ISSN: 2320 - 4230

Journal of Drug Discovery and Therapeutics 1 (5) 2013, 37-41

RESEARCH ARTICLE

EVALUATION OF PKD2 GENE (G/C) POLYMORPHISM IN PATIENTS WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE AMONG SOUTH INDIANS (MADURAI).

***P. Veeramuthumari, K. Srividhya, W.Isabel.**

PG & Research Department of Zoology & Biotechnology, Lady Doak College, Madurai, Tamil Nadu, India.

ABSTRACT

Polycystic kidney disease (PKD) is the leading causes of end-stage renal failure (ESRD) and a common indication of dialysis / renal transplantation. Autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD) are the two forms of PKD. ADPKD is one of the most common monogenic inherited disorders with an approximate frequency of 1:500 individuals in different population. The PKD1 and PKD2 are on chromosomes 16p13.3 and 4q 13-23 and the most common cause of ADPKD is mutation in PKD1 and PKD2 genes. Hence the study aimed to analyze PKD2 (G/C) polymorphism. The study comprised of 50 ADPKD patients and 50 age, sex matched healthy individuals as a control subjects were selected among South Indian (Madurai) population. Single nucleotide polymorphism (SNP) study was carried out by isolating DNA which was subjected to PCR and RFLP analysis. The results showed to be G/C polymorphism at position 83 in exon 1 of the PKD2 gene among South Indian (Madurai) population with ADPKD and it also revealed that the "CC" "GC" mutant genotype and mutant "C" allelic frequency were found to be higher in patients (0.65) than in control subjects (0.38). The statistical analysis (chi – square test) showed that the frequency of mutant allele was significantly (at $p<0.05$) higher in ADPKD patients when compared to control subjects. Therefore, the current study found that an association of PKD2 gene polymorphism and ADPKD among South Indian (Madurai) population.

KEY WORDS: PKD 1 gene, allelic frequency, genotype, ARPKD, polycystin.

INTRODUCTION:

Polycystic kidney disease (PKD) is the most common genetic, life threatening disease, affecting more than 12.5 million people worldwide. It also has been reported that 21, 14, 478 persons were affected by PKD among Indian population (1). Therefore, the current study is to be focused on polycystic kidney disease. There are two forms of PKD, i) Autosomal recessive polycystic kidney disease (ARPKD) and ii) Autosomal dominant polycystic kidney disease (ADPKD). ADPKD occurs in worldwide and in all races. ADPKD disease is one of the most commonly inherited conditions in human with an incidence of 1:500 to 1:1000 compared to ARPKD (2) (3). It is genetically heterogeneous with two genes identified, PKD1 (16P13.3) and PKD2 (4q21) (4) (5). The proteins encoded by the PKD1 and PKD2 genes are polycystin 1 and polycystin 2, interact with each other in the primary cilia of renal epithelial cells and participate in complex signal transduction pathways, which seems to be involved in chemosensory/mechanic functions and has some role in cell proliferation and maturation (5). Linkage analysis studies have revealed that the mutation of PKD1 is responsible for 85%, whereas mutation in PKD2 15% of the familial ADPKD (6) (7). Several research reports were stating that, gene polymorphism either in PKD1 or PKD2 leads to ADPKD. Hence, our study also focused on PKD2 gene.

The PKD2 gene is located on the long (q) arm of chromosome 4 at position 22.1. More precisely, the PKD2 gene is located from base pair 88,928,798 to base pair 88,998,930 on chromosome 4. It encodes a 5.3kb mRNA transcript that is translated into a 968 amino acid protein. Polycystin-2 is a protein that in humans is encoded by the *PKD2* gene (8) (9). The protein may be an integral membrane protein involved in cell-cell/matrix interactions. Transmembrane protein polycystin 2 (TRPP2) may function in renal tubular development, morphology, and function, and may modulate intracellular calcium homoeostasis and other signal transduction pathways. TRPP2 interacts with transmembrane protein polycystin 1 (TRPP1) to produce cation-permeable currents.

The common complications includes Hypertension, Urinary tract infection, Renal calculi, Cardiac valve abnormalities, Hernia of the anterior abdominal wall, Diabetes, Cerebral berry aneurysms (10). Systemic hypertension is also very common occurring in more than 75% of the renin-angiotensin system. The End stage renal disease usually occurs within 5 to 10 years after development of renal failure. ADPKD patients were found to be developing ESRD in the mean age of 75.5 years (11).

* ***Corresponding author:*** *P. Veeramuthumari* ***/ Email:*** *muthusdream@gmail.com*

Therefore the study focused on association of PKD2 gene polymorphism and autosomal dominant polycystic kidney disease in ADPKD patients and control group in the age group of 10-80 years among South Indian (Madurai) population.

MATERIALS AND METHODS:

In the preliminary study undertaken by us, a hundred clinically proven Autosomal dominant polycystic kidney disease (ADPKD) patients within the age group of 10-80 years were selected. Blood samples and related data were collected from the Department of Nephrology, Madurai Rajaji hospital, Madurai Kidney transplantation and Research Centre Madurai (TN). The age and the sex matched healthy individuals as a control subjects were selected from the general population. The blood samples were collected in EDTA coated tubes, and were stored at 4^{o}C.

The study was carried out with the permission of Institutional biosafety ethical clearance committee (IBSE), Lady Doak College and also it got Ethical clearance committee certificate from Government Rajaji hospital, Madurai (TN).

GENOTYPING OF PKD 2 GENE G/C POLYMORPHISM:

Genomic DNA was isolated from all the healthy individuals and ADPKD patients from peripheral red blood cells (12, 13). The presence of DNA was confirmed with 0.7% Agarose gel electrophoresis and the amount of DNA was quantified using UV-Spectrophotometer (Systronics). PCR-based restriction enzyme analysis was performed in the isolated DNA.

POLYMERASE CHAIN REACTION (PCR) ANALYSIS (13- 15):

The DNA sequence (279 bp) of PKD 2 gene was amplified by PCR. The genomic DNA (0.2ng) was incubated in a total reaction volume of 50µl containing 10 pmol of both forward primer 5'-TGA GCT CCG TGG GCG CGC GGA GCC-3' and reverse 5'- CTG GGC TGG GGC ACG GCG GG -3' primer (Fermentas Life Sciences) for the PKD 2gene G/C Single nucleotide polymorphism(SNP) (G83C), using 2.5 units of *Taq DNA polymerase* (Bangaloe genei, India) and dNTPs (200µM). Amplification for the PKD2 gene G83C SNP was performed with an initial denaturation of 94^{0}C for 5 min PCR thermsl cycler. The PCR amplification conditions were as follows: 30 cycles consisting of 30s denaturation at 94^{0}C, 45s annealing at 61^{0}C, 30s extension at 72^{0}C. The PCR amplified product was confirmed by 2% agarose gel electrophoresis under UV-transilluminator (13).

RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) ANALYSIS (13- 15):

The PKD 2 G83C SNP creates *a Bsp12861* (Fermentas Life Sciences, Germany) restriction enzyme recognition sequence site. The SNP was detected by digestion of PCR amplified product (10µl) with *Bsp12861* (1.0µl) for 3hrs at 37^{0}C and inactivated by incubation at 65^{o}C for 15 min (13). Restriction fragment size was performed by visualization of digested PCR product by 2% agarose gel electrophoresis.

STATISTICAL ANALYSIS:

Allelic frequency was calculated by using Hardy-Weinberg equilibrium. p + q=1, p = frequency of dominant allele, q = frequency of recessive allele.

The significant level of genotype and allelic frequency was tested by chi square (χ^2) test. Odds ratio at 95% confidence intervals were calculated.

RESULTS AND DISCUSSION:

The study it was observed that (50%) males and (50%) females were equally affected by ADPKD and most of the patients were found to be in the age group of 30-50 years. This might be due to the gene inheritance in both the sexes equally and some of the normal individuals are also affected by ADPKD, it might be a follow up of simple Mendelian co-inheritance (14). Autosomal dominant polycystic kidney disease most often initially presents in adults aged 20-40 years (16). The study also showed that 72% of the patients have high blood pressure (Hypertension) and also ADPKD patients were prone to have common associated complications like hematuria (12%), renal calculi (10%), urinary tract infection (13%) diabetic nephropathy (17%) cardiovascular problems (21%), renal osteodystrophy (13%) and anemia (14%) **(Table: 1)**. The complications were noted in all patients compared to control subjects, which might be due to changes in the levels of Lipid profile (total cholesterol, triglycerides, variable low density lipoprotein, low density lipoprotein) and trace elements like calcium, sodium, potassium, iron, manganese, zinc, selenium (17, 18). Several study reported on PKD2 gene polymorphism, C/G insertion, C/T substation, deletion) among Caucasian, spain and Netherland population (Koptides *et al.* 1999, Hateboer *et al*., 2000)

In the study the amplified PCR product (279bp) was digested with BSP12861. The restriction enzyme acts on the "C" variation, but not on the "G" variation. The presence of "C" allele at position 83 creates restriction sites for Bsp12861, thereby resulting in either 3 fragments (279bp, 170bp, 109bp – Heterozygous mutant) or 2 fragments (170bp, 109bp – Homozygous mutant). If there is no transversion of "C" allele at position then the restriction site for BSP12861 is abolished, as a results of

which there is no change of the PCR product (279bp – wild type – Homozygous).

The allelic frequency was calculated by using Hardy-Weinberg equation and the study group showed the mutant "C" allelic frequency (0.65) to be significantly higher in ADPKD patients than in control subjects (0.38). There was a significant difference (P=0.001 at 5%) was found in genotype and allelic frequency between the ADPKD patients and control group between the control subjects and ADPKD subjects in both genotype and allele frequency among South Indian (Madurai) population (**Table: 2).** Koptides *et al.,* (1999) (14) work also identified this polymorphism at position 83 among Caucasians which was occupied by either G or C encoding either arginine or proline (R28P) this polymorphism enabled us to verify the disease was co-inherited with allele "C". The calculated odds ratio (OR) showed that individuals with mutant genotype (G/C, C/C) have 3.6 fold risk for Autosomal dominant polycystic kidney disease (95% confidence interval = 1.630 – 6.532)

Table 1: ADPKD associated complications in ADPKD patients among South Indian (Madurai) population.

Complications	Occurrence of frequency in percentage
Hematuria	12%
Renal calculi	10%
Urinary tract infection	13%
Diabetic nephropathy	17%
Arthrosclerosis	21%
Anemia	14%
Osteodystrophy	13%

Table 2: Prevalence of genotype and allelic frequency of PKD2 (Arg/Pro 83 G/C) in ADPKD patients and control subjects among South Indian (Madurai) population:

Genotype	Control subjects (n = 50)	ADPKD subjects (n = 50)
C/C Homozygous mutant	10 (20%)	23 (46%)
G/C Heterozygous mutant	18 (36%)	19 (38%)
G/G Homozygous normal	22 (44%)	8 (16%)
Allele frequency		
C (Mutant)	0.38	0.65
G (Normal)	0.62	0.35

(at $p<0.001$, $p<0.05$ significant)

Table 3: International reports on PKD1 & 2 gene polymorphism in different population:

Author and Year	gene	Population	Mutation
Xenophontos *et al.,* (1997) (19)	PKD2	Cyprus	SSCP (C insertion) Frameshift mutation
Pei *et al.,* (1998) (20)	PKD2	Canadian Kinderds	Frameshift mutation
Watnick *et al.,* (1998) (21)	PKD1	Maryland population	Polymorphism
Kopides *et al.,* (1999) (14)	PKD2	Caucasians	C/G insertion polymorphism
Constantinides *et al.,* (1999) (22)	PKD1	Caucasians, Greek Greek-Cypriot	A/G polymorphism C/T polymorphism
Torra *et al.,* (1999) (23)	PKD2	Spain	Nonsense mutation, Frameshift, Missense, polymorphism in exon 1
Hateboer *et al.,* (1999, 2000) (24, 25)	PKD2	Spain, Netherlands, UK, Bulgaria, Australia	C-T substitution, deletion, Nonsense mutation, Frameshift, Missense, Splice mutation.

Koptides *et al.,* (2000) (26)	PKD1 PKD2	Cyprus	Mutation in exon 24 Mutation in exon 1
Katja Vouk *et al.,* (2006) (27)	PKD1 PKD2	Slovenia	Frameshift/ Missense mutation Nonsense mutation

The distribution of genotypes (G/C, C/C, G/G) for PKD2 gene polymorphism varies among different populations (Caucasian, Japanese, Canadian, Cyprus, Spain and UK populations) (19-26). Till date, there is no study available for the prevalence of PKD2 gene polymorphism among ADPKD patients in South Indian population.

Susceptibility to ADPKD has significant genetic components. The PKD2 gene provides instruction for making a protein called poolycystin-2 this protein is found in the kidneys before birth and in many adult kidneys. Polklycystin-2 would be functioning as a channel spanning the cell membrane of the kidney cells. In conjugation with polycystin-1, the channel transcripts positively charged atoms (ions) particularly calcium ions into the cells. The influx of calcium ions triggers a cascade of chemical reactions inside the cell that might instruct the cell to undergo certain changes polycyustin-1 and polycystin-2 work together to help regulate cell growth and division, cell movement and interaction with other cells. The interaction of polycystin-1 and polycystin-2 in the renal tubules promotes a normal development and function of the kidneys (27). Hence, the study associating PKD2 gene polymorphism and autosomal polycystic kidney disease was conducted among South Indian (Madurai) population.

Therefore, in accordance with the previously published reports the present study also demonstrated that an association between the PKD2 (mutant genotype) gene polymorphism and autosomal dominant polycystic kidney disease among South Indian (Madurai) population The future study will be focused on PKD1 and 3 gene polymorphism and sequencing the same population and further research on the DNA based drug design by using bioinformatics databases which might help the physicians in providing the better treatment for polycystic kidney disease patients.

REFERENCES:

1. Nagpal S, Pankaj (2009): Polycystic kidney treatment Inbdia, Polycystic Kidney Surgery hospital, India.
2. Persu A, Stoenoiu T, Messiaen S (2002): Modifier effect of ENOS in autosomal polycystic kidney disease. *Hum Mol. Genet* 11:229-241.
3. Grantham JJ and Calvet PJ (2001): Polycystin-2 the protein mutated in autosomal dominant polycystic kidney disease (ADPKD), is a Ca2+- permeable non selective cation channel. *Proc Natl Acad Sci USA* 98 (3): 790-792.
4. *R*advind D, Walker R, Gibson R,Foresst S, Richard R, Friend K, Shieffied L, Kincaid Smith A,Dank D(1992): Phenotype and genotype heterogenecity in Autosomal dominant polycystic kidney disease. *Lancet* 340; 1330-1333.
5. Igarishi P, Somlo S (2002): Genetics and pathogenesis of Polycystic Kidney Disease. *J Am Soc Nephrol* 13:2384-2398.
6. European polycystic kidney disease consortium: The polycystic kidney disease 1 gene encodes a 14kb transcript and lies within a duplicated region on chromosome 16.
7. Gogusev J, Murakami I, Doussau M, Telvi L, Stojkoski A, Lesavre P, Droz D (2003)- Molecular cytogenic aberration in Autosomal dominant polycystic kidney disease tissue, J Am Nephrol 14:359-356.
8. Hayashi T, Mochizuki T, Reynolds DM, Somlo S (1992): Characterization of Autosomal Dominant Polycystic Kidney disease. *Science* 272: 1339 – 1342.
9. Yoder BK, Hou X, Guay- Woodford LM (2002): The polycystic kidney disease proteins, polycystin-1,polycystin-2, Polaris,cystin co-localized in renal cilia, *J Am Soc Nephrol* 13:2508-2516.
10. Hateboer N, Veldhusen B, Peters D, Breuning MH, Dijk MA, Afzal AR, Jeffery S, Saggar AK, Torra R, Dimitrakov D, Manitez I, Sanz S, Krawczak M, and Ravine D (2000): Locations of mutations within the PKD2 gene influences clinical outcome *Kidney int* 57: 1444 - 1451.
11. Torres VE, Harris PC (2007): Polycystic kidney disease, genes, proteins, animal models, disease mechanism and therapeutic oppurtunities *J. int med* 10:1365-2789.
12. Sambrook J and Russel DW (1993), Molecular cloning, a laboratory manual, III Edition, Blackwell publisher, A16.
13. Veeramuthumari P, Isabel W, Kannan K (2011): A atudy on the level of T_3, T_4, TSH and the association of A/G polymorphism with CTLA-4 gene in Graves' hyperthyroidism among south Indian population.
14. Koptides M, Hadjimichael C, Koupepidou P, Pierides A, Constantinou Deltas C (1999): Germinal and somatic mutatiin the PKD2 gene of renal cysts in abnormal dominant polycystic kidney disease. *Hum Mol Genet* 8(3):509-513.
15. Chauvet V, Quian F, Boute N (2002): Expression of PKD1 and PKD2 transcripts the proteins in human

embryo and during normal kidney development. *Am J Pathol* 160:973-983.

16. Verghese P, Jordan M, Henrique S, Lederman M, Peter J, H (2008) : Polycystic Kidney Diseases- department of nephrology, University of Washington and University of Pennsylvania WWW.emedicinespecialtus >pediatrics:General medicine > Nephrology.
17. Dumm NT, Giammona AM, Touceda LA and Raimondi C (2003): Lipid abnormalities in chronic renal failure patients undergoing hemodialysis**.** *Lip and health des* 2:1-6.
18. Dumm NT, Giammona AM, Touceda LA and Raimondi C (2001): Lipid abnormalities in chronic renal failure patients undergoing hemodialysis. *Medicina (Buenos Aires)* 61: 142-146.
19. Xenophontos S, Constantinides R, Hayashi T, Mochizuki T, Somlo S, Pierides A, and Delta CC (1997): A translation frameshift mutation induces by a cytosine insertion in the Polycystic Kidney Disease 2 gene (PKD2). *Hum Mol Genet* 2:949 – 953.
20. Pei Y, Wang K, Kasenda M, Paterson AD, Chan G, Liang Y, Roscoe J, Brissenden J, Hefferton D, Parfrey P, Somlo S, ST. George – Hyslop P (1998): A spectrum of mutation in polycystic kidney disease – 2(PKD2) gene from eight Canadian kindred. *J Am Soc Nephrol* 9:1853 – 1860.
21. Watnick T, He N, Wang K, Liang Y, Parfrey P, Hefferton D, St George-Hyslop P, Germino G, Pei Y (2000): Mutations of *PKD1*in ADPKD2 cysts suggest a pathogenic effect of trans-heterozygous mutations. Nat Genet 25:143–144.
22. Constantinides R, Xenophontos S, Nepytou P, Nomura S, Pierides A, Constantinou C (1999): New amino acid polymorphism:Evolution OF alleles. *Hum Genet* 99:644-647.
23. Torra R, Viribay M, Tellaria D, Badenas C, Watson M, Harris P, Darnell A, San Millan JL(1999): Seven novel mutations of the PKD2 gene families with autosomal dominant polycyctic kidney disease. *Kidney international* 56:28 – 33.
24. Hateboer N, Dijk MA, Bogdanova N (1999): Comparison of phenotypes of polycystic kidney disease types 1 and 2. *Lancet*: 353**:** 103–07.
25. Koptides M, Mean R, Demetriou K, Pierides A, Deltas CC (2000): Genetic evidence for a trans-heterozygous model for cystogenesis in autosomal dominant polycystic kidney disease. Hum Mol Genet 9:447–452.
26. Hateboer N, Veldhusen B, Peters D, Breuning MH, Dijk MA, Afzal AR, Jeffery S, Saggar AK, Torra R, Dimitrakov D, Matinez I, Sanz S, Krawczak M, and Ravine D (2000): Location of mutations within the PKD2 gene influences clinical outcome. *Kidney Int* 57: 1444-1451.
27. Vouk K**,** Strmecki L**,** Stekrova J**,** Reiterova J**,** Bidovec M**,** Hudler P**,** Kenig A**,** Jereb S**,** Zupanic-Pajnic I**,** Balazic J**,** Haarpaintner G**,** Leskovar B**,** Adamlje A**,** Skoflic A**,** Dovc R**,** Hojs R and Komel R(2006): PKD1 and PKD2 mutations in Slovenian families with autosomal dominant polycystic kidney disease. *BMC Medical Genetics*, **7**:6.
28. Foggensteiner L, Beven AP, Thomos R, Boulter C, Bradley J, Klinger K, Sandford R (2000): Celular and sub cellular distribution of Polycystin-2, the protein product of PKD gene*Am Soc Nephrol* 11:814-827.