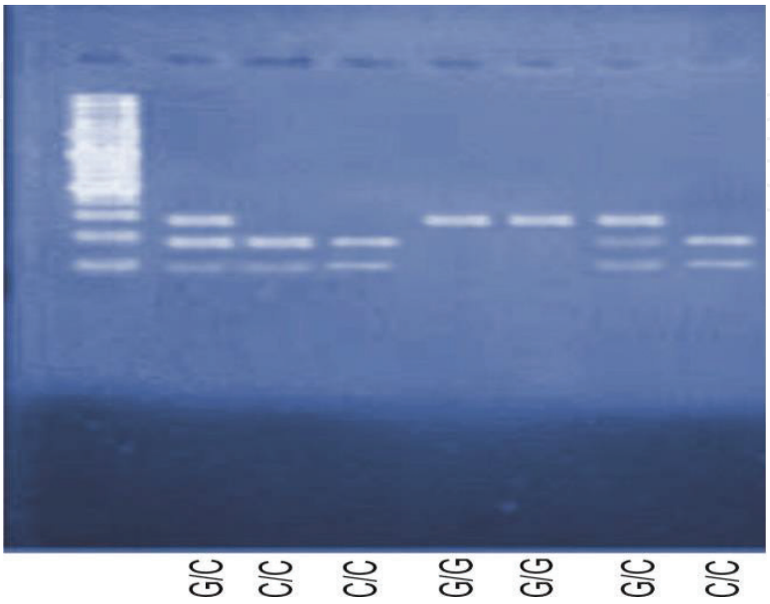

Figure 6. Confirmation of PKD 2 gene polymorphism using 1.2% agarose gel electrophoresis. C/C: homozygous mutant (170 bp, 109 bp); G/C: heterozygous mutant (279 bp, 170, 109); G/G: homozygous normal (279 bp); Lane 1: ladder (100 bp).

	Genotype			Allele frequency		χ^2 value	*p* value
	T/T	**C/T**	**C/C**	**T**	**C**		
Control group **N = 300**	87 (29%)	82 (27.33%)	131 (43.67%)	0.425	0.575	14.16	P < 0.05 9.488
ADPKD patients **N = 300**	143 (47.67%)	99 (33%)	58 (19.33%)	0.642	0.358	13.93	

T/T: homozygous mutant; /T: heterozygous mutant; C/C: homozygous normal.

Table 3. Comparison of genotype and allelic frequency of PKD1 gene in control group and ADPKD patients among South Indian (Madurai) population.

	Genotype			Allele frequency		χ^2 value	*p* value
	C/C	**G/C**	**G/G**	**C**	**G**		
Control group **N = 300**	79 (26.33%)	84 (28%)	137 (45.67%)	0.403	0.662	20.79	*P* < 0.005, 14.860
ADPKD patients **N = 300**	152 (50.67%)	93 (31%)	55 (18.33%)	0.597	0.338	20.10	

C/C: homozygous mutant; G/C: heterozygous mutant; G/G–homozygous normal.

Table 4. Comparison of genotype and allelic frequency of PKD2 gene in control group and ADPKD patients among South Indian (Madurai) population.

5.4. PKD1 (C/T) and PKD2 (G/C) SNP sequencing

The PKD1 (C/T) and PKD2 (G/C) single nucleotide polymorphism was also confirmed by sequencing the PCR amplified gene products of PKD1 and PKD2.

PKD1 (C/T) – Ala/Val. 4058 in Exon 45:

Ala

A. 5′-AAG CTG TAC G**C**C CTC ACT GG-3′—Wild type Allele

Val

5′-AAG CTG TAC G**T**C CTC ACT GG-3′—Mutant Allele

PKD2 (G/C) – Arg/Pro.28 in Exon 1

Arg

B. 5′-CG CGC C**G**G ACG CCA CTG ACC-3′—Wild type Allele

Pro

5′-CG CGC C**C**G ACG CCA CTG ACC-3′—Mutant Allele

Underlined sequence denotes change in allele leads to new amino acid formation, which is known to be polymorphism.

The study coincides with the work of Constantinides et al. [70], Watnick et al. [79] and Koptides et al., [30] in Caucasians, Greek-Cypriot populations. The present study reveals that these mutation/polymorphism leads to evolution of new alleles and formation of new amino acids among South Indian population.

6. Conclusion

Polymorphic DNA markers could be used for presymptomatic and prenatal diagnosis of ADPKD. Breuning et al. [80] and Balcells and Criach [81] recommended that prenatal diagnosis of PKD by chorionic villi sampling and linkage phase of the DNA markers has been established by haplotyping the index family. This testing offers the chance of performing prenatal or preimplantation testing in families with severe cases of the disease. Hence the current study suggests that genetic testing is very important in determining the severity and progression of the disease and could possibly be treated with effective drug and delay the end-stage renal disease (ESRD). Further research of this study is on DNA based on drug design using bioinformatics databases that might help the physicians in providing better treatment for polycystic kidney disease patients.

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